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Letters

Sodium Azide Deemed Unnecessary for Evaluating Proteins by Coomassie Blue Dye-Binding Method

Our method for the use of Coomassie Blue G dye binding to evaluate heat-damage to protein (Kratzer et al., 1990a) was based upon the method of Bradford (1976) with the buffer which contained 0.2g of sodium azide per liter, as described by Read and Northcote (1981). Sodium azide is reported to be an explosive chemical which is dangerous to handle and is not available in many laboratories which have been interested in using this method to evaluate proteins. For this reason, the necessity of using sodium azide in the buffer has been questioned.

A sample of NaCl/P_i buffer was prepared with Na_2HPO_4 2.0 g/L, KH_2PO_4 0.6 g/L, and NaCl 7.0 g/L with and without the addition of sodium azide 0.2 g/L. These buffers were held in the laboratory at room temperature and compared at various times over a 10-month period to evaluate samples of soybean meal as described by Kratzer et al. (1990b). The average values obtained for 10 samples by using the two different buffers agreed within 2% and there was no evidence of change in the response with time.

We suggest that the use of sodium azide in the buffer is *not* necessary and that in the interests of safety it not be used.

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Read, S.M. and Northcote, D.H. 1981. Minimization of variation in the response to different proteins of the Coomassie Blue G dye-binding assay for protein. Anal. Biochem. 116: 53–64.

—F. H. Kratzer and P. Vohra, Dept. of Avian Sciences, Univ. of California, Davis, CA 95616

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Memo *FROM THE SCIENTIFIC EDITOR*

It's Not Easy—But Worth It!

Getting published by our Journal, that is! Over the past year about one of every two manuscripts submitted to our editorial and review process was finally accepted and printed. Our current acceptance rate is about 45%. This is primarily due to the thorough operation and effectiveness of our Scientific Editors and our peer review system which is just completing its third year. The system is based on having an Associate Scientific Editor for specific discipline/commodity areas and it has brought another facet of expertise to our overall scientific review system.

In effect, besides having two peer scientists review each prospective manuscript, a manuscript is also closely scrutinized by an Associate Scientific Editor who has specific expertise in the field of the study of that manuscript. That expertise also results in a much higher likelihood that each manuscript will be reviewed by an expert in its subject matter, due to the Associate Editor's personal knowledge and acquaintance with experts in fields closely related to their own. That in turn leads to more thorough and effective reviews. We believe it shows in the high quality of the final product. Look over this issue and judge for yourself.

We know that a principal and critical element of scientific publication is the time it takes to get into print. That has also been shortened thanks to our effective editorial/reviewer system. Our manuscripts now average about 9 months from date of first submission to appearing in print. We have a few that are in print within 20 to 24 weeks and a few that may take as long as 18 months. The latter are the exceptions. You can help us shorten this time and be more efficient in this respect. Our recent studies of the review system have shown the main cause of delay in publication is UNNOTICED DELINQUENT REVIEWERS! We try to follow up on review assignments and contact and remind reviewers when they are late. Unfortunately, every now and then, one slips by without being noticed. There is no substitute for conscientious, diligent, on-time reviews. Many of you have been called upon in this respect and most of you have been outstanding in your responsiveness. If you consider it as one of your professional duties, as most of you seem to, then it just becomes another element of your job.

We also know there are controversial issues related to the anonymous scientific peer review system, but it seems to be the best that is available. We have had a few instances in the past year or so, of authors accusing reviewers of being unduly biased. In several specific instances authors have advised us of certain individuals (or institutions) whom they thought had served as reviewers of their work and had been unfairly biased because they were doing related work and wished to suppress the reports of others in that field. In every case, we found they were wrong about both the reviewer and the institution they suspected. Of course at times this may be an attempt of the authors to avoid the embarrassment of a rejection and uphold their professional pride. By far the great majority of you have shown a strong sense of professionalism, pride, dignity and integrity in constructive reviews. We couldn't achieve and continue the high quality of scientific publication we have reached without that fact. Thanks to all of you on the accompanying list who have been so helpful to us and to your fellow scientists over the past year.

—*Robert E. Berry*, Scientific Editor
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Microbial and Oxidative Changes in Low-Fat Ground Beef During Simulated Retail Distribution

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ABSTRACT

A low-fat ground beef product containing water, carrageenan, encapsulated salt and hydrolyzed vegetable protein (carrageenan-based) was compared to a low-fat, all-beef control during simulated commercial manufacturing and retail distribution. Few differences were found in oxidative stability and microbial growth of the two. No differences ($P > 0.05$) were found in aerobic plate counts, populations of psychrotrophs or mold and yeast populations for the two products. Hunter color 'a' values (redness) and overall color scores for surface color were higher ($P < 0.05$) for the carrageenan-based product than the all-beef product. Retail display of low-fat products in aerobic packaging resulted in greater ($P < 0.05$) product discoloration than anaerobic retail display.

Key Words: beef, microbes, oxidation, color, storage, distribution

INTRODUCTION

MANY CONSUMERS are concerned about the amount of fat in their diets (Yankelovich, 1985) and limit their dietary intake of fat and calories (Burke, 1987). They eliminate those perceived as being higher in fat, such as ground beef. Research efforts have focused on development of low-fat ground beef products which have sensory properties similar to higher fat products. Through such research efforts, technology has been developed for the production of low-fat ground beef products designed for the health conscious segment of the population. Products from such technology have sensory properties similar to higher fat (20% fat) ground beef products. The technology employs the use of flavor enhancers (encapsulated salt and hydrolyzed vegetable protein), carrageenan and added moisture for enhancement of sensory properties of low-fat ground beef (Egbert et al., 1991).

No research has focused on determining the refrigerated storage stability of such low-fat ground beef products manufactured using the carrageenan-base. It has been suggested that the addition of 10% water to the carrageenan-based, low-fat ground beef product might result in decreased stability of such products under refrigerated conditions encountered during normal retail distribution.

Ground beef may be coarsely or finely ground and then packaged in oxygen impermeable films for refrigerated distribution. This method of packaging increases the storage stability and may encourage lactic acid-producing bacterial growth (Jaye et al., 1962; Roth and Clark, 1972). The finely ground products are marketed as "chub" packs in the original oxygen impermeable packaging. In contrast, the coarsely ground products are more finely ground at retail and repackaged in oxygen permeable films. Under such conditions, the finely ground product develops the bright cherry-red color consumers associate with freshness. This procedure is designed to enhance the red oxymyoglobin color rather than extend shelf-life. The aerobic conditions produced by grinding and oxygen permeable packaging enhances the growth of proteolytic microflora, such as *Pseudomonas* spp., often resulting in more rapid product spoilage (Harrison et al., 1981; Pierson et al., 1970). This aerobic environment can also promote more rapid myoglobin and lipid oxidation.

Our objective was to determine the effect of low-fat ground beef production using water, carrageenan, salt and hydrolyzed vegetable protein on the microbial growth and oxidative (color and lipid) stability of commercially manufactured low-fat ground beef during simulated retail distribution.

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MATERIALS & METHODS

MEAT PROCESSORS employ two packaging systems for the distribution of ground beef: (1) case-ready, retail chub packaging (chub pack); and (2) coarse ground product distributed in "keeper" casings, finely (0.32 cm plate) ground at the retail outlet and merchandised in white plastic foam trays with oxygen permeable film overlay. For our study, two low-fat ground beef products were commercially manufactured and packaged using the two systems. The products were then placed in conditions to simulate retail distribution and finally evaluated for microbial growth and oxidative stability after such simulated retail display.

Commercial manufacture

Low-fat (8% fat) ground beef and a low-fat, carrageenan-based ground beef product (Egbert et al., 1991) were manufactured and packaged at a commercial grinding operation (Miller Meat Co., Chino, CA). Low-fat ground beef products were manufactured from 95% lean beef trimmings. The carrageenan-based product was formulated to contain 10% water, 0.5% carrageenan (Viscarin SD 389, FMC Corp., Philadelphia, PA), 0.4% encapsulated salt (150-85 TFC, Van den Bergh Food Co., Lisle, IL) and 0.2% hydrolyzed vegetable protein (A.C. Legg Packing Co. Birmingham, AL). For each of three replications, the lean beef trimmings were coarsely ground (1.27 cm plate) using a fresh/frozen block grinder (Weiler, Model 1109, Chino, CA). Coarse ground lean trimmings were transferred to a ribbon blender (Food Processing Equipment Co., Cerritos, CA) and blended for about 5 min. Nonmeat ingredients, used in manufacture of the carrageenan-based, low-fat product were incorporated with the coarse ground trimmings during the blending process. After blending, about half of each of the blended coarse-ground products were further processed and packaged as follows: (1) ground through a 0.64 cm grinder plate and packaged (Hanetmann Stuffer, Model VF20, T.W. Kutter, Boston, MA in combination with a Kartridge Pak machine, Model 42 chub maker, Davenport, IA) as coarsely ground product in oxygen-impermeable (oxygen transmission rate < 10 cc/M^2/24 hr/atm at 25°C) film ("keeper" casings, after refrigerated storage to be finely ground and packaged in oxygen permeable packaging for retail display) and/or (2) ground through a 0.32 cm grinder plate and packaged as finely ground product in oxygen-impermeable film (chub pack, no further processing, retail display in anaerobic chub packaging).

After packaging, all products were crust frozen (internal product temperature about -5°C) in a blast freezer (12 hr at -25°C), packed in dry ice and shipped overnight to the Auburn University Meat Science Laboratory. Initial data for each product/packaging system combination were measured upon product arrival at Auburn (within 8 hr).

Simulated product distribution

The products were held at -2 to 0°C for 0, 7 or 14 days to simulate the optimal temperature and holding times encountered during refrig-

Table 1—TBARS values and microbial populations of ground beef products during simulated retail distribution.

	TBARS values[b]	Microbial populations[a]			
		Aerobic	Psychro-trophic	Coliform	Molds & Yeasts
Product[c]					
Control	1.0[x]	6.3[x]	7.9[x]	3.5[x]	2.1[x]
Developed	0.9[x]	6.4[x]	7.8[x]	3.7[y]	2.1[x]
SEM[d]	0.03	0.03	0.02	0.02	0.06
Packaging system[e]					
Impermeable	0.9[x]	6.4[x]	7.9[x]	3.6[x]	2.0[x]
Permeable	1.0[x]	6.4[x]	7.8[x]	3.5[x]	2.2[y]
SEM	0.03	0.03	0.02	0.02	0.06
Storage period[f]					
0 days	0.9[x]	6.4[x]	7.9[x]	3.9[x]	2.3[x]
7 days	0.9[x]	6.4[x]	7.8[x]	3.5[y]	1.9[x]
14 days	0.9[x]	6.3[x]	7.8[x]	3.3[y]	2.0[x]
SEM	0.04	0.08	0.08	0.13	0.14
Retail display[g]					
0 hr	0.9[x]	6.4[x]	7.8[x]	3.5[x]	2.2[x]
24 hr	0.9[x]	6.4[x]	7.8[x]	3.6[x]	2.1[x]
48 hr	0.9[x]	6.4[x]	7.9[x]	3.6[x]	2.0[x]
SEM	0.02	0.02	0.06	0.05	0.05

[a] Microbial counts expressed as the $\log_{10}$ CFU/g.

[b] Thiobarbituric acid reactive substances values expressed as mg malonaldehyde/kg meat.

[c] Ground beef products: Control = low-fat (8% fat) all-beef; Developed = low-fat product with 10% added water, 0.5% carrageenan, 0.4% encapsulated salt and 0.2% hydrolyzed vegetable protein.

[d] SEM = Standard error of the mean.

[e] Impermeable = products prepared as fine ground and packaged as chub packs when manufactured, held in chub packaging during storage period and retail display; Permeable = products prepared as coarse ground (1.27 cm), packaged and held during storage period in chub packaging, after storage were finely ground (0.32 cm), placed in white plastic foam trays and overwrapped with oxygen-permeable film for retail display.

[f] Ground beef products held at −2° to 0°C.

[g] Display at 5 to 7°C under retail lighting conditions.

[xyz] Means within a column with different superscripts differ ($P<0.05$).

Table 2—Hunter color 'a' values and metmyoglobin concentrations of ground beef products during simulated retail distribution

	'a' Values[a]		Metmyoglobin (%)	
	Total[b]	Surface[c]	Total	Surface
Product[d]				
Control	11.1[x]	9.5[x]	33.5[x]	41.0[x]
Developed	11.6[x]	10.3[y]	29.9[x]	36.0[x]
SEM[e]	0.15	0.22	1.22	2.62
Packaging system[f]				
Impermeable	12.6[x]	11.7[x]	23.8[x]	25.3[x]
Permeable	10.1[y]	8.1[y]	39.7[y]	51.7[y]
SEM	0.15	0.22	1.22	2.62
Storage period[g]				
0 days	12.7[x]	11.2[x]	23.5[x]	29.8[x]
7 days	10.6[y]	9.2[y]	31.9[y]	41.5[y]
14 days	10.8[y]	9.2[y]	39.7[z]	44.3[y]
SEM	0.35	0.33	2.48	2.60
Retail display[h]				
0 hr	12.3[x]	12.4[x]	26.6[x]	24.4[x]
24 hr	11.3[y]	8.9[y]	33.5[y]	44.0[y]
48 hr	10.5[z]	8.3[y]	35.0[y]	47.2[y]
SEM	0.18	0.63	1.83	0.05

[a] Hunter color 'a' value = redness/greeness (+ = red, − = green).

[b] Total product color as determined from representative ground sample.

[c] Surface color as determined from representative samples removed from surface (≈3 mm) of ground beef products.

[d] Ground beef products: Control = low-fat (8% fat) all-beef; Developed = low-fat product with 10% added water, 0.5% carrageenan, 0.4% encapsulated salt and 0.2% hydrolyzed vegetable protein.

[e] SEM = Standard error of the mean.

[f] Impermeable = products prepared as fine ground and packaged as chub packs when manufactured, held in chub packaging during storage period and retail display; Permeable = products prepared as course ground (0.64 cm), packaged and held during storage period in chub packaging, after storage were finely ground (0.32 cm), placed in white plastic foam trays and overwrapped with oxygen-permeable film for retail display.

[g] Ground beef productsf held at −2° to 0°C.

[h] Displayed at 5 to 7°C under retail lighting conditions.

[xyz] Means within a column with different superscripts differ ($P<0.05$).

erated storage at processing plants. At each time period (0, 7 and 14 days), four packages from each product/packaging system combination were transferred to and stored at 4°C for 4 days. This was intended to simulate the approximate temperatures and holding times encountered in distribution and storage of ground beef products at retail outlets. After the 4 day holding period, four packages (packaging system 1) of coarse-ground items from each product were finely ground (0.32 cm grinder plate, equipment thoroughly cleaned and sanitized prior to grinding), placed on white plastic foam trays and overwrapped with oxygen-permeable polyvinyl chloride (oxygen transmission rate = 6500 cc/M^2/24 hr/atm at 25°C) film. These packages as well as chub packs (packaging system 2) of each product were then held under retail lighting conditions (fluorescent lighting with 75 ft-c at the meat surface) at 4°C for 0, 24 or 48 hr. At each time interval (0, 24 and 48 hr), one package from each product/packaging system combination was evaluated for microbial populations and oxidative stability (color and lipid). The pH of samples from each product/packaging system combination was also determined after 48 hr of retail display.

Composition and pH

Moisture, fat (petroleum ether extraction) and protein (Kjeldahl) content of the raw ground beef products was determined using AOAC methods (1980).

The pH of each ground beef product/packaging combination was measured initially and at each storage period after 48 hr of retail display. Representative 10g ground beef samples (prepared as described for total color) from each product/packaging combination were homogenized for 30 sec with 100 mL distilled water using a blender (John Oster Mfg. Co., Milwaukee, WI). The pH of prepared homogenates was determined with a Fisher Accumet (Model 805) pH meter equipped with a combination electrode.

Color evaluation

Color of the low-fat ground beef products was determined initially and after 0, 24 and 48 hr of retail display. Products were evaluated at the time periods described above for color stability using the following methods: (1) visual appraisal panel, (2) Hunter Lab Digital Color Difference Meter, and (3) spectrophotometric determination of metmyoglobin concentration. Surface (sample removed from product surface, ~3 mm) and total color were evaluated for each product/packaging combination after 0, 24 and 48 hr of retail display. Samples for total color, pH and thiobarbituric acid reactive substances (TBARS) determination were ground using a mixer (Kitchenaid, Dayton, OH) with grinder attachments to produce representative homogeneous samples. Color samples (surface and total) were placed in petri dishes (100 X 15 mm), covered with oxygen-permeable plastic interleave sheets (1300 cc o_2/M^2/24 hr/atm at 25°C) and allowed to bloom (develop typical bright cherry-red color) at 4°C for 2 hr.

Visual appraisal panel. A trained visual appraisal panel was used for subjective color determination. Visual appraisal panelists (10 individuals) evaluated prepared color samples for overall color (8 = extremely red and 1 = extremely brown) and for percent discoloration (0–100%). Visual appraisal panelists were trained in 20 min sessions where panelists evaluated ground beef samples that varied in color and degree of discoloration. Samples were evaluated under Warm White fluorescent lighting positioned to provide 70 ft-c at the counter surface.

Hunter color measurements. Color samples (total and surface) were evaluated for Hunter color 'L', 'a' and 'b' values using a Hunter Lab Digital Color Difference Meter (Model D25D2A, Reston, VA) following visual panel appraisal. Standardization of the Hunter Color Meter was accomplished with the pink C2 3311 standard plate ('L' = 68.7, 'a' = 23.0 and 'b' = 9.4). Delta E values were calculated using the 'L', 'a' and 'b' values as described by Yen et al. (1988).

Metmyoglobin determination. Surface and total (prepared as described previously) metmyoglobin concentrations were determined using the procedure as described by Chu et al. (1987). Percent metmyoglobin was calculated as described by Krzywicki (1979).

Thiobarbituric acid reactive substances (TBARS)

Lipid oxidation of each homogeneous sample (prepared as described for total color) was assessed using the procedure outlined by Ke et al. (1984). TBARS values were determined using a representative sample as prepared for total color. A standard curve (0 to 2.0

Table 3—Visual appraisal color scores of ground beef products during simulated retail distribution

	Overall color[a]		Discoloration (%)	
	Total[b]	Surface[c]	Total	Surface
Product[d]				
Control	5.2[x]	4.0[x]	20.8[x]	47.7[x]
Developed	5.8[y]	5.1[y]	17.5[x]	36.2[x]
SEM[e]	0.05	0.28	1.29	4.35
Packaging system[f]				
Impermeable	6.2[x]	5.8[x]	8.4[x]	19.6[x]
Permeable	4.8[y]	3.3[y]	30.0[y]	64.4[y]
SEM	0.05	0.28	1.29	4.35
Storage period[g]				
0 days	5.7[x]	4.9[x]	13.1[x]	36.6[x]
7 days	5.6[x]	4.6[x]	17.8[x]	42.7[x]
14 days	5.2[x]	4.3[x]	26.7[y]	46.6[x]
SEM	0.16	0.35	2.60	5.13
Retail display[h]				
0 hr	5.6[x]	5.7[x]	16.6[x]	17.2[x]
24 hr	5.6[x]	4.2[y]	19.3[xy]	52.3[y]
48 hr	5.3[y]	3.9[y]	21.6[y]	56.4[y]
SEM	0.07	0.12	1.10	2.02

[a] Overall color of ground beef samples evaluated on 8 point scale (8 = extremely red, 1 = extremely brown).
[b] Total product color as determined from representative ground sample.
[c] Surface color as determined from representative samples removed from surface (≈3 mm) of ground beef products.
[d] Ground beef products: Control = low-fat (8% fat) all-beef; Developed = low-fat product with 10% added water, 0.5% carrageenan, 0.4% encapsulated salt and 0.2% hydrolyzed vegetable protein.
[e] SEM = Standard error of the mean.
[f] Impermeable = products prepared as fine ground and packaged as chub packs when manufactured, held in chub packaging during storage period and retail display; Permeable = products prepared as course ground (1.27 cm), packaged and held during storage period in chub packaging, after storage were finely ground (0.32 cm), placed in white plastic foam trays and overwrapped with oxygen-permeable film for retail display.
[g] Ground beef products held at −2° to 0°C.
[h] Displayed at 5 to 7°C under retail lighting conditions.
[xyz] Means within a column with different superscripts differ ($P<0.05$).

X 10^{-6}M) was prepared using 1,1,3,3-tetraethoxypropane (TEP) [malonaldehyde bis(diethyl acetal)]. Absorbance readings were taken at 538 nm (Lambda 4A, model CO99-1232 spectrophotometer, Perkin-Elmer, Norwalk, CT) and compared to a standard curve to determine malonaldehyde concentration. TBARS values were expressed as mg malonaldehyde/kg meat.

Microbial evaluation

Microbial evaluation of ground beef products held at 4°C was completed initially and at 0, 24 and 48 hr of retail display. Samples were evaluated for populations of aerobic (aerobic plate count, APC), psychrotrophic and coliform bacteria as well as for molds and yeasts. Representative samples (11g) from each product/packaging combination were aseptically weighed, placed in sterile lab bags (Tekmar, Cincinnati, OH) with 99 mL of 0.1M (pH 7.2) potassium phosphate-buffered diluent and mixed for 2 min using a Colworth stomacher (Tekmar, Cincinnati, OH). Serial dilutions were prepared in phosphate-buffered diluent and inoculated (1 mL) into appropriate media using pour-plate technique.

Aerobic (48 hr at 32°C) and psychrotrophic (14 d at 5°C) bacteria were cultivated on plate count agar (Difco, Detroit, MI) described by the Food and Drug Administration (1978). Coliforms were enumerated in violet red bile agar (Difco, Detroit, MI) after incubation at 35°C for 24 hr (Fishbein et al., 1976). Mold and yeast populations were determined on acidified (pH 3.5) potato dextrose agar (Difco, Detroit, MI) after incubation for 5 days at 25°C (Koburger and Marth, 1984). All microbial populations were expressed as $\log_{10}$ colony forming units (CFU)/g.

Statistical analysis

A split-split plot factorial analysis (Cochran and Cox, 1966) was used to evaluate oxidative stability and microbial data. When analysis of variance revealed a significant ($P<0.05$) effect, means were separated using Fisher's least significant difference procedure (Steel and Torrie, 1980). Correlations were determined using MANOVA procedure of SAS Institute, Inc. (1985).

RESULTS & DISCUSSION

Initial product evaluation

Low-fat carrageenan-based products contained less ($P<0.05$) fat (5.7% ca. 6.2%; SEM = 0.13) and protein (18.9% ca. 20.4; SEM = 0.22) and more ($P<0.05$) moisture (74.4% ca. 73.0%; SEM - 0.30) than the low-fat all-beef control. These compositional differences were in agreement with previous reports by Egbert et al. (1991). Differences ($P<0.05$; SEM = 0.02) were also found among the low-fat products for initial product pH with the carrageenan-based product (pH 5.7) having a slightly higher pH than the all-beef control product (pH 5.5). This slightly higher pH for the low-fat carrageenan-based

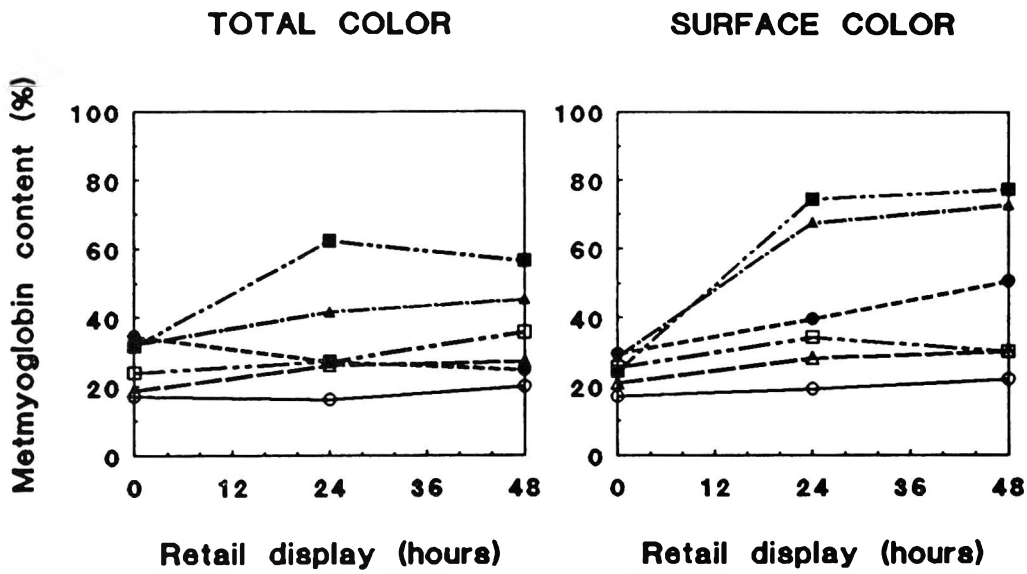

Fig. 1—Effect of retail display on metmyoglobin concentrations (%) of aerobically and anaerobically packaged low-fat ground beef after refrigerated (5–7°C) storage. Total color determined from representative ground samples of low-fat products. Surface color determined from representative samples removed from surface (~3 mm) of ground beef products. [Symbols: ○ ——— ○ 0AN; ● -·- ● 0AR; △ ——— △ 7AN; ▲ ——— ▲ 7AR; □ ——— □ 14AN; ■ -···- ■ 14AR.] Numbers indicate days storage 0, 7 or 14. AR = aerobic, AN - anaerobic retail display.

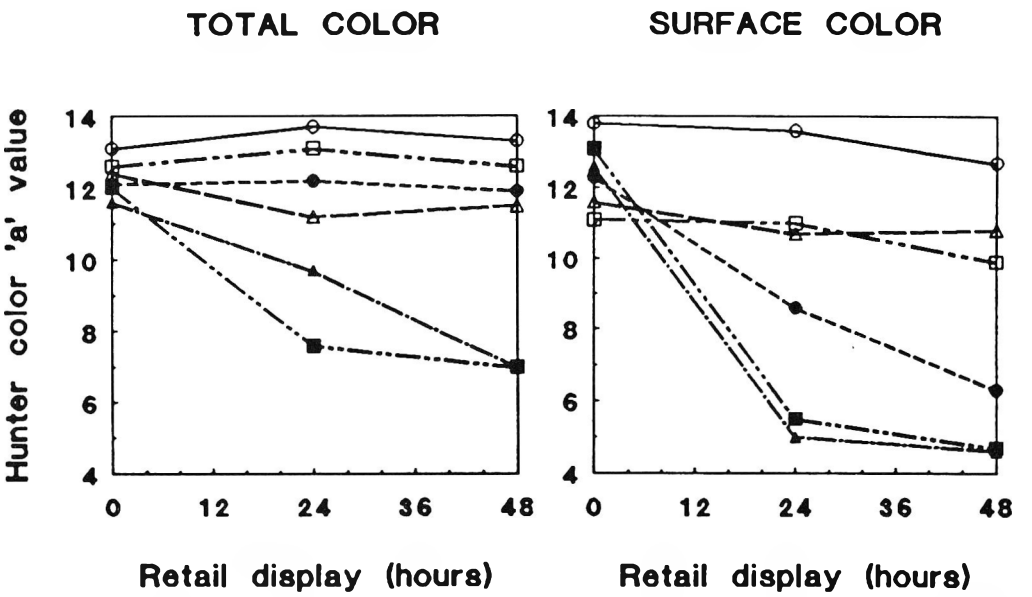

Fig. 2—Effect of retail display on Hunter color 'a' values (+ = redness, - = greeness) of aerobically and anaerobically packaged low-fat ground beef after refrigerated (5–7°C) storage. Total color determined from representative ground samples of low-fat products. Surface color determined from representative samples removed from surface (~3 mm) of ground beef products. [Symbols: ○ ——— ○ 0AN; ● -·- ● 0AR; △ ——— △ 7AN; ▲ ——— ▲ 7AR; □ ——— □ 14AN; ■ -·-· ■ 14 AR.] Numbers indicate days storage 0, 7 or 14. AR = aerobic, AN = anaerobic retail display.

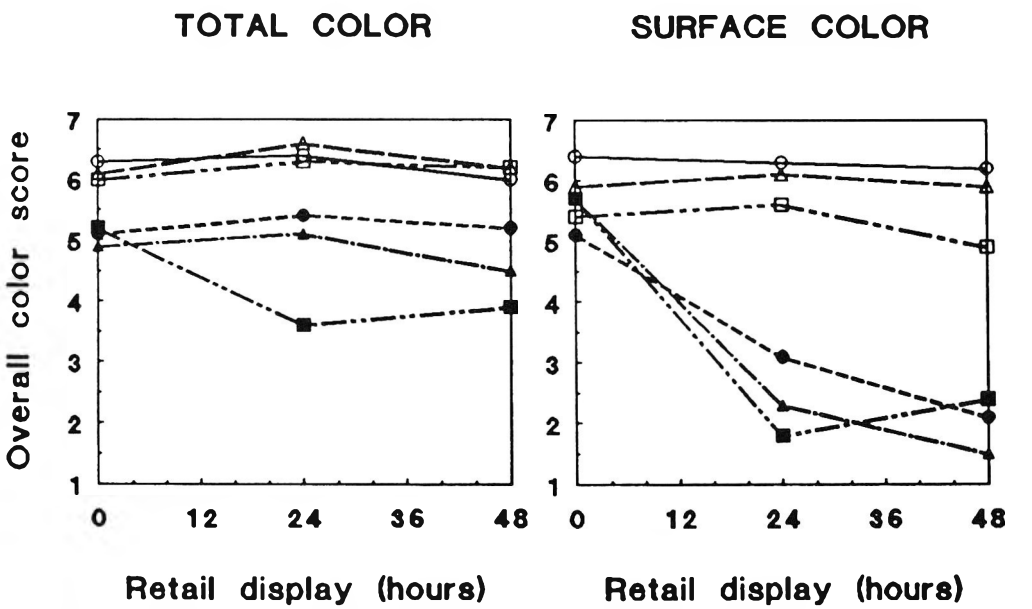

Fig. 3—Effect of retail display on visual appraisal overall color scores (8 = extremely red, 1 = extremely brown) of aerobically and anaerobically packaged low-fat ground beef after refrigerated (5–7°C) storage. Total color determined from representative ground samples of low-fat products. Surface color determined from representative samples removed from surface (~3 mm) of ground beef products. [Symbols: ○ ——— ○ 0AN; ● -·- ● 0AR; △ ——— △ 7AN; ▲ ——— ▲ 7AR; □ ——— □ 14AN; ■ -·-· ■ 14 AR.] Numbers indicate days storage 0, 7 or 14. AR = aerobic, AN = anaerobic retail display.

product was likely the result of the alkaline nature of the carrageenan (FMC, 1988).

No differences ($P>0.05$) were found initially between the low-fat, all-beef product and the low-fat, carrageenan-based product for the following: TBARS values (0.7 ca. 0.7, respectively); Hunter color 'L' (surface color 32.5 ca. 33.0; total color 33.5 ca. 34.0), 'a' (surface 16.9 ca. 17.0; total 17.4 ca. 17.3) and 'b' (surface 11.3 ca. 11.3; total 11.4 ca. 11.5) values; metmyoglobin content (surface 8% ca. 7%; total 10% ca. 9%); visual appraisal overall color (surface 6.4 ca. 6.5; total 6.6 ca. 6.8) and percent discoloration (surface 2% ca. 1%; total 2% ca. 2%) scores; and microbial populations (aerobic 5.7 ca. 5.5; psychrotrophic 6.8 ca. 6.7; coliform 3.8 ca. 3.7; molds & yeasts 2.0 ca. 2.0).

Microbial growth

There has been concern regarding the possibility of increased microbial growth in the low-fat, carrageenan-based ground beef product as a result of the addition of 10% water to the product. However, few differences were found in microbial populations. No interactions ($P>0.05$) were found among the main effects (product, packaging system, refrigerated stor-

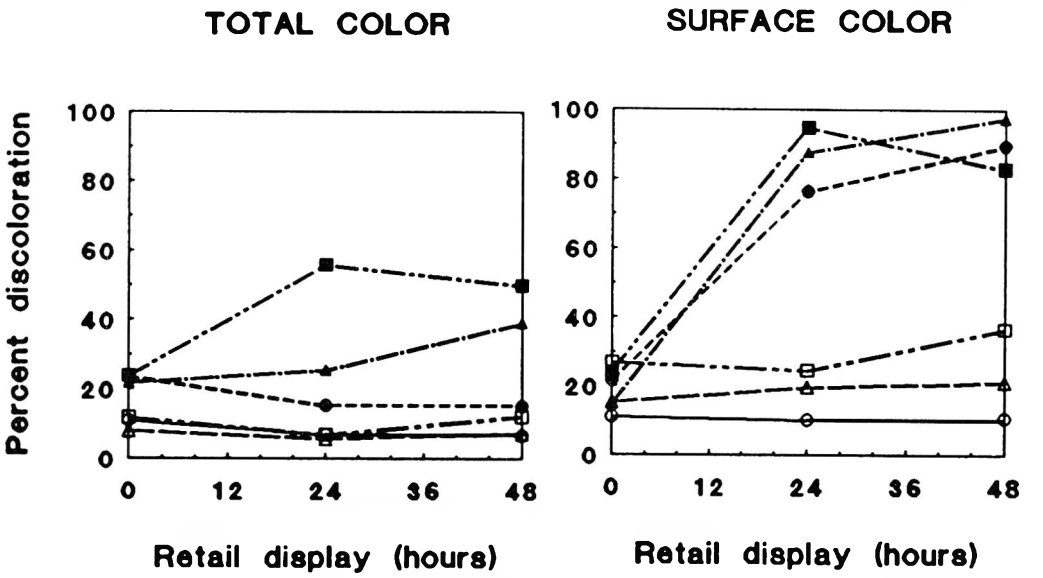

Fig. 4—Effect of retail display on visual appraisal scores for percent discoloration of aerobically and anaerobically packaged low-fat ground beef after refrigerated (5–7°C) storage. Total color determined from representative ground samples of low-fat products. Surface color determined from representative samples removed from surface (~ mm) of ground beef products. [Symbols: ○ ——— ○ 0AN; ● -·- ● 0AR; △ ——— △ 7AN; ▲ ——— ▲ 7AR; □ ——— □ 14AN; ■ -·-·- ■ 14AR.] Numbers indicate days storage 0, 7 or 14. AR = aerobic, AN = anaerobic retail display.

Table 4—Hunter color 'L', 'b' and ΔE values of ground beef products during simulated retail distribution.

	'L' values[a]		'b' values[a]		ΔE values[a]	
	Total[b]	Surface[c]	Total	Surface	Total	Surface
Product[d]						
Control	37.1[x]	36.4[x]	10.6[x]	10.3[x]	30.3[x]	30.8[x]
Developed	35.9[y]	35.1[x]	10.2[y]	9.9[x]	29.0[x]	30.1[x]
SEM[e]	0.25	0.51	0.04	0.12	2.62	2.95
Packaging system[f]						
Impermeable	35.5[x]	34.0[x]	10.3[x]	10.0[x]	26.1[x]	30.3[x]
Permeable	37.6[y]	37.5[y]	10.4[x]	10.3[x]	33.2[x]	30.7[x]
SEM	0.25	0.51	0.05	0.12	2.62	2.95
Storage periods[g]						
0 days	35.5[x]	34.6[x]	10.4[x]	10.0[x]	0.0[x]	0.0[x]
7 days	37.2[x]	36.5[x]	10.3[x]	10.1[x]	44.5[y]	47.1[y]
14 days	36.9[x]	36.1[x]	10.4[x]	10.2[x]	44.7[y]	44.2[y]
SEM	0.83	0.92	2.48	0.14	5.34	6.38
Retail display[h]						
0 hr	36.0[x]	34.9[x]	10.5[x]	10.2[x]	29.1[x]	31.1[x]
24 hr	36.3[x]	35.9[y]	10.4[x]	10.1[x]	30.4[x]	35.8[x]
48 hr	37.3[y]	36.4[y]	10.4[x]	10.0[x]	29.5[x]	34.5[x]
SEM	0.29	0.63	0.07	0.08	1.95	1.98

[a] Hunter color values: 'L' = lightness (0 = black, 100 = white); 'b' = yellowness/blueness (+ = yellow, − = blue); ΔE = estimation of total color change (Yen et al., 1988).

[b] Total product color as determined from representative ground samples.

[c] Surface color as determined from representative samples removed from surface (≈3 mm) of ground beef products.

[d] Ground beef products: Control = low-fat (8% fat) all-beef; Developed = low-fat product with 10% added water, 0.5% carrageenan, 0.4% encapsulated salt and 0.2% hydrolyzed vegetable protein.

[e] SEM = Standard error of the mean.

[f] Impermeable = products prepared as fine ground and packaged as chub packs when manufactured, held in chub packaging during storage period and retail display; Permeable = products prepared as course ground (1.27 cm), packaged and held duirng storage period in chub packaging, after storage were finely ground (0.32 cm), placed in white plastic foam trays and overwrapped with oxygen-permeable film for retail display.

[g] Ground beef products held at −2° to 0°C.

[h] Display at 5 to 7°C under retail lighting conditions.

[xy z] Means within a column with different superscripts differ (P<0.05).

age and retail display) for aerobic, psychrotrophic, coliform or mold and yeast populations.

No differences (P>0.05) were found between the low-fat, all-beef product and the low-fat carrageenan product for aerobic plate counts, populations of psychrotrophs or mold and yeast populations (Table 1). However, a small difference (P<0.05) was found between the products for coliform populations with the low-fat carrageenan product having slightly higher counts (Table 1). Thus the addition of water to the low-fat, carrageenan-based product had little effect on microbial growth. This could possibly be explained by the small difference in ratio of moisture to dry matter (DM) for the carrageenan-based product compared to the low-fat, all-beef control product (3.73g H_2O/g DM ca. 3.61g H_2O, respectively).

Retail packaging method had no (P>0.05) effect on aerobic, psychrotrophic or coliform bacteria populations. Aerobically packaged low-fat products had slightly higher (P<0.05) mold and yeast populations than anaerobically packaged products. This could indicate growth of these microorganisms during aerobic storage or, more likely, this difference could be due to different levels of contamination of the aerobically stored product. Increased contamination could have occurred during the fine grinding of the coarse ground product prior to retail display though measures were taken to minimize contamination. Length of refrigerated storage or retail display had no effect (P>0.05) on aerobic plate counts, psychrotrophic bacteria or mold and yeast populations (Table 1). However, coliform populations appeared to decrease slightly during refrigerated storage.

pH values

No differences (P>0.05) were found in product pH as a result of refrigerated storage time or packaging system during retail display. The low-fat, carrageenan product continued to maintain a pH slightly higher than that of the low-fat, all-beef product through 48 hr of retail display (pH values 5.4 ca. 5.3, SEM = 0.01). This slightly elevated pH was probably the result of the alkaline nature of the carrageenan (FMC, 1988).

TBARS values

TBARS values, a measure of lipid oxidation were not different (P>0.05) among any of the main effects (products, packaging systems, refrigerated storage and retail display) (Table 1). This was an indication that the anaerobic environment produced by packaging low-fat products in oxygen impermeable films was effective in exluding oxygen and as a result,

oxygen-promoted lipid oxidation could not proceed (Greene, 1969). Retail display of the low-fat ground beef products in oxygen-permeable packaging for 48 hr did not result in increased ($P > 0.05$) oxidation in the low-fat products.

Color stability

Decreases in Hunter color 'a' values or overall color scores and increases in metmyoglobin concentrations or percent discoloration scores are indications of fresh meat discoloration. Hunter color 'a' values were highly correlated ($P < 0.01$) with metmyoglobin concentrations ('a' value ca. metmyoglobin content for total color, $r = -0.63$; 'a' value ca. metmyoglobin content for surface color, $r = -0.70$). Hunter color 'a' values and metmyoglobin concentrations for surface color also moderately to highly correlated ($P < 0.05$) with subjective visual appraisal scores (overall color and percent discoloration) for surface color (Hunter color 'a' for surface color ca. overall color score, $r = 0.60$ and percent discoloration, $r = -0.65$; metmyoglobin concentration for surface color ca. overall color score, $r = -0.54$ and percent discoloration, $r = 0.53$). Hunter color 'a' values for total product color also moderately correlated ($P < 0.01$) with visual appraisal percent discoloration scores ($r = -0.46$) for total product color.

Product color stability. No interactions ($P > 0.05$) were found between the low-fat ground beef products for color stability and other main effects (packaging system, refrigerated storage or retail display). This indicated that the two low-fat products reacted similarly under the 2 packaging systems and during refrigerated storage and retail display.

No differences ($P > 0.05$) were found among the two low-fat products for total color as indicated by Hunter color 'a' values, metmyoglobin concentrations or percent discoloration scores, and for surface color as indicated by metmyoglobin concentrations or percent discoloration (Tables 2, 3). However, Hunter color 'a' values and overall color scores for surface color suggested that the low-fat, carrageenan-based product was more ($P < 0.05$) red than the low-fat control product. Visual appraisal overall color scores for total color also supported the observation that low-fat, carrageenan-based ground beef had greater ($P < 0.05$) redness than the low-fat control product (Table 3).

Retail packaging effects of color stability. Both low-fat products appeared to react similarly during retail display under the two packaging systems. No interaction ($P > 0.05$) was found between the main effects of product and packaging system for any of the measures of color stability. Results indicated that the packaging system used for retail display could have a notable effect on color stability of low-fat ground beef. Upon exposure of the low-fat products to air (under refrigerated conditions) for the evaluation of total and surface color, products packaged under oxygen-permeable (aerobic) conditions during retail display had less redness than those packaged under oxygen impermeable (anaerobic) conditions. Anaerobically packaged product had greater ($P < 0.05$) Hunter color 'a' values (Table 2) and overall color scores (Table 3) for both total and surface color. Discoloration measurements (metmyoglobin concentrations, Table 2 and visual appraisal percent discoloration scores, Table 3) were lower for anaerobically packaged low-fat ground beef than for aerobically packaged product. These color changes were as expected, since extended exposure of fresh meat, displayed in oxygen permeable (aerobic) packaging under retail lighting conditions, has resulted in meat discoloration (Hood, 1980; Jeremiah et al., 1972). Product discoloration of aerobically packaged red meat is the result of the oxidation of the red meat pigments oxymyoglobin and myoglobin to brown colored ferric metmyoglobin (Greene, 1969). Stewart et al. (1965) have shown that enzymes in raw ground beef can effectively reduce the metmyoglobin pigment to myoglobin under anaerobic conditions. Once the metmyoglobin pigment has been reduced it will remain in the reduced state provided no additional oxygen enters the package.

Effect of storage on color stability. The methods for objective measurement of color stability were more sensitive than subjective color measurements of the low-fat ground beef color stability as affected by refrigerated storage. Hunter color 'a' values and metmyoglobin concentrations indicate a reduction ($P < 0.05$) in the red color of the low-fat products during refrigerated storage between day 0 and day 7 (Table 2). There was a further increase in metmyoglobin concentrations for total color (decreased redness) between day 7 and day 14. Decreased color stability of the low-fat products during refrigerated storage was consistent with previous reports by Pierson et al. (1970), which showed similar decreases in color stability of fresh beef slices with extended storage.

Visual appraisal percent discoloration scores for total color indicated a decrease ($P < 0.05$) in total product redness between day 7 and day 14 (Table 3). No other differences ($P < 0.05$) were found for objective color measurements during refrigerated storage.

Color stability during retail display. As expected, the redness of the low-fat ground beef products decreased during retail display. Hunter color 'a' values and visual appraisal overall color scores decreased ($P < 0.05$). Metmyoglobin concentrations and visual appraisal percent discoloration scores increased ($P < 0.05$) for both total and surface color of low-fat ground beef during retail display (Tables 2 and 3).

Display by storage by packaging interaction. An interaction ($P < 0.05$) was found between the main effects retail display, refrigerated storage and packaging system for the following measures of total and surface color stability: metmyoglobin concentration (Fig. 1), Hunter color 'a' value (Fig. 2), overall color score (Fig. 3) and percent discoloration (Fig. 4). In general, color deterioration during retail display was less severe in total color evaluation than for surface color. Also, color stability appeared to decrease with increased refrigerated storage time for the low-fat products regardless of the packaging system employed during retail display. Greater ($P < 0.05$) changes (decreased redness) in color were observed for aerobically packaged products than for anaerobically packaged products during retail display (Fig. 1 - 4). This was expected, since red meat pigment oxidation proceeds rapidly upon exposure of fresh meat products to an aerobic environment (Greene, 1969). Metmyoglobin concentrations (Fig. 1) and Hunter color 'a' values (Fig. 2) for surface and total color indicated that the rate of product discoloration (increased metmyoglobin content and decreased 'a' values) during retail display was greater after 7 and 14 d of refrigerated storage than after 0 day storage. These differences were less notable based on objective measures of product color (visual appraisal overall color and percent discoloration scores, Fig. 3 and 4, respectively).

Hunter color 'L', 'b' and ΔE values

No interactions ($P > 0.05$) were found among the main effects for Hunter color 'L' (lightness), 'b' (yellowness) or ΔE values (calculated estimation of total color change). Few notable differences were found for these Hunter color values (Table 4). The low-fat, carrageenan-based product (total color) was slightly darker ($P < 0.05$) and less ($P < 0.05$) yellow in color than the low-fat all-beef product. Upon exposure to air, Hunter color 'L' values (total and surface color) were higher ($P < 0.05$) for products which had been packaged in aerobic conditions compared to anaerobically packaged products. ΔE values indicated an overall color change for the low-fat ground beef products during refrigerated storage, which may be another indication of decreased redness in low-fat products. There was also a slight increase ($P < 0.05$) in lightness of products (total and surface color) during retail display. No other differences ($P > 0.05$) were found for Hunter color 'L', 'b' and ΔE values.

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Volatile Concentration and Flavor of Beef as Influenced by Diet

J. L. MARURI and D. K. LARICK

ABSTRACT

Yearling steers (48) were finished on one of three intense pasture rotation systems: (1) Tifleaf pearl millet; (2) millet with restricted grain; or (3) millet followed by grain ad libitum in the feedlot. In both 20% fat ground beef and subcutaneous fat, gamey/stale off-flavor decreased and roasted beef flavor increased when steers were fed grain. Compounds (59) including acids, alcohols, aldehydes, diterpenoids, hydrocarbons, and lactones, were identified in the purge-and-trap volatiles. Lactones positively correlated with roasted beef flavor and negatively correlated with gamey/stale off-flavor, whereas diterpenoids positively correlated with gamey/stale off-flavor and negatively correlated with roasted beef flavor.

Key Words: volatiles, beef, flavor, sensory, low fat

INTRODUCTION

RESEARCH STUDIES have suggested that changing consumer preferences influence beef consumption (Hahn, 1987; Skaggs and Menkhaus, 1986). Because of increased concern over dietary fat, numerous researchers have evaluated the effect of different feeding regimens on carcass composition and flavor characteristics of beef. Researchers (Bidner et al., 1981; Schupp et al., 1979) have reported that carcasses from cattle finished on forage had less subcutaneous fat and less marbling than those from cattle finished on grain.

Trained panelists have characterized the less desirable flavor associated with beef from forage-fed cattle as grassy (Larick et al., 1987), gamey (Larick and Turner, 1990) and milky-oily (Melton et al., 1982). Beef from grain-fed cattle has been characterized as having intense fatty flavor (Larick and Turner, 1990; Melton et al., 1982; Schroeder et al., 1980). Consumer studies have not confirmed studies which utilized trained sensory panelists in that several studies (Bidner et al., 1981; Chastain et al., 1985; Schupp et al., 1979) have reported that consumers were not able to detect significant differences in flavor, tenderness or juiciness in beef from forage- and grain-fed cattle.

Larick et al. (1987) identified numerous compounds in beef fat which correlated with the characteristic flavor of beef fat from forage- and grain-fed cattle. The compounds 2, 3-octanedione and 3-hydroxy-octan-2-one, heptanal and 2-decenal, which were also identified in lamb fat (Suzuki and Bailey, 1985), decreased when cattle were fed grain. The concentration of δ-tetradecalactone and δ-hexadecalactone produced by heating beef fat increased and the concentration of γ-dodecalactone decreased when cattle were finished on grain for varying lengths of time. The concentration of diterpenoid compounds, which included phytane, phyt-1-ene, phyt-2-ene, neophytadiene, an isomer of neophytadiene, phytol and dihydrophytol, were reported to decrease when cattle were finished on grain. Because of the importance of fat in the overall flavor of meat, additional research is necessary to identify those compounds present in fat that are responsible for the grassy flavor of fat from forage-fed cattle.

The objectives of our research were to: (1) identify and quantify purge-and-trap volatiles present in heated beef fat from forage- and grain-fed steers, (2) characterize the flavor of 20% fat ground beef and cooked beef fat form forage- and grain-fed steers using descriptive analysis, (3) to determine if a relationship existed between the concentration of identified volatiles and the sensory characteristics of the cooked fat and the 20% fat ground beef.

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MATERIALS & METHODS

Sample preparation

Yearling steers (32 Angus and 16 Hereford Angus crosses) were first fed on clover-fescue from April till June after which, 28 steers were foraged on Tifleaf pearl millet (GRASS) for 101 days. The remaining 20 steers were finished on a millet with restricted grain diet (90% corn and 10% animal fat, GOG) for 101 days. Pastures were divided into eight subplots to allow for intensive pasture management. Steers were foraged on millet for 3 to 5 days or until the leafy portion had been grazed and then rotated to another subplot. At the end of the grazing period, 16 of the millet fed steers and the 20 steers fed millet with restricted grain were commercially slaughtered. The remaining 12 millet-fed steers were placed in the feedlot and fed an ad libitum diet consisting of 20.8% corn silage, 4.8% soybean meal, 1.1% limestone and 73.3% rolled oats (GRAIN) for 63 days and then slaughtered. Carcasses were evaluated 24 hr postmortem to determine USDA yield and quality grades.

A 4-rib section, from the ninth to the twelfth thoracic vertebra was removed from the right side of each carcass. The Longissimus dorsi (L. dorsi) muscle was removed, trimmed and sliced into 2.5 cm thick steaks using a Hobart slicer. A sample of the subcutaneous fat from each rib section and the steaks were vacuum packaged (27.5 inches of vacuum) in oxygen impermeable pouches (Cryovac Corp., Duncan, SC) and over-wrapped with white polyethylene coated freezer paper. The steaks were labeled from 1 to 7 starting at the twelfth rib and held at 2°C. After 7 days postmortem, samples were frozen and held at −20°C until analyzed.

Crude fat (AOAC, 1980) and crude moisture (AOAC, 1980) were determined using steak 5. To measure pH of raw L. dorsi and cooked 20% fat ground beef, 10g of sample was blended with 100 mL of distilled deionized water for 3 min. to prepare the 20% fat ground beef sample, it was cooked at 120°C for 10 min, with stirring after 5 min, and then quantitatively transferred to a blender jar. The pH of the meat slurries was measured using a tris electrode.

Purge-and trap analysis

Subcutaneous fat (50g ground through a 0.48 cm plate) was placed in 400 mL Pyrex beakers and cooked in a forced air oven at 175°C for 20 min. The fat drippings were poured into Pyrex test tubes, flushed with nitrogen gas and capped with Teflon-lined caps. Samples were stored at −20°C until analyzed.

To prepare samples for chromatographic analysis, the fat drippings were melted at 80°C and 1g of melted fat was weighed into a Pyrex test tube. The sample was injected with 3 μ L of hexane containing 357.15 ng of internal standard (2,3,4-trimethylpentane) per μ L. The test tube was sealed with a teflon-lined cap and vortexed for 2 min to ensure an even distribution of the standard. A 200 mg sample of fat, with standard added, was weighed into a 9 by 85 mm glass tube which was loosely packed with pesticide grade glass wool (Supelco,

Inc., Bellefonte, PA) and then covered with another small plug of glass wool.

The glass tubes were preconditioned for 12 hr and the glass wool was preconditioned for 24 hr at 200°C and then stored in glass jars until needed. To further condition the glass wool, it was flushed with helium at 150°C for 90 min immediately prior to use.

Volatiles were stripped from the fat using an External Closed Inlet Device (Scientific Instrumentation Service, River Ridge, LA) equipped with a 6-port rotary valve. The inlet, valve and carrier line, which transported volatiles to the inlet of the chromatograph, were maintained at 150°C, 160°C, and 170°C, respectively. The valve was placed in the position to stop gas flow. The tube of fat was placed into the inlet of the purge-and-trap sampler. The sampler was closed, the valve was turned to the inject position and volatiles were flushed onto a 30 m DB-5 fused silica capillary column (J and W Scientific, Folsom, CA) with an internal diameter of 0.32 mm and a film thickness of 1.0 micron for 5 min. The chromatograph (Varian model 3700, Palo Alto, CA) was held at −30°C for 1 min after sampling and then increased to 290°C at 4°C/min. The carrier gas was helium. The injector and detector were maintained at 310°C and 320°C, respectively. The column head pressure was 35 psi with a column flow of 5.5 mL/min and a split ratio of 20 to 1. Data were collected and analyzed using the Maxima 820 Chromatography Workstation (Millipore, Waters Chromatography Division, Milford, MA).

Mass spectrometry

Volatile compounds were extracted and concentrated in methylene chloride using a Likens-Nickerson liquid-liquid extractor. Briefly, 200g of subcutaneous fat was ground using a 0.48 cm plate and then placed in a 500 mL boiling flask with 225 mL of distilled water. The flask of fat and water was placed on one side of the extractor and a 100 mL boiling flask containing 75 mL of methylene chloride was attached to the other side. Volatiles were collected by heating both flasks at a gentle boil for 4 hr. At the end of extraction, the methylene chloride was concentrated under vacuum (10 psi) using a rotary evaporator at 20°C to ≈ 2 mL. The sample was quantitatively transferred to a graduated Pyrex tube, and then the sample was concentrated to 0.1 mL under a gentle (10 mL/min) stream of nitrogen.

Concentrated sample (1 μL) was directly injected onto a capillary column identical to that described above. The column was held at initial temperature −30°C for 1 min and then increased to 290°C at 4°C/min. Volatiles were analyzed using a Hewlett Packard 5987 gas chromatograph/mass spectrometer with an ionization potential of 70 eV and a scanning range of 30 to 300 m/e. Peaks were tentatively identified by comparing the mass spectrometry data to that of the NIH/EPA reference spectra (NIH/EPA, 1978).

Descriptive analysis of ground beef

A group of 20 experienced sensory panelists were asked to participate in three triangle tests, during which time they were asked to differentiate between samples from the following treatment combinations; (1) ground beef from GRASS cattle vs ground beef from GRAIN cattle and (2) ground beef from GRASS cattle vs ground beef GOG cattle. From that group, 11 panelists were selected based on their ability to differentiate between samples of 20% fat ground beef during triangle tests. Each of the panelists was an employee of the Dept. of Food Science at North Carolina State Univ. Panelists had an average of 2 to 3 yr experience on meat flavor panels.

During a series of training sessions, panelists selected five terms to characterize the flavor of beef from forage and grain-fed steers. Selected terms included sour, roasted beef, metallic, tallowy, and gamey/stale-off flavor. Each characteristic was ranked for intensity using a 10 cm line anchored at the ends with the terms "none" and "intense."

Steaks 2 and 4 from each carcass were tempered for 12 hr at 4°C. The steaks were trimmed free of subcutaneous fat and the same fat was added back to the lean tissue to make a 20% fat ground beef product. Intramuscular fat content was previously determined by Soxhlet extraction, and the total fat content of the subcutaneous fat was estimated at 90% (Ockerman, 1976). The lean tissue and the fat were cubed and then ground twice through a 0.48 cm plate. Duplicate 150g aliquots of 20% ground beef were placed in heavy duty Pyrex beakers which were covered with watch glasses. The samples were baked 20 min in a conventional oven that was preheated to 177°C. The duplicate beakers of cooked sample were combined, mixed thoroughly and spooned into the labeled, prewarmed jars. The jars were capped and kept warm by placing them in preheated sand until use. Samples (5) were evaluated during each session.

Table 1—Comparison of yield grade and quality grade characteristics of carcasses from GRASS-, GOG- and GRAIN-finished steers

Characteristic	Diet		
	GRASS	GOG	GRAIN
Fat thickness (mm)	5.00[b]	10.50[a]	12.50[a]
Hot carcass weight (kg)	244.26[c]	275.72[b]	304.55[a]
% KPH[d]	0.97[b]	1.70[a]	1.62[a]
Ribeye area (cm^2)	71.48[a]	72.64[a]	75.94[a]
Yield Grade	1.70[b]	2.57[a]	2.78[a]
Marbling[e]	8.31[b]	12.45[a]	10.67[b]
Fat color[f]	2.69[a]	2.65[a]	2.08[b]
Quality grade[g]	13.38[b]	15.60[a]	15.00[a]

[abc] Means within a given row bearing different superscripts differ (P≤0.05).

[d] %KPH = percent kidney, pelvic, and heart fat.

[e] Marbling score: Traces$^+$ = 7, Slight$^-$ = 8, Slight^0 = 9, Slight$^+$ = 10, Small$^-$ = 11, Small^0 = 12, Small$^+$ = 13.

[f] Fat color: 1 = white; 5 = yellow.

[g] Quality grade: Select$^-$ = 13, Select^0 = 14, Select$^+$ = 15, Choice$^-$ = 16.

Testing was done in an air conditioned panel room equipped with individual booths, which had pass through windows for sample distribution. Panelists were provided with warm weak tea for rinsing prior to tasting each sample. Samples were presented under red lights, in random order.

Additional triangle tests were conducted using the trained panel to determine if fat contributed significantly to the overall flavor of the ground beef. Three triangle tests were conducted during which time panelists were asked to differentiate between samples of ground L. dorsi with no added fat from GRASS steers vs GRAIN steers, and GRASS steers vs GOG steers.

Descriptive analysis of subcutaneous fat

Panelists (10) from the previous study were willing to continue with this phase. Additional training sessions were conducted, during which time the panelists selected two terms to describe the flavor of subcutaneous fat; roasted beef and gamey/stale off-flavor. Characteristics were scored separately using a 10 cm line anchored at the ends with the terms "none" and "intense."

Subcutaneous fat from over the rib was tempered at room temperature for 10 min after which 50 to 75g was cut into cubes. The cubes were ground once through a 0.48 cm plate. The ground sample was placed in 600 mL Pyrex beakers, covered with watch glasses and baked 20 min at 177°C in a preheated conventional oven. Prewarmed disposable borosilicate pipettes were used to pipette 3.0 mL of liquid fat into labeled prewarmed vials, kept warm by placing them in prewarmed sand until analyzed. Testing conditions were the same as those used during analysis of 20% fat ground beef.

Statistical analyses

The data were analyzed as a completely randomized design with three treatments (diets). The analysis of variance was calculated for each data set using the general linear model procedure (SAS, 1985). Waller-Duncan k-ratio t-tests were calculated for significantly different main effect means (P≤0.05). Correlation coefficients were calculated to determine the relationship between purge-and-trap volatiles and sensory characteristics of cooked beef fat. Stepwise discriminant analysis (SAS Institute, Inc., 1985) was used to select terms which could be included into a model to predict diet using either purge-and-

Table 2—Influence of diet on sensory characteristics of 20% fat ground beef and cooked beef fat

Flavor descriptor	Diet		
	GRASS	GOG	GRAIN
20% Fat ground beef			
Sour	3.36[a]	3.20[a]	3.78[a]
Bloodlike	2.60[c]	3.26[b]	4.00[a]
Tallowy	3.67[b]	4.02[a,b]	4.40[a]
Roasted beef	2.55[b]	3.36[a]	3.80[a]
Gamey/Stale Off-flavor	4.38[a]	2.20[b]	2.49[b]
Subcutaneous fat			
Roasted beef	3.15[b]	4.84[a]	5.02[a]
Gamey/Stale Off-flavor	5.50[a]	2.73[b]	2.65[b]

[a,b,c] Means within the same row bearing different superscipts differ (P≤0.05).

trap volatiles or sensory characteristics of 20% fat ground beef. The ability of the model to predict diet was then determined using the discriminant procedure (SAS Institute, Inc., 1985).

RESULTS & DISCUSSION

USDA carcass characteristics (Table 1), were greater for carcasses that were finished on grain in the feedlot (GRAIN) or while on pasture (GOG) than for carcasses from forage-finished (GRASS) steers. Marbling was greater in carcasses from GOG steers. Overall carcass fat color from GRAIN steers was whiter than that from GOG or GRASS cattle. Carcasses from GRASS steers were the lightest in weight whereas those from GRAIN steers were heaviest. The carcass data were in general agreement with previous research (Dolezal et al., 1982; Larick et al., 1987; May et al., 1989; Schroeder et al., 1980; Shinn et al., 1976), which reported yield and quality grade characteristics improved when cattle were fed a high energy grain finishing diet.

L. dorsi from GRASS steers, which had an average fat content of 2.3%, was lower in fat than L. dorsi from GOG or GRAIN steers, which had an average fat content of 4.0 and 3.4%, respectively. These values were consistent with the higher marbling scores (Table 1) for GOG and GRAIN steers. Moisture content, which averaged 72.6% among all three treatments, did not differ as a result of diet. Other researchers (Dinius and Cross, 1978; Leander et al., 1978; Moody et al., 1970; Schroeder et al., 1980) have previously reported that lipid content increased when cattle received grain as part of the finishing diet.

The pH of raw L. dorsi muscle and cooked 20% fat ground beef, which had average values of 5.42 and 5.55, respectively, were not influenced by finishing diet. May et al. (1989) indicated that carcasses from grass-fed steers had higher pH than carcasses from steers finished on concentrate for a minimum of 84 days. The higher pH in carcasses from grass-fed cattle was attributed to two factors: (1) grass-fed steers are thought to be more susceptible to pre-slaughter stress (Bowling et al., 1977), (2) grass-fed steers tend to have less glycogen in their muscle which results in a smaller decrease in pH during rigor (Dube et al., 1971). Data by other researchers, however, suggested that, as demonstrated in our study, diet did not influence pH. Schupp et al. (1979) and Bidner et al. (1981) reported that muscle pH was not affected by diet when steers were finished on forage, forage with grain or grain in the feedlot.

Triangle tests (data not shown) were utilized to determine if there were differences in lean or 20% fat ground beef that could be attributed to diet. Panelists asked to differentiate between samples of cooked ground lean from GRASS and GRAIN steers, and GRASS and GOG steers were unable to differentiate between diets ($P \geq 0.05$). Thus there was no difference in flavor of lean tissue as a result of diet.

Triangle testing was also conducted to determine if flavor differences due to diet could be detected if ground beef was formulated to contain 20% fat. When experienced panelists were asked to differentiate between samples of cooked 20% fat ground beef from GRASS vs GRAIN steers and GRASS vs GOG steers, differences in flavor were found since the samples could be differentiated ($P \leq 0.05$) according to diet. This combination of triangle tests demonstrated that differences in flavor of 20% fat ground beef, as a result of diets, were due to differences in composition of the fat.

Quantitative descriptive analysis was used to characterize differences in flavor of samples of 20% fat ground beef from the 3 treatments (Table 2). Sour flavor did not differ across diets. This was expected since pH was also not influenced by

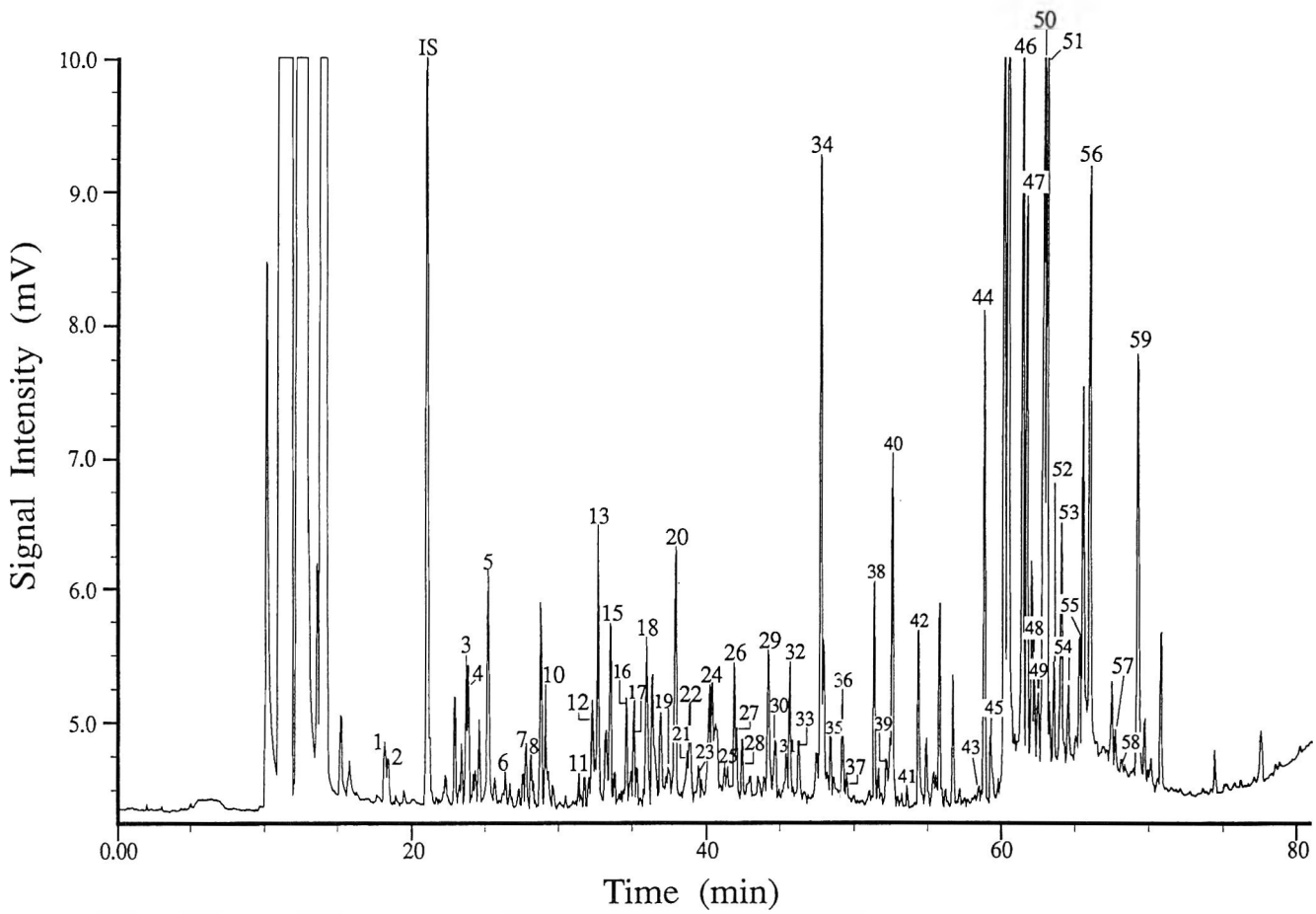

Fig. 1—A representative chromatogram of purge-and-trap volatiles from cooked beef fat.

Table 3—Concentration (ppm) of purge-and-trap volatiles of melted beef fat

Peak #	Compound Name	Diet GRASS	GOG	GRAIN
Acids and alcohols				
12	Octanol	0.073[b]	0.166[ab]	0.212[a]
24	2-Ethyl-1-decenol	0.034[a]	0.036[a]	0.054[a]
52	Pentadecanoic acid	0.049[a]	0.040[a]	0.013[b]
56	Hexadecanoic acid	0.186[a]	0.421[a]	0.623[a]
59	Octadecanoic acid	0.120[b]	0.124[b]	0.260[a]
Aldehydes and ketones				
1	Pentanal	0.088[a]	0.046[b]	0.071[ab]
3	Hexanal	0.096[a]	0.121[a]	0.173[a]
10	2-Heptenal	0.024[b]	0.046[a]	0.050[a]
11	Benzaldehyde	0.018[a]	0.015[ab]	0.008[b]
20	Nonanal	0.139[ab]	0.121[b]	0.212[a]
27	Decanal	0.021[a]	0.019[a]	0.015[a]
29	2-Decenal (E)	0.095[b]	0.085[b]	0.142[a]
31	2,4-Decadienal (E,Z)	0.033[a]	0.036[a]	0.041[a]
33	2,4-Decadienal (E,E)	0.031[b]	0.047[b]	0.076[a]
37	Dodecanal	0.014[a]	0.003[b]	0.003[b]
39	2-Tridecanone	0.041[a]	0.056[a]	0.041[a]
41	Tetradecanal	0.018[ab]	0.026[a]	0.010[b]
44	2-Pentadecanone	0.299[a]	0.189[b]	0.235[ab]
48	17-Octadecenal	0.054[a]	0.023[b]	0.033[ab]
54	2-Heptadecanone	0.064[a]	0.142[a]	0.079[a]
Diterpenoids				
46	Phyt-1-ene	1.570[a]	0.409[c]	0.945[b]
49	Phytane	0.041[a]	0.015[b]	0.045[a]
50	Neophytadiene	3.516[a]	1.301[c]	2.250[b]
51	Phyt-2-ene	1.934[a]	0.327[c]	0.658[b]
53	Phytadiene	0.161[a]	0.070[a]	0.152[a]
57	Dihydrophytol	0.248[a]	0.130[b]	0.012[c]
Hydrocarbons				
2	Heptane	0.048[b]	0.047[b]	0.086[a]
4	Octane	0.088[a]	0.092[a]	0.121[a]
5	2-Octene	0.029[a]	0.030[a]	0.003[b]
6	2,5-Dimethylheptane	0.067[a]	0.062[a]	0.068[a]
7	2,2,4-Trimethylheptane	0.011[a]	0.012[a]	0.017[a]
8	1,4-Dimethylbenzene	0.011[b]	0.012[ab]	0.017[a]
9	Nonane	0.127[a]	0.092[a]	0.125[a]
13	2,7-Dimethyloctane	0.032[b]	0.078[b]	0.185[a]
14	1,3,5-Trimethylbenzene	0.028[b]	0.049[b]	0.209[a]
15	Decane	0.098[a]	0.097[a]	0.105[a]
16	2,6-Dimethyloctane	0.035[a]	0.036[a]	0.004[b]
17	3-Methyl-5-propylnonane	0.037[a]	0.036[a]	0.006[b]
18	3-Methyldecane	0.038[a]	0.033[a]	0.026[a]
19	Undecane	0.071[a]	0.104[a]	0.020[b]
21	3,6-Dimethyldecane	0.005[a]	0.003[a]	0.002[a]
22	2,2,5-Trimethyldecane	0.036[a]	0.032[a]	0.004[b]
25	3-Methylundecane	0.030[a]	0.032[a]	0.001[b]
26	Dodecane	0.057[a]	0.072[a]	0.011[b]
28	2,6-Dimethylundecane	0.036[a]	0.062[a]	0.011[a]
30	7-Methyltridecane	0.023[a]	0.029[a]	0.000[b]
32	Tridecane	0.076[a]	0.080[a]	0.005[b]
34	Heptylcyclohexane	0.226[b]	0.265[b]	0.681[a]
35	2,6,10-Trimethyldodecane	0.012[a]	0.013[a]	0.004[a]
36	Tetradecane	0.068[a]	0.064[a]	0.007[b]
38	2,6,10-Trimethyltridecane	0.118[a]	0.054[b]	0.064[b]
40	Pentadecane	0.159[a]	0.058[c]	0.087[b]
42	Hexadecane	0.098[a]	0.025[c]	0.059[b]
47	Octadecane	0.428[a]	0.088[b]	0.185[b]
Lactones				
43	γ-Dodecalactone	0.002[b]	0.559[a]	0.037[b]
45	δ-Dodecalactone	0.053[b]	0.089[a]	0.107[a]
55	δ-Tetradecalactone	0.088[c]	0.152[b]	0.242[a]
58	δ-Hexadecalactone	0.007[b]	0.039[b]	0.094[a]

[a,b,c] Means within the same row bearing different superscripts differ ($P \leq 0.05$).

diet. Bloodlike, which was defined to include metallic or serum-like flavors, was scored highest for beef from GRAIN steers. Tallowy and roasted beef flavors describe the fatty roasted characteristic of acceptable beef and were scored lowest for GRASS steers. Gamey/stale off-flavor characterizes the grassy flavor associated with beef from forage-fed cattle and beef from GRASS cattle was scored higher for that.

Cooked subcutaneous fat from over the L. dorsi muscle was evaluated using descriptive analysis (Table 2) to further characterize the influence of fat on off-flavor of grass-fed beef. Cooked fat from GRASS steers was scored lower for roasted beef flavor and higher in gamey/stale off-flavor. The intensity of gamey/stale off-flavor was greater for cooked beef fat than for 20% fat ground beef. These data, confirmed that fat contributes significantly to off-flavor of beef from grass-fed cattle.

Composition of volatiles in cooked beef fat, analyzed using purge-and-trap gas chromatography, gave a representative chromatogram as presented in Fig. 1. Of 89 compounds quantified, 59 were identified. They included 6 diterpenoids, 26 hydrocarbons, 11 aldehydes, 3 methyl ketones, 3 lactones, 3 aromatic compounds, 2 alcohols, 3 acids, and decamethyl cyclopentasiloxane. The concentration of total volatiles was greater for fat from GRASS and GRAIN steers than for GOG steers. Diterpenoid compounds, among the major volatiles quantified, were present in greatest concentration in fat from GRASS cattle. The concentration of lactones was higher in fat from GOG and GRAIN steers than GRASS steers. The concentration of total aldehydes was greater in fat from GRAIN steers. The concentration of alcohols and acids showed similar trends, to that observed for lactones in that the concentration increased when steers received grain as part of their finishing diet.

The concentration of 41 of the identified compounds were influenced by diet (Table 3). A series of n-homologous hydrocarbons ranging from 7 to 18 carbons in length were identified. The concentrations of heptane, undecane, dodecane, tridecane, tetradecane, pentadecane, hexadecane, and octadecane were influenced by diet. One unsaturated hydrocarbon, 2-octene, was identified and present in a higher concentration in fat from GRASS steers. Twelve branched chain hydrocarbons were identified in the volatile fraction of cooked beef fat, but only six were influenced by diet. One cyclic hydrocarbon, heptylcyclohexane, was identified and determined to be greater in concentration in fat from GRAIN steers.

Identified alcohols included octanol and 2-ethyl-1-decanol, however, only octanol varied in concentration as a result of diet. Cooked fat from GRAIN steers had more octanol than fat from GRASS steers. The formation of n-hydrocarbons and primary alcohols can be explained in an analogous manner (Selke et al., 1975). It has been suggested that n-hydrocarbons are formed by the reaction of alkyl-free radicals, which are products of thermally decomposed hydroperoxides, with hydrogen-free radicals. Primary alcohols are suspected to be formed from the reaction of hydroxyl-free radicals with the alkyl-free radicals. Many of these branched chain hydrocarbons appear to originate as decomposition products of the diterpenoids present in the fat of GRASS steers. Their contribution to gamey/stale off-flavor needs further characterization.

Three high molecular weight acids, including pentadecanoic acid, hexadecanoic acid, and octadecanoic acid were identified. Kawada et al. (1967) suggested several schemes by which the acids may be formed from triglycerides. The postulated schemes were: (1) hydrolysis of ester linkages; (2) oxidation of fatty acids of longer chain length; (3) thermal degradation of esters; (4) thermal oxidation of an alpha carbon atom of the acids in triglycerides; and (5) autoxidation of aldehydes and ketones. Selke et al. (1975) postulated that aliphatic acids were formed by oxidizing their corresponding aldehydes. These concentrations reflect differences in initial fatty acid composition of the diet in that corn contains a high concentration of octadecanoic acid (Blank, 1955).

Aromatic compounds (1,4-dimethyl benzene, benzaldehyde, and 1,3,5-trimethylbenzene) were identified from cooked beef fat. One possible source of aromatic compounds is the thermal oxidation of aromatic amino acids. Kato et al. (1971) reported on the occurrence of volatile aromatic compounds including toluene, benzaldehyde, ethyl benzene and vinyl benzene, that were formed when phenylalanine was heated at 300°C. Kawada et al.

Table 4—Correlation between purge-and-trap volatiles and sensory characteristics of 20% fat ground beef

Peak #	Compound Name	Flavor Descriptor: Sour	Blood-like	Tallowy	Roasted	Gamey/stale off-flavor
Acids and alcohols						
12	Octanol	−0.002	0.263	0.123	0.307*	−0.303*
52	Pentadecanoic acid	0.170	−0.254	−0.138	−0.244	0.300*
56	Hexadecanoic acid	0.002	0.095	0.042	0.314*	−0.261
59	Octadecanoic acid	0.021	0.304*	0.110	0.503**	−0.306*
Aldehydes and ketones						
1	Pentanal	0.136	−0.092	−0.080	−0.175	0.331*
11	Benzaldehyde	−0.098	−0.242	−0.019	−0.291*	0.248
33	2,4-Decadienal (E,E)	0.181	0.347*	0.031	0.280*	−0.288*
37	Dodecanal	0.141	−0.240	−0.065	−0.212	0.372**
44	2-Pentadecanone	0.050	−0.089	−0.126	−0.168	0.330*
48	17-Octadecenal	−0.030	−0.240	−0.193	−0.186	0.388**
Diterpenoids						
46	Phyt-1-ene	0.063	−0.171	−0.275	−0.348*	0.505**
50	Neophytadiene	−0.098	−0.429**	−0.254	−0.181	0.370**
51	Phyt-2-ene	−0.091	−0.179	−0.171	−0.166	0.438**
57	Dihydrophytol	−0.192	−0.321*	−0.188	−0.062	0.163
Hydrocarbons						
2	Heptane	0.100	0.369**	0.178	0.336*	−0.204
4	Octane	0.024	0.290*	0.213	0.219	−0.196
5	2-Octene	−0.175	−0.469**	−0.225	−0.304*	0.119
47	Octadecane	−0.094	−0.306*	−0.234	−0.260	0.494**
8	1,4-Dimethylbenzene	0.183	0.353*	0.167	0.036	0.009
13	2,7-Dimethyloctane	0.071	0.296*	−0.022	0.331*	−0.270
14	1,3,5-Trimethylbenzene	0.109	0.413**	0.171	0.357*	−0.199
16	2,6-Dimethyloctane	−0.243	−0.390**	−0.250	−0.396**	0.185
17	3-Methyl-5-propylnonane	−0.290*	−0.496**	−0.264	−0.452**	0.286*
21	3,6-Dimethyldecane	−0.035	−0.322*	−0.300*	−0.248	0.195
22	2,2,5-Trimethyldecane	−0.261	−0.487**	−0.298*	−0.447**	0.298*
25	3-Methylundecane	−0.183	−0.348**	−0.248**	−0.430**	0.208
32	Tridecane	−0.166	−0.284*	−0.143	−0.268	0.246
34	Heptylcyclohexane	0.122	0.290*	0.214	0.442**	−0.316*
36	Tetradecane	−0.138	−0.308*	−0.198	−0.291*	0.309*
38	2,6,10-Trimethyltridecane	0.009	−0.262	−0.120	−0.283*	0.497**
40	Pentadecane	0.038	−0.299*	−0.137	−0.333*	0.614**
42	Hexadecane	0.007	−0.286*	−0.137	−0.310*	0.640**
Lactones						
43	γ-Dodecalactone	−0.093	0.052	0.061	0.149	−0.455**
45	δ-Dodecalactone	0.000	0.292	0.165	0.462**	−0.478**
55	δ-Tetradecalactone	0.034	0.394**	0.188	0.496**	−0.468**
58	δ-Hexadecalactone	0.073	0.195	0.085	0.425**	−0.246

* Significant at the $P \leq 0.05$ level.
** Significant at the $P \leq 0.01$ level.

(1967) proposed that benzaldehyde was formed as a result of the thermal oxidation of an aromatic ring structure formed as a result of the dehydration of a keto acid intermediate.

Three methyl ketones were identified, but only 2-pentadecanone was influenced by finishing diet. Finishing diet did not affect the concentration of 2-tridecanone or 2-heptadecanone in subcutaneous fat. Formation of methyl ketones is thought to result from the β-oxidation of the carbon chain and the subsequent decarboxylation of the fatty acid (Selke et al., 1975).

Eleven aldehydes, which included pentanal, hexanal, 2-heptenal, nonanal, decanal, 2-decenal, 2,4-decadienal (E,E), 2,4-decadienal (E,Z), dodecanal, tetradecanal and 17-octadecenal, were identified in the volatile component of cooked beef fat. Eight of these were influenced by finishing diet. In general, the presence of aldehydes has been attributed to the thermal decomposition of monohydroperoxides hypothesized to be initial products of thermally oxidized fats (Selke et al., 1975).

The four lactones identified in the volatile fraction of cooked beef fat were influenced by finishing diet. In general, fat from cattle fed grain, either on grass or in the feedlot, had higher concentrations of lactones. Lactones have been identified as volatile decomposition products of trilinolein (Selke et al., 1977; Thompson et al., 1978), tristearin (Selke et al., 1975, 1977), esterified olein-stearin (Selke et al., 1977) and also long chain fatty acids, including lauric, palmitic, stearic and oleic acids (Watanabe and Sato, 1971). Oxidation of fatty acids or aldehydes is a proposed source of both saturated and unsaturated lactones (Watanabe and Sato, 1969). For fatty acids, it has been suggested that hydroperoxides, formed as a result of addition of heat and oxygen, are decomposed to form hydroxy-free radicals, which are subsequently oxidized to form hydroxy acids. The hydroxy acids then undergo a cyclization reaction to form lactones. Watanabe and Sato (1969) have also suggested an alternate mechanism for formation of saturated lactones involving oxidation of aldehydes to form corresponding acids, which undergo a cyclization reaction to form lactones. Several researchers (Stark et al., 1978; Urbach, 1990) have presented mechanisms for the biosynthetic production of lactones. These pathways involve production from unsaturated fatty acids, particularly linoleic acid and oleic acid, via formation of corresponding 9-hydroxy fatty acids followed by several β-oxidations.

Six diterpenoid compounds, which included phyt-1-ene, phytane, neophytadiene, phyt-2-ene, phytadiene, and dihydrophytol, were identified in cooked fat volatiles. Diterpenoids comprised 55.7% of the total volatile concentration of fat from GRASS steers. With exception of dihydrophytol, the diterpenoids were all influenced by diet in a similar manner. That is, the concentration of the diterpenoids in fat from GOG steers was less than the concentration in fat from GRASS steers. This was consistent with the trend observed in marbling and quality grades in that beef from GOG steers was scored higher for these characteristics which indicates a dilution of diterpenoids due to increased marbling (triglyceride) deposition. This study confirmed findings pertaining to terpenoid compounds identi-

Table 5—Correlation between purge-and-trap volatiles and sensory characteristics of cooked beef fat

Peak #	Compound Name	Flavor descriptors: Roasted	Flavor descriptors: Gamey/style off-flavor
Acids			
59	Octadecanoic acid	0.355*	−0.224
Aldehydes and ketones			
1	Pentanal	−0.276	0.467**
3	Hexanal	0.475**	−0.338*
33	2,4-Decadienal (E,E)	0.362*	−0.173
37	Dodecanal	−0.405**	0.485**
44	2-Pentadecanone	−0.345*	0.500**
54	2-Heptadecanone	0.532**	−0.421**
Diterpenoids			
46	Phyt-1-ene	−0.591**	0.628**
50	Neophytadiene	−0.316*	0.462**
51	Phyt-2-ene	−0.374**	0.349*
53	Phytadiene	−0.325*	0.332*
57	Dihydrophytol	−0.240	0.326*
Hydrocarbons			
2	Heptane	0.297*	−0.129
13	2,7-Dimethyloctane	0.405**	−0.219
22	2,2,5-Trimethyldecane	−0.171	0.307*
34	Heptylcyclohexane	0.375**	−0.236
36	Tetradecane	−0.207	0.317*
38	2,6,10-Trimethy tridecane	−0.482**	0.574**
40	Pentadecane	−0.575**	0.702**
42	Hexadecane	−0.619**	0.685**
47	Octadecane	−0.307*	0.302*
Lactones			
43	γ-Dodecalactone	0.399**	−0.476**
45	δ-Dodecalactone	0.486**	−0.409**

* Significant at the P≤ 0.05 level.
** Significant at the P≤ 0.01 level.

Table 6—Sensory characteristics of 20% ground beef selected by stepwise discriminant analysis for inclusion into the model used to predict diet[a]

Roasted beef
Bloodlike
Gamey/stale off-flavor

[a] This model had an average squared canonical correlation (ASCC) of 0.4615.

fied by Larick et al. (1987) in that phyt-1-ene, phytane, phyt-2-ene, neophytadiene and dihydrophytol content decreased when cattle were finished on grain. Phytene and phytadiene were identified in several types of rabbit tissue, which included the kidneys, muscle, and adipose tissue (Ferretti and Flanagan, 1977). Presence of these compounds in rabbit tissues has been attributed to the feed, which also contained phytene and phytadiene.

The presence of isoprenoid hydrocarbons in animal products has not been studied extensively, but two precursors have been reported responsible for their occurrence. Urbach and Stark (1975) reported on the occurrence of neophytadiene and the absence of phyt-1-ene and phyt-2-ene in pasture grass. They concluded, therefore, that neophytadiene was the precursor of phyt-1-ene and phyt-2-ene in butterfat, since butterfat contains neophytadiene and also phyt-1-ene and phyt-2-ene. They postulated that neophytadiene was hydrogenated in the rumen to form phyt-1-ene. Body (1977), however, suggested that dietary phytol was the precursor of isoprenoid hydrocarbons. He showed that the dehydration of dihydrophytol, formed by biohydrogenation of phytol in the rumen, results in formation of phyt-1-ene, phyt-2-ene, and phytane.

Correlation coefficients were calculated to establish the relationship between purge-and-trap volatiles from beef fat and sensory characteristics of 20% fat ground beef (Table 4). Compounds (15 positive and 7 negative) were shown related to the gamey/stale off-flavor note which characterizes grassy flavor

Table 7—Purge-and-trap volatiles from melted fat selected by stepwise discriminant analysis for inclusion into the model to predict diet[a]

hexanal	γ-dodecalactone
2-octene	δ-dodecalactone
heptanal	phyt-1-ene
1,3,5-trimethylbenzene	neophytadiene
unknown 14	phyt-2-ene
2,6,10-trimethyltridecane	pentadecanoic acid
pentadecane	dihydrophytol
tetradecanal	octadecanoic acid
hexadecane	

[a] This model had an average squared canonical correlation (ASCC) of 0.9292.

associated with beef from forage-fed steers. Eight compounds positively correlated with the bloodlike and roasted terms and seven compounds negatively correlated with those terms.

Correlation coefficients were also calculated to determine if there was a relationship between purge-and-trap volatiles identified in melted fat and sensory characteristics of the cooked fat (Table 5). Trends were similar to those observed when evaluating relationships between volatiles from beef fat and sensory characteristics of 20% fat ground beef. Several branched hydrocarbons and each of the diterpenoids positively correlated with the gamey/stale off-flavor characteristic and negatively correlated with the roasted characteristic.

The forward selection procedure of stepwise discriminant analysis (SAS Institute, Inc., 1985) was used to select sensory characteristics of 20% fat ground beef and purge-and-trap volatiles from melted fat for inclusion into models to predict diet. Three sensory characteristics were selected for inclusion into a model to predict diet (Table 6). The model had an average squared canonical correlation (ASCC) of 0.4615. The ASCC is close to 1.0 if all groups (finishing diet fed to cattle) are well separated and if all or most directions in the discriminant space show good separation, for at least two groups (SAS Institute, Inc., 1985). Using this model, it was possible to correctly classify 74% of the samples. Eleven samples were incorrectly classified. Six GOG samples were classified as GRAIN samples. One GRASS sample was misclassified as a GOG sample and one was incorrectly identified as a GRAIN sample. Three GRAIN samples were incorrectly categorized. Two were identified as GOG samples and one was predicted to be a GRASS sample.

Purge-and-trap volatiles identified in melted fat, that were selected for inclusion into a model to predict diet are listed in Table 7. Seventeen compounds were selected. The model had an ASCC of 0.9292 and was capable of correctly classifying 100% of the samples into the proper diet treatment.

This procedure has been used previously to select a model to predict diet using volatile compounds identified in fat from cattle finished on grain for up to 112 days (Larick et al., 1987). Phyt-2-ene was the only compound found to have a commonality between our results and that presented by Larick et al. (1987).

The lack of significance in triangle tests in which trained panelists were unable to differentiate between samples of lean L. dorsi emphasizes the importance of fat in overall flavor of beef from forage- and grain-fed cattle. The data presented demonstrates that diterpenoid compounds are statistically associated with gamey/stale off-flavor in beef from GRASS cattle. This is evidenced by the higher concentrations of diterpenoid compounds, which included phyt-1-ene, neophytadiene, phyt-2-ene, and dihydrophytol, and also the positive correlation values between these compounds and the gamey/stale off-flavor of both cooked fat and 20% fat ground beef. There is also statistical evidence that lactones contribute to overall flavor of beef from grain-fed cattle, since these compounds were present

in a greater concentration in fat from GRAIN steers. Also they positively correlated with the roasted and bloodlike characteristics and negatively correlated with the gamey/stale off-flavor.

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Thermophysical Properties of Extruded Beef/Corn Flour Blends

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ABSTRACT

Thermal properties, specific heat, and thermal conductivity of three beef/corn meal blends extruded to reach end product temperatures of ≈91°C and 109°C were analyzed. Density was also measured and used to calculate thermal diffusivity. The extruded products had densities of 1,054 to 1,091 kg/m^3; specific heat was 2.944 to 3.055 kJ/kgK; and thermal conductivity, 0.26 to 0.39 W/mK. Calculated values of thermal diffusivity were 0.831 to 1.200 ($\times 10^{-7}$m^2/sec). With the exception of specific heat, extrusion conditions and formulation affected some of the products physical characteristics and thermal properties.

Key Words: corn, beef, extrusion, thermal properties, conductivity

INTRODUCTION

DATA on thermal properties of new food products are needed to understand their thermal behavior and to control heat transfer processes. In analysis of transient heating or cooling, data on thermal properties (specific heat, thermal conductivity, and thermal diffusivity) are needed (Rice et al., 1988).

Sweat (1986) emphasized the need to know how thermal properties change during a process, especially when products of more uniform composition are produced. Two major conditions that influence extrusion processing are composition of the raw material and process conditions. Minor deviations in process conditions and variations in formula can cause major changes in the quality of the finished product (Davidson et al., 1984). Thermal conductivity depends mostly on composition, but also on any factor that affects the heat flow paths through the material (Sweat, 1986). Differences in thermal conductivity among samples of the same food blends may reflect unintentional changes in extrusion conditions. Thus, thermal conductivity may also be used as a quality control indicator in an extrusion process. Some extruded products are used in food formulations mixed with other ingredients. Differences in temperature response among such ingredients could have adverse effects on the quality of the final product. Thermal properties must be known to properly identify undesirable heating conditions.

Contributing to the development of a beef/corn flour extruded product (Unklesbay et al., 1992), our objective was to: (1) measure the moisture and lipids content and thermal properties of three beef/corn flour blends, and (2) determine the effects of extrusion variables on those thermal properties. Since the product is not shelf stable, it would be stored, either frozen or chilled, and heated for consumption. Thermal property data would be needed to optimize these post-extrusion operations.

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MATERIALS & METHODS

THE PRODUCT formulations and extrusion conditions were described by Unklesbay et al. (1992). Formulations included ground beef with either white corn flour (30%), yellow corn flour (30%), or white corn flour (25%) combined with soy fiber (5%). Data in Table 1 give the mean extruder conditions. The higher temperature extrusion required significantly ($p < 0.05$) higher torque, specific energy and die pressure. Samples were securely wrapped with freezer wrap (Loxol freezer wrap, polyethylene coated; James River Corp., Parchment, MI) and placed in a low temperature freezer (−70°C). The samples were tempered at 4°C for 24 hr prior to analysis. All thermal property determinations were at ambient temperature (22 ± 1°C).

Analyses

Moisture content was determined by the vacuum oven method (AOAC, 1984). Lipids were determined using the Folch procedure (Folch et al., 1957) by extracting 5g samples with chloroform:methanol (2:1, v/v).

Density

A known mass (≈ 5g) of sample was added to a 60 mL volumetric flask which was filled to volume with distilled water (22°C). Density was calculated using equation 1:

$$\rho = \frac{M}{60 - V} \quad (1)$$

where ρ = density (kg/m^3); M = mass of sample (kg); and V = volume of water added (m^3) (Szczesniak, 1983). This procedure was recommended for meats.

Prior to using these procedures, absorption experiments were done with water to determine if the product structure contained too many void spaces to use this technique. When 10 samples (5g) were individually submerged in water for 60 sec (the mean time for the density experiment), the average moisture absorption was 1.75 ± 0.25%. Previous research with density and pork/soy hull mixtures (Muzilla et al., 1990) demonstrated that differences in moisture contents as great as 9.2% did not significantly ($p < 0.05$) alter density values. Further, accuracies >2–5% are not required for thermal property measurements (Sweat, 1986). Thus, the conclusion was reached that the procedure (Eq. 1) was appropriate for this rather compact, extruded product.

Specific Heat

A 1L Dewar flask fitted with a plastic foam lid was used. A thermocouple (Type K) was placed through the lid to the midpoint of a Thermos® which had been calibrated with known amounts of hot and cold distilled water. A known mass (≈100g) of heated distilled water (≈80°C) was placed into the Thermos and equilibrated. This temperature was recorded (Omega digital thermometer, Model 2176A). A known mass of sample (≈50g) at a known temperature (≈22°C) was added to the water and its temperature recorded after equilibration. The equation for calculating specific heat was given by McProud and Lund (1983):

$$C_p = \frac{(C_p,H_2O)\,(M_w)\,(T_i - T_e)}{(M_s)\,(T_e - T_s)} \quad (2)$$

where C_p = specific heat of sample (cal/g°C); C_p,H_2O = specific heat of water (cal/g°C) obtained from the calibration; M_w = mass of water (g); T_i = initial temperature of water in thermos (°C); T_e = equilibrium temperature of water and sample (°C); M_s = mass of

Table 1—Mean extruder conditions

Beef Product	Mean barrel temperature (°C)[a]				Mean parameters[b]				
	Zone 3	Zone 4	Zone 5	Zone 6	Feed rate (kg/h)	Screw speed (rpm)	Torque (%)	Specific energy (kJ/kg)	Die pressure (kPa)
WCFF[c] (LT)[d]	27.5	102.3	102.4	102.3	15	100.3	6.3[B]	84.1[D]	279.0[D]
WCFF (HT)[e]	27.7	130.2	129.2	131.5	15	100.3	7.1[A]	95.6[B]	308.8[B]
WCF (LT)	27.4	101.3	101.0	101.6	15	100.3	5.7[B]	75.9[E]	267.5[E]
WCF (HT)	27.8	131.1	131.4	132.2	15	100.3	7.0[A]	94.0[C]	321.9[A]
YCF (LT)	27.1	99.5	99.6	99.7	15	100.3	5.6[B]	75.8[E]	261.1[F]
YCF (HT)	28.3	132.1	131.4	132.3	15	100.3	6.9[A]	92.5[A]	296.0[C]

[a] *Each mean barrel temperature was the mean of 77 readings per product, measured every 15 seconds.*
[b] Values with the same superscripts [ABCDEF] in a column are not significantly different at the 5% level.
[c] WCFF = white corn flour and soy fiber; WCF = white corn flour; YCF = yellow corn flour.
[d] Low extrusion temperature with barrel temperatures for Zones 1 and 3–6 set at ca. 29°C and 100°C, respectively.
[e] High extrusion temperature with barrel temperatures for Zones 1 and 3–6 set at ca. 29°C and 133°C, respectively.

Table 2—Thermophysical Properties and Proximate Analysis of Extruded Beef/Corn Four Blends[a,b]

Product	End product temp. (°C)	Moisture (%)	Lipids (%; M.F.)	Density (kg/m³)		Specific heat (kJ/kgK)		Thermal conductivity (W/mK)		Thermal diffusivity (x10⁻⁷m²/s)
		Mean	Mean	Mean	S.E.	Mean	S.E.	Mean	S.E.	
WCFF (Raw)		48.8	20.13	1,090	10	3.687	0.13	0.32	0.01	0.796
WCFF (LT)	92.1[A]	43.7	14.92	1,054[B]	8.2	2.970	0.03	0.32[AB]	0.01	1.022
WCFF (HT)	109.0[B]	43.3	14.22	1,079[A]	7.4	3.010	0.06	0.39[A]	0.02	1.200
WCF (Raw)		49.0	20.00	1,150	10	3.021	0.01	0.37	0.02	1.065
WCF (LT)	91.0[A]	44.1	16.12	1,055[B]	7.7	2.944	0.03	0.32[AB]	0.02	1.030
WCF (HT)	109.4[B]	43.4	14.25	1,081[A]	1.4	3.055	0.06	0.30[B]	0.01	0.900
YCF (Raw)		48.9	19.97	1,150	10	3.590	0.14	0.29[B]	0.03	0.702
YCF (LT)	91.0[A]	44.9	18.71	1,036[B]	7.0	3.017	0.08	0.26[B]	0.01	0.831
YCF (HT)	109.1[B]	44.2	16.30	1,070[AB]	10	3.042	0.06	0.38[A]	0.04	1.167

[a] N = 5 except moisture and lipids (duplicate samples).
[b] For heat processed samples, values with the same superscript[AB] in a column are not significantly different at the 5% level.

sample (g); and T_s = temperature of sample (°C). Values were converted to kJ/kg°C by multiplying by the factor 4.1868 (Toledo, 1991).

Thermal conductivity

A small thermal conductivity probe identical to those used by Sweat et al. (1973) was used. It contained a resistance heater and was powered by a DC power supply set at 4.0 V with current in the heater circuit ranging from 235–240 mA. An ice water filled Thermos® functioned as the reference junction for the probe thermocouple (Type T). After each sample (ca. 43g; 45 × 20 × 27 mm) was placed into a 30 mL Pyrex beaker, the probe was positioned in the geometric center of a piece to cover the full length of the probe. Before initiating these experiments, we had established that when the probe was activated, no heat transferred to the surface of the extruded product. For this experiment, the power supply was activated and the temperature rise recorded. Elapsed time and temperature values were sampled at 6 s intervals for ≈4 min and the slope between each pair of points was calculated. By examining the values of slopes, the nonlinear segments of the curve were rejected. Linear regression was performed on the remaining values to determine the best fit which was used to compute thermal conductivity (Sweat and Haugh, 1974).

Thermal diffusivity

Thermal diffusivity was calculated from density, specific heat, and thermal conductivity using Eq. (3):

$$\alpha = \frac{K}{\rho C_p} \qquad (3)$$

where α = thermal diffusivity (m^2/sec); K = thermal conductivity (W/mK); ρ = density (kg/m^3); and C_p = heat capacity (kJ/kgK) (Singh, 1982).

Statistical Analysis

Each thermal property measurement was replicated five times. Data were analyzed by ANOVA (Snedecor and Cochran, 1980) based on a 2 × 3 factorial design. Means were separated using Fisher's least significant difference (Chew, 1976). The SAS statistical package was used (SAS Institute, Inc., 1985).

RESULTS & DISCUSSION

Moisture content

Water has the major influence on temperature dependence of thermal properties (Morley, 1972). Moisture was lost by evaporative cooling as extrudates exited from the die. The extent of moisture loss increased with increasing difference in temperature behind the die and the boiling point of water at ambient pressure. Thus, each of the blends exhibited a downward trend in moisture content with increase in extrusion temperature (Table 2).

Lipid Content

When calculated on a moisture-free basis, the lipid content (%) after low and high extrusion ranged from 14.92 to 18.71% and 14.22 to 16.30%, respectively. When the beef/corn flour blends exited the die, some lipids in the liquid state could be visually observed escaping from the product. The data (Table 2) revealed this occurred to a slightly greater extent with the higher extrusion temperatures.

Density

Density reflects the degree of the product expansion on exiting the extruder die. Values of density are also needed to determine the thermal diffusivity. After extrusion processing, density values ranged from 1,054 to 1,091 kg/m^3 (Table 2). These values agreed with the range for beef of 1,030 to 1,090 kg/m^3 reported by Dickerson and Read (1975). Blends containing white corn flour, produced significantly ($p < 0.05$) higher density extrudates at the high temperature compared to the lower temperature extrusion.

Specific Heat

Specific heat is the enthalpy change associated with a change in temperature. Specific heat may be considered constant over a narrow temperature range. No published values for specific heat of extruded products exist. The specific heat of fresh beef is 2.930 kJ/kgK and 3.515 kJ/kgK at 48 and 72% moisture contents, respectively (Szczesniak, 1983). No significant ($p < 0.05$) differences in heat capacity values were observed (Table 2). Specific heat values ranged from 2.944 to 3.055 kJ/kgK. Water content affects the specific heat more than the other formula components (Lewis, 1987). The moisture content range of 43.3 to 44.9% did not reflect a large enough difference to influence specific heat values.

Thermal conductivity

Thermal conductivity varies with moisture content, composition, structural orientation, temperature, and pressure (Mohsenin, 1980). The extruded yellow corn flower blends made at low extrusion temperatures had thermal conductivity values significantly ($p < 0.05$) different from those made at high extrusion temperatures. Although Sweat (1986) suggested that thermal conductivity increases with moisture content, our data (Table 2) indicated the opposite. Thermal conductivity however, is also directly related to the structural orientation relative to the direction of heat flow (Lentz, 1961). We observed no pattern of fiber orientation in the samples. However, extrusion physically converts protein bodies into a homogenous matrix (Stanley, 1989). Starch may stabilize meat particles into a specific structure, therefore differences in structure may exist. Such structural differences could have more of an effect on thermal conductivity than moisture.

Thermal diffusivity

Thermal diffusivity values (Table 2) of the extruded products varied over a relatively narrow range. Water content, temperature, composition, and porosity are the major factors affecting thermal diffusivity. A strong dependence on water content has been shown (ASHRAE, 1981). Because thermal diffusivity is dependent on thermal conductivity, specific heat and density, trends in calculated values reflect the same trend as those thermal properties. Thermal diffusivity values tended to increase when: (1) high end product temperatures were used (Table 1); (2) higher torque (%), specific energy, and die pressure occurred during extrusion (Table 1); and (3) density and thermal conductivity values increased (Table 2).

CONCLUSIONS

WHEN FORMULATIONS were altered to include either white or yellow corn flour and soy fiber, several differences among thermophysical properties were observed. A high end point product temperature (109°C) after extrusion resulted in less moisture in the extrudate which was reflected by a lower product density, compared to those extruded at the lower temperature (91°C). Except for the formulation containing yellow corn flour, extrusion conditions had no significant effect on thermal conductivity values. This indicated that, for the formulations with white corn flour, greater precision in the thermal conductivity measurements would be needed before this measurement could be used as a quality control criterion in extrusion. Specific heat values of extruded products showed no significant differences attributable to formulation or extrusion conditions.

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Flavor Compounds of Beef Broth as Affected by Cooking Temperature

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ABSTRACT

Of various cooking temperatures studied, broth from beef obtained on heating at 85°C for 60 min had the most intense brothy flavor. The components of the fractions with brothy flavor were isolated by gel filtration chromatography and characterized. A significant correlation between cooking temperature of the broth and the concentration of certain compounds such as free amino acids (FAA) ($r=0.88$, $p<0.005$), carnosine ($r=0.83$, $p<0.005$) and inosine 5'-monophosphate (IMP) ($r=0.94$, $p<0.005$) was detected. Because these results correspond to those of sensory evaluation, it was concluded that a wide mixture of FAA, peptides of low molecular weight ($\leq$ 300 daltons) and IMP played an important role in the flavor intensity of beef broth.

Key Words: beef, broth, flavor, cook temperature

INTRODUCTION

FLAVOR is one of the most important characteristics of beef broth. Taste-active substances are, in principle, nonvolatile compounds. Many of these such as free amino acids, peptides, amines, proteins, sugars, organic acids and nucleotides are present in foods. Since lean meat/muscle tissue is primarily a proteinaceous product, it represents the most pronounced source of "peptidic" flavor principles (Spanier and Edwards, 1987). The role of free amino acids in various foods has been extensively investigated (Kato et al., 1989), but the role of peptides has not been studied in detail. In recent years, however, a number of reports have been published dealing with both synthetic and naturally occurring flavor-active peptides (Nosho et al., 1985; Otagiri et al., 1985; Rodriguez et al., 1985).

Monosodium glutamate (MSG) is known as a flavor enhancer. MSG and 5'-ribonucleotides are mutually synergistic and are important in taste (Kirimura et al., 1969). Inosine 5'-monophosphate (IMP) and guanosine 5'-monophosphate (GMP) have been attributed to enhance meaty flavor (Koehler and Jacobson, 1967). Arya and Parihar (1979) studied the changes in free nucleotides, nucleosides and purine bases during thermal processing of goat and sheep meats. The major changes took place during the first 30 min of heating and the rates of changes were maximum at 60°C and minimum at 100°C. Nucleotides and nucleosides are also potential precursors of free ribose and ribose phosphate, which have been implicated in Maillard reactions during processing and storage of flesh foods (Macy et al., 1964).

Heating of muscle during processing or cooking results in both chemical and physical changes of many compounds. With the temperature as low as 30°C protein degradation is initiated, and small fragments may be split from some proteins (Wasserman, 1972). Between 35°C and 50°C actomyosin fibers unfold, increasing the number of available imidazolium groups. Above 80°C the -SH groups are oxidized to disulfide bonds and above 90°C, H_2S is released from the -SH groups of the myofibrillar protein (Wasserman, 1972). Cheng and Parrish (1979) studied heat-induced changes in myofibrillar protein solubility and as Wasserman (1972) reported most of the proteins are coagulated between 55°C and 80°C. Spanier et al. (1990) studied the activity of some hydrolytic enzymes in beef at temperatures similar to those during cooking. They stated that the transition temperature for most of the major changes in fresh meat flavor appeared to be near 68°C. Although chemical changes in protein during cooking have been well documented (Davis and Anderson, 1984), few studies deal with the role of peptides in determining the flavor of cooked meat products. Spanier and Edwards (1987) and Spanier et al. (1988) have studied the potential of low-molecular-weight peptides on flavor from red meat. The greatest initial increase in low molecular weight peptides appeared at 70°C (Spanier et al., 1990). While numerous peptides, which exhibit bitter taste, are known, only a few products of protein hydrolysis are described as broth-like. Examples are the so-called "delicious peptides," which were isolated from beef treated with papain (Yamasaki and Maekawa, 1978), and some dipeptides, containing aspartic acid and/or glutamic acid (Arai et al., 1973; Noguchi et al., 1975; Ohyama et al., 1988). Arai et al. (1972) isolated four peptides from a proteinase modified soybean protein, which had a brothy taste. Nishimura et al. (1988) suggest that the increase in free amino acids and peptides contributed to improvement of brothy, meaty taste with storage.

The objectives of this study were to determine the effects of cooking temperature on the flavor of beef broth and to identify the compounds responsible for improvement of the brothy flavor due to heating temperature. The concentration of water-soluble components especially free amino acids, peptides and 5'-ribonucleotides was determined to clarify the relationship between taste intensity and the concentration of taste-active compounds.

MATERIALS & METHODS

Preparation of broths

Beef (*M. sternomastoideus*) stored at 15°C for 1 day an then at 0°C for 5 days after slaughtering was used. Meat samples were minced in a grinder (Bizerba FW 70, West Germany) using a plate with holes of 3 mm diameter. Portions of the minced meat were mixed with an equal volume of saline solution (0.75% NaCl). These homogenates were heated in a water bath for 60 min at 55°, 65°, 75°, 85° and 95°C (from 20 to 30 min were necessary to reach the end-point temperature). After the specified heating time, samples were filtered (Whatman No. 4) and centrifuged (3,000 rpm.; 10 min; 15°C) to obtain a clear broth. The final volume of each broth was adjusted with 0.75% NaCl solution to its original volume (meat plus saline solution).

For chemical analysis and gel filtration chromatography an acid-soluble fraction was prepared by addition of trichloroacetic acid (TCA) to a final concentration of 10%. Nitrogen concentration of TCA-soluble broth was determined by the Kjeldhal method (928.08, AOAC, 1990). Lactic acid, creatine and creatinine were determined by enzyme assay from Boehringer Mannheim (Anonymous, 1984).

Gel filtration chromatography

Sephadex G-10 was packed into a column (2.5 cm by 100 cm) and equilibrated with 0.01M LiCl. Elution (eluent: 0.01 M LiCl) was at room temperature, at a flow rate of about 0.4 mL/min. For sensory

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Table 1—Sensory evaluation of the relative strength of the brothy beef flavor obtained in heating at different temperatures for 60 min

Cooking temperature	Number of samples judged in order of intensity of brothy beef flavor				
	Score				
(°C)	1 (minimum	2	3	4	5 maximum)
55[a]	20				
65[a]		19	1		
75[a]		1	19		
85[a]				2	18
95[a]				18	2

[a] $p < 0.005$, $n = 20$.

Table 2—Concentration (μmol/100 mL) of free amino acids (FAA), amino acids after acid hydrolysis (PAA)[a] and ATP-metabolites in TCA-soluble fraction of beef broth obtained by heating at 55°, 65°, 75°, 85° and 95°C for 60 min[b]

Temperature °C	FAA[c]	PAA[d]	ATP-Metabolite[e]	PAA/FAA
55	257.1 ± 21.8	512.5 ± 27.9	51.9 ± 0.4	1.99
65	334.6 ± 20.9	686.1 ± 47.4	63.6 ± 1.1	2.05
75	390.2 ± 33.4	779.2 ± 17.9	82.2 ± 5.1	1.99
85	645.9 ± 42.1	1064.3 ± 28.8	173.9 ± 0.5	1.65
95	600.7 ± 18.2	1227.8 ± 63.4	223.9 ± 4.3	2.04
			Mean:	1.94

[a] Potential amino acid were estimated from the differences between the values of FAA and amino acids after hydrolysis.

[b] Values in table are means ± standard error from four different samples.

[c] Significant correlation between cooking temperature of broth and FAA concentration: $r = 0.88$, $p < 0.005$, $n = 20$.

[d] Significant correlation between cooking temperature of broth and PAA concentration: $r = 0.96$, $p < 0.005$, $n = 20$.

[e] Significant correlation between cooking temperature of broth and ATP metabolites concentration: $r = 0.95$, $p < 0.005$, $n = 20$.

evaluation elution was made with 0.01M NaCl. Samples of 2 mL of the TCA-soluble fraction from broth were evaporated in a rotary evaporator (RE 111 Büchi) and resuspended in 2 mL column eluant and applied to Sephadex G-10 column. Each fraction was detected by absorbance at 220, 254 and 280 nm.

Amino acid analysis

In broth or in TCA supernatant, carnosine, anserine, free amino acids (FAA) before and after hydrolysis with 6N HCl at 120°C for 24 hr (under nitrogen atmosphere) were analyzed with a Beckman Multichrom M amino acid analyzer. The amino acids were separated on the Ultrapac 11 Resin Lithium form, LKB (LKB Biochrom LTB. Cambridge. England). The quantities of "potential" amino acids (PAA) were estimated from the differences between the values of FAA and amino acids after hydrolysis. The PAA are considered to be amino acids from peptides and/or other amino acid derivatives.

ATP metabolites analysis

Adenosine 5'-triphosphate (ATP), adenosine 5'-diphosphate (ADP), adenosine 5'-monophosphate (AMP), guanosine 5'-monophosphate (GMP), uridine 5'-monophosphate (UMP), inosine 5'-monophosphate (IMP)(adenosine, inosine, xanthine, adenine and hypoxanthine (all called "ATP-metabolites") were analyzed by high performance liquid chromatography (HPLC) using an anion exchange column (Chromsep HPLC column, 10 cm, packed with Ionospher-A from Chrompack) at 60°C with a linear gradient from 90% KH_2PO_4(0.006M), pH 3 and 10% KH_2PO_4 (0.3 M), pH 6 at 0 min to 5% KH_2PO_4 (0.006M), pH 3 and 95% KH_2PO_4 (0.3M), pH 6 at 25 min.

Sensory evaluation

Four trained panelists judged the relative strength of the brothy beef flavor between different heating temperatures using a rank order-test. A group of five beef broth samples at 40–45°C were presented simultaneously in 5 different experiments. Every broth was prepared immediately before sensory evaluation. The judge was instructed to rank them in order of the intensity of brothy beef taste using a 5-point scale (1-minimum and 5-maximum taste intensity).

The flavor from each fraction eluted by gel filtration chromatography of the TCA-soluble fraction of beef broths was also evaluated for taste quality (sweet, bitter, sour, salty, brothy and astringent impression) and taste intensity using a 4 point scale (+ weak, + + medium, + + + strong and + + + + very strong). The sensory analyses of gel filtration chromatography fractions were performed at room temperature (20–22°C).

Statistical analysis

Simple linear regression analysis was performed with a "StatView®" program from Macintosh (Feldman and Gagnon, 1985).

RESULTS & DISCUSSION

Sensory evaluation and analytical data

As the heating temperature increased to 95°C, the brothy flavor intensity became significantly stronger (Table 1). The brothy flavor at 85°C, however, was stronger than that at 95°C. Wassermazn (1972) showed that boiled beef flavor was produced in the presence of water whereby the temperature during boiling did not exceed 100°C. Apparently, flavor precursors of meat are extractable with cold water and lyophilized cold water extracts of raw beef and pork produce meaty flavor upon heating (Bailey, 1983). Bowers et al. (1987) described the change of sensory attributes during heating (Bailey, 1983). Bowers et al. (1987) described the change of sensory attributes during heating of meat between 55°C and 85°C by two heating methods and the results were in general agreement with those obtained in our present study. Bowers et al. (1987) stated that meaty flavor increased with increasing temperature whereas bloody-serumy, metallic and sour flower decreased with increased temperature. Protein denaturation appeared to be mostly complete after muscle samples were heated to 80°C (Bowers et al., 1987; Spanier et al., 1990).

The concentration of FAA, PAA and ATP metabolites (sum of 5'-monoribonucleotides and their breakdown products detected by HPLC) increased with increasing endpoint temperature of heating (Table 2). The levels of these three compounds are considered markers of hydrolytic activity. The results may be compared with other reported studies. Bowers et al. (1987) studied flavor, color and related changes that occur as beef muscle was heated between 55° and 85°C. They concluded that the cooking losses probably resulted from protein denaturation completed at 75° to 80°C and may account for the flavor change detected. Spanier et al. (1990) studied the activity of beef endogenous proteinases at 20° to 80°C. They stated that the greatest initial increase in low molecular weight peptides appeared at 70°C. These low molecular weight peptides are potential flavor principles (Spanier et al., 1990).

The PAA and ATP-metabolites reached a maximum in broth from meat heated to 95°C (Table 2). However, the FAA content was highest in broth obtained at 85°C. FAA ($r = 0.88$, $p < 0.005$), PAA ($r = 0.96$, $p < 0.005$) and ATP-metabolites ($r = 0.95$, $p < 0.005$) concentrations showed a significant correlation with the cooking temperature of broths (Table 2). These results corresponded to results of the sensory evaluation studies, indicating that FAA together with PAA and some ATP metabolites were very important to the improvement of beef broth taste. Nishimura et al. (1988) also observed that the increase in FAA and oligopeptides contributed to improvement of brothy taste of meat during storage.

The average value of the PAA/FAA relation was 1.94 (Table 2). This value was similar at all heating temperatures (about 2, except for 85°C, which was about 1.6). These results suggest a similar increase of peptide (PAA) and FAA levels with increasing temperature. The small differences observed between the broth at 85°C and 95°C may be attributed to different components (such as sugars) and amino acids reactions (Maillard-type) made possible at higher heating temperatures (Bailey, 1988; Bowers et al., 1987). Wasserman (1972) reported that browning began at about 90°C and increased with time

Table 3—Concentration (μmol/100 mL) of each amino acid before (FAA) and after acid hydrolysis (PAA)[a] in TCA-soluble fraction of beef broths obtained by heating at 55°, 65°, 75°, 85° and 95°C for 60 min[b]

	Heating temperatures									
	55°C		65°C		75°C		85°C		95°C	
Amino Acid	FAA	PAA	FAA	PAA	FAA	PAA	FAA	PAA	FAA	PAA
Cysteic acid	c	0.9± 0.5	0.5± 0.4	0.1±0.09	0.2± 0.1	c	0.5± 0.1	7.0± 3.6	0.3±0.1	0.5± 0.3
Taurine	25.0±3.1	0.4± 0.2	32.8± 3.1	3.4± 0.3	16.1± 0.9	7.7± 0.9	106.1± 1.7	46.9± 3.5	96.5±5.4	25.7± 3.2
Aspartic acid		16.0± 1.3	22.2± 4.6	21.7± 2.4	0.9± 0.1	24.3± 5.5	4.0± 0.8	25.8± 3.3	6.5±2.1	28.9± 2.0
Hydroxyproline		133.4±27.3		47.0±16.2		176.5±56.8		91.9±22.4		178.7±17.3
Threonine	2.5±0.4	16.4± 0.7	2.1± 0.4	6.9± 1.7	2.4± 0.2	6.9± 1.1	10.4± 0.6	12.7± 5.4	10.7±0.8	12.1± 3.6
Serine	6.8±1.2	26.7± 5.1	5.0± 0.8	20.7± 6.9	52.5±11.6	19.0± 3.5	20.7± 3.8	34.7± 6.1	24.4±3.9	27.5± 4.0
Glutamic acid	10.6±0.9	86.3± 5.9	13.2± 4.7	64.5± 0.4	7.1± 1.8	63.0± 0.4	18.5± 0.3	131.3±12.2	26.0±1.4	127.2±11.0
Glutamine	112.6±8.4		138.5±10.7		168.7±16.5		147.1±26.7		99.1±1.5	
Sarcosine		4.3± 0.5		5.6± 1.1		5.3± 0.7		34.3± 9.4		27.5± 6.6
Proline	c	12.3± 4.7	1.3± 1.1	4.3± 2.7	0.4± 0.3	10.6± 2.1	10.5± 4.9	12.5± 4.8	8.3±1.1	98.9±32.3
Glycine	36.6±3.4	63.9± 0.4	21.1± 0.3	69.4± 2.2	15.0± 1.3	62.3± 6.0	72.7± 4.5	123.6± 9.9	70.0±1.7	152.0±11.2
Alanine	48.6±4.9	17.0± 3.5	28.6± 1.3	26.1± 5.3	33.5± 7.8	16.5± 4.5	98.4± 2.4	46.7± 7.0	95.1±1.1	39.5± 2.5
Valine	4.4±0.5	c	c	1.0± 0.6	0.9± 0.6	0.6± 0.3	10.1± 0.6	7.7± 0.9	7.2±1.2	8.5± 1.0
Cysteine	c	c	c	1.0± 0.6		0.3± 0.1		11.2± 4.0		10.3± 1.4
Methionine	c	c	c	0.3± 0.2	0.3± 0.2	b	0.3± 0.2	9.0± 2.7	0.2±0.1	9.1± 3.4
Isoleucine	4.7±0.5	1.7± 0.1	4.0± 1.3	77.6± 6.3	4.4± 0.9	3.6± 0.4	15.5± 0.2	41.0± 4.1	14.3±2.1	24.0± 4.2
Leucine	c	18.3± 3.4	10.1± 1.9	10.9± 4.0	14.3± 1.8	10.1± 2.1	18.5± 0.3	90.7±10.7	48.4±6.0	29.5± 0.8
Tyrosine	c	0.4± 0.2	1.5± 0.9	0.5± 0.4	c	0.5± 0.3	16.4± 2.5	0.5± 0.3	15.5±2.2	0.6± 0.4
Phenylalanine	c	0.6± 0.3	2.4± 0.6	0.7± 0.5	0.2± 0.1	1.0± 0.7	16.1± 2.6	1.0± 0.7	16.3±3.1	0.7± 0.4
B-Alanine	3.2±0.3	36.7± 2.9	26.6± 5.4	157.4±20.9	29.5± 6.1	157.1± 2.7	15.9± 2.6	157.2± 2.8	18.3±4.3	178.3±16.3
Ornithine	0.5±0.3	c	17.2± 3.1	0.3± 0.2	0.6± 0.1	3.8± 1.7	44.5±16.2	15.4± 5.8	19.5±6.4	18.4± 4.8
Lysine	1.6±0.2	3.8± 0.5	1.0± 0.3	4.5± 2.0	6.4± 1.1	7.0± 2.3	5.5± 1.2	13.5± 3.3	6.9±0.8	23.5± 2.7
3-Methylhistidine							0.5± 0.3		0.3±0.2	
1-Methylhistidine	c	12.3± 1.2	4.1± 0.6	9.4± 1.1	0.8± 0.5	10.3± 1.9	3.3± 0.9	40.4± 4.8	2.8±0.2	43.2± 5.7
Histidine	c	57.9± 3.5	1.5± 0.3	150.7±19.8	34.9± 9.2	189.9±19.9	8.8± 0.3	98.7±12.3	12.4±0.9	146.5±18.5
Arginine	c	0.5± 0.4	c	0.7± 0.4	0.5± 0.3	0.8± 0.5	0.4± 0.3	8.2± 1.9	0.5±0.2	15.6± 3.6

[a] Potential aminos acid were estimated from the differences between the values of FAA and amino acids after hydrolysis.
[b] Values in table are means ± standard error from four different samples.
[c] Trace amounts, less than 0.01 μmol/100 mL.

Table 4—Concentration (μmol/100 mL) of each amino acid before (FAA) and after acid hydrolysis (PAA)[a] in fractions eluted by gel filtration chromatography of TCA-soluble fraction of beef broths obtained by heating at 55° and 85°C for 60 min[b]

	Heating temperature											
	55°C						85°C					
	FRACTION						FRACTION					
	1	3 +	4	5	6	7	1	3 +	4	5	6	7
Amino Acid	PAA	FAA	PAA	PAA	PAA	PAA	PAA	FAA	PAA	PAA	PAA	PAA
Cysteic acid	-	-	3.9	-	-	-	13.9	0.8	-	-	-	-
Taurine	-	30.0	-	-	-	-	20.9	109.3	10.6	1.8	11.9	7.0
Aspartic acid	-	-	7.4	-	-	6.4	6.6	5.4	8.8	3.2	2.9	-
Hydroxyproline	-	-	-	66.2	118.5	6.0	6.4	-	32.4	5.9	-	-
Threonine	1.6	3.1	2.0	4.8	7.3	2.0	4.2	9.8	3.9	2.0	3.1	3.9
Serine	10.0	7.3	2.7	7.6	13.7	1.5	9.3	27.3	8.3	3.8	7.8	7.6
Glutamic acid	-	11.3	58.3	14.9	12.2	8.0	53.5	8.6	60.9	5.2	11.4	8.9
Glutamine	-	118.1	-	-	-	-	-	193.0	-	-	-	-
Sarcosine	-	-	3.6	-	-	-	-	-	15.0	35.7	-	-
Proline	-	-	-	-	5.1	-	-	20.7	3.1	-	-	-
Glycine	10.0	40.5	-	10.3	18.6	25.3	59.4	79.8	12.1	10.0	22.7	33.7
Alanine	-	56.8	-	3.2	3.9	3.9	16.0	102.6	12.1	8.0	10.5	8.4
Valine	-	4.7	-	-	-	-	-	10.9	3.8	-	2.4	-
Cysteine	-	-	-	-	-	-	8.1	-	-	-	4.9	-
Methionine	-	-	-	-	-	-	9.2	-	-	-	4.7	-
Isoleucine	-	5.3	-	-	-	1.8	15.2	15.3	6.1	13.1	6.4	7.3
Leucine	1.0	-	10.3	-	-	2.2	3.8	18.3	26.1	21.2	23.6	22.5
Tyrosine	-	-	-	-	-	-	-	20.7	-	-	-	-
Phenylalanine	-	-	-	-	-	-	-	20.6	-	-	-	-
β-Alanine	-	3.6	24.7	6.9	-	-	25.6	19.6	110.2	9.2	4.4	4.6
Ornithine	-	0.6	-	-	-	-	7.5	71.3	4.6	4.6	5.5	-
Lysine	-	1.6	2.2	-	-	2.5	5.8	3.5	3.6	2.5	4.2	3.1
3-Methylhistidine	-	-	-	-	-	-	-	1.0	-	-	-	-
1-Methylhistidine	-	-	14.5	-	-	-	7.1	4.8	31.9	5.5	4.3	-
Histidine	-	-	61.6	-	-	2.4	5.2	8.6	89.5	0.9	7.4	3.6
Arginine	-	-	-	-	-	-	-	-	-	-	6.5	-
Percent of total	4.0[c]	99.6[d]	33.5[c]	20.0[c]	31.4[c]	10.8[c]	24.9[c]	98.5[d]	39.7[c]	11.9[c]	13.1[c]	10.0[c]

[a] Potential amino acids were estimated from the differences between the values of FAA and amino acids after hydrolysis.
[b] Values in table are the means of duplicate samples.
[c] Percent of total PAA.
[d] Percent of total FAA.

and temperature. It was also observed that peptides and amino acids were formed and removed during processing of cooked and stored meat by Spanier et al., 1988. They also (1988, 1990) reported that both peptides and FAA served as flavor principles and/or precursors for the formation of Maillard products (Bailey, 1938).

Contents of each FAA and each amino acid after acid hydrolysis (PAA) in TCA-soluble fraction of beef broth were

Table 5—Concentration (μmol/100 mL) of anserine, carnosine, creatine, creatinine and lactic acid in TCA-solubles of beef broths obtained by heating at 55°, 65°, 75°, 85° and 95°C for 60 min[a]

Temp °C	Anserine	Carnosine[b]	Creatine	Creatinine[c]	Lactic acid
55	6.9 ± 1.3	34.9 ± 2.5	0.7 ± 0.1	0.05 ± 0.004	1.3 ± 0.1
65	7.4 ± 1.0	56.5 ± 3.6	1.0 ± 0.1	0.05 ± 0.005	1.8 ± 0.2
75	8.4 ± 0.4	53.7 ± 5.6	0.9 ± 0.1	0.07 ± 0.004	2.0 ± 0.4
85	42.3 ± 2.0	108.5 ± 4.1	1.0 ± 0.04	0.2 ± 0.02	2.2 ± 0.1
95	16.4 ± 2.1	134.4 ± 24.9	0.8 ± 0.03	0.3 ± 0.03	1.9 ± 0.1

[a] Values in table are means ± standard error from four different samples.
[b] Significant correlation between carnosine concentration and cooking temperature: $r = 0.83$, $p < 0.005$; $n = 20$.
[c] Significant correlation between creatinine concentration and cooking temperature: $r = 0.89$, $p < 0.005$; $n = 20$.

recorded (Table 3). FAA detected were Tau, Asp, Thr, Ser, Glu, Gln, Pro, Gly, Ala, Val, Ile, Leu, Tyr, Phe, β-Ala, Orn, Lys and His. No significant correlation was detected between any free amino acid concentration and cooking temperature of broth.

Separation of broths by gel filtration

The elution profiles by gel filtration were similar for different broths. Seven identifiable fractions were detected at 220 nm with approximate retention times of 195 (1); 255 (2); 270 (3); 280.5 (4); 312 (5); 375 (6), and 465 (7) min. Fraction (1) eluted at the void volume as molecules with molecular weight higher than 700 daltons. According to the elution time, (2) might be considered as molecular weights smaller than 600 daltons. Fractions (3) and (4) showed similar chromatographic behavior as standard free non-aromatic amino acids, carnosine and anserine. The retention time for (5) was similar to that found for a standard peptide of about 400 daltons (as succinyl-alanine-alanine-alanine-4 nitroanilide, m. w = 451). Fractions (6) and (7) were eluted before standard aromatic amino acid tryptophan and later than standard peptides of molecular weight < 300 daltons (as alanine-alanine-alanine-alanine, m. w. 302). The analysis for each fraction indicated that gel filtration chromatography on Sephadex G-10 was valid to combine and separate broth compounds to associate the fraction with a similar chromatographic behavior for further analyses. Thus, it was possible to eliminate a number of broth-compounds, which do not make an important contribution to flavor development.

Free amino acids (FAA) and those obtained after acid hydrolysis (PAA)

Amino acid analysis of each fraction was carried out with and without acid hydrolysis to get information about the content and kind of FAA and PAA in each chromatographic fraction. The amount of each individual FAA and each amino acid in the PAA of the fractions of broth obtained at 55°C and 85°C is listed in (Table 4). Results from broths heated at 65°, 75° and 95°C are not shown because they were similar to those of 85°C. Because of the small area of peak 4 and the similar elution time with peak 3, fractions 3 + 4 were combined. Fractions 3 + 4 contained almost all of FAA (more than 98%) in broths. The most abundant free amino acids were glutamine, alanine, glycine and taurine at 55° C and additionally proline, ornithine, serine and β-alanine at 85°C. It is also important that the PAA of fractions 3 + 4 ranged between 30–40% of total PAA in broth. The most abundant amino acids in PAA of fractions 3 + 4 were β-alanine, histidine and glutamic acid. The rest of PAA were distributed in fractions 1,5,6 and 7. No amino acid was detected in fraction 2 before and after acid hydrolysis.

Anserine and carnosine eluted together with FAA and were also detected in fractions 3 + 4. These dipeptides were analyzed by amino acid analyzer (Table 5). The concentrations of carnosine increased in general when heating temperature increased and a significant correlation between this variable was found. The same is true for the content of anserine up to 85°C but only carnosine concentration showed a significant correlation to cooking temperature of broths with correlation coefficient 0.83 ($p < 0.005$). Anserine and carnosine have been found in high concentrations in different meat soups (Nishimura et al., 1988) but their contributions have not been clarified. It has been suggested (Suyama and Shimizu, 1982) that carnosine and anserine have a notable buffer action in the pH range above 6.0. In this way they may contribute to the improvement of food taste. Because these results corresponded to results of our sensory evaluation studies, this dipeptide may contribute to improvement in beef broth taste.

ATP metabolites

Inosine, hypoxanthine, adenosine, adenine, IMP, UMP and GMP were found in all beef broths. Other ATP metabolites such as xanthine and uric acid were not detected. Levels of ATP metabolites were determined on each fraction obtained via gel filtration chromatography from each extract (Table 6). The nucleotides, UMP, IMP and GMP eluted with fraction 2 and fractions 3 + 4. Inosine, adenosine, hypoxanthine and adenine eluted in the same range as fractions 6 and 7. No ATP metabolites were detected in fractions 1 and 5. IMP was found mainly in fractions 3 + 4 and reached 41.4 and 58.2 μmol/100 mL sample of extract obtained at 85°C and 95°C, respectively. Other nucleotides were low in comparison, but inosine and hypoxanthine had similar or higher concentrations at all temperatures of heating. Only IMP concentration showed a significant correlation to cooking temperature of broth, (correlation coefficient 0.94, $p < 0.005$).

Sensory evaluation of the chromatographic fractions

A sensory evaluation of the different fractions obtained by gel filtration chromatography from beef broths was carried out. Because the broths obtained at 85°C and 95°C were evaluated as having the major brothy taste intensity only the chromatographic fractions corresponding to those broths were considered. The results from broths heated at 55°, 65° and 75°C were similar but with weak intensity. The impressions perceived were sour (fractions 6 and 7), salty (fraction 7), bitter (fractions 5 and 7) and brothy (fractions 3 + 4). Fractions 1 and 2 were judged tasteless. Fraction 1 was composed of peptides and/or some other types of amino acid derivatives of molecular weight higher than 700 daltons. The PAA of fraction 1 was quantitatively higher from 85°C and 95°C (23.8 and 12.9% of total-PAA, respectively) but at lower temperatures this PAA clearly decreased representing only 3.9-7.4%. Fractions 3 + 4 contained nearly all FAA, carnosine, anserine and other peptides and/or some other type of amino acid derivatives of low molecular weight (≤ 300 daltons) and IMP. Some GMP and AMP were detected in these fractions of broth heated at 85°C and 95°C (Table 6). These fractions presented a remarkable brothy taste (+ + +). Fujimaki et al. (1973) observed that the brothy taste of a fish protein hydrolysate was mostly due to the fraction of molecular weight about 500 daltons. In this way, Kazeniac (1961) reported that heating the raw chicken dialysate developed some flavor that might be associated with chicken, but is was not typical of fresh chicken broth and was relatively weak. These results also suggest that low molecular weight compounds were important to meat flavor development.

Fraction 5 was composed of PAA, which accounted for 5-20% of total PAA (Table 4). It was judged as bitter (+) and astringent (+). Further analysis of this fraction proved the presence of creatine and creatinine, which increased when heating temperature increased (Table 5). Moreover, creatinine concentration showed a significant correlation ($r = 0.89$, $p < 0.005$) to the cooking temperature of broths. These results agreed with those reported by Kazeniac (1961) who stated that

Table 6—Concentration (μmol/100 mL) of each ATP metabolite detected in fractions eluted by gel filtration chromatography of TCA-soluble fraction of beef broths obtained by heating at 55°, 65°, 75°, 85° and 95°C for 60 min[a]

Fraction[b]	T°C	I[c]	H	AD	A	IMP	GMP	UMP	AMP	ADP	TOTAL	% of sum
2	55	-	-	-	-	0.5	-	1.9	-	-	2.4	4.7
	65	-	-	-	-	0.4	-	0.3	-	-	0.7	1.2
	75	-	-	-	-	0.2	-	0.2	-	-	0.4	0.5
	85	-	-	-	-	0.3	-	0.6	-	-	0.9	0.5
	95	-	-	-	-	0.9	-	0.6	-	1.2	2.7	1.1
3+4	55	-	-	-	-	7.2	-	-	-	-	7.2	14.0
	65	-	-	-	-	34.6	-	-	-	-	34.6	58.4
	75	-	-	-	-	30.0	-	-	-	-	30.0	40.7
	85	-	-	-	-	41.4	2.9	-	5.7	-	50.0	28.6
	95	-	-	-	-	58.2	0.4	-	11.2	-	69.8	30.2
6	55	4.0	-	11.0	-	-	-	-	-	-	15.0	29.3
	65	10.5	-	-	-	-	-	-	-	-	10.5	17.7
	75	26.9	-	-	-	-	-	-	-	-	26.9	36.5
	85	77.2	-	4.4	-	-	-	-	-	-	81.6	46.7
	95	86.3	-	6.4	-	-	-	-	-	-	92.7	40.1
7	55	-	26.5	-	-	-	-	-	-	-	26.5	51.8
	65	-	12.6	-	0.8	-	-	-	-	-	13.4	22.6
	75	0.5	15.8	-	-	-	-	-	-	-	16.3	22.1
	85	0.9	30.1	-	11.0	-	-	-	-	-	42.0	24.0
	95	0.3	57.7	-	7.6	-	-	-	-	-	65.6	28.4
Sum	55	4.0	26.5	11.0	-	7.7	-	1.9	-	-	51.1	
	65	10.5	12.6	-	0.8	35.0	-	0.3	-	-	59.2	
	75	27.4	15.8	-	-	30.3	-	0.2	-	-	73.6	
	85	78.1	30.1	4.4	11.0	41.7	2.9	0.6	5.7	-	174.5	
	95	86.6	57.7	6.4	7.6	59.1	0.4	0.6	11.2	1.2	230.8	

[a] Values in table are the means of duplicate samples.
[b] No ATP metabolites were detected in fractions 1 and 5.
[c] I: inosine; H: hypoxanthine; AD: adenosine; A: adenine; AMP: adenosine 5'-monophosphate; ADP: adenosine 5'-diphosphate; GMP: guanosine 5'-monophosphate; IMP: inosine 5'-monophosphate; UMP: uridine 5'-monophosphate.

on heating creatine was readily converted to creatinine. Both creatine and creatinine appear stable to heat but too weak basically to form salts of ionic species to give mouth satisfaction. These compounds have slightly bitter taste (Kazeniac, 1961).

Fraction 6 was rich in inosine but also contained adenosine (Table 6). Sensory evaluation of this fraction turned out a sour-salty taste (+ +). The concentration of inosine increased with heating temperature. Further analysis of this fraction was carried out and indicated the presence of lactic acid. Some investigators reported that lactic acid had no effect on flavor (Dryden et al., 1969; Hayashi et al., 1981). However, there was conflicting results (Pearson, 1968). In broth, the amount of lactic acid increased with increasing heating temperatures up to 85°C (Table 5).

In fraction 7 hypoxanthine content was noteworthy (Table 6). The concentration of hypoxanthine was higher in broths obtained at 85 and 95°C (Table 6). Arya and Parihar (1979) have also reported significant increase in inosine and hypoxanthine during heating of goat and sheep meats. On the other hand, it has been found that hypoxanthine was stable at canning temperatures (Hughes and Jones, 1966). Fraction 7 tasted strongly acid, salty and bitter. This sour and salty taste may be due to several substances of low molecular weight coming from preparations of broths and from obtaining their acid-soluble fractions (as TCA, HCl residual).

The results of sensory analysis suggested that compounds of fractions 3 and 4 were those potentially involved in taste eliciting responses. The concentration of several FAA (taurine, glycine, alanine) was higher as the heating temperature increased in the range of 55–95°C (Table 3), although the maximum values were obtained at 85°C. Other FAA, such as leucine also increased depending on heating temperature, but the highest concentrations were reached at the maximum temperature assayed (95°C). In the temperature range of 55–75°C, an increase of glutamine was also detected, but above that range of temperature a decrease was observed. This profile may be due to the heat-sensitivity of glutamine which probably decomposed during heating.

Nishimura et al. (1988) reported that the concentrations of aspartic acid and tyrosine in heated beef broth were significantly higher in those from stored meat than from fresh. These results corresponded to the higher brothy taste intensity. Our results cannot be compared directly with theirs since, as mentioned, they studied changes during meat storage. However, this agreement suggests that the increase in FAA contributed to the improvement of meat taste. Similarly, Jarboe and Mabrouk (1974) studied the low molecular weight compounds of the aqueous beef broth. In the ninhydrin-positive fraction, they identified the same amino acids we found.

On the other hand, Zaika et al. (1968) studied the water-soluble low-molecular-weight beef aroma precursors by gel filtration and ion exchange chromatography and concluded that the separation of the amino acids in the aroma producing fractions was not sufficiently clear to evaluate their individual contributions to meat flavor. However, tyrosine, phenylalanine, taurine and glutamine acid may be removed without seriously affecting this flavor. In our present work no significant correlation was found between any free amino acid concentration and cooking temperature of broth. However, the total concentration of FAA increased with increasing temperature of heating and showed a significant correlation ($r = 0.88$, $p < 0.005$). These results corresponded to the brothy taste intensity (Table 1). This agreement suggested that the increase in FAA contributed to the improvement of beef broth taste but it has not been established which one had the overwhelming effects on this taste. It appears that the combination of all free amino acids determines the complex brothy taste sensation.

The level of IMP also increased with heating temperature (Table 6). Piskarev et al. (1972) have also observed that these ATP metabolites increased during thermal sterilisation of freshly slaughtered meat but decreased in aged meat. Although Nishimura et al. (1988) considered that IMP was not a particularly important component contributing to the improvement of meat taste, IMP and GMP have been reported to enhance the meaty flavor (Jones, 1969). In addition to it flavor potentiating action, IMP has also been reported to be essential for development of meaty aroma during cooking (Koehler and Jacobson, 1967). Yamaguchi (1967) reported a synergistic reaction be-

tween IMP and glutamic acid. Kuchiba-Manebe et al. (1991) reported that the thermal degradation of IMP during usual cooking scarcely affects the taste of foods.

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Moisture Retention and Textural Properties of Ground Chicken Meat as Affected by Sodium Tripolyphosphate, Ionic Strength and pH

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ABSTRACT

Batches of ground chicken meat were mixed with 10% solutions containing 0.0—5.0% sodium tripolyphosphate and sufficient NaCl to adjust the ionic strength (IS) of the solutions from <0.7 to 6.0 at pH 6.5 to 9.5 in a 6 × 4 × 7 factorial experimental design. Patties prepared from the mixtures were evaluated for pH, cooking loss, and objective texture characteristics. Most effects of STPP were attributable to the direct effect of STPP on IS. However the ability of the phosphate to alter textural properties was greater at a pH value near the pK_a of the phosphate.

Key Words: chicken, ionic strength, acidity, texture, moisture retention, STPP

INTRODUCTION

POLYPHOSPHATES have been used in meat and poultry products for many years to improve yields, texture, and moisture retention. Consumer interest in reducing dietary sodium has further encouraged the use of such compounds. Low salt meat products, which tend to have unsatisfactory cooking yields and textural attributes, have improved quality and consumer acceptance when treated with polyphosphates. Phosphates are often used with low levels of NaCl since it has been shown that the NaCl enhances the effectiveness of the phosphates (Hamm, 1970; Poulanne and Terrell, 1983; Trout and Schmidt, 1984). Hamm (1970) ascribed the effects of the polyphosphates on meat products to increasing meat pH, increasing ionic strength (IS), binding of proteins by the phosphates, and dissociation of actomyosin into actin and myosin. It is well known that the first two effects, increasing meat pH and IS, increase moisture binding (Hamm, 1970; Lawrie 1985). However, in protein systems, pH and IS are not always independent. Increasing ionic strength of protein systems, even with neutral salts, tends to depress the pH of the system. In the case of salts of weak acids (e.g., those of polyphosphoric acids), the pH of the system can also be altered by the buffering effect of the salts. In turn, the buffering capacity of the salts is determined by both the concentration of the buffer salt, it's pK_a and the pH of the medium. Thus, it is very difficult to ascribe the effects of polyphosphates in meat systems individually to pH changes, phosphate concentration, or IS changes. Rather, all three factors must be considered. The objective of our study was to determine how polyphosphate concentration, pH and ionic strength interact to affect texture and moisture binding characteristics of chicken patties.

MATERIALS & METHODS

Experimental design

Boneless, skinless broiler chicken breast meat was obtained from a local processing plant on the day of slaughter. Proximate composition of the meat was 75.1% moisture, 21.6% protein, and 3.3% fat (AOAC, 1980) and pH was 5.6. The meat was ground once through a 0.95 cm plate.

Batches of the meat weighing 600g were mixed for 3 min by hand in a plastic bag with 10% of each of the solutions described below. After measuring the pH with an Orion model EA 940 pH meter (Orion Research, Inc., Cambridge, MA), each mixture was stuffed into a 10-cm moisture-proof fibrous casing and frozen in a −34°C room. Four 2.54 cm thick patties of about 100g were cut from each batch with a band saw for cooking yield and texture evaluation. The patties were stored at −34° C. Within 1 wk, they were tempered at 4°C for 24 hr then individually cooked in a 177°C rotary oven (Dispatch, 611 S. E. 8th St., Minneapolis, MN 55440-1320) to an endpoint of 74°C. Each patty was reweighed. Cooked yield of each patty was calculated relative to initial weight.

The patties were sampled for moisture and protein analysis (AOAC, 1980) and objective texture differences by Texture Profile Analysis. Since subsample variations were small relative to treatment variations, the results were pooled over the 4 patties for statistical analysis.

Experimental solutions

The experimental solutions were calculated to contain 0.0, 1.0, 2.0, 3.0, 4.0 or 5.0% STPP and have IS of 0.0, 2.0, 4.0 or 6.0. Calculated ionic strength (IS) was adjusted with NaCl. pH of the solutions was adjusted to 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, or 9.5 with HCl. Thus, there were six STPP concentrations, four IS values, and seven pH values for a total of 168 combinations in a 6 × 4 × 7 factorial arrangement. All solutions also contained 0.05 M tris (hydroxymethyl) aminomethane hydrochloride (Tris, Sigma Chemican Company, P. O. Box 14508, St. Louis MO 63178). This added buffer was necessary to adjust the pH of the 0.0% STPP solutions and was accounted for in IS calculations. The IS of those solutions which contained no STPP and no NaCl is designated as 0.0 IS but note that the IS of those solutions was between 0.0 and 0.7.

Texture profile analysis

Texture Profile Analysis (TPA) was performed using the procedure reported by Lyon et al. (1980). The objective portion of the texture profile was partitioned into hardness, cohesiveness, springiness and chewiness attributes.

Statistical analysis

The effects of IS, solvent pH and STPP concentration on meat pH, cooking loss, and TPA were fitted to a response surface using the solution pH, IS, and STPP content as main effects and including all first order interactions. Main effect and interaction regression estimates were tested for statistical significance using the lack of fit mean squares. Significant interactions were plotted against percentage STPP and IS at solution pH 6.5, 8.0 and 9.5 using the SAS G3D procedure (SAS Institute, 1987).

RESULTS & DISCUSSION

MEAT pH increased as solution pH and STPP concentration increased and decreased with increasing IS but these effects were not independent; i.e., the solution pH × STPP interaction was statistically significant (Table 1, Fig. 1.) At pH 6.5, STPP had little effect on meat pH at low IS. As solution IS

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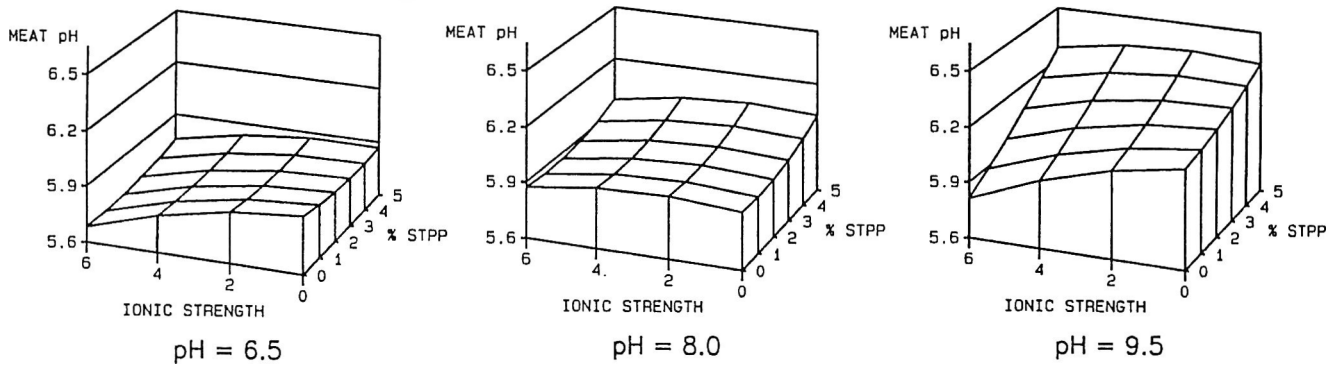

Fig. 1—Response surface showing interaction of solution sodium tripolyphosphate (STPP) concentration and ionic strength at pH 6.5, 8.0 and 9.5 on pH of chicken patties.

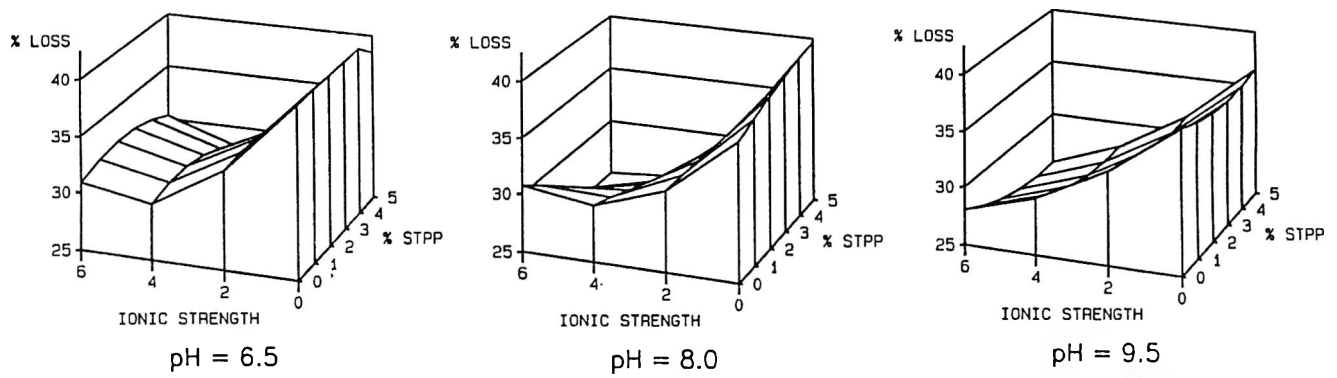

Fig. 2—Response surface showing interaction of solution sodium triploy phosphate (STTP) concentration and ionic strength at pH 6.5, 8.0, and 9.5 on cooking loss of ground chicken meat.

Table 1—Regression estimates for chicken meat pH, cooking loss and texture profile attributes on solution pH, ionic strength and STPP content and on their first order interactions

	Dependent variables					
Independent variables	Meat pH	Cooking loss	Hardness (kg)	Cohesive-ness	Springi-ness (cm)	Chewi-ness (kg-cm)
Intercept	5.50*	41.67*	20.84*	0.44*	0.67*	6.90*
Solution pH	0.06*	−0.61	−0.84*	< −0.01	0.01	−0.31*
IS[a]	−0.54*	−1.21*	−15.41*	−0.12	0.07	−4.39*
pH X IS	0.03	−1.74*	0.74	0.01	0.01	0.21
STPP[b]	1.05*	−11.55*	−8.24	<0.01	0.08	−3.65*
pH X STPP	0.17*	1.27	0.99	<0.01	−0.01	0.43*
IS X STPP	0.24	−6.41	1.93	.09	0.05	1.36

[a] Calculated ionic strength.
[b] Sodium tripolyphosphate
* Significantly > 0.0, P<0.05

increased at low STPP concentration, meat pH decreased. If STPP concentration was elevated to 0.5% then increasing IS had little effect on meat pH.

At elevated solution pH, increasing STPP concentration raised pH, even at the highest IS. The effectiveness of STPP in increasing pH of ground chicken meat appeared optimum at higher pH. A pK_a of STPP is reported to be 9.25 (Smith and Martell, 1976). At that pH, the buffering capacity of the phosphate is maximum. Thus, the ability of the STPP to resist pH decreases due to increasing IS or postmortem metabolic activity is maximum when the pH of the solution is near 9.25.

Ionic Strength and STPP concentration significantly affected cooking loss (Table 1). For each unit increase in IS or percentage increase in STPP, cooking loss decreased by 1.21 and 11.55%, respectively. Solution pH *per se* did not significantly affect cooking loss. These observations were consistent with those of Trout and Schmidt (1984). However, the pH × IS interaction significantly affected cooking loss (Fig. 2). The ability of the STPP to reduce cooking loss was dependent on pH of the solution and IS. At low IS, increasing STPP concentration had little effect on cooking loss regardless of IS. Elevated IS alone decreased cooking loss. If solution pH was high, IS also increased the ability of STPP to induce improved moisture binding by the meat. At pH 6.5, STPP had no effect on cooking loss. The data confirmed the conclusion of Trout and Schmidt (1984) that the ability of polyphosphates to improve moisture binding characteristics of meats was largely the result of the effect of the phosphate on IS and that the direct effect of phosphate on pH was of little importance. However, the data also indicated that pH and IS effects were not totally independent. The effectiveness of the phosphate on moisture binding could be reduced if the solution pH is low and the IS is high.

Objective hardness values increased as solution pH or IS increased and these effects were independent (Table 1). None of the solution characteristics affected cohesiveness or springiness. However, chewiness (the product of hardness, cohesiveness and springiness) was affected by solution pH and IS. As pH increased each unit, chewiness decreased by 0.31 kg-cm, as IS increased each unit, chewiness decreased 4.39 kg-cm, and as STPP concentration increased by 1%, chewiness decreased by 3.65 kg-cm. Moreover, these effects were not independent. The effectiveness of the STPP to alter hardness was dependent on solution pH (Fig. 3). As STPP concentration increased, chewiness also increased, but only at solution pH levels 8.0 and 9.5. Reduced chewiness may be related to the decreased ability of the STPP to improve moisture binding at low pH.

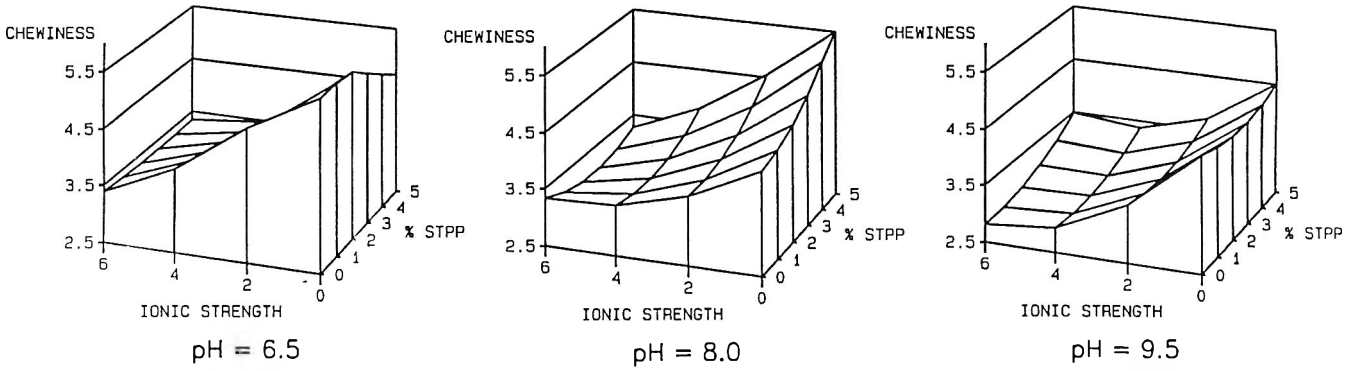

Fig. 3—Response surface showing interaction of solution sodium tripolyphosphate (STPP) concentration and ionic strength at pH 6.5, 8.0, and 9.5 on objective chewiness of ground chicken meat.

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*CHANGES IN LOW-FAT GROUND BEEF IN DISTRIBUTION. . .*From page 1274

CONCLUSIONS

NO DIFFERENCES were found in the aerobic plate counts, populations of psychrotrophic bacteria or mold and yeast populations for low-fat carrageenan-based ground beef and a low-fat all-beef control. Retail display of the low-fat products in oxygen-permeable (aerobic) packaging resulted in greater product discoloration than that of products in oxygen-impermeable packaging. Color stability of the low-fat ground beef products decreased with increased refrigerated storage time. Overall, few differences were found in oxidative stability and microbial populations of the two low-fat products.

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Physical, Chemical and Sensory Changes in Irradiated Fresh Pork Packaged in Modified Atmosphere

ANNE D. LAMBERT, JAMES P. SMITH and KAREN L. DODDS

ABSTRACT

The effects of irradiation dose (0, 0.5 and 1.0 kGy), headspace oxygen (0, 10 and 20% O_2, balance nitrogen) and storage temperature (5, 15 and 25°C) on the physical, chemical and sensory changes in fresh pork were studied using factorial design experiments. Irradiation in the absence of oxygen extended the sensory shelf life of pork from 9 to 26 days at 5°C and from < 2 to 2 days at 25°C. Oxygen in the package headspace combined with irradiation adversely affected physical, chemical and sensory characteristics of the end product.

Key Words: pork, irradiation, sensory, flavor, texture

INTRODUCTION

CONSUMERS judge the quality of fresh meat by color, appearance, presence of exudate in the package and odor when the package is opened. Under refrigerated conditions (≤ 4°C), the shelf life of fresh meat is limited to 3–4 days by the growth of aerobic, psychrotrophic spoilage microorganisms. A total number of 10^7 CFU/g of meat is considered to be the upper limit of microbiological acceptability (Niemand et al., 1981). However, before those numbers are reached, discoloration and presence of excess exudate in the package may cause product rejection by the consumer.

Modified atmosphere packaging (MAP), involving both vacuum and gas packaging, has been used extensively to extend shelf life and keeping quality of fresh meat (Young et al., 1988). Under MAP, a shelf life of 4–8 wk is possible for pork and beef at 0–2°C, depending on the microbiological quality at the time of packaging and the gas mixture used. With fresh meat, O_2 is often included in the gas mixture to maintain red color or bloom, by keeping myoglobin in its oxygenated form (Clark and Lentz, 1973; Bartkowski et al., 1982). However, some studies have shown that, for the longest shelf life, O_2 should be excluded from the package headspace (Christopher et al., 1979).

Low-dose irradiation, i.e., dose levels < 10 kGy, also can be used effectively to increase shelf life of fresh meat. Lea et al. (1960) reported that when beef samples were subjected to low doses of irradiation (0.25 to 1 kGy) in the presence of air, microbial spoilage was considerably retarded. However, this treatment adversely affected appearance and odor of the fat. Low-dose irradiation enhances the shelf life of meat by inactivation of spoilage microorganisms (Urbain, 1986). Other additional means are needed to control chemical spoilage, such as rancidity, in fresh meat. One possible treatment is MAP in combination with irradiation. While the combined effect of MAP/refrigeration and MAP/irradiation/refrigeration may extend the shelf life of the product, little is known about the combined effect of these treatments on pH, lipid oxidation, color, odor, and exudate loss of fresh pork.

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The objectives of our study were to monitor the combined effect of MAP/irradiation on the physical, chemical and sensory characteristics of fresh pork stored at refrigerated storage temperature, slight temperature abuse, and at average room temperature; and to determine the maximum shelf life of MAP/irradiated pork based on sensory changes.

MATERIALS & METHODS

Experimental design

The experimental design was a 2×2×2 factorial (Table 1). Factors evaluated included O_2 concentration (0 and 20% headspace gas, balance N_2), irradiation dose (0 and 1.0 kGy), and storage temperature (5 and 25°C). An additional combination of factors (center point) consisting of 10% O_2, 0.5 kGy and storage at 15°C was studied. Levels of each factor were selected from previous MAP/irradiation studies on pork (Ehioba et al., 1987; Mattison et al., 1986; Seideman et al., 1979). The experiment was repeated a second time.

Preparation of meat samples

Fresh, trimmed pork loins (12) were obtained from Provigo meat distribution center (Laval, Québec) within 72 hr after slaughtering and cut into 1.5 cm-thick slices of ≈100g each. Each slice was placed in a 210×210 mm Cryovac barrier bag (O_2 transmission rate, 3–6 mL/m^2, 24 hr, atm at 4.4°C, 0% RH) and randomly assigned to one of the 9 treatments (Table 1). For treatments requiring 0% O_2, an O_2 absorbent (type FX-200, Mitsubishi Gas Corporation, Japan) was placed at the base of a petri dish inside the bag, and samples were packaged with 100% N_2 prior to heat sealing. For treatments requiring 20% O_2, samples were packaged in air. The gas mixture was composed of 10% O_2 and 90% N_2 for treatments requiring 10% O_2. Packaging and sealing of samples were done using a Multivac chamber-type, heat-seal packaging machine (Model A300/42, Multivac, D-8941 Wolfertschwenden, Germany). A Smith's proportional gas mixer (Model 299-028, Tescom Corp., Minneapolis, MN) was used to give desired proportions of O_2 and N_2 in the package headspace.

Irradiation and storage

Samples were transported to the irradiation facility in Styrofoam™ coolers with ice packs to prevent temperature abuse. Samples were maintained at 0°C until irradiation (which took place at room temperature) and placed in the cooler immediately after irradiation. Samples were irradiated to average doses of 0.5 and 1.0 kGy using a Gamma-Cell (Model 220, Atomic Energy of Canada, Limited, Ottawa). The dose rate was 3.95 kGy/hr, and the duration, 7.6 min and 15.2 min for the 0.5 and 1.0 kGy doses, respectively. Samples were placed in trays and incubated at 5, 15 or 25°C (±1°C) for up to 35 days.

Physicochemical analyses

Sampling times were day 0, day 3, day 7 and subsequently on a weekly basis until day 35. All physicochemical analyses were conducted on one 100g slice. Another slice was used for the sensory evaluation.

Color

The product L (lightness), a (redness), and b (yellowness) color values (DeMan, 1980) were measured using the Minolta Chroma Meter colorimeter (Model CR-100, Minolta Corp., Ramsey, NJ) with illuminant C (6774K) as a light source. The measurement was taken

Table 1 – Conditions for treatments

Treatment	Factor		
	Oxygen (%)	Irradiation dose (kGy)	Temperature (°C)
1	20[a]	0	5
2	0[b]	0	5
3	20	1	5
4	0	1	5
5	20	0	25
6	0	0	25
7	20	1	25
8	0	1	25
9[c]	10[d]	0.5	15

[a]Samples packaged in air.
[b]Samples packaged in 100% N_2 with an oxygen absorbent.
[c]Central point; used for comparison of shelf life only.
[d]Balance N_2.

3 times at the center of the slice and the averages reported. The colorimeter was calibrated using a standard-white reflector plate prior to measuring meat color.

Exudate

At the end of each storage period, individual bags were opened aseptically, the meat weighed and the accompanying exudate measured in a graduated cylinder. The exudate loss was reported in mL/100g meat.

pH

Meat pH was measured with a previously calibrated Corning pH-meter (Model 220, Corning Glass Works, Corning, NY) by plunging a spear-tip electrode (Ross Combination Spear-tip pH electrode, Model 81-63, Orion Research Inc., Boston, MA) into the meat.

TBA value

Oxidative rancidity was assessed using the modified thiobarbituric acid (TBA) method for fresh meat described by Tarladgis et al. (1960). Meat (10g) was blended with distilled water (50 mL) in a Waring Blendor for 2 min. The slurry was transferred to a distillation vessel using 47.5 mL distilled water, and 2.5 mL 4N HCl was added. Distillate (50 mL) was collected in a graduated cylinder, 5 mL TBA reagent added to 5 mL distillate and the mixture immersed in a boiling water bath for 35 min. A blank, consisting of 5 mL distilled water and 5 mL TBA reagent, was prepared in the same manner. After cooling in tap water for 10 min, the absorbance (A) was read in a spectrophotometer (Coleman, model 295, Perkin-Elmer Corp., Oakbrook, IL) at 538 mμ. The instrument was previously calibrated to read 0% A using the blank solution. The concentration of malonaldehyde was calculated from a standard curve using solutions of 1,1,3,3-tetraethoxypropane. Results were reported as mg malonaldehyde/kg meat.

Sensory evaluation

At day 0 and at each subsequent sampling time, samples were subjectively evaluated for color and odor by a 10-member untrained panel. Samples were identified by a three digit code number and evaluated visually for surface discoloration under a white fluorescent light using a 10-cm unstructured scale (0 cm = no surface discoloration: 10 cm = complete surface discoloration; Larmond, 1979). A color chart from Agriculture Canada (Publication 5180/B) served as reference for the normal color of meat. Off-odor was evaluated under a red fluorescent light (to prevent influence by color of the sample) using a 10-cm unstructured scale (0 cm = no off-odor; 10 cm = extreme off-odor; Larmond, 1979). A fresh meat sample was used as a control for odor evaluation. All samples were evaluated in a random sequence. For each attribute, a score of 6 was considered the upper limit of acceptability, implying that shelf life was terminated when this score was reached (Poole et al., 1990).

Statistical analyses

Results of the two experiments were pooled and then analyzed by the Statistical Analysis System (SAS Institute, Inc., 1988) using the General Linear Model Procedure, and comparison of means was done using Duncan's multiple range test and least square means.

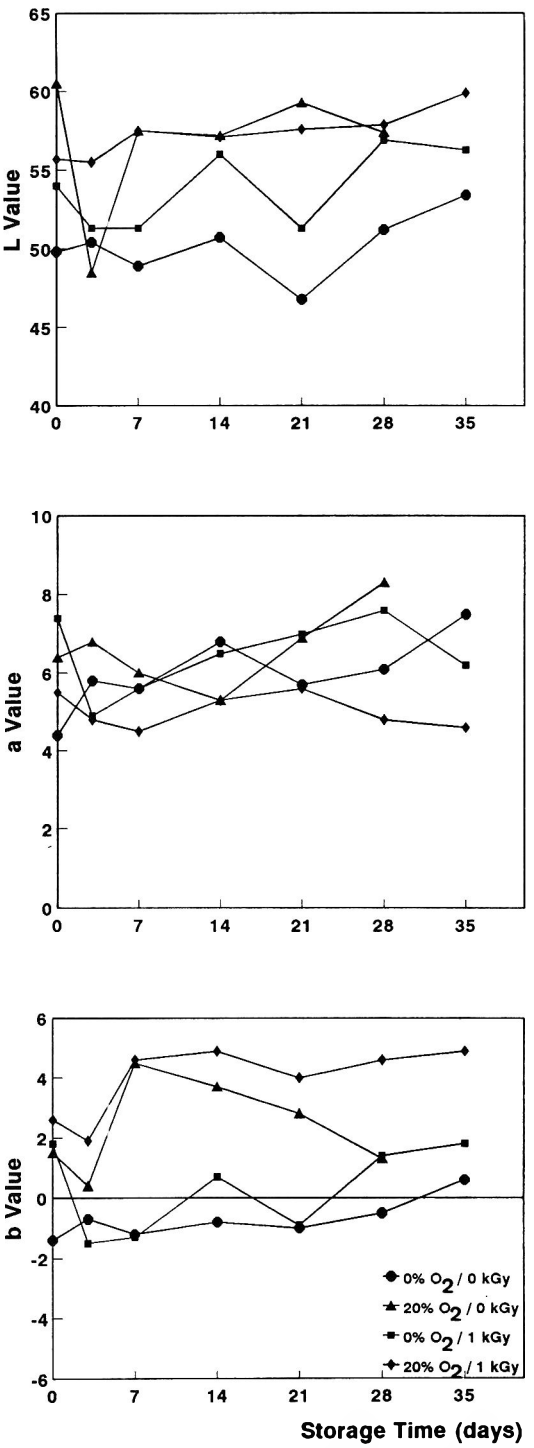

Fig. 1 – Effect of O_2 level and irradiation dose on the measured color of MAP fresh pork stored at 5°C. With a standard error of the mean ranging between: L value: 0.4 and 2.4; a value: 0.1 and 0.6; b value: 0.3 and 1.0.

RESULTS & DISCUSSION

Color measurement (L, a, b color values)

Generally, the L, a and b color values of samples increased slightly throughout storage (Fig. 1), indicating that meat became more pale and that the meat color changed from red to yellow. In general, samples stored at 25°C (results not shown) were more pale than those stored at 5°C. At day 0, the L value

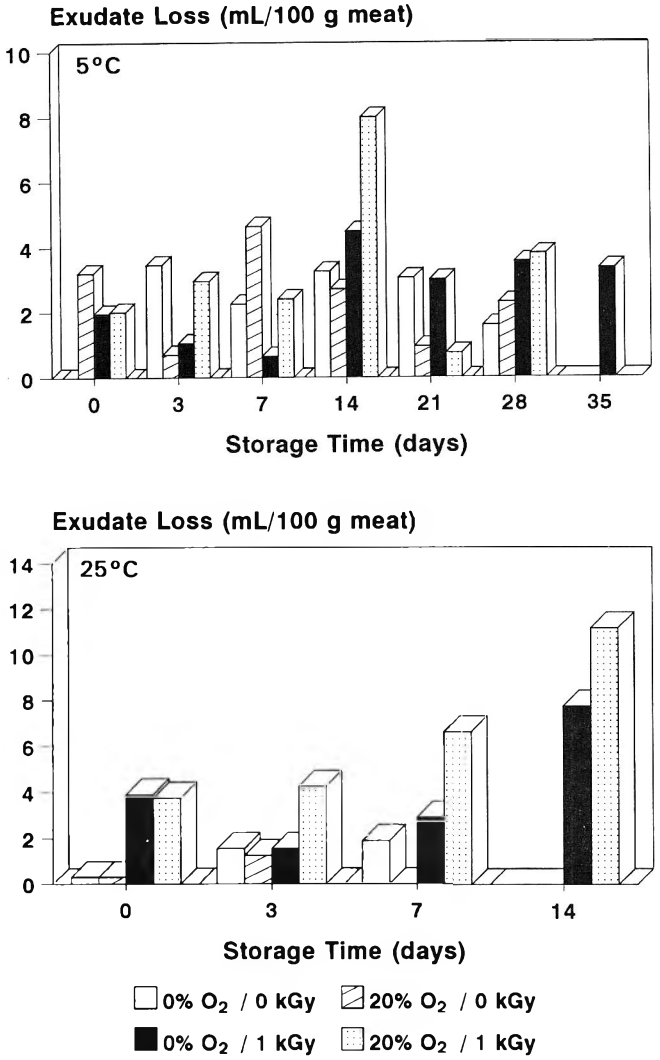

Fig. 2—Effect of O_2 level and irradiation dose on the exudate loss of MAP fresh pork samples stored at 5°C and 25°C.

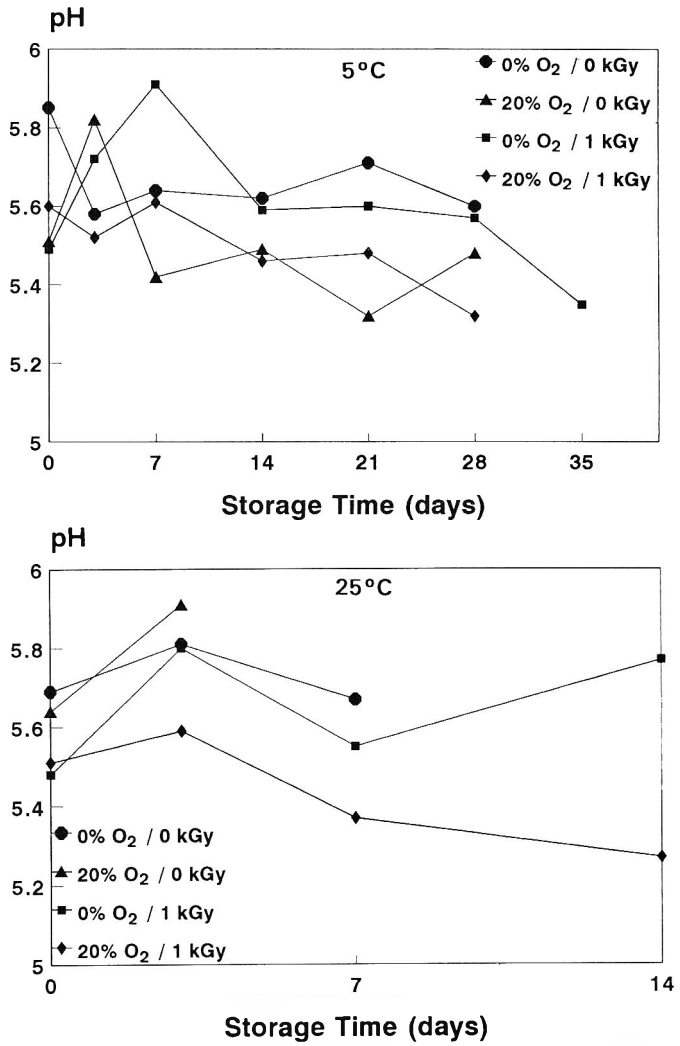

Fig. 3—Effect of O_2 level and irradiation dose on the pH of MAP fresh pork samples stored at 5°C and 25°C.

of irradiated samples was higher ($P < 0.01$) than that of non-irradiated samples. After 7-21 days, all samples packaged with 20% O_2 (irradiated and nonirradiated) stored at 5°C had higher ($P < 0.01$) values than those packaged with 100% N_2 and an O_2 absorbent (Fig. 1). The synergistic effect of irradiation and O_2 on the L value was more noticeable towards the end of the storage period. After 28 days, only non-irradiated pork packaged in 100% N_2 had a lower L value ($P < 0.01$), i.e., was darker than all other treatments. At the end of storage (day 35), there was a difference ($P < 0.01$) between all treatments stored at 5°C. Samples packaged with 20% O_2 and irradiated at 1 kGy had the highest L value, followed by irradiated, N_2-packaged products and then by non-irradiated N_2-packaged samples. Similar changes in L values were observed for treatments stored at 25°C (results not shown).

Changes in a values were not as clearly defined (Fig. 1). After 21 days, there was no difference between all treatments stored at 5°C. At day 28 and 35, irradiated samples packaged with 20% O_2 and stored at 5°C had a lower ($P < 0.01$) a value compared to all other treatments. After 35 days at 5°C, non-irradiated samples packaged with 100% N_2 and an O_2 absorbent had the highest a value, followed by irradiated, N_2-packaged products. Irradiated samples stored at 25°C had higher a values ($P < 0.01$) compared to similarly treated samples stored at 5°C (results not shown). This may be attributed to the conversion of myoglobin into a more stable bright red pigment by the combined action of irradiation and high temperature storage (Ginger et al., 1955).

Differences in b values (yellowness) were noticeable from day 3 (Fig. 1). Nearly all samples packaged with 20% O_2 had a higher b value ($P < 0.01$) compared to other treatments. After 28–35 days, irradiated, aerobically-packaged samples had the highest b value, followed by irradiated, N_2-packaged samples, with non-irradiated N_2-packaged product having the lowest b value.

All three factors studied, i.e., O_2, irradiation and temperature, had an affect on the L, a, and b values. Irradiation was the most important factor influencing the L value from day 0 until the end of storage, while O_2 and temperature affected the L value only after days 3 and 7, respectively. The combined effect of O_2 and irradiation on the a value was notable from day 0 and throughout storage. O_2 was a factor ($P < 0.01$) influencing the b value throughout the storage period while irradiation was significant only at day 0. A significant interaction occurred between O_2 and irradiation affecting the b value at day 3 and 7. Packaging with air or storage at 25°C resulted in an increased L value, possibly because of the enhanced growth of spoilage microorganisms which favored the oxidation of myoglobin by reducing the O_2 tension at the meat surface (Seideman et al., 1984). Overall, samples packaged with 20% O_2 and irradiated at 1 kGy had higher L values, lower a values and higher b values compared to all other treatments. This indicated that O_2, irradiation and their combination resulted in more pale (or whiter), less red and more yellow pork. This may be attributed not only to the presence of O_2, which oxidizes myoglobin into metmyoglobin, but also to the en-

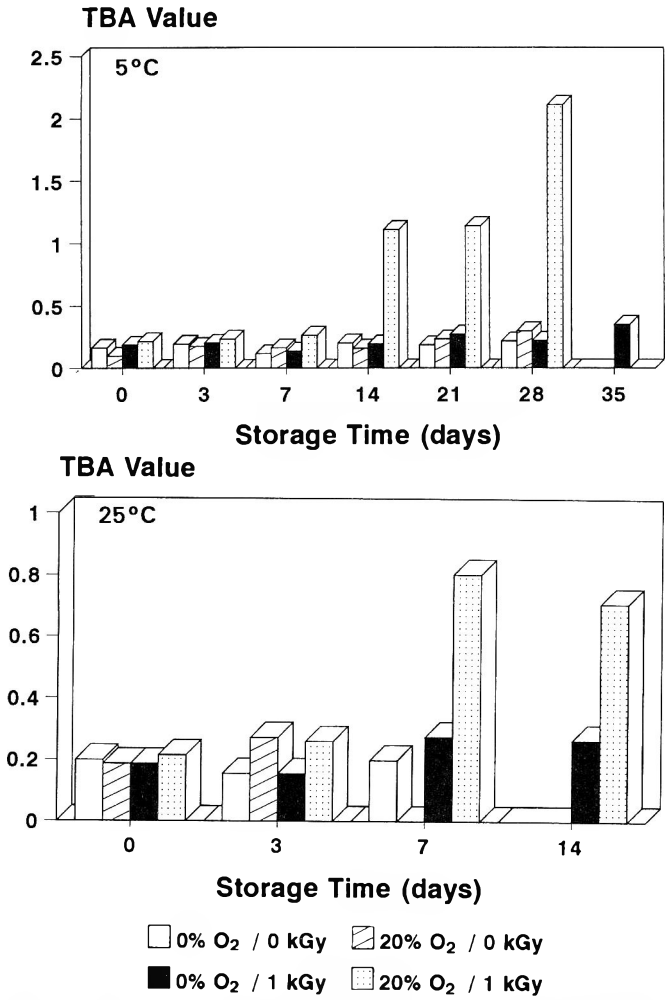

Fig. 4—Effect of O_2 level and irradiation dose on the TBA value of MAP fresh pork samples stored at 5°C and 25°C. With a standard error of the mean ranging between 0.02 and 0.37.

hanced oxidation of the meat pigment when samples are irradiated in the presence of O_2. Irradiation in the presence of O_2 generates oxidizing substances such as peroxides which promote oxidation of meat fat and pigment (Urbain, 1986). Lea et al. (1960) reported that the appearance of beef irradiated in air was adversely affected. In our study, no significant difference was detected between L, a and b values of nonirradiated and irradiated N_2-packaged samples stored at 5°C from day 0 until day 28, indicating that irradiation in the presence of 100% N_2 did not adversely affect meat color. However, after 28 and 35 days of storage at 5°C, irradiated samples had a higher L value compared to non-irradiated samples. Lebepe et al. (1990) observed that after 2, 3 and 6 wk at 2–4°C, the L values of irradiated (3 kGy) vacuum-packaged pork were higher ($P < 0.05$) compared to those of non-irradiated samples. However, they obtained inconsistent L and b values at other sampling times. They also reported that the a values of irradiated pork were higher ($P < 0.05$) throughout storage at 2–4°C compared to those of non-irradiated pork.

Exudate loss

The effects of storage time on exudate loss in each treatment (Fig. 2) indicated irradiation had an effect ($P < 0.05$) at day 0. The average exudate loss for all irradiated samples, immediately after irradiation, was 2.9 mL /100 g meat compared to 0.8 mL/100 g meat for all non-irradiated treatments. However, at day 3, no differences were observed in exudate losses among treatments. All samples stored at 25°C had a greater amount ($P < 0.05$) of exudate than similarly treated samples stored at 5°C. Furthermore, samples packaged with 20% O_2 and irradiated at 1 kGy had a higher amount of exudate compared to samples of all other treatments.

These results confirmed the reports of Urbain (1986) and Shay et al. (1988) that irradiation increased exudate loss of fresh meat. This may result from a change in secondary and tertiary structures of protein (Urbain, 1986), influencing the water-holding capacity of muscle tissue. Furthermore, Lakritz et al. (1987) reported that irradiation at doses > 10 kGy resulted in myofibril fragmentation and a decrease in tensile strength of muscles. Lawrie et al. (1961) reported a decrease in water-holding capacity of beef and pork irradiated at 50 kGy.

pH changes

Measure of pH (Fig. 3) showed no difference between pH of all treatments up to day 21. However, after day 21, the pH of non-irradiated samples packaged with 20% O_2 and stored at 5°C were lower ($P < 0.05$) than those from non-irradiated N_2-packaged samples. This can be attributed to a higher level of headspace CO_2, 22%, in the samples packaged with 20% O_2, compared to 0.5% in the N_2-packaged samples, and dissolution of CO_2 in the aqueous phase of the product (Lambert et al., 1991). The lower pH in the samples initially packaged with O_2 may also be attributed to the growth of lactic acid bacteria, which are favored by an atmosphere containing ≥ 20% CO_2, and the consequent production of lactic acid (Lambert et al., 1991). However, Lee et al. (1985) did not report changes in pH values of vacuum- or N_2-packaged pork throughout storage at refrigeration temperatures. Irradiation did not affect the pH of pork. This confirmed observations of Lebepe et al. (1990) who reported that the pH of pork loin was not affected by irradiation at 3 kGy, a higher dose than we used.

TBA value

Measure of TBA values (Fig. 4) showed for samples stored at 5°C (Fig. 4), there was no difference between treatments until day 7. At day 7, TBA values from samples packaged with 20% O_2 were higher ($P < 0.05$) than those from N_2-packaged samples. Storage temperature also was a significant ($P < 0.05$) factor at day 7 with the TBA values of samples stored at 25°C being higher than those from samples stored at 5°C. For the remainder of storage TBA values remained approximately constant and there was no difference between all non-irradiated samples and N_2-packaged samples irradiated at 1 kGy. These observations were in confirmation of those of Mattison et al. (1986) and Ehioba et al. (1987) who showed that the TBA values of vacuum-packaged pork loin and ground pork were not affected by irradiation at 1 kGy. Similarly, Lebepe et al. (1990) reported that an irradiation dose of 3 kGy did not affect TBA values of vacuum-packaged pork loin. However, the TBA values of irradiated samples packaged with 20% O_2 increased from day 7 until the end of the storage period and were higher ($P < 0.05$) than those from all other treatments. The higher TBA value of samples packaged with 20% O_2 and irradiated at 1 kGy may be explained by the fact that autoxidation of fat is accelerated if O_2 is present during or after irradiation (Urbain, 1986). Lea et al. (1960) reported that rapid fat oxidation occurred in beef samples packaged in O_2-permeable bags and irradiated at doses ranging from 0.25 to 1 kGy. This may be attributed to the combination of free radicals with O_2 to form hydroperoxides which break down into various decomposition products including aldehydes (Nawar, 1985; Urbain, 1986). Malonaldehyde and aldehydes are the major TBA reactive substances (Hoyland and Taylor, 1991).

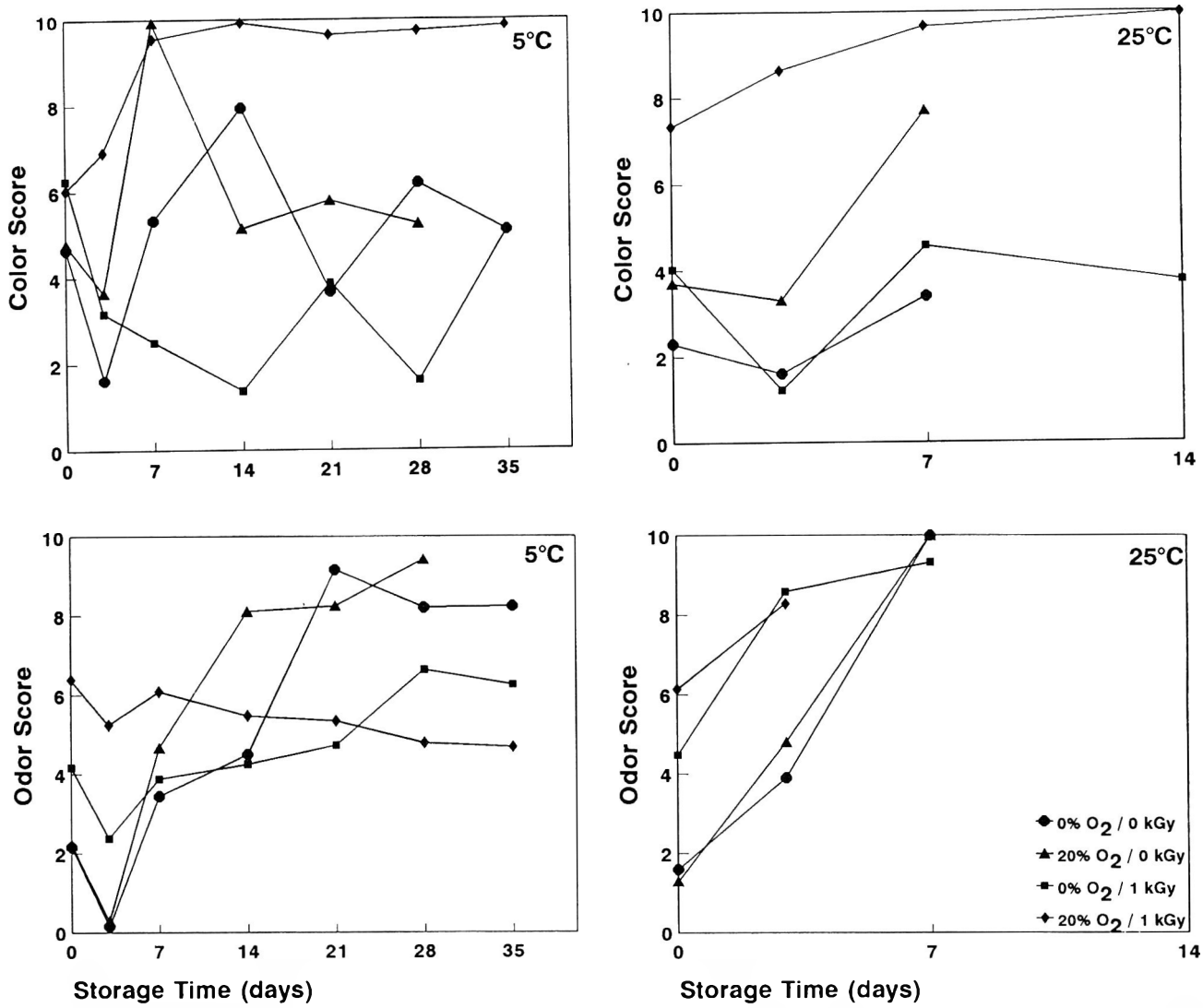

Fig. 5—Changes in sensory color and odor scores in MAP, irradiated and non-irradiated pork samples stored at 5°C and 25°C. With a standard error of the mean ranging between 0.2 and 1.4.

Sensory evaluation

Irradiation, O_2 and irradiation/O_2 significantly affected color (Fig. 5). Irradiated (1 kGy), aerobically-packaged samples stored at 5°C were more discolored ($P < 0.01$) than other treatments. After 7 days storage at 5°C or at 25°C, all aerobically-packaged treatments (irradiated and non-irradiated) were more discolored ($P < 0.01$) than the corresponding N_2-packaged treatments. These results agreed with the higher L and b values observed for aerobically-packaged samples after 7 days. Furthermore, after 14 days at 5°C, all non-irradiated samples were more discolored ($P < 0.01$) compared to those of the N_2-packaged, irradiated treatments. From day 7 until the end of the storage period, N_2-packaged samples irradiated at 1 kGy were less discolored ($P < 0.02$) than those of other treatments. At 25°C, all irradiated samples packaged with 20% O_2 were more discolored than irradiated, N_2-packaged samples immediately after irradiation and throughout storage.

Initially (day 0), samples packaged with 20% O_2 and irradiated at 1 kGy had a stronger off-odor (higher score) ($P < 0.01$) compared to all non-irradiated treatments (Fig. 5). No difference was found between non-irradiated treatments and N_2-packaged samples irradiated at 1 kGy. After 3-14 days of storage at 5°C, no difference was observed among treatments (Fig. 5). However, after 21 days at 5°C, irradiated, N_2-packaged samples had a weaker off-odor (lower score) ($P < 0.01$) compared to nonirradiated samples but were not different from irradiated samples packaged with 20% O_2. At day 28, irradiated samples packaged with 20% O_2 had a weaker off-odor ($P < 0.01$) compared to similarly packaged, non-irradiated product. No difference was observed between any other treatments. No difference in off-odor was detected among treatments after 35 days at 5°C. After 3–7 days storage at 25°C, temperature had a significant effect ($P < 0.01$), with samples stored at 25°C having stronger off-odors than product stored at 5°C. There was no difference among treatments stored at 25°C (Fig.

Table 2—Estimated sensory shelf life of MAP, irradiated pork loin

O_2[a] (%)	Irradiation dose (kGy)	Storage temp (°C)	Rejection point/color (days)[b]	Rejection point/odor (days)[b]	Sensory shelf life (days)[c]
20	0	5	4	10	4
0	0	5	9	16	9
20	1	5	0	0	0
0	1	5	35	26	26
10	0.5	15	>14	7	7
20	0	25	<2	<2	<2
0	0	25	<2	<2	<2
20	1	25	0	0	0
0	1	25	>14	2	2

[a]Balance N_2. 0% O_2: samples packaged with 100% N_2 and an O_2 absorbent.
[b]Rejection point: time to reach a score of 6.
[c]Earliest rejection point on color or odor scale.

5). The observed decrease in odor for samples packaged with 20% O_2 and irradiated at 1 kGy may be due to the fact that upon storage at 5°C, the irradiation odor was dissipating. In addition, the microbiological population of these samples was mainly composed of lactic acid bacteria (Lambert et al., 1992) which do not product strong off-odors (McMeekin, 1982). Comparatively, samples irradiated in the absence of O_2 had a weaker initial off-odor, but the score for odor increased during storage due to the growth of spoilage microorganisms.

These results clearly showed the important effect of O_2 on both color and odor of samples. The presence of O_2, while initially promoting a bright red color do to the oxygenation of meat myoglobin to oxymyoglobin, would eventually favor the growth of aerobic spoilage microorganisms. The resulting consumption of O_2 and reduction in the O_2 tension at the meat surface favor the oxidation of myoglobin to brown metmyoglobin (Seideman et al., 1984). Indeed, total aerobic counts on the same samples we used were higher in aerobically-packaged samples compared to N_2-packaged samples (Lambert et al., 1992).

During irradiation, the presence of O_2 favors the oxidation of myoglobin to brown metmyoglobin through the formation of strong oxidizing agents such as peroxides (Coleby et al., 1961). Lea et al. (1960) reported that irradiation of beef with 2 kGy in air resulted in detrimental changes in appearance and odor of product compared to beef irradiated under anaerobic conditions (100% N_2).

The panelists reported a weak off-odor (score of 4) in irradiated, N_2-packaged samples immediately after irradiation but after 3 days, these samples scored lower, indicating that the odor had dissipated or was less pronounced. While irradiated samples packaged with 20% O_2 had a strong off-odor (score > 6) immediately after irradiation, those samples subsequently had lower scores, indicating that the odor was dissipating or was weaker. A similar trend was reported by Mattison et al. (1986).

Shelf life

The sensory shelf life of pork was estimated from sensory evaluation scores for color and odor (Table 2). The time (in days) to reach either a color or odor score of 6 (rejection point) was used as an indicator of shelf life. For example, nonirradiated meat packaged with 20% O_2 and stored at 5°C had a sensory shelf life of 4 days. However, note that similarly packaged meat, irradiated at 1 kGy was immediately rejected on the basis of strong off-odor and complete discoloration, although that treatment was microbiologically acceptable until day 31 (Lambert et al., 1992). The odor score does not correlate, however, with the low TBA value obtained at day 0 and 3 for that treatment (Fig. 4) indicating that other chemical anaylses such as the peroxide value might have proven more indicative of the unacceptability of the odor. Nonirradiated meat packaged in 100% N_2 and with an O_2 absorbent had a shelf life of 9 days at 5°C and was rejected on the basis of discoloration. The longest sensory shelf life at 5°C was 26 days. This was on samples irradiated at 1 kGy in the complete absence of O_2.

CONCLUSIONS

WE CONFIRMED that a substantial extension in sensory shelf life of fresh pork could be achieved using modified atmosphere packaging (MAP) in conjunction with low-dose irradiation. However, to obtain maximum shelf life, product must be stored at 5°C or less. For maximum extension of shelf life, product should be irradiated under anaerobic conditions since irradiation in presence of O_2 had a detrimental effect on physical, chemical and sensory characteristics, resulting in rejection of the product.

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Physical and Sensory Characteristics of Fresh Pork Leg Roasts Cooked at Low Temperatures

E.M. LARSON, E.T. HOLM, M.J. MARCHELLO, and W.D. SLANGER

ABSTRACT

The effects of oven temperatures of 82, 93, 121 and 163°C were studied using 31 fresh pork leg roasts cooked to 74°C in oven bags. Warmed-over-flavor (WOF) was measured by sensory analysis after holding 3 hr at 68°C and after holding 24 hr at 2°C and then reheating to 68°C prior to serving. Roasts cooked at 82 and 93°C tended to have less WOF after refrigeration and reheating than roasts cooked at 121 and 163°C. Roasts cooked at 93°C had fewer aerobic mesophilic bacteria than those cooked at 121 and 163°C. In general, objective and sensory perception measurements indicated that roasts cooked at 82 and 93°C were of higher quality.

Key Words: pork, leg roast, flavor, texture, tenderness

INTRODUCTION

THE TYPICAL METHOD of cooking fresh pork is at an oven temperature of 163°C to an internal temperature of 77°C. Recently the Meat Board Test Kitchens and Pork Industry Group (1989) recommended an optional end-point temperature of 71°C for certain pork cuts and an optional cooking temperature of 149°C. Research by Heymann et al. (1990) and Siemens et al. (1990), in part responsible for these new recommendations, found lower end-point temperatures decreased cooking losses, increased juiciness, and generally resulted in roasts that were preferred by consumers over roasts cooked to higher end-point temperatures.

Moist-heat cooking of large cuts of meat at much lower oven temperatures (77°C) resulted in juicier, more tender and flavorful meat (Marchello, 1989), as well as a decrease in shrinkage and an increase in meat yields. Tuomy and Lechnir (1964) reported that when pork was cooked in tubes using water bath temperatures below 66°C, no increase in tenderness occurred with extended cooking time. At cooking temperatures between 66–99°C, an increase in tenderness as determined by the shear and sensory methods was found as cooking time increased. Jones et al. (1980) found that when pork loins were oven-roasted at 93, 121, 149 and 163°C to an end-point of 77°C, roasts were most tender when cooked at 93°C. The researchers concluded this occurred because the roasts were maintained at higher internal temperatures for longer periods of time. Roasts cooked at 121°C resulted in the least evaporation losses and highest juiciness scores. Meadows (1974) found that shear forces were greater for oven film-wrapped-pork roasts than for those that were open-roasted. Ferger et al. (1972) reported little difference in shear values between the two cooking methods.

It is sometimes necessary to hold meat at serving temperatures for several hours in restaurants and institutional food services. Warmed-over-flavors (WOF) or off-flavors may develop under these circumstances or after meat has been refrigerated and reheated. The most probable cause of WOF is oxidation of polyunsaturated fatty acids in polar lipids, especially phospholipids, to form volatile compounds such as carbonyls, alcohols, and furans (Bailey et al., 1980; Igene et al., 1985).

Cooking has been found to speed lipid oxidation in meats (Younathan and Watts, 1960; Sato and Hegarty, 1971; Keller and Kinsella, 1973), and the intensity of the heat treatment increases the degree of lipid oxidation (Lillard, 1985; Tanchotikul et al., 1989).

Our objective was to assess the benefits of low-temperature, moist heat cooking at oven temperatures of 82, 93, 121 and 163°C on various chemical and sensory characteristics of fresh pork leg roasts. In addition, aerobic mesophilic bacterial counts were taken on uncooked and cooked roasts to determine differences in bacterial growth at different oven temperatures.

MATERIALS & METHODS

BONELESS rolled fresh pork leg roasts (# 402C, NAMP, 1988) from 31 hogs of known breeding and feeding history from the North Dakota State University Animal and Range Sciences' swine herd were used. Each fresh pork leg weighed between 4.9 and 5.9 kg. For each of four trials, eight thawed fresh pork leg roasts were cooked two per oven, using four still air ovens, operating at one of four temperatures: 82, 93, 121 and 163°C. Each oven could only hold two roasts. Individual roasts were weighed and inserted in ovenproof film bags with fat side down in individual glass Pyrex baking pans (both of known weight). The open ends of the bags were tied with plastic twists and five slits were cut in the top of the bag to allow steam to escape during cooking. Each pair of roasts was cooked in the center of a household-model electric 76 cm Whirlpool oven. An iron-constantant thermocouple inserted in the center of each roast was used to determine when an endpoint temperature of 74°C was reached. The temperature of each roast was recorded continuously during cooking by a Honeywell Electronik Model 112 potentiometer. When the end-point temperature of 74°C was reached, the pork roasts were individually weighed and drip loss recorded, and the semimembranousus muscle was removed for sensory examination.

Objective measurements

Cooking time and losses. For each roast, total cooking time, cooking rate, and cooked meat weight were recorded. The percentage of dripping losses, volatile losses, and total cooking losses based on raw weights of the roasts was calculated. Volatile losses were calculated as the difference between the initial weight and the combined cooked meat plus drippings weight.

Moisture. Percent moisture of the cooked meat was determined according to AOAC (1984) procedures.

Tenderness. A Warner-Bratzler (WB) shear device was used to measure tenderness. Six measurements were taken from one core cut, 2.54 cm in diameter, from the semitendinosus muscle of each roast.

Table 1—Scale of sensory attributes evaluated and the end-point descriptors

Min = 0	Attribute	Max = 15
Very unacceptable	← appearance →	acceptable
Not juicy	← juiciness →	juicy
Not tender	← tenderness →	tender
Very undesirable	← flavor →	very desirable
WOF	← WOF →	no WOF

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Table 2—Significance levels of physical and sensory characteristics of fresh pork leg roasts cooked at four different temperatures

Characteristics[h]	P values Temp	Trial	Temp*trial	Mean square	Least squares mean ± standard error 82°C	93°C	121°C	163°C
CT min	0.0001	0.0017	0.0002	224	1078 ± 5.9[c]	630 ± 5.3[d]	366 ± 5.3[e]	212 ± 5.3[f]
CR min/kg	0.0001	0.0935	0.0973	352	477 ± 7.4[c]	256 ± 6.6[d]	153 ± 6.6[e]	92 ± 6.3[f]
WB[a,b] kg	0.0001	0.1572	0.0840	2.06	3.13 ± 0.231[c]	4.02 ± 0.207[d]	4.36 ± 0.207[d]	5.40 ± 0.207[e]
TCL %	0.0136	0.0085	0.2698	3.90	29.2 ± 0.78[c]	28.9 ± 0.70[c]	30.4 ± 0.70[cd]	32.4 ± 0.70[d]
VL %	0.3671	0.0952	0.2533	5.67	7.4 ± 0.94	5.5 ± 0.84	7.5 ± 0.84	6.9 ± 0.84
MOIS %	0.0133	0.1102	0.1961	2.00	60.4 ± 0.56[cd]	61.0 ± 0.50[d]	59.0 ± 0.50[cd]	58.6 ± 0.50[c]
DL %	0.0255	0.2829	0.8011	4.53	21.9 ± 0.84[c]	23.4 ± 0.75[cd]	22.9 ± 0.75[cd]	25.6 ± 0.75[d]
WT[a] kg	0.1391	0.7661	0.9923	0.0267	2.27 ± 0.065	2.47 ± 0.058	2.40 ± 0.058	2.32 ± 0.058
BACTD[g] $\log_{10}$	0.0475	0.6558	0.5636	0.235	0.67 ± 0.198[g]	1.23 ± 0.198	0.43 ± 0.198	0.45 ± 0.198

[a] After cooking.
[b] Six WB readings taken on each roast for degrees of freedom of 155 and mean square of 0.432.
[c,d,e,f] Means in same row with different superscripts are different ($P<0.05$).
[g] Total of 24 roasts, eight per trial for three trials instead of four.
[h] Cooking time (CT), cooking rate (CR), Warner-Bratzler shear (WB), total cooking loss (TCL), volatile loss (VL), moisture (MOIS), drip loss (DL), weight (WT), and difference in bacterial counts before and after cooking (BACTD).

Table 3—Means and P values of tests for linear, quadratic and cubic effects of oven temperature for taste panel sensory characteristics of 31 cooked fresh pork leg roasts

Oven temp	N	Appearance	Juiciness	Tenderness	Flavor	WOF
82°C	158	8.28[b]	6.12[b]	9.76[b]	7.34	9.84
93°C	181	8.07[b,c]	6.07[b]	9.22[b]	7.47	9.86
121°C	182	8.05[b,c]	5.16[b,c]	6.87[c]	6.67	9.29
163°C	179	6.43[c]	4.35[c]	5.82[c]	6.53	9.19
Roast (temp*trial) mean square		29.34	13.64	31.06	13.39	25.39
		P values of tests for linear, quadratic and cubic temperature effects				
Linear		0.0043	0.0001	0.0000	0.0176	0.1591
Quadratic		0.2234	0.8122	0.0904	0.5006	0.6666
Cubic		0.2277	0.8139	0.0933	0.5047	0.6695

[a] After 24 hr refrigeration at 2°C and microwave reheating.
[b,c] Means in same column with different superscript are different ($P<0.05$).

The core was cut parallel to the muscle fiber after the roasts had been held at 2°C for 24 hr.

Bacterial counts. Aerobic mesophilic bacterial counts were determined by taking 15 g samples from the gracilis muscle of each roast before and after cooking. A sterile knife and tongs were used to remove lean surface tissue within a 5 cm diameter template to a depth of ≈5 mm. The excised tissue was placed in a sterile blender jar containing 135 mL sterile 0.1% peptone water and blended in an Oster blender 2 min. Serial dilutions and pour plates using trypticase soy agar were prepared according to standard methods. After incubation at 35°C for 48 hr, colony-forming units were counted.

Sensory measurements

An eight member taste panel was selected from North Dakota State University personnel (faculty, staff, and students). One week before the subjective evaluation of the cooked pork began, the panel members received a general explanation of the study and became familiar with the evaluation procedures. The characteristics listed on the evaluation form were defined. The taste panel evaluated the cooked pork on appearance, juiciness, tenderness, flavor, and warmed-over-flavor (WOF). The panelists were to assume that they were being served fresh pork roast as an entree. They were to evaluate each sample for appearance (overall color appeal, uniformity of color, wetness or dryness, brownness–general overall appetite appeal); tenderness (what degree of force was required to bite through the sample, how many chews were required to prepare the sample for swallowing), juiciness (was the lean perceived as dry or moist as it was chewed) and flavor (did the lean have typical pork flavor, was there a noticeable difference in the level of flavor development, were there off-flavors such as burned, rancid, or raw serumy notes). In training for evaluation of WOF, panelists were given freshly prepared samples and samples which had been prepared 2 days previously, refrigerated, and reheated in a microwave oven. When test samples were evaluated for WOF, a freshly prepared sample was presented as a reference each time.

All subjective evaluations were conducted in individual booths spe-

Table 4—Univariate and multivariate analysis of variance P value and test mean squares for taste panel sensory characteristics of 31 fresh pork cooked leg roasts

Source	Degrees of freedom	Appearance	Juiciness	Tenderness	Flavor	WOF[a]	Multivariate (Wilk's lambda) P value
Temp	3	0.0248	0.0010	0.0001	0.0646	0.6946	0.0058
Trial	3	0.4036	0.9327	0.8012	0.3661	0.2556	0.0052
Temp*trial	9	0.7367	0.1015	0.6589	0.4651	0.3561	0.1986
Roast (temp*trial) mean square	30	29.34	13.64	31.06	13.39	25.39	
Time	2	0.0012	0.0100	0.3986	0.0001	0.0001	0.0001
Temp*time	6	0.2000	0.7413	0.1306	0.1243	0.6132	0.0637
Time*trial	6	0.8115	0.0742	0.1467	0.2446	0.0542	0.0879
Temp*time*trial	18	0.4779	0.1856	0.0909	0.8383	0.3488	0.3289
Time*roast (temp*trial) mean square	30	8.23	11.08	11.33	8.73	13.89	
Panelist	7	0.0001	0.0001	0.0040	0.2736	0.0001	0.0001
Temp*pan	21	0.0030	0.0001	0.0023	0.5335	0.4781	0.0010
Pan*trial	20	0.0001	0.0217	0.9426	0.4532	0.0031	0.0001
Temp*pan*trial	60	0.0930	0.3929	0.9303	0.1166	0.3780	0.1406
Pan*roast (temp*trial) mean square	101	5.96	6.01	10.19	9.54	16.43	
Time*pan	14	0.0230	0.0141	0.0369	0.0198	0.0127	0.0001
Time*pan*trial	38	0.0719	0.0013	0.2677	0.0164	0.0166	0.0001
Temp*time*pan	42	0.8748	0.0060	0.1454	0.0581	0.4982	0.0277
Temp*time*pan*trial	114	0.5202	0.2569	0.6582	0.5319	0.5151	0.4027
Error mean square	190	7.26	5.40	7.29	5.86	12.52	

[a] After 24 hr refrigeration at 2°C and microwave heating.

Table 5—Estimates of variance for taste panel sensory characteristics of 31 fresh pork cooked leg roasts

Characteristic	σ^2 error	σ^2 time*roast (temp*trial)	σ^2 panelist*roast (temp*trial)	σ^2 roast (temp*trial)
Appearance	7.26 (86.8%)[a]	0.132 (1.58%)	−0.449 (0.0%)	1.02 (11.6%)
Juiciness	5.40 (83.3%)	0.774 (12.0%)	0.211 (3.26%)	0.091 (1.40%)
Tenderness	7.29 (75.8%)	0.550 (5.72%)	1.00 (10.4%)	0.772 (8.03%)
Flavor	5.86 (77.4%)	0.391 (5.16%)	1.28 (16.8%)	0.0486 (0.641%)
Warmed-over-flavor[b]	12.5 (86.8%)	0.187 (1.30%)	1.36 (9.42%)	0.350 (2.43%)

[a] Percentages of total variation.
[b] After 24 hr refrigeration at 2°C and microwave heating.

Table 6—Time means for taste panel sensory characteristics of 31 fresh pork cooked leg roasts

Time	N	Appearance	Juiciness	Tenderness	Flavor	WOF[a]
1[b]	240	8.13[d]	6.01[d]	8.12	7.71[d]	11.50[d]
2	227	7.95[d]	5.05[e]	7.74	6.94[e]	9.28[e]
3	233	7.00[e]	5.13[e]	7.70	6.31[e]	7.75[f]
TMS[c](df = 30)		8.23	11.08	11.33	8.73	13.89

[a] Warmed-over-flavor after microwave reheating after 24 hr refrigeration at 2°C.
[b] 1 = immediately after cooking, 2 = after 3 hr-68°C holding period, 3 = after 24 hr refrigeration at 2°C and microwave reheating.
[c] Test mean squares.
[d,e,f] Means in same column with different letters are different (P < 0.05).

cifically designed for sensory evaluation. Panelists were provided with room-temperature spring water during evaluations. Evaluations of a 0.64 cm cross section of the semimembranosus muscle of each roast were conducted immediately after cooking, after a 3 hr holding period at 68°C, and upon microwave reheating after refrigeration at 2°C for 24 hr. Eight meat samples (one from each roast) were judged during each evaluation period. Four meat samples were presented simultaneously on Styrofoam™ (Dow Chemical Co.) plates coded with three-digit random numbers. After the panelists had evaluated the first set of meat samples, a second set was presented for evaluation. The panelists were asked to evaluate the samples by placing a vertical line across the 15 cm horizontal line of the unstructured scale at a point which best described that attribute. The scale for sensory testing and end-point descriptors is described in Table 1.

The objective measurements of WB, cooked weight, moisture, cooking times and cooking losses were analyzed as a four (temperature) by four (trial) factorial with two (or in one case one) replications (roasts) per each of the 16 temperature-trial combinations. Each trial consisted of eight roasts, two per temperature. The subsampling of the WB cores allowed for within sample variation to be estimated in that case. Temperature least squares means were calculated and compared with the Tukey-Kramer (Sokal and Rohlf, 1981) multiple means comparison procedure. Bacterial counts were not available for trial four, so the analysis in that case was 4 by 3 factorial. The bacterial counts were transformed by $\log_{10}$ and differences obtained before and after cooking. Analysis of variance was applied to these differences.

The model of the analysis of variance for the measurements of the 5 sensory variables included the main effects of temperature, trial, time between cooking and sensory evaluation, taste panelist, roasts within temperature and trial and all possible interactions. Roasts were considered random. A multivariate analysis was also obtained. These analyses used Type III sums of squares of SAS/STAT™ (1988) General Linear Models procedure. The design was near balanced, i.e., there were only 5 incidents throughout the four trials where measurements were not available from one of the panelists, and all five involved only two of the eight panelists. Temperature and time averages were compared using Tukey's multiple means comparison procedure using the harmonic mean of the four individual temperature sample sizes as the sample size (Sokal and Rohlf, 1981). Estimates of variance components were obtained using the expectations of the Type III sums of squares. Temperature by time averages were compared using the Tukey-Kramer procedure. Orthogonal polynomials were used to test for linear, quadratic and cubic effects of temperature on the sensory perception characteristics (Gill, 1978).

RESULTS & DISCUSSION

Objective measurements

Cooking times (Table 2) of roasts were most variable at oven temperature 82°C. This variability decreased with increasing oven temperatures. The arithmetic average, minimum, maximum and standard deviation for this lowest temperature were 1078 min = 17.8 hr, 1006 min = 16.8 hr, 1163 min = 19.4 hr, and 52 min. These summary statistics for 93, 121 and 163°C were 630 = 10.5, 600 = 10, 675 = 11.25,25; 366 = 6.1, 331 = 5.5, 400 = 6.7, 22; and 212 = 3.5, 200 = 3.3, 231 = 3.8, 10; respectively. Jones et al. (1980) also indicated extremely variable cooking times when cooking roasts with mean weights of 900g at low temperatures; at 93°C, cooking time ranged from 372 to 482 min. In the present study, the total cooking time and min/kg cooking time significantly decreased as oven temperature increased (Table 2). Despite the great differences in cooking time from one temperature to the next, cooked final weights were similar (Table 2). Volatile losses were not significantly different even though cooking times were quite different. This could be explained in part because the roasts were cooked in bags.

Mean shear force values (Table 2) indicated greatest tenderness for roasts cooked at 82°C (3.13 kg) and least tenderness for roasts cooked at 163°C (5.40 kg). Tuomy and Lechnir (1964) found similar results at cooking temperatures between 66 and 99°C; pork roasts became more tender at the lower temperatures. Jones et al. (1980) reported an increase in tenderness at 93°C when compared to higher cooking temperatures.

Total cooking losses were less for pork roasts cooked at 82 and 93°C than for roasts cooked at 163°C, but volatile losses

Table 7—Time-by-temperature means of sensory characteristics from 31 fresh pork cooked leg roasts

Time	82°	93°	121°	163°C	82°	93°	121°	163°C	82°	93°	121°	163°C
	Appearance (TMS[a] = 8.23)				Juiciness (TMS = 11.08)				Tenderness (TMS = 11.33)			
1[b]	8.86^{s}	8.74^{s}_{x}	8.31^{s}	6.72^{t}	6.40	6.81	5.64	5.22	10.16^{s}	9.70^{s}	6.56^{t}	6.32^{t}
2	$8.25^{s,t}$	8.74^{s}_{x}	$7.95^{s,t}$	6.85^{t}	6.04^{s}	5.46^{s}	$5.02^{s,t}$	3.75^{t}	9.78^{s}	$8.58^{s,t}$	7.61^{t}	5.20^{u}
3	7.71^{s}	$6.74^{s,t}_{y}$	7.89^{s}	5.73^{t}	5.93^{s}	5.89^{s}	$4.79^{s,t}$	4.02^{t}	9.32^{s}	9.35^{s}	6.45^{t}	5.88^{t}
		P value[c] = 0.0556				P value = 0.2974				P value = 0.3079		
	Flavor (TMS = 8.73)				WOF[d] (TMS = 13.89)				Sample sizes			
1	7.20	8.48_{x}	7.71_{x}	7.38_{x}	11.39_{x}	11.83_{x}	11.38_{x}	11.39_{x}	54	62	62	62
2	7.91^{s}	$7.11^{s,t}_{y}$	$6.62^{s,t}_{x,y}$	$6.24^{t}_{x,y}$	$9.92_{x,y}$	9.18_{y}	9.01_{y}	9.12_{y}	51	59	60	57
3	6.94	6.79_{y}	5.66_{y}	5.93_{y}	8.18_{y}	8.50_{y}	7.42_{y}	6.97_{z}	53	60	60	60
		P value = 0.0832				P value = 0.0947						

[a] Test mean squares, df = 30.
[b] 1 = immediately after cooking, 2 = after 3 hr-68°C holding period, 3 = after 24 hr refrigeration at 2°C and microwave reheating.
[c] One-tailed test of the difference between [(8.86-7.71) + (8.74-6.74)]/2 and [(8.31-7.89) + (6.72-5.73)]/2 is significant at P = 0.0556. Other P = 's are analogous.
[d] Warmed-over-flavor after 24 hr refrigeration at 2°C and microwave reheating.
[s,t,u] Means in same row for each attribute that do not have same superscript are different (P < 0.05).
[x,y,z] means in same column for each attribute that do not have same subscript are different (P < 0.05).

were not significantly different (Table 1). Jones et al. (1980) found significantly increased cooking losses in pork roasted at higher oven temperatures of 121, 149 and 163°C at end-point temperatures of 77°C. The use of oven film bags in our study may have reduced the amount of cooking losses in roasts cooked at low temperatures. Meadows (1974) found decreased total cooking losses using oven film to cook pork at oven temperatures of 163°C when compared to open-roasted pork at the same temperature.

Moisture determinations in our study showed roasts cooked at 93°C contained significantly more moisture than those cooked at 163°C (Table 2). There were no temperature differences for volatile losses but drip loss at 163°C was significantly more than at 82°C (Table 2).

Aerobic mesophilic bacterial counts showed roasts cooked at 93°C had the greatest average cycle reduction in numbers with a reduction of 1.23 log cycles (Table 2). Fewer aerobic mesophilic bacteria were destroyed at the other cooking temperatures, 0.67, 0.43 and 0.45 for 82, 121 and 163°C. The reduction at 93°C was different from the mean reductions at the other 3 cooking temperatures ($P = 0.0086$). The average reduction at the 2 lower temperatures was greater than that at the 2 higher temperatures ($P = 0.0247$). The mean reduction in aerobic bacteria in our study was in general agreement with studies using slow cookers as a low-temperature method of cooking. Slow cookery studies have shown greater reductions in aerobic bacterial counts at temperatures of 93°C compared to conventional oven heating at 163 and 176°C (Peters et al., 1983; Sundberg and Carlin, 1978). A possible explanation for the greater reduction in aerobic plate counts at the lower temperatures for roasts in our study was the length of time the roasts were in the temperature range of 66–74°C. At oven temperatures of 82 and 93°C, roasts were in that range for 400 min (6.5 hr) and 200 min (3.3 hr), respectively, compared to 80 min and 60 min at oven temperatures of 121 and 163°C, respectively. Thus, a cumulative time-temperature effect may be responsible for reduced aerobic mesophilic bacterial numbers at 93°C.

Sensory characteristics

Table 3 summarizes the temperature sensory averages and the P values of the orthogonal polynomials that tested for linear, quadratic and cubic temperature effects. Roasts cooked at 163°C scored lower in appearance than roasts cooked at 82°C. No statistical difference occurred at the other temperatures studied. However, there was strong evidence of a linear effect for appearance. Juiciness scored lower at the 163°C higher temperature than at the two lower temperatures. There was strong evidence for a linear effect on juiciness but no evidence for quadratic or cubic effects. Roasts were less tender at the two higher temperatures than at the two lower temperatures. The tests for linear, quadratic and cubic effects were all significant. There were no statistical differences among the means for flavor and WOF; however, both trends were in favor of the lower temperatures. For both variables there was evidence for linear effects (much more so for flavor) but no evidence for quadratic or cubic effects.

The univariate and multivariate analysis of variance P values and univariate test mean squares for the sensory characteristics are in Table 4. In general, the univariate results show large temperature, time and panelists main effects with the majority of the significant interactions being those associated with panelists. The results seemed consistent over the four trials. Practically all of the multivariate tests were significant. About the only effect that did not seem to affect at least one of the sensory perception characteristics was the four-way interaction among temperature, time, panelist and trial. Note that the overall effect of temperature on WOF was not significant.

Table 5 contains the variance component estimates obtained from the univariate analyses of variance of Table 4. Roasts cooked together in the same oven were similar. There was some variability among the three times and among the 8 panelists; however, most of the experimental error was in the error variance. The error variance includes variability of time by panelists within temperature and roast.

Mean evaluation time ratings of sensory characteristics are shown in Table 6. Scores for appearance, juiciness, tenderness, and flavor decreased in value with time. WOF increased significantly at each time of testing. Individual attributes for time and temperature (Table 7) indicated that roasts cooked at lower temperatures developed less WOF. Roasts cooked at the 2 lowest temperatures developed slightly less WOF after refrigeration and reheating than those cooked at the two highest temperatures ($P = 0.0947$ of Table 7). Tanchotikul et al. (1989) reported that although objective testing indicated an increase in lipid oxidation at increased cooking temperatures, significant differences in WOF at those temperatures were not evident in sensory evaluation trials.

Analogous information for the other sensory characteristics is shown in Table 7. The higher temperature had a negative effect on appearance and tenderness at the initial cooking time but did not affect overall flavor. When the roasts were held for 3 hr or cooked and reheated, the same effects were observed. Flavor was affected to a greater extent ($P = 0.0832$) at the higher temperatures compared to the lower temperatures. The first time period scored highest for each sensory characteristic and cooking temperature. These results are in contrast with the findings of Jones et al. (1980) who reported no difference in sensory tenderness scores in pork loin roasts at roasting temperatures between 93°C and 163°C.

CONCLUSION

FRESH PORK leg roasts cooked at low temperatures yielded a juicier, more tender and flavorful product. Cooking pork at low temperatures for extended periods of time decreased total cooking losses, thereby increasing meat yields and minimizing cost/serving. Pork cooked slowly may develop less WOF after refrigeration and reheating than pork cooked by conventional methods. Slowly cooked pork held for several hours at 68°C developed no more WOF than meats cooked at higher temperatures for shorter times. In large-group feeding situations, this would be important when meals are served over a period of several hours.

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Phosphates and Muscle Fiber Type Influence Thermal Transitions in Porcine Salt-Soluble Protein Aggregation

GERALD H. ROBE and YOULING L. XIONG

ABSTRACT

Salt-soluble proteins (SSP) were extracted from porcine Serratus ventralis (red), Vastus intermedius (red) and Longissimus dorsi (white) muscles and heated to examine dynamic changes and transitions in protein aggregation. At pH 6.0, red muscle SSP typically showed 1 or 2 transitions and white muscle SSP exhibited 3 transitions. Addition of ortho-, pyro-, tripoly- and hexametaphosphate up to 1% increased SSP transition temperatures and altered transition patterns; NaCl at comparable ionic strengths did not show this effect. SSP transitions were most affected by 0.15–0.25% tripolyphosphate and low pH (<6.0). Red and white SSP exhibited different thermal properties and responses to phosphate treatments. These findings indicate red and white muscle types should undergo different processing treatments for optimum quality meat products.

Key Words: porcine, salt-soluble protein, aggregation, transition

INTRODUCTION

PHOSPHATE compounds are commonly added during meat processing to modify texture, water binding, and other quality factors in finished products. Phosphates improved water binding and emulsion stability, and reduced cooking losses in turkey frankfurters (Barbut et al., 1988) and in chicken rolls (Froning, 1965). Phosphates are also added to meat products as meat and water binding agents, partially substituting for NaCl in the production of low-sodium meat products (Trout and Schmidt, 1983). Whiting (1984) and Knipe et al. (1990) reported increased water binding and gel strengths in reduced NaCl frankfurters by pyro- or tripolyphosphate. The improved muscle functionality has been attributed to changes in pH and ionic strength caused by addition of phosphates (Trout and Schmidt, 1983). However, Trout and Schmidt (1986a) also showed an improvement in protein functionality by phosphates which could not be explained fully by pH or ionic strength changes.

Muscles generally consist of different types of fibers, and the functional properties of proteins obtained from different muscle fiber types may differ. Asghar et al. (1984) and Morita et al. (1987) reported that thermally induced gels from chicken white fiber myosin had greater strengths than gels made from red fiber myosin under pH conditions similar to those used during meat processing. Similar studies of the effects of fiber type on muscle protein funcitionality have been conducted using myofibrillar proteins isolated from beef (Fretheim et al., 1986) and turkey (Foegeding, 1987).

The different functionalities of white and red muscle proteins could be related to different thermal stabilities of the proteins. Xiong et al. (1987) demonstrated different thermal denaturation patterns for white and red chicken muscle proteins and those from different animal species. The transition patterns in thermal denaturation could be modified by phosphates (Kijowski and Mast, 1988). Furthermore, variations in thermal aggregation of salt-soluble proteins were observed between chicken red and white muscles (Acton and Dick, 1986; Xiong and Brekke, 1990).

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Our objective was to study the thermal aggregation of porcine red and white fiber muscle proteins in relation to various phosphates, phosphate concentrations, and pH.

MATERIALS & METHODS

Muscle source and preparation

Seven Hampshire-Yorkshire crossbred barrows (91–95 kg live wt) were slaughtered at the University of Kentucky Meats Laboratory. Longissimus dorsi (LD, 75% white fiber), Serratus ventralis (SV, 43% red fiber) and Vastus intermedius (VI, 76% red fiber) (Beecher et al., 1965; Suzuki and Cassens, 1980) muscles were excised from the carcasses after 48 hr postmortem storage at 2°C. Red and white muscle samples were placed in plastic bags, vacuum packaged, and stored frozen at −29°C for less than 3 months before use.

Salt-soluble protein preparation

Muscle samples were thawed at 4°C overnight. After removal of connective tissue and external fat, samples were minced prior to protein preparations. Salt-soluble protein (SSP) was prepared using the methods previously described (Xiong and Brekke, 1989). The minced muscle was washed four times with a buffer (4 vol) consisting of 0.1M NaCl, 2.0 mM $MgCl_2$, 1.0 mM EGTA, and 20.0 mM Na_2HPO_4 (pH 7.0). The myofibril pellet was purified by washing three times with 0.1M NaCl containing 1.0 mM NaN_3. SSP was extracted from the myofibril pellet in 0.6 M NaCl, 50 mM piperazine-N-N'-bis-(2-ethanesulfonic acid) (PIPES) at pH 6.0. Protein samples were stored in ice and used within 3 days of isolation. Protein concentration was determined by the biuret method (Torten and Whitaker, 1963) using bovine serum albumin as the standard.

SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was carried out according to the method described by Laemmli (1970) using an SE 250 Mighty Small II slab gel electro-

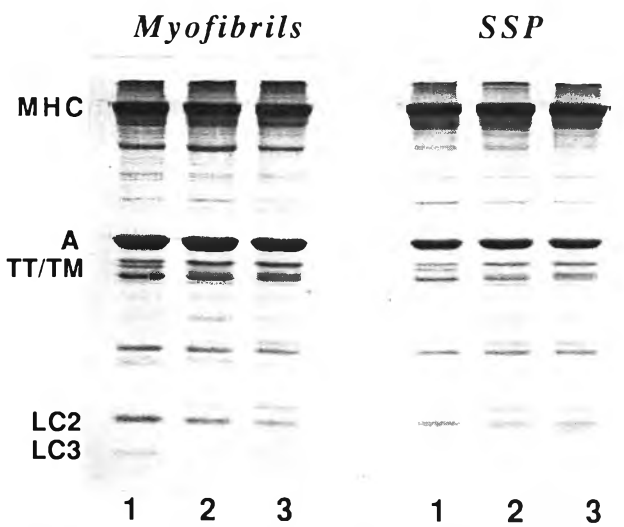

Fig. 1—SDS-PAGE results for myofibrils and salt-soluble proteins isolated from Longissimus dorsi (1), Serratus vantralis (2), and Vastus intermedius (3) muscles. MHC = myosin heavy chain; A = actin; TT = troponin-T; TM = tropomyosin; LC2, LC3 = myosin light chains 2 and 3.

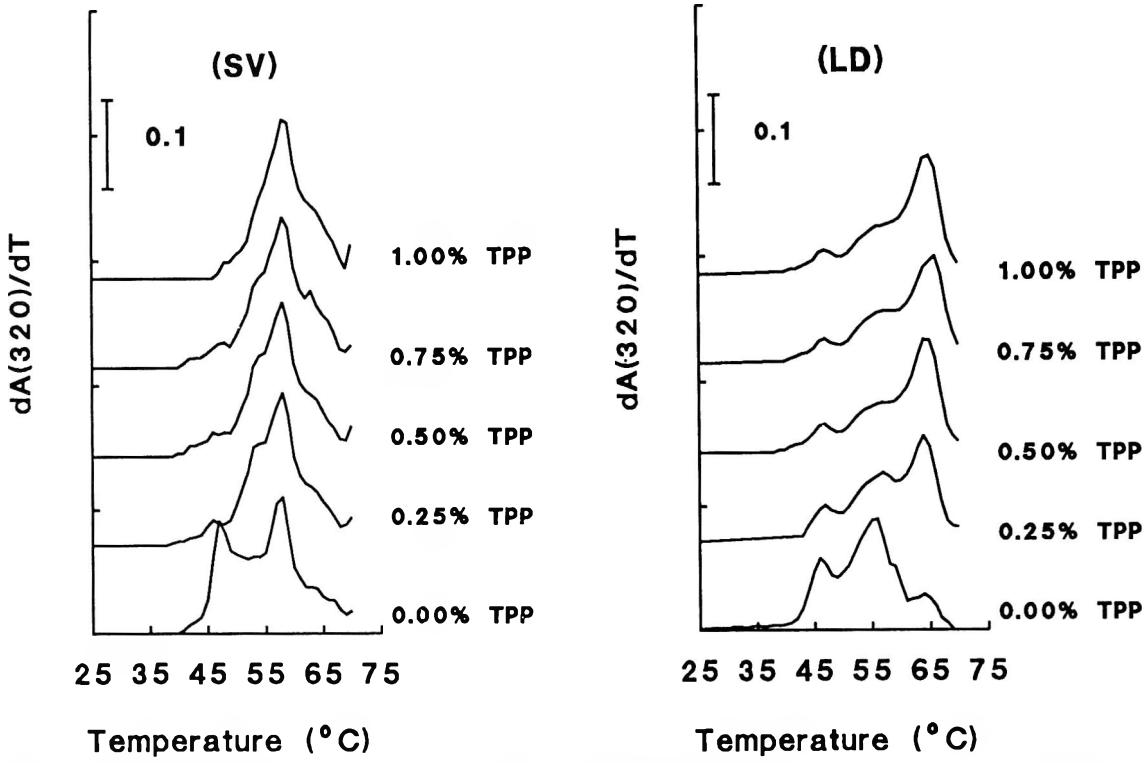

Fig. 2— Influence of sodium tripolyphosphate (TPP) concentration on thermal aggregation of Serratus ventralis (SV) and Longissimus dorsi (LD) muscle salt-soluble proteins.

phoresis unit (Hoefer Scientific Instruments, San Francisco, CA). The stacking gel and the resolving gel were made of 3% and 10% acrylamide, respectively. All electrophoretic samples contained 1 μg/μL protein and 10% β-mercaptoethanol, and 20 μg of protein was loaded in each lane. Protein bands were tentatively identified according to Porzio and Pearson (1977).

Protein-protein interaction

Samples used for the measurement of protein-protein interaction were prepared by suspending SSP to a 1 mg/mL concentration in 0.6M (final) NaCl, 50 mM (final) PIPES ("base buffer", pH 6.0) containing various phosphate treatments as described below. Unless otherwise specified, the pH of all protein solutions (treatments) was adjusted to 6.00 ± 0.01 using 0.1 to 0.5N NaOH or HCl. The amount of NaOH or HCl consumed during pH adjustments (titration) for all protein solutions was less than 0.015M; therefore, the contribution of the titrants to the total ionic strength of protein solutions (≥ 0.6M) was considered negligible.

Phosphate concentration effect. SSP solutions were formulated to contain 0, 0.25, 0.50, 0.75, or 1.00% sodium tripoolyphosphate (TPP) (Sigma Chemical Co., St. Louis, MO).

Ionic strength effect. Two sets of SSP solutions were prepared to determine whether the phosphate effect on protein aggregation was due to an increase in ionic strength. The first set consisted of 4 SSP solutions (in base buffer) with 0.15, 0.30, 0.45, or 0.60% added TPP (equivalent to ionic strengths of 0.03, 0.05, 0.08, and 0.10 at pH 6.0, respectively). The second set was comprised of 4 SSP solutions (in base buffer) which contained no phosphates but contained additional NaCl at ionic strengths equivalent to those of the added TPP in the first set. The ionic strengths of TPP and NaCl were calculated according to Trout and Schmidt (1986b).

pH effect. SSP solutions containing 0.5% TPP in base buffer were adjusted to pH 5.5, 6.0, 6.5, and 7.0 using 0.1 to 0.5N HCl or NaOH.

Phosphate type effect. Pre-determined amounts of sodium ortho- (P), pyro- (PP), TPP (Sigma Chemical Co., St. Louis, MO), or hexametaphosphate (HMP) (EM Science, Gibbstown, NJ) were dissolved in base buffer, and mixed with SSP to produce a final 0.5% phosphate concentration.

Turbidity measurement. SSP solutions were transferred to 0.5 mL quartz cuvettes, capped loosely to prevent pressure build-up and dehydration during heating, and placed in a six-position thermoset cuvette holder attached to a Gilford Response II UV/vis spectrophotometer (Gilford Instrument Laboratories Inc., Oberlin, OH). Cuvettes filled with base buffer were used as blanks. After equilibration at the initial temperature (25°C for 1 min), samples were heated to 80°C at 1.6°C/min. Due to the small volume of the cuvettes, the temperature of the solutions was assumed to be equal to the temperature recorded for the heated cuvette holder.

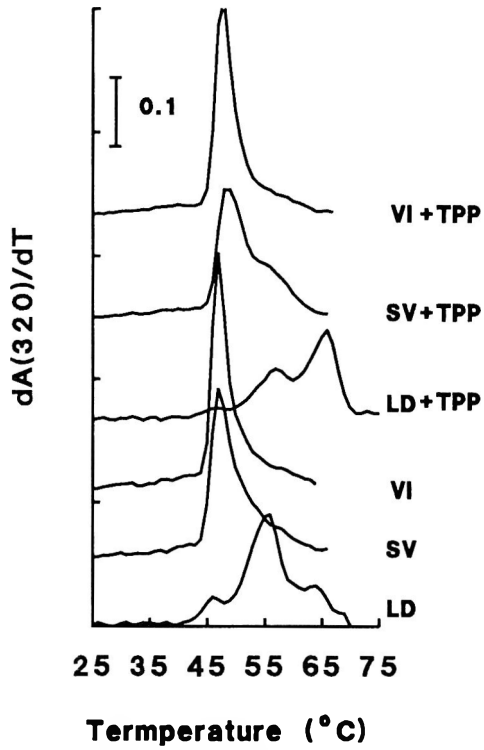

Fig. 3—Effect of muscle fiber type and 0.5% sodium tripolyphosphate (TPP) on thermal aggregation of salt-soluble proteins. LD = Longissimus dorsi; SV = Serratus ventralis; and VI = Vastus intermedius.

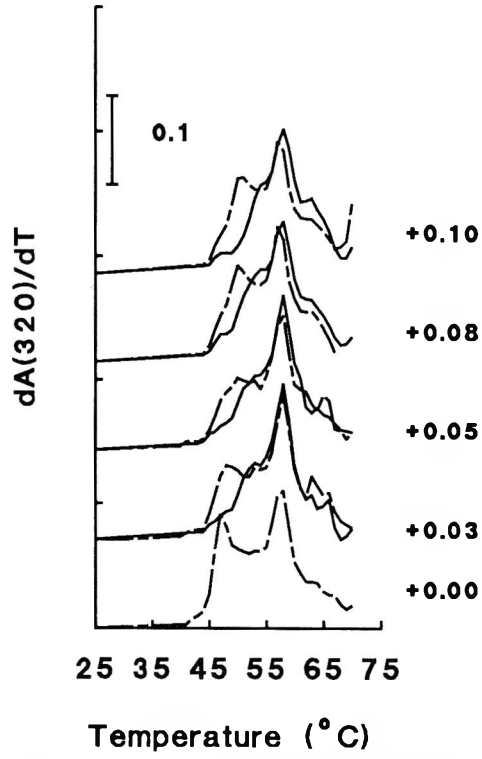

Fig. 4—Effect of NaCl and sodium tripolyphosphate (TPP) at equivalent ionic strengths on thermal aggregation of Serratus ventralis muscle salt-soluble protein. The numbers next to the curves indicate ionic strengths corresponding to the added NaCl or TPP. Solid lines indicate TPP treatments; broken lines indicate NaCl treatments.

The dynamic changes in solution turbidity resulting from protein aggregation were recorded for each 1°C increase by determining the optical density of the solution at wavelength 320 nm. The differential changes in optical density as a function of temperature (dA_{320}/dT), calculated by the spectrophotometer software, were used to identify the maximum rates and transitions in protein-protein interaction (Acton and Dick, 1986). The turbidity measurements were replicated at least four times using SSP isolated from different animals. Figures represent typical thermal curves of the four replications.

RESULTS & DISCUSSION

SDS-PAGE showed essentially identical electrophoretic patterns for SV and VI myofibril and SSP samples (Fig. 1). However, the LD sample differed considerably from SV and VI, particularly in the low molecular weight region (below the actin band). LD exhibited a greater amount of myosin light chain 2 and light chain 3 than SV and VI, as judged by the band intensity. In addition, LD showed three distinct bands in the region of troponin-T and tropomyosin, where only two bands were observed for SV and VI. The differences in protein compositions between LD, SV and VI muscles were probably related to the content of white and red fibers. Protein compositional variations between different fiber types are well documented (Young and Davey, 1981; Foegeding, 1987; Xiong and Brekke, 1989).

The effect of TPP at varying concentrations is shown in Fig. 2. In the absence of TPP, SV muscle SSP typically had two thermal transitions at about 47 and 57°C, respectively. However, occurrence of the second thermal transition was inconsistent; occasionally it was unresolved or absent. This inconsistency in thermal transitions was probably due to variations in the red fiber content of SV muscles from different animals. Addition of 0.25% TPP elimated or reduced the first transition and enhanced the second; further increases in TPP concentration had little effect on transition patterns. LD muscle SSP always exhibited three transitions (Fig. 2), but an increase in the phosphate concentration suppressed the first (about 47°C) and second (57°C) transitions and augmented the the third transition (65°C). Furthermore, the transition temperatures increased slightly with the added phosphate.

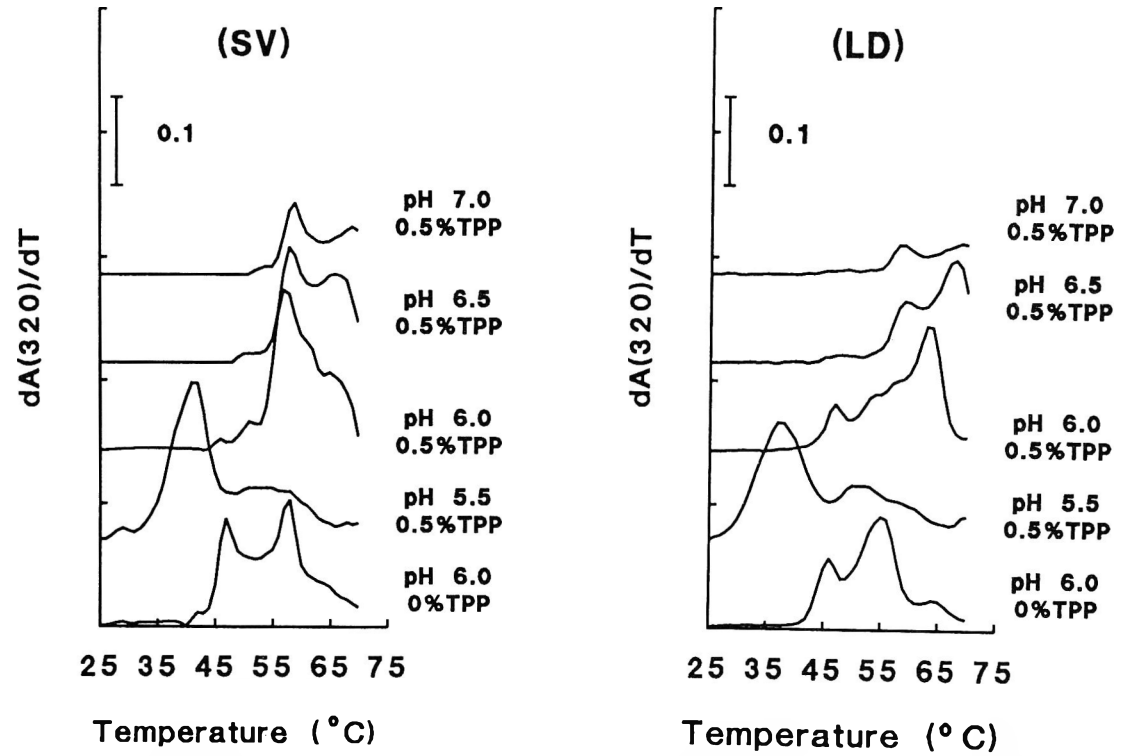

Fig. 5—Effect of pH on thermal aggregation of Serratus ventralis (SV) and Longissimus dorsi (LD) muscle salt-soluble proteins in the presence of 0.5% sodium tripolyphosphate (TPP).

The disappearance or reduction of thermal transitions caused by the addition of TPP probably resulted from an increased thermal stability in the protein domains (Kijowski and Mast, 1988). Some transitions in the SV samples probably still occurred, but their presence was masked by the major transition occurring around 57°C when TPP was added. Based on the relative thermal stability of the major SSP components (Wright and Wilding, 1984), we assumed that the two transition peaks in SV muscle SSP and the first two peaks in LD muscle SSP resulted from the aggregation of myosin and actin (or actomyosin), while the third peak in LD muscle SSP was due to the association of the tail portions of myosin. However, possibly other myofibrillar components were involved in these thermal transitions.

The marked differences between SV and LD muscle proteins in thermal behavior and response to the TPP treatment may be related to the different muscle fiber types from which SV and LD proteins were extracted. Red and white muscle fibers and myofibrillar protein isomers obtained from the two fiber types differ in biochemical and functional characteristics (Asghar et al., 1984; Fretheim et al., 1986). Our study revealed considerable differences in protein composition between red and white fibers (Fig. 1). Xiong and Brekke (1990) previously observed two thermal transitions in chicken hen leg (red) SSP but three in breast (white) SSP. SV muscle has a greater content of red fibers (43% red fibers) than LD muscle (less than 25% red fibers) (Beecher et al., 1965). This difference may contribute to variations in SSP aggregation patterns. To verify this possibility, SSP was extracted also from VI muscle, which mainly contained red fibers (76%). SSP of VI muscle exhibited essentially a single thermal transition (Fig. 3) even in the absence of phosphate, and the addition of 0.5% TPP did not alter the transition pattern. This result suggests that the protein-protein interactions were influenced by the muscle fiber types and were dependant upon the relative content of the specific fiber types in the muscle.

The effect of phosphates on meat products has often been attributed to increases in ionic strength. To test this possibility, the effects of NaCl and TPP at equal ionic strengths were compared. Two distinct transitions were observed for SV SSP at all NaCl concentrations (ionic strengths), but a single transition was observed for the TPP treatments at comparable ionic strengths (Fig. 4). These results indicated that the effects of TPP on SSP thermal transition patterns were not solely due to increased ionic strength. TPP seemed to be more effective than NaCl in modifying the electrostatic forces in proteins, thereby altering SSP aggregation patterns.

Protein-protein interaction for both fiber types was markedly influenced by pH of the solutions. In general, transition temperatures increased with pH. A major transition at pH 5.5 occurred at 40°C for SV muscle SSP and 37°C for LD muscle SSP (Fig. 5). Furthermore, peak height generally decreased at higher pH, reaching a minimum at pH 7.0. This apparently resulted from electrostatic repulsion between negatively charged protein molecules at high pH, and, consequently, either the rate or the extent of protein-protein interaction was reduced. Presumably at high pH, more homogeneous protein aggregates were formed, resulting in decreased optical density. When the pH approaches the isoelectric point, at which protein molecules bear no net charges, maximum protein-protein interaction through hydrophobic forces would occur. As pH increased, TPP also becomes more deprotonated and, when bound to SSP, would facilitate intermolecular repulsions.

TPP is a major phosphate compounds used in meat processing as a meat and water binder and partial salt replacement. Other phosphate compounds of varying chain lengths are also used in meat processing, ranging from single unit phosphates, such as orthophosphate, to long-chain or cyclic phosphates, such as hexametaphosphate (Trout and Schmidt, 1983). It is therefore important to examine the effects of different phosphate compounds on SSP aggregation. As illustrated (Fig. 6),

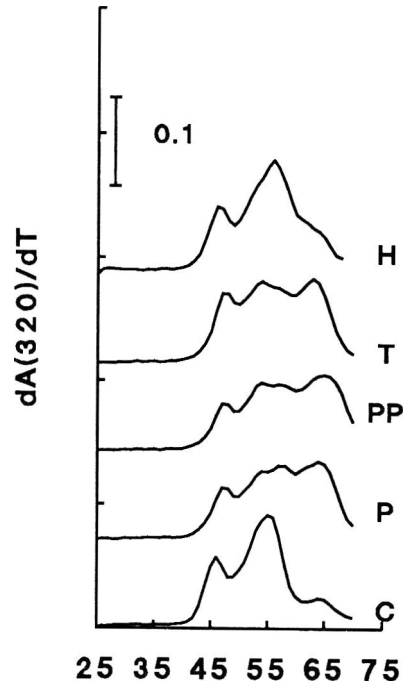

Fig. 6—Effects of various sodium phosphates (0.5%) on thermal aggregation of Longissimus dorsi *salt-soluble protein. C = control, P = orthophosphate, PP = pyrophosphate, T = tripolyphosphate, H = hexametaphosphate.*

the addition of P, PP, and TPP all produced similar effects on protein-protein interaction of LD muscle SSP. The addition of these phosphates generally facilitated the thermal transition occurring around 65°C, while the thermal transitions occurring around 47°C and 57°C were slightly suppressed. The HMP treatment and the control (no phosphate) produced similar thermal transition patterns, indicating that HMP was not as effective as the short-chain phosphates at altering reactions involved in porcine protein-protein interactions. Muhlrad (1991) and Muhlrad et al. (1991) conducted myosin-phosphate binding studies, and reported that P, PP, and TPP bound to myosin subfragment 1 at the same binding sites. HMP is a large, cyclic molecule compared with P, PP, and TPP. Possibly HMP had less affinity for myosin, and hence, less effect on thermal transitions of SSP. Furthermore, because all 4 phosphates were applied on an equal weight concentration basis (0.5%, w/v), the molar concentration of HMP would be lower than that of the other 3 phosphates. This difference might also contribute to the observed difference in effectiveness of HMP relative to the other phosphates.

Thus, myofibrils and SSP from red and white porcine muscle fibers exhibited markedly different thermal behaviors and different responses to phosphate treatments. The phosphate effects were not exclusively due to changes in ionic strength and pH, and phosphate type affected the thermal behavior of SSP. Because protein aggregation is closely related to other protein functional properties, such as gelation (Ziegler and Acton, 1984), these results suggest that salt, TPP, and other types of phosphates may not be completely interchangeable in pork product formulations. It would therefore be advisable during meat processing to treat red and white muscles differently with respect to temperature, concentration of phosphate and salt, and pH level to maximize quality and functionality of processed muscle foods. *—Continued on page 1310*

Detection of Proteolytic Activity in Microorganisms Isolated from Dry-Cured Ham

IRENE MOLINA and FIDEL TOLDRÁ

ABSTRACT

Different assays were tested for the detection of proteolytic activity in both *Pediococcus pentosaceus* and *Staphylococcus xylosus*, two major microorganisms isolated from dry-cured ham. Absence of endopeptidase activity was found even when using raw sarcoplasmic and myofibrillar proteins. However, *P. pentosaceus* showed strong leucine and valine arylamidase activities while *St. xylosus* showed a very weak leucine arylamidase activity.

Key Words: ham, meat, microbes, proteolytic, dry cured

INTRODUCTION

INTENSE proteolysis has been observed during the successive stages of the dry-curing process of Italian Parma ham (Bellattti et al., 1983), Spanish Serrano ham (Aristoy and Toldrá, 1991; Toldrá et al., 1992a) and American Country-Style ham (McCain et al., 1968). In all cases there was found to be a progressive increase in the level of non-protein nitrogen and, more specifically, in the free amino acids. Thus, proteolysis of muscle proteins seems to be one of the most important biochemical changes during the dry-curing but its origin remains unclear. Toldrá and Etherington (1988) did not find large microbiological counts in the interior of the hams. Those values were sufficiently low to suggest a non significant contribution to proteolysis during the process. They attributed the observed proteolysis to muscle proteinases. In fact, the role of those enzymes and exopeptidases in dry-cured meat products has been extensively studied (Toldrá, 1992; Toldrá et al., 1992b, c).

Microbial flora in dry-cured ham has been recently isolated and studied (Silla et al., 1989). Identification of the microorganisms showed that the most important were *Staphylococcus xylosus* (Molina et al., 1989a) and *Pediococcus pentosaceus* (Molina et al., 1989b). *Pediococcus pentosaceus* and most strains of *Staphylococcus xylosus* have been found nonproteolytic (Garvie, 1986; Kloos and Schleifer, 1986). A preliminary study did not show proteolytic activity in these microorganisms (Bermell et al., 1992) although the assays were not very sensitive. The objective of our work was to test different assays for the detection of microbial proteolytic activity and confirm the absence of proteolysis from microbial origin during the processing of dry-cured hams.

MATERIALS & METHODS

Materials

The microorganisms were isolated during the processing of Spanish Serrano dry-cured ham as previously described (Silla et al., 1989) and identified as *Staphylococcus xylosus* (Molina et al., 1989a) and *Pediococcus pentosaceus* (Molina et al., 1989b). All proteolytic assays were verified with the respective strains *Staphylococcus xylosus* CECT 237 and *Pediococcus pentosaceus* CECT 923 (Dawson et al., 1990).

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A microorganism with well characterized proteolytic activity, *Bacillus polymyxa* CECT 153 (Dawson et al., 1990), was used as reference.

Standard methods for detecting proteolytic activity

Agar plate assay. Each microorganism was tested for proteolytic activity as described by Harrigan and McCance (1979) by harvesting in standard solid culture media (10 g/L peptone, 10 g/L meat extract, 5 g/L NaCl and 15 g/L agar) containing 10% gelatin and 10% powdered milk as substrates. After incubation at 30°C for 48 hr, the plates were scanned for clear zones confirming proteolytic activity.

Apizym system. Each microorganism was suspended in sterile distilled water to a final turbidity between MacFarland No. 3 and No. 4 standards. Each microbial suspension (60μL) was then added to each of the galleries in the Apizym strip (Api system, Montalieu-Vercieu, France), three strips/microorganism. The strips were incubated at 37°C for 4 hr. Color intensity values, ranging from 0 to 5, were determined in accordance with the manufacturers instructions.

Proteolytic activity against sarcoplasmic proteins

Extraction of sarcoplasmic proteins. Muscle *B. femoris* (20g) diluted 1:10 with 200 mL of 0.03N phosphate buffer pH 7.4, was homogenized in a Stomacher 400 blender (London, UK) for 3 min and centrifuged at 10000 x *g* for 20 min at 4°C (Toldrá et al., 1992a). The supernatant (sarcoplasmic fraction) was sterilized by tangential filtration through a 0.45 μm pore size filter using a Minitan system (Millipore, Bedford, MA) in aseptic conditions. Sterility of the filtered protein extract was confirmed by checking the absence of microbial growth after incubation at 30°C for 48 hr. The extract contained 5 mg protein/mL.

Qualitative assay. Each microorganism was harvested in a solid culture medium containing MRS-Oxoid agar in the case of *P. pentosaceus* or nutritive agar when testing *St. xylosus* and *B. polymyxa* CECT 153. In all cases, the solid culture medium contained 20% (v/v) of the sarcoplasmic protein extract. The plates were incubated for 48 hr at 30°C, stained with 0.05% (w/v) Coomassic Brilliant Blue R-250 in methanol:acetic acid:water (50:40:10), destained by soaking in several changes of 10% methanol-7.5% acetic acid and scanned for clear zones confirming proteolytic activity.

Proteolytic activity against myofibrillar proteins

Extraction of myofibrillar proteins. The pellet resulting from sarcoplasmic protein extraction was resuspended in 200 mL of 0.03N phosphate buffer, pH 7.4, containing 0.1% (v/v) Triton X-100, (which ensured maximal extraction of muscle proteinases (Okitani et al., 1981) and homogenized for 2 min in a Stomacher 400 blender. After centrifugation at 10000g for 20 min at 4°C, the pellet was washed three times by resuspending in the same buffer for a complete elimination of muscle proteinases. The resulting pellet was resuspended in 200 mL of 0.1N phosphate buffer and 1.1M KI, pH 7.4, homogenized for 2 min in a Stomacher 400 blender and centrifuged at 10000 x *g* for 20 min in refrigeration. The supernatant was filtered through a 0.45 μm pore size filter using a Minitan tangential filtration system in aseptic conditions. The sterility of the filtered extract was also confirmed by checking the absence of microbial growth at 30°C for 48 hr. The extract contained 0.43 mg protein/mL.

Microbial cultures. Each microorganism was grown in its respective liquid culture media (LB and MRS). Culture (1mL) was taken at the end of the exponential phase of growth and centrifuged at 10000 x *g* for 5 min. The supernatant was used as microbial extract for further assays.

Qualitative assay. Myofibrillar protein (1mL) extract (pH = 7.4)

Table 1—Color intensity values of proteolytic enzymes detected in microbial cultures of P. pentosaceus *and* St. xylosus *isolated from dry-cured ham and respective CECT collection strains*

Microorganism	Enzymes detected[a,b]				
	A	B	C	D	E
P. pentosaceus	5	4	0	0	0
P. pentosaceus CECT 923	5	4	0	0	0
St. xylosus	1	0	0	0	0
St. xylosus CECT 237	1	0	0	0	0

[a] A (*leucina arilamidase*, EC 3.4.11.1.), B (*valine arilamidase*, EC 3.4.11.–), C (*cystine arilamidase*, EC 3.4.11.3.), D (*trypsin*, EC 3.4.21.4.), and E (*chimotrypsin*, EC 3.4.21.1.).
[b] Color intensity values: <1 (trace), 1–2 (low, 5–10 nM), 3 (medium, 10–30 nM), and 4–5 (maximum intensity, 30–40 nM).

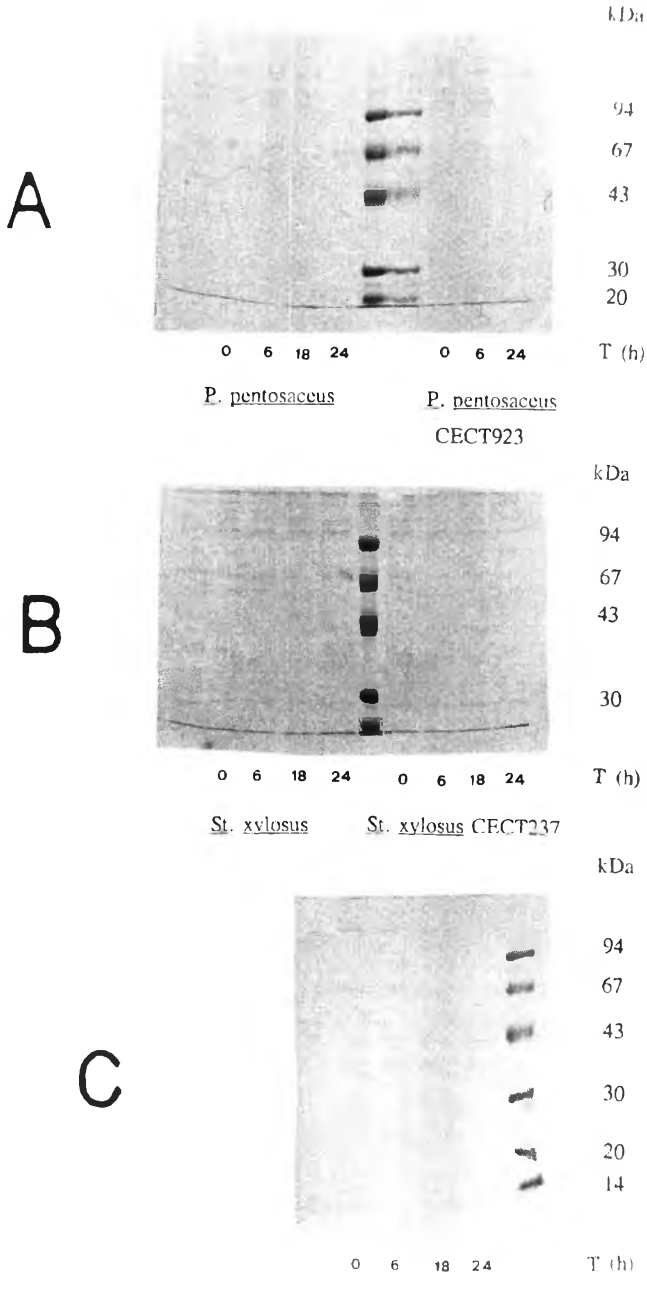

Fig. 1.—SDS-PAGE electrophoregrams of myofibrillar proteins at different times of incubation of 37°C with (A) P. pentosaceus, (B) *St. xylosus*, and (C) *B. Polymixa* CECT 153.

was added to 200 μL of each microbial extract and incubated at 37°C in a shaken water bath. Distilled water instead of microbial extract was used as control. Samples (100 μL) were taken at 0, 6, 18 and 24 hr and immediately prepared for electrophoresis.

Electrophoresis. Protein samples were prepared for electrophoresis as described by Toldrá et al. (1992a). Each sample was mixed in a ratio 2.5:1 (v:v) with 0.5M Tris/HCl, pH 8.0, containing 5% (w/v) Sodium dodecyl sulphate (SDS), 14.5% (v/v) β-mercaptoethanol, 30% (v/v), glycerol and 0.03% (v/v) bromophenol blue. The mixture was boiled for 4 min before loading samples onto the gels. SDS-Polyacrylamide gel electrophoresis was performed as described by Laemmli (1970) using 10% polyacrylamide gels. Electrophoresis was carried out with a current of 25 mA/gel until the bromophenol blue marker reached the bottom of the gel. The proteins in the gel were stained by soaking in a solution containing 0.05% (w/v) Coomassie brilliant blue (R-250), 50% (v/v) methanol and 10% (v/v) acetic acid for 2 hr at room temperature. Gels were then washed and destained with 10% methanol/7.5% acetic acid until clear background. Standard proteins (Pharmacia, Uppsala, Sweden) were simultaneously run for protein identification. These standards were: thyroglobulin (330,000), ferritin (220,000), phosphorylase b (94,000), serum albumin (67,000), catalase (60,000), ovalbumin (43,000), lactate dehydrogenase (36,000), carbonic anhydrase (30,000), trypsin inhibitor (20,100) and 1-lactalbumin (14,400).

Protein concentration. The concentration of protein in both sarcoplasmic and myofibrillar extracts was determined by the method of Bradford (Bradford, 1976) using bovine serum albumin (BSA) as standard.

RESULTS & DISCUSSION

No proteolytic activity was observed with the agar plate assay when using nutritive gelatin, powdered milk or sarcoplasmic proteins as substrate. Only the *B. polymixa*, the reference microorganism with strong proteolytic activity, showed a clear zone indicating proteolysis. The Apizym system also confirmed the absence of proteinase (trypsin and chymotrypsin) activity (Table 1). However, *P. pentosaceus* showed a strong leucine and valine arilamidase activities in accordance with the biochemical profile of *P. pentosaceus* reported in milk and cheese (Tzanetakis and Litopoulou-Tzanetaki, 1989). With dry-cured ham, where there is a very important generation of free amino acids (Aristoy and Toldrá, 1991), the observed exopeptidase activity might have some effect on muscle proteins and peptides in combination with muscle aminopeptidases (Toldrá et al., 1992c) and, thus, have an influence on ripening and flavor development. *St. xylosus* only showed a very weak leucine arylamidase activity (Table 1).

A further confirmation of the absence of endopeptidase activity was achieved using myofibrillar proteins as substrate. The electrophoretic patterns did not show any alteration in protein bands after 24 hr of incubation at 37°C (Fig. 1). *B. polymixa* (reference microorganism with proteolytic activity) produced the disappearance of myosin (MW 200 KDa) and other bands in the range 30-60 KDa after 24 hr incubation, confirming its proteolytic activity against some of the myofibrillar proteins and the validity of the assay. No alterations were observed in the controls without added microbial cultures confirming also the absence of endogenous muscle proteinases. Thus, the assay could be useful for research to test endopeptidase activity of microbial cultures against myofibrillar proteins obtained in sterile conditions in the absence of endogenous muscle proteinases.

The absence of proteolytic activity in the assayed strains, (the most important during the processing of dry-cured ham) confirmed other proteinases as responsible for hydrolytic changes in muscle proteins. In fact, a small proteolytic activity due to an endocellular protease from yeast has been reported in dry-cured ham (Comi et al., 1982). However, yeasts have been mainly found on the surface of dry-cured hams (Motilva et al., 1991). They were also inside (Silla et al., 1989) but in very small counts, less than 0.3–1.0% of the total Micrococcaceae and lactic acid bacteria. All these facts confirm the main role of muscle proteinases in the major breakdown of muscle proteins during dry-curing. Relatively high activities of cathepsins B, D, H and L (Toldrá and Etherington, 1988) and muscle aminopeptidases (Toldrá et al., 1992c) have been recovered even after 8 mo processing, indicating good stability to long-term processing and storage (Toldrá et al., 1991).

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Post-exsanguination Infusion of Ovine Carcasses: Effect on Tenderness Indicators and Muscle Microstructure

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ABSTRACT

Lambs were assigned to 3 treatment groups: control (Ctr), infused with 10% volume (by wt) of a tenderizing blend (NCa), and NCa plus 0.015M $CaCl_2$ (WCa). Compared to Ctr and WCa, NCa-treated samples had lower shear force values ($P<0.05$) and higher percent change in myofibrillar fragmentation index ($P<0.05$). SDS-PAGE of infused samples revealed the appearance at 24 hr postmortem of 22–30 kd protein components. Scanning electron micrographs of NCa myofibrils showed they were more fragmented, fractured, or split and had wider interfibrillar spaces compared to Ctr and WCa. The fracture plane of muscles immediately postmortem was along the endomysial-sarcolemmal sheath, while at 24 hr postmortem the sheath was weakened enough for the fracture to occur along the surface of the myofibrils.

Key Words: tenderness, infusion, SDS-PAGE, ultrastructure, interfibrillar space

INTRODUCTION

THE IMPORTANCE of tenderness in meat is the reason that it has been so extensively studied (Calkins and Seideman, 1988; Koohmaraie et al., 1988; Cross, 1987; Lawrie, 1985; Karmas, 1970). Several theories have been advanced to explain tenderness-toughness in meat. However, the most widely accepted views of tenderness-toughness are those that relate to myofibrillar contraction and/or connective tissue characteristics (Bailey and Light, 1989; Stanley, 1983; Marsh, 1977). Postmortem conditions and treatment are hypothesized to have far more effect than live animal factors in determining tenderness in meat (Locker, 1985; Cross, 1979). Some postmortem conditions affecting tenderness include muscle pH and temperature (Dutson, 1983; Marsh, 1983), muscle fiber contraction (Jaime et al., 1989; Bouton et al., 1973), rate of glycolysis (Smulders et al., 1990), muscle fiber fracture (Marsh et al., 1981), proteolysis (Asghar and Bhatti, 1987; Goll et al., 1983; Davey and Gilbert, 1973), myofibrillar fragmentation index (Olson and Parrish, 1977) and infusion of $CaCl_2$ (Koohmaraie et al., 1988, 1989, 1990; Pearson et al., 1973). Tenderness has been assessed by shear force measurement (Shackleford et al., 1991), and myofibrillar fragmentation index (Culler et al., 1978; Olson and Parrish, 1977). Both transmission and scanning electron microscopy (Eino and Stanley, 1973; Schmidt and Parrish, 1971; Cassens et al., 1963) and SDS-PAGE (Ducastaing et al., 1985; Yates et al., 1983; Olson et al., 1977) have been applied to identify factors related to tenderness.

In a previous study (Farouk et al., 1992), the infusion of dextrose, maltose, glycerin and polyphosphates significantly improved tenderness in cows, compared to control animals. The objectives of our present study were to further ascertain the impact of infusing these ingredients and $CaCl_2$ on tenderness and to relate the effect of the process to changes in rate of glycolysis and to changes in muscle ultrastructure.

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MATERIALS & METHODS

LAMBS (24, 6–8 mo old; 40–52 kg live wt) were randomly assigned, 8 for each of 3 treatments: Ctr, Control (lambs slaughtered and dressed according to MSU Meat Laboratory procedures); NCa, infused with 10% of live weight with a tap water solution of 0.1% maltose, 0.21% glycerin, 0.23% dextrose, 0.14% blend of sodium and potassium tripolyphosphate; WCa, infused with same ingredients as NCa adding 0.17% (0.015M) $CaCl_2$. These concentrations were selected based on preliminary trials.

Lambs to be infused were stunned, bled and then transferred to a pelting cradle. One branch of the carotid artery was exposed and clamped. The other branch was partially severed with a sanitized knife. A sanitized nozzle of a brine pump (Koch No. 011227) (Koch Company, Kansas City, MO) was inserted and secured. Solution was pumped (3.2 kg/cm^2) into the artery until 10% by weight of the animal was dispensed. After the completion of infusion, carcasses were dressed and transferred to a blast chill cooler (2°C).

After the carcasses were dressed and before they were transferred to the cooler, pH and temperature were measured (Solomat Modumeter^R; Solomat Corporation, Stanford, CT) in the longissimus thoracis et lumborum (LTL) at 0 hr after the completion of infusion process and at 1, 3, 6, 12 and 24 hr postmortem. A small cut was made on 3 locations in the muscle sample at each sampling period, and the electrode was inserted in a way that proper contact was established between the surfaces of the electrode and the muscle. The surface of the electrode was washed between measurements and left slightly coated with distilled water to avoid dryness. Samples of LTL were also taken at 0 and 24 hr for scanning electron microscopy (SEM), myofibrillar fragmentation index (MFI) measurement and for SDS polyacrylamide gel electrophoresis (SDS-PAGE). Loin chops were prepared by cutting chilled loins on a bandsaw taken at 24 hrs for Warner-Bratzler shear force (WBS) and sarcomere length measurements. LTL used in this section describes the portion of the musculus longissimus that combine longissimus thoracis and longissimus lumborum (Kaufmann et al., 1990).

Loin chops (3, 2.2 cm thick) were taken from each carcass between the 11th rib and 2nd lumbar vertebra for shear force determination. These chops were blast frozen (−28°C), held for ≤ 30 days (−20°C or less) and thawed (2 to 3°C overnight) before cooking. Chops were cooked in convection oven (Honeywell Model 1860.2; Market Forge Co., Everett, MA) at 107°C oven temperature to internal temperature 71°C. Chops were cooled at 2–4°C for 24 hr and 2 cores (1.27 cm diameter) parallel to the fiber direction of the muscle were removed from each chop. Each core was sheared 3 times with a Warner-Bratzler shear device, and average reading per core recorded such that 6 core averages/carcass were obtained (n = 8 [48/6]/treatment).

Sarcomere length was determined by measuring a total of 15 sarcomeres from 10 myofibrils in each loin sample (11th to 13th rib) with a Wild microscope that had a phase contrast setting (Wild Heerbrugg, Switzerland) at a magnification of 400X by use of an eye piece micrometer standardized with a stage micrometer. Samples for sarcomere measurement were prepared as described by Smith et al. (1976).

MFIs were determined on fresh loin muscles (11th to 13th rib) according to the procedure of Olson et al. (1976) as modified by Culler et al. (1978). However, EGTA (Sigma Chemical Company, St. Louis, MO) was used in the isolating medium instead of EDTA and absorbance (540nm) was read with a Spectronic 20 (Milton Roy Company, Rochester, NY) spectrophotometer.

For SDS-PAGE, the isolation of myofibrils was according to Goll et al. (1974). Each sample (0.5 mL) of myofibrils isolated according to the procedure was dissolved in 2 mL of sample buffer (62.5% Tris-HCl, pH 6.8, 2% SDS, 5% MCE, 10% glycerol and 0.2% bromophenol blue). The samples were then heated in boiling water bath for 5 min and stored frozen at −30°C until used. Electrophoresis was conducted on slab gels according to the procedure of Laemmli (1970) as modified by Babiker (1989), except 70 μg of protein were loaded

Table 1—Effect of infusion on tenderness in ovine longissimus muscle

Measure[a]	Treatments[c] Ctr	NCa	WCa	SEM[d]
Shear force (kg)	8.19[e]	5.96[f]	9.22[e]	0.52
MFI[b] (%) (520nm * 200)	64.71[e]	105.08[f]	51.37[e]	16.9
Sarcomere length (μm)	1.82[e]	1.85[e]	1.63[e]	0.07

[a]Values are means of: 48 observations, n = 8 for Shear force; 16 observations, n = 8 for MFI; 120 sarcomeres, n = 8 for Sarcomere length.
[b]MFI = Myofibrillar fragmentation index change over 24 hr period.
[c]Ctr = Control, NCa = No $CaCl_2$, WCa = with CaCl.
[d]SEM = Standard error of measurement.
[e-f]Any two means in the same row with the same superscripts are not significantly different (P< .05), by Bonferoni t-statistics.

to each 2 cm wide lane of the gel. Gels containing 4% (stacking gels) and 12.5% (separating gels) were used for the separation.

Sample preparation for scanning electron microscopy (SEM) was a combination of the procedures described by Klomparens et al. (1986) and Cheng and Parrish (1976). Fresh infraspinatus muscle samples were placed in petri dishes containing 2.5% glutaraldehyde (in 0.1M phosphate, pH 7.2) and diced into cubes 1mm × 1mm × 4mm with a sharp razor and fixed in 2.5% glutaraldehyde for 1-1/2 hr at room temperature. Samples were then washed in buffer (0.1M phosphate, pH 7.2) 2× for 30 min each. Postfixation was carried out in 1% O_sO_4 in 0.1M phosphate buffer at pH 7.2 for 2 hr at room temperature. Samples were then washed and dehydrated in graded (25%, 50%, 75%, 95%, 100%) ethanol for 10 min each. Cryofracture was carried out by freezing in liquid nitrogen and fracturing with a razor blade. The fractured fragments were allowed to thaw in 100% ethanol. The samples were critical point dried in liquid carbon dioxide with a Balzer's critical point dehydrator, and the dried fragments mounted on holders with adhesive mounting tabs and then grounded with a conducting graphite paint. The samples were coated with gold using a sputter coater. Samples were examined and photographed in a JEOL JSM-35C scanning electron microscope using 10kv accelerating voltage, 15mm working distance and 250 condenser lens aperture.

The design employed for collection and analysis of samples for WBS, MFI and sarcomere length measurements was a complete randomized design (Gill, 1978). One way ANOVA table was generated with a Microstat statistical package (Ecosoft, 1984). Comparisons (nonorthogonal) of means was by Bonferoni t-statistics at 95% level of confidence.

RESULTS & DISCUSSION

SHEAR FORCE values (WBS), myofibrillar fragmentation index (MFI) and sarcomere length (SL) of ovine longissimus muscle were compared (Table 1) from the various treatments. Infused samples without $CaCl_2$ (NCa) had lower ($P<0.05$) WBS and a higher ($P<0.05$) percent increase in MFI over 24 hr compared to samples infused with $CaCl_2$ (WCa) and Controls (Ctr). No difference ($P>0.05$) was observed between WCa and Ctr samples. A simple correlation coefficient, (r) of -0.5 ($P<0.05$) was found between WBS and % change in MFI. The greater tenderness (27%) observed in NCa samples compared to Ctr could be attributed to the faster rate of glycolytic changes (Fig. 1a and 1b) in those samples as well as the combined effect of ingredients infused into the carcasses. The importance of accelerating glycolysis for tenderness improvement has been demonstrated (Smulders et al., 1990; Ducastaing et al., 1985). The combination of high temperature and low pH might have favored some of the enzymes responsible for meat tenderization (Dutson, 1983). The added phosphate could have contributed to acceleration of glycolysis, solubilization of actomyosin, chelation of divalent cations and consequently increased tenderness (Lewis et al., 1986; Pearson and Tauber, 1984; Trout and Schmidt, 1983; Hamm, 1977). The other ingredients in addition to phosphate have been used in various combinations for tenderizing (Karmas, 1970). The disruption of muscle cells caused by infusion could lead to improved tenderness as a result of the process alone or in addition to the stimulation of glycolytic and proteolytic enzymes (Stanton and Light, 1990b; Hamm, 1982). As opposed to previous reports (Taylor and Etherington, 1991; Koohmaraie et al., 1988, 1990, 1991; Alarcon-Rojo and Dransfield, 1989), infusion of $CaCl_2$ increased toughness of the muscles by about 35% and 13% compared to NCa and Ctr treated samples, respectively. The lower concentration (0.015M vs 0.3M) of $CaCl_2$ we used (20 × less than that used by Koohmaraie et al. 1989, 1990) could explain the difference observed. The infusion of $CaCl_2$ at concentrations we employed resulted in calcium induced shortening (shorter SL, 12%) of the muscle fibers in WCA treated samples compared to NCa and Ctr (Pearson et al., 1973). The muscle fiber shortening could be the reason for the observed lower tenderness in WCA samples (Jaime et al., 1991; Smulders et al., 1990; Bouton et al., 1973).

The hypothesis that the toughness of WCA samples was mainly because of muscle fiber shortening was supported by the result of the SDS-PAGE (Fig. 2). The appearance of 28, 29 and 30 kd protein components in the gels of the myofibrillar proteins of NCa and WCa at 24 hr postmortem indicated that proteolytic changes occurred faster in those samples than in Ctr. Since the appearance of 30 kd is used as an index of

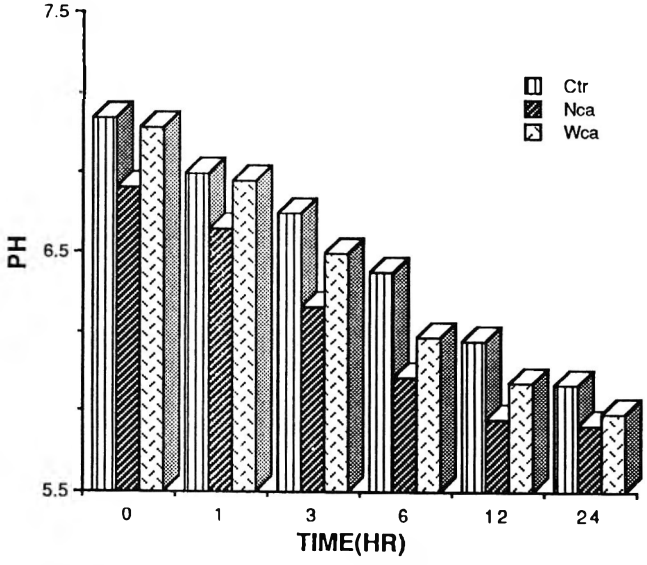

Fig. 1a.—Effect of infusion on ph changes in ovine infraspinatus muscle.

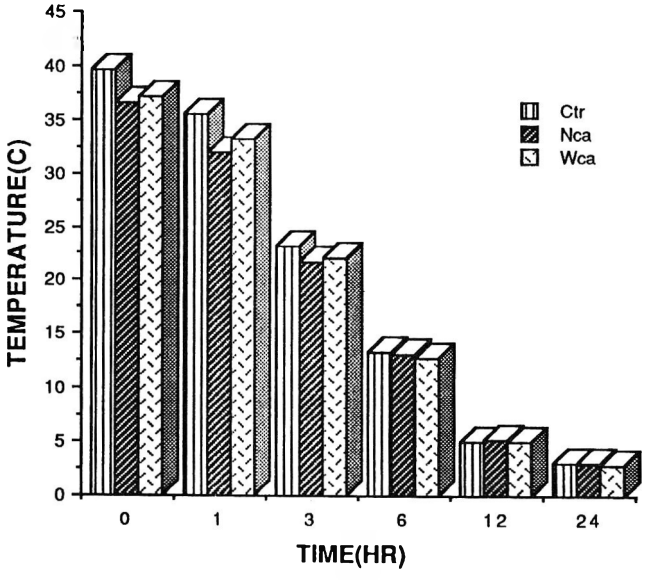

Fig. 1b.—Chilling rate of infused vs control ovine carcasses.

myofibrillar proteolysis and tenderness and it was not affected by shortening (Babiker et al., 1989; Koohmaraie et al., 1984a,b; Yates et al., 1983; McBride and Parrish, 1977; Samejima and Wolfe, 1976), then the toughness of WCa samples could be attributed to shortening of fibers and not to absence of proteolysis, per se. Thus our results demonstrated that it is possible to have a 30 kd protein in the gels of extracts from tough meat. Therefore, based on our results, the appearance of 30 kd may be used as an index of the rate of proteolysis only but not of tenderness.

Other bands observed in the gels of infused samples but not in the controls included the appearance at 24 hr of a 21–22 kd and 24 kd protein component. The appearance of a 24 kd protein component in the longissimus muscle of a lamb aged 7 days was shown by Whipple and Koohmaraie (1992). When gels prepared from 0 hr samples were compared to 24 hr samples several changes were also observed at 24 hr: A protein band appeared with a molecular weight > 66 kd; the intensity of 55–56 kd protein increased; the 34–35 kd and 17 kd protein bands completely disappeared. These observations were made on 12 animals and are shown more clearly in the gels than in the gel photographs. For that reason, asterisks and letters were included on the pictures to indicate areas of interest. Some of these changes could be indicators of early steps in tenderization. Previous studies (Whipple and Koohmaraie, 1992; Whipple et al., 1990; Koohmaraie et al., 1988, 1984b; Cheng and Parrish, 1978) did not show these changes, probably a result of differences in methods of gel preparation or because the quantity of proteins loaded (in relation to width of the lanes

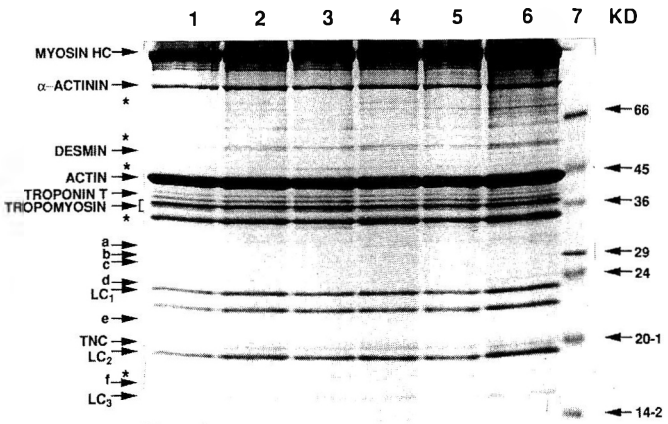

*Fig. 2—Electrophoretic pattern of myofibrillar proteins from infused and control ovine longissimus muscles. Lanes 1, 3 & 5 = Ctr, WCa & NCa samples at 0 hr, respectively. Lanes 2, 4 & 6 = Ctr, WCa & NCa samples at 24 hr, respectively. Lane 7 = Molecular weight markers. a, b, c, d, e & f = 30, 29, 28, 24, 22 & 17 kd bands. * = Bands that changed in appearance or intensities at 24 hr postmortem.*

of the gels) resulted in bands heavy enough to mask other bands. However, with the widely accepted view that the tenderizing process starts early postmortem (Mills et al., 1989; Koohmaraie et al., 1986; Zeece et al., 1986; Marsh, 1983), it

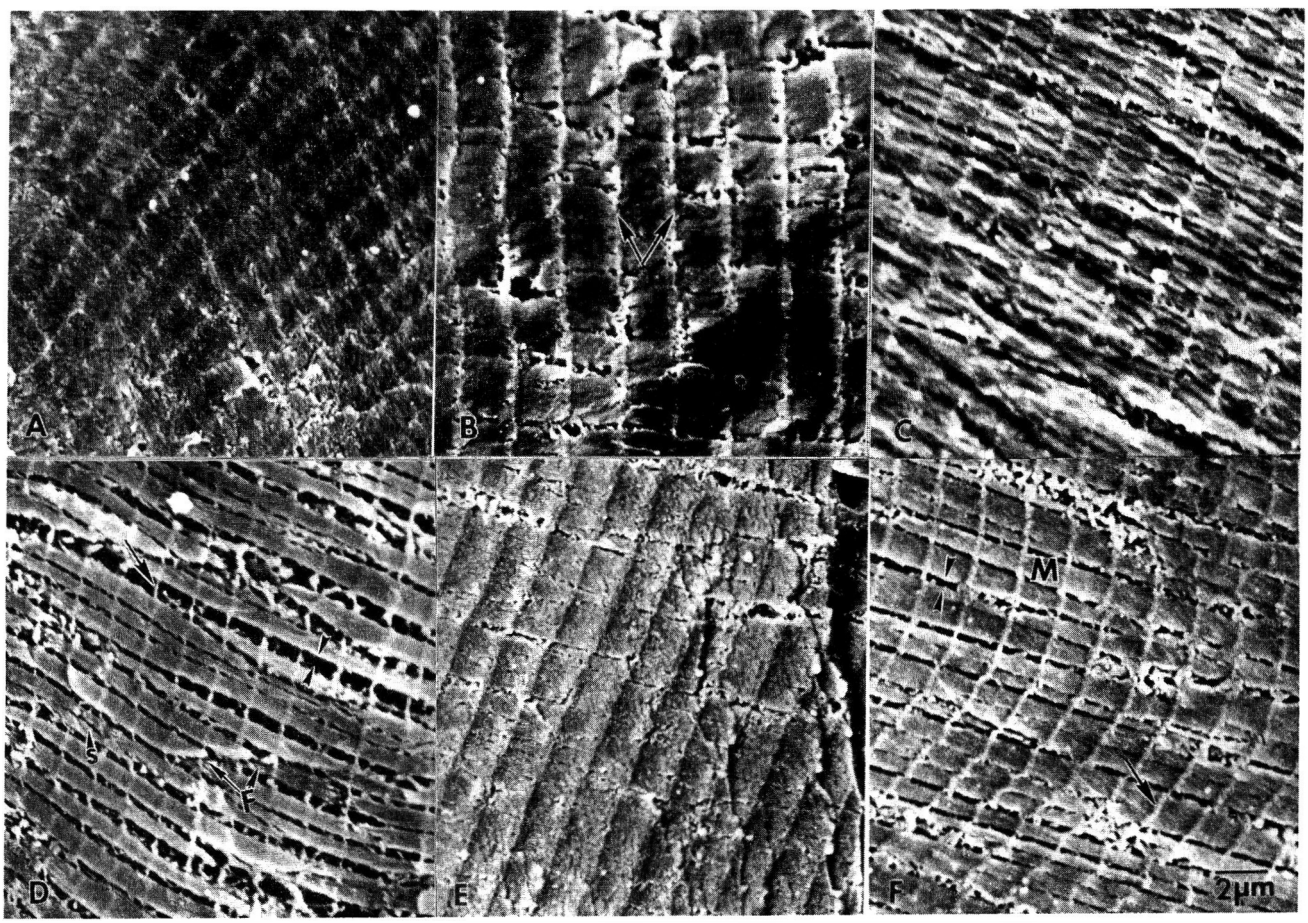

Fig. 3A-F—Scanning electron micrographs of freeze fractured ovine infraspinatus muscle. A & B (Ctr), C & D (NCa) and E & F (WCa) are at 0 and 24 hr postmortem, respectively. Z-line (arrows), interfibrillar spaces (arrow heads), fracture (F), split (S) and myofibril (M). Note the presence of a layer of endomysial-sarcolemmal sheath covering the surface of M in A, C, E. All the micrographs are the same magnification and were printed on the same paper with the same polycontrast filter. The bar at the bottom right represents 2μm.

is essential whenever SDS-PAGE is employed to assure that preparation allows changes taking place within the first 24 hr to be observed.

The SEM micrographs of the infraspinatus muscle (Fig. 3 A-F) are representative examples of several from which our interpretations were drawn. Results indicated that 0 hr samples fractured (Fig. 3A, C and E) along the endomysial-sarcolemmal sheath; whereas 24 hr samples fractured along the surface of myofibrils. Subtle proteolytic changes in the endomysial-sarcolemmal tissues might have weakened the tissue resulting in the differences shown in the fracture planes (Jones et al., 1976; Eino and Stanley, 1973; Schaller and Powrie, 1971). Endomysium is the layer of connective tissue which lies immediately over the sarcolemma (basement membrane and plasmalemma) and the two tissues hardly ever separate under external pressure (Rowe, 1989; Orcutt et al., 1986). The endomysial complex is composed of a mixture of types I, III and V collagen (Bailey and Light, 1989). Recent studies indicated that proteolytic changes (though subtle) take place in the collagen component of the endomysial complex (Stanton and Light, 1990a; Bailey and Light, 1989; Mills et al., 1989). These changes might explain the differences observed in the fracture planes of 0 and 24 hr freeze fractured muscles. When the exposed myofibrillar surfaces were compared, the myofibrils of NCa (Fig. 3D) were more splintered and had wider interfibrillar spaces, compared to WCa (3F) and Ctr (3B). MFI accounted for more than 50% of the variation observed in aged bovine steaks (Culler et al., 1978; Olson and Parrish, 1977). A collagenolytic enzyme bound to skeletal muscle collagen caused splitting of collagen fibrils and presumably disintegration of muscle fibers related to tenderization (Suzuki et al., 1985). Although electron micrographs are difficult to interpret (Lewis et al., 1986; Cheng and Parrish, 1976), these changes suggest that the interfibrillar distance, myofibrillar splitting and fracturing might be one way the process of tenderization takes place in conditioned muscles. Very little fracturing of myofibrils was required to produce marked tenderization in electrically stimulated carcasses (Marsh et al., 1981).

CONCLUSIONS

AT CONCENTRATIONS used, a blend of maltose, dextrose, glycerin and polyphosphates infused into carcasses achieved marked improvement in tenderness within 24 hr postmortem. But infusion of the blend with $CaCl_2$ (at the concentration used) could result in increased toughness. The appearance of, or changes in myofibrillar proteins with molecular weights > 66, 55–56, 34–35, 30, 29, 28, 24, 21–22 and 17 kd's in addition to splitting, fracturing or differences in relative distances between myofibrils, may help explain the process of tenderization.

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Thermal Aggregation Properties of Duck Salt-Soluble Proteins at Selected pH Values

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ABSTRACT

Thermal aggregation properties of duck breast and leg salt-soluble proteins (SSP) were studied at pH 5.50, 5.75 and 6.00. At pH 5.50, a major transition for breast was observed at 60.3°C and for leg at 41.8°C. At pH 5.75, major transitions at 44.6 and 43.2°C were obtained, respectively, for the breast and leg SSP. Three transitions at 46.0, 53.0 and 59.0°C were exhibited by breast SSP at pH 6.00, whereas only two major transitions at 47.4 and 54.0°C were identified in leg SSP. Changes in transition peak heights and shifts in transition temperatures as a result of pH changes indicated that, depending on fiber type, pH may enhance or suppress the aggregation behavior of specific constituents of the myosin/actomyosin complex, thereby altering the overall aggregation pattern of the protein preparation.

Key Words: duck meat, myofibrillar proteins, thermal aggregation, red/white muscle, protein aggregation

INTRODUCTION

SINCE thermally induced protein-protein interaction is important in the manufacture of gelation-based meat products, it is imperative to understand the gelation mechanism and the relationship of gelation properties and muscle type. Ferry (1948) stated that gelation is a two-step process involving the denaturation and subsequent aggregation of polypeptide chains. Salt-soluble proteins (SSP), specifically the myofibrillar proteins, have been shown to be the major functional component in the binding of meat pieces in formed or comminuted meat products (Siegel and Schmidt, 1979).

Transition temperatures of protein systems usually denote events of major conformational changes associated with different protein molecules or with different segments within the same protein molecule. Transition temperatures associated with protein aggregation have been reported for chicken SSP (Xiong and Brekke, 1990), poultry breast and thigh muscles (Acton and Dick, 1986) and bovine natural actomyosin (Ziegler and Acton, 1984). Using light scattering techniques, Acton and Dick (1986) found two distinct thermal transitions for chicken breast actomyosin at 49.2 and 60.2°C, and for leg actomyosin at 52.6 and 57.9°C. The first transition was attributed to the association of the myosin heads of actomyosin, and also to early stages of the transition of myosin tail regions from helix to coil. The second transition was attributed to the aggregation of the myosin rod segments.

Solution pH has been shown to affect thermally induced protein-protein aggregation and transition temperatures (Goodno and Swenson, 1975b; Ziegler and Acton, 1984; Morita et al., 1987; Xiong and Brekke, 1990). Ziegler and Acton (1984), also using light scattering techniques on bovine natural actomyosin, found two distinct transitions at 43.0 and 56.0°C for pH 5.50, and 48.5 and 57.5°C for pH 6.00. The increase in transition temperature with increasing pH was associated with a proton-coupling mechanism. Xiong and Brekke (1990) also observed differences in transition temperatures of chicken breast and leg SSP as affected by solution pH, and associated the dissimilarity to the presence of myosin isoforms present in the different muscle systems (red myosin in leg muscles and white myosin in breast muscles). Red and white muscle fibers obtained from leg and breast muscles, repectively, of chicken and turkey have also been shown to exhibit different aggregation patterns under indentical pH conditions (Acton and Dick, 1986; Foegeding, 1987; Morita et al., 1987; Xiong and Brekke, 1990). Xiong and Brekke (1990) reported differences in the transition temperatures of SSP extracted from chicken postrigor breast and leg muscles.

Limited work has been done on the thermal aggregation behavior of duck breast and leg muscle proteins, both of which are predominantly made up of red fibers. It has not been determined, however, whether anatomical location, or fiber type, is more important in accounting for observed differences in the aggregation behavior of chicken or turkey breast and leg proteins. The objectives of this study were, therefore, (1) to investigate the thermal aggregation behavior of duck breast and leg SSP as affected by pH, and (2) to make comparisons with previous work on chicken breast and leg SSP (Xiong and Brekke, 1990) to determine whether anatomical origin affects aggregation.

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MATERIALS & METHODS

Muscle source

The six ducks used in this study were female domestic mallards, (6 to 7 mo old). They were purchased from a nearby farm and maintained in the Washington State University Experimental Animal Laboratory Building until they were used.

Birds were bled to death and carcasses were immediately skinned and eviscerated. Carcasses were then chilled in crushed ice for 30 min. The unboned breast and legs of each bird were then excised from the carcass and immediately wrapped in paper towels moistened with 1 mM sodium azide to prevent microbial growth, packed in polyethylene bags and stored in ice for 48 hr prior to myofibril preparation. At the end of the storage period, the combined major breast muscles (*pectoralis* and *supracoracoideus*) and all the combined muscles of the leg (thigh and drumstick), from each of the 6 birds, were obtained.

Preparation of "Crude" myofibrillar proteins

Breast and leg muscles were deboned, trimmed of gross connective tissue and fat, and weighed prior to grinding and blending. Crude myofibrils and SSP were prepared according to Xiong and Brekke (1989). After isolating the crude myofibrils, the myofibril pellet was stored in ice and used within 3 days of isolation. The weight of the myofibril pellet was measured, and the protein concentration determined using the biuret method (Gornall et al., 1949) and appropriate protein standards (bovine serum albumin, 10 mg/mL).

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

Discontinous SDS-PAGE (Laemmli, 1970) of duck breast and leg myofibrillar proteins was conducted with a 8–16% linear gradient of acrylamide in the resolving gel (pH 8.8), and 3.8% acrylamide in the stacking gel (pH 6.8). A vertical slab was used to prepare gels 1.5-mm thick. A 20-μL aliquot of protein (1 mg/mL) dissolved in the sample buffer was loaded into each sample slot. A constant current of 50 mA was maintained for 10 hr at 15°C.

Gels were fixed and stained overnight in a 45% (v/v) methanol:9% glacial acetic acid solution containing 0.002 mg/mL Coomassie Brilliant Blue R-250, then destained in a 7.5% glacial acetic acid:25% methanol solution. The protein bands were identified by comparing

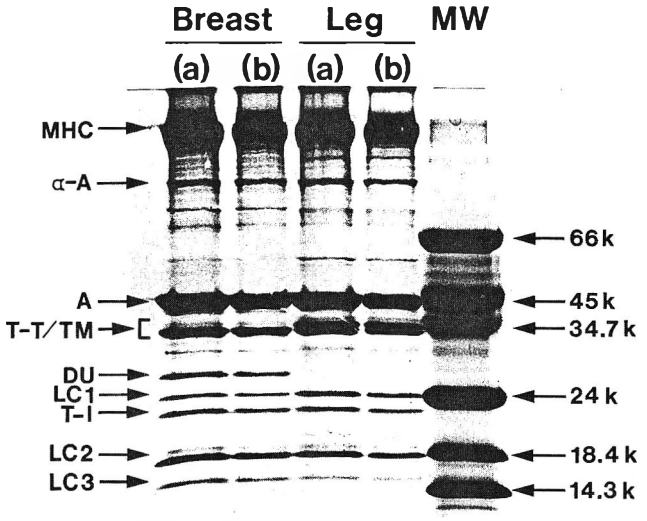

Fig. 1—SDS-polyacrylamide gel electrophoresis of duck breast and leg myofibrillar proteins. MHC=Myosin heavy chain; α—A=Alpha-actinin; A=actin; T-T=Troponin-T; TM=Tropomyosin; DU=30,000 dalton unit; T-I=Troponin-I; LC-1, LC-2 and LC-3 are myosin light chains 1, 2 and 3, respectively. Protein concentration: lane (a)=20μg/mL; lane (b)=10μg/mL.

their relative mobility and order of migration with those of molecular weight standards (MW-SDS-70, Sigma Chemical Co., St. Louis, MO).

Salt-soluble protein extraction and turbidity measurement

A pre-calculated amount of NaCl-solution, containing piperazine-N, N bis (2-ethane sulfonic acid), (PIPES), and sodium azide, was used to suspend the crude myofibrils. The final protein concentration achieved was 5 mg/mL, in 0.6M NaCl, 50 mM PIPES, 1 mM sodium azide, at pH 5.50, 5.75 and 6.00. Solutions were mixed manually with a stirring rod until the protein was fully dispersed, then stored at 4°C for 24 hr prior to subsequent studies.

To measure protein aggregation, aliquots of myofibril suspension containing 5 mg/mL (pH 5.50, 5.75 and 6.00) were placed in 15-mL plastic centrifuge tubes and centrifuged at 5000 x *g* for 15 min at 2°C. The supernatant containing SSP was carefully decanted with a Pasteur pipette, and the protein concentration of the supernatant, determined by the biuret method, was adjusted to 0.3 mg/mL with a 50 mM PIPES buffer (containing 1 mM sodium azide) of corresponding pH.

Thermally induced protein-protein interaction was determined by monitoring the change in absorbance at 320 nm during heating of the SSP preparation at 1°C/min from 20 to 70°C. SSP suspension (3 mL) of 0.3 mg/mL at about 0°C was placed in a quartz cuvette. The cuvette was inserted into a temperature-controlled, water-jacketed sample holder (Lamda 5 UV/VIS spectrophotometer; Perkin-Elmer Corp., Norwalk, CT), and allowed to equilibrate for 5 min at 20°C. Absorbance was recorded every minute. A first derivative plot, taking the change of optical density as a function of a degree change in heating temperature, was calculated from the turbidity curve, and used to determine transition temperatures. These temperatures were defined at points of maximum peak heights detected in the first derivative plot. A correlation equation obtained in a previous study from thermocouple measurements of the sample temperature during heating was used to adjust experimental temperatures. Six different trials, from each of the six birds, on six different protein preparations, were done, representing six replications. In order to avoid premature coagulation that occurred as a result of prolonged storage, only one trial was done on each SSP preparation. Data from the six trials were used to calculate the mean transition temperatures.

Statistical analyses

Replication here refers to the experimental work from different protein preparations done at different times (days) and not to repeated measurements done on the same protein preparations. Transition temperatures reported are mean values obtained from the first derivative curves of the six different replications. Significance of the difference between means was determined by the student's t-test (Steel and Torrie, 1960). Means of turbidity curves were not taken; therefore, the turbidity curves shown were only representative curves of the pH values of 5.50, 5.75 or 6.00. Turbidity curves were not subjected to statistical analysis; we selected typical curves to represent the effects of pH values of 5.50, 5.75 or 6.00.

RESULTS & DISCUSSION

SDS-PAGE

The electrophoretic pattern of the myofibrillar proteins of duck breast differed from that of leg in several respects (Fig. 1). First, there was a doublet protein band in the tropomyosin region of the leg electrophoretogram only. A similar doublet protein band, characteristic of red muscles, has been reported by other researchers. Hay et al. (1973) noticed the presence of a 40,000-dalton component in chicken leg muscle, of higher molecular weight than tropomyosin, which they identified as troponin-B. That component was absent from the breast. Salviati et al. (1982) also observed a doublet band in the tropomyosin region of rabbit red muscles. They identified the high-molecular-weight fragment as beta-tropomyosin and the low-molecular-weight component as an isoform of troponin. Electrophoretic patterns of chicken leg myofibrillar proteins have also shown this doublet (Xiong, 1989). The results of our study, which are consistent with those of previous work on chicken, provide evidence that duck leg muscles are predominantly of the slow type (red), and are different from breast.

Secondly, a prominent band of about 30,000 daltons was present only in the duck breast. A band corresponding to this 30,000-dalton fragment has also been reported in postrigor chicken, turkey and bovine white muscles (Wu and Sayre, 1971; Hay et al., 1973; Foegeding, 1987; Yu and Lee, 1986; Dudziak and Foegeding, 1988). However, other researchers working on postmortem white muscles did not find this component (Acton and Dick, 1986; Xiong, 1989). Wu and Sayre (1971) reported that myosin extracted from postrigor red and white chicken muscles differed in that only the white muscle contained a "T" component of about 30,000 daltons. Hay et al. (1973) also found this 30,000-dalton component in 48-hr aged chicken breast muscle, but not in the leg muscle. However, this component appeared in the leg after 168 hr of aging. Yu and Lee (1986) also observed this 30,000-dalton component from bovine *longissimus* muscles. This component was suggested to originate from proteolytic action (acidic proteases) on the heavy chain of myosin. Olson and Parrish (1977) showed that the 30,000-dalton component in beef muscles resulted from proteolysis of the calcium-activated factor, a neutral protease, on troponin-T. The presence of this 30,000-dalton unit in duck breast after 48 hr postmortem suggested some characteristics, probably enzyme content or enzyme activity, typical of white muscle fibers.

Banding patterns of duck leg (red muscle) proteins observed in our study were identical to those observed in previous work done on chicken leg (red muscle) proteins, while duck breast and chicken breast exhibited differences, especially the presence of the 30,000-dalton component in the duck breast. We could not explain exactly why this 30,000-dalton component was found in duck breast, but apparently not in chicken breast (Xiong, 1989). Differences in enzyme content and activity in the muscle may partly explain the disparity. The electrophorectic results are not conclusive, but allow the possibility that duck leg is predominantly a slow-type red muscle, whereas duck breast is either a "mixed" or intermediate muscle composed of fast-type white and slow-type red fibers, or it is a muscle composed primarily of fast-red muscle fibers. Chicken has been shown to have predominantly white breast muscles and predominantly red (slow-type) leg muscles. The presence of red fibers in duck breast (10–13%) has been documented by Smith et al. (1990) using a more sensitive histochemical fiber-staining technique. Based on our data it was not possible to ascertain the exact relative composition with respect to fiber-types in the different muscles. Further work using histochemical methods would be necessary for that purpose.

Table 1—Transition temperatures (Tm) of duck breast and leg salt-soluble proteins (SSP) obtained from the first derivative curves of turbidity at pH 5.50, 5.75 and 6.00

Transition Peak	pH 5.50		pH 5.75		pH 6.0	
	Breast	Leg	Breast	Leg	Breast	Leg
Tm1	N/D* (46.3)**	$41.8^{a} \pm 0.55^{m}$ (38.1)	$44.6^{b} \pm 0.54$	$43.2^{c} \pm 0.23$	$46.0^{d} \pm 0.97$ (43.7)	$47.4^{e} \pm 0.26$ (43.6)
Tm2	N/D	N/D (49.1)	N/D	$54.9^{f} \pm 0.52$	$53.0^{g} \pm 0.69$ (52.4)	$54.0^{h} \pm 0.57$ (53.2)
Tm3	$60.3^{i} \pm 0.88$ (61.5)	N/D	$60.7^{ij} \pm 1.81$	$60.0^{ij} \pm 1.16$	$59.0^{i} \pm 0.68$ (59.5)	N/D

[a-i] Means (± standard deviation; n=6) with no common superscripts are significantly different (P<0.05).
* N/D = No transition peak detected.
** Comparative values for chicken SSP, from Xiong and Brekke (1990), are presented in parentheses.

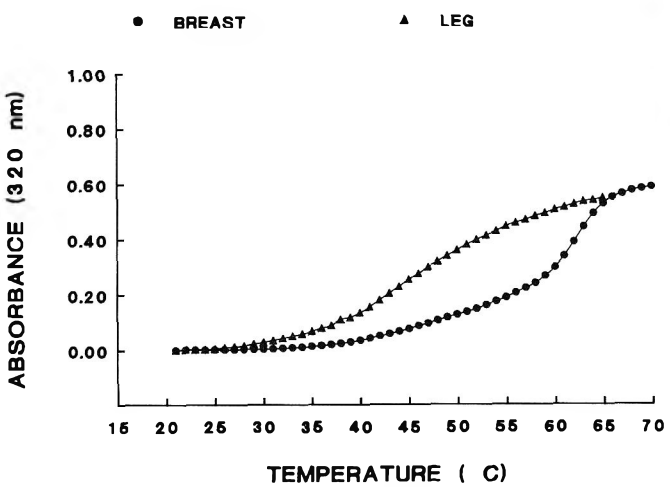

Fig. 2a—Turbidity measurement for isolated salt-soluble proteins (0.3 mg/mL) of duck breast and leg at pH 5.50, 0.6 M NaCl, 50 mM PIPES, and 1 mM sodium azide; heating rate = 1°C/min. ● Breast; ▲ leg.

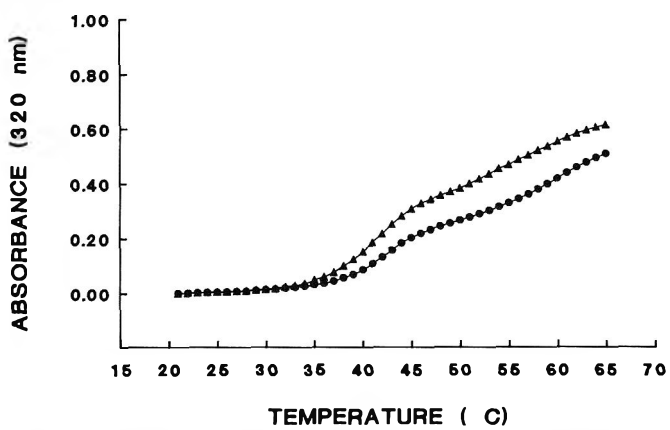

Fig. 2b—Turbidity measurement for salt-soluble proteins (0.3 mg/mL) of duck breast and leg at pH 5.75, 0.6 M NaCl, 50 mM PIPES, and 1 mM sodium azide; Heating rate = 1°C/min. ● Breast; ▲ leg.

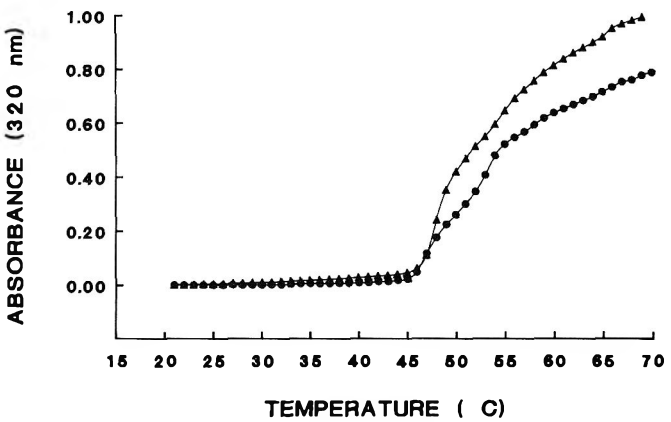

Fig. 2c.—Turbidity measurement of salt-soluble proteins (0.3 mg/mL) of duck breast and leg at pH 6.00, 0.6 M NaCl, 50 mM PIPES, and 1 mM sodium azide; Heating rate = 1°C/min. ● Breast; ▲ leg.

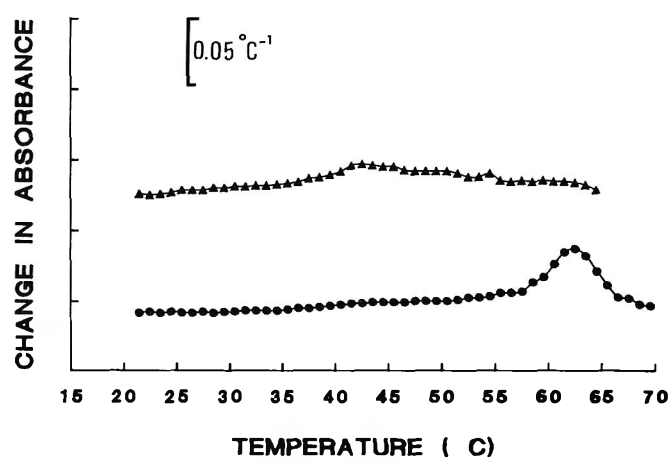

Fig. 3a.—First derivative plot of turbidity for duck breast and leg salt-soluble proteins at pH 5.50. ● Breast; ▲ leg.

Protein-protein interaction

pH effects. Protein-protein interactions upon heating were pH-dependent, with a marked difference in aggregation behavior observed between the leg and the breast at different pH values (Fig. 2a-c). The SSP extracted from duck breast and leg muscles at pH 5.50, 5.75 and 6.00 showed aggregation patterns (Fig. 2a-c) that differed from each other, with the leg proteins generally having a greater turbidity than those of the breast, expecially at temperatures greater than 40°C (Fig. 2a-c). Similar results, indicating greater turbidity in the leg relative to the breast, have also been reported by Samejima et al. (1989). They suggested that different protein-protein interactions occured in duck leg and breast as a function of temperature.

In comparison to chicken breast and leg SSP (Xiong and Brekke, 1990), duck breast was more similar in its pattern of aggregation to duck leg (red) than was chicken breast (white) to chicken leg (red). Duck leg was essentially similar to chicken leg, but the aggregation patterns of duck and chicken breast, though similar at pH 5.50, were different at pH 6.00. That breast showed properties common to red and white muscles was somewhat expected because electrophoretic results showed duck breast to exhibit properties of white muscles, while histochemical techniques have

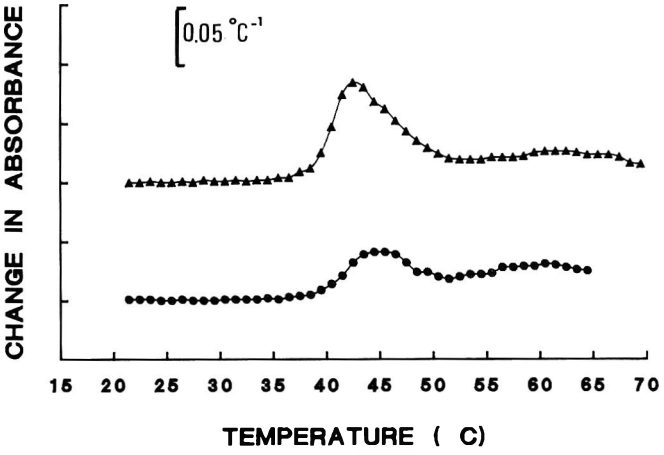

Fig. 3b.—First derivative plot of turbidity for duck breast and leg salt-soluble proteins at pH 5.75. • Breast; ▲ leg.

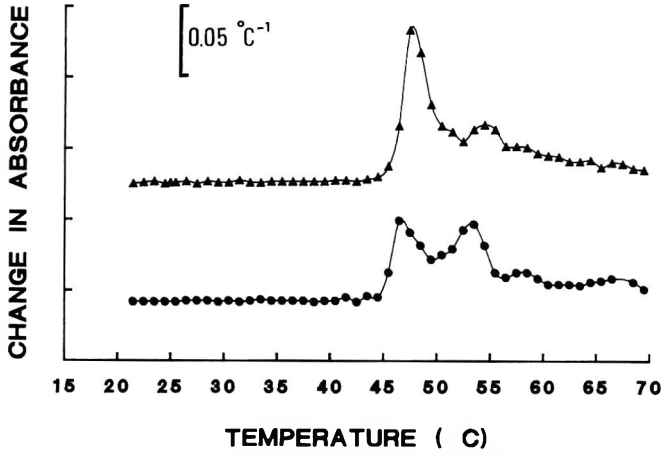

Fig. 3c—First derivative plot of turbidity for duck breast and leg salt-soluble proteins at pH 6.00. • Breast; ▲ leg.

shown it to contain red muscle fibers as well (Smith et al., 1990). These results were consistent with the hypothesis that anatomical location of the muscle is less important than the preponderance of fiber-type regarding subsequent aggregation of the proteins from the respective muscles.

Transition temperatures. Transition temperatures (Tm) were derived from the first derivative plots (Fig. 3a-c). In general, changes in pH affected the Tm, and this effect was different for breast and leg (Table 1). An increase in pH has been shown to have an effect by shifting the first and second transitions to higher temperatures (Goodno and Swenson, 1975a,b; Wright et al., 1977; Stabursvik and Martens, 1980; Wright and Wilding, 1984; Xiong and Brekke, 1990). These observations indicate that electrostatic interactions are involved in the aggregation process.

Duck breast SSP showed one, two, or three major transitions, depending on the pH (Fig. 3a-c, Table 1). At pH 5.50, the breast showed only one major transition at 60.3°C (Fig. 3a), whereas at pH 5.75 a major transition at 44.6°C and a minor transition at 60.7°C were observed. When the pH was increased to 6.00, 3 majors transitions were observed (46.0, 53.0 and 59.0°C), with the first two peaks of almost equal height and greater than the third (Fig. 3c). The peak height reflects the maximum rate of turbidity (protein-protein interaction) that was attained at a given transition. The difference in peak heights suggests that different segments of the myosin molecule affected the aggregation process (ease of aggregation) differently depending on pH, since transition peaks represent major conformational changes that occur within specific segments of the myosin/actomyosin molecule.

Duck leg SSP also showed differences in transition temperatures with changes in pH. At pH 5.50, only one major transition was observed at 41.8°C (Fig. 3a), while at pH 5.75 one major transition was identified at 43.2°C with two minor transitions at 54.9 and 60.0°C (Fig. 3b). At pH 6.00, the leg had two major transitions at 47.4 and 54.0°C, with a minor shoulder at about 60.0°C (Fig. 3c).

The number of transitions at pH 6.00 (Table 1) was similar to that reported by Xiong and Brekke (1990), who reported three transitions for chicken breast-SSP at 43.7, 52.4 and 59.5°C and two transitions for chicken leg-SSP at 43.6 and 53.2°C (pH 6.0, 0.6 M NaCl). At pH 5.50, Xiong and Brekke (1990) obtained one major transition for chicken breast-SSP at 61.5°C and a minor transition at 49.1°C. The number of transition peaks for duck breast and leg obtained at pH 5.50 was similar to that obtained for chicken at pH 5.50, i.e., both breast and leg proteins exhibited only one major transition each. At pH 6.00, the number of transition peaks was also similar. Slight variations, however, which do exist in transition temperatures—for chicken, usually lower—and peak heights may be a result of species differences in postmortem biochemical changes.

Acton and Dick (1986) observed two transitions each for chicken thigh and breast actomyosin at pH 6.00; first and second transitions were observed at 49.2 and 60.2°C for chicken breast actomyosin and at 52.6 and 57.9°C for thigh. They appararently did not observe a third transition which was reported for the SSP of both chicken breast (Xiong and Brekke, 1990) and duck breast. The discrepancy between these results may be due to the different protein preparations used.

The height of the peaks on the derivative curves varied with pH. In breast, the height of the first transition was reduced as pH was reduced from 6.00 to 5.75, and almost disappeared at pH 5.50. However, the height of the third transition peak, which was relatively low at pH 6.00 and 5.75, became the major transition at pH 5.50 (Fig. 3a-c). At pH 5.75, the second transition for breast almost disappeared as compared to that at pH 6.00. In the leg, almost the same trend for the height of the first transition was observed as with breast, except that the first transition of the leg became dominant at pH 5.50. The reduction of peak height of the second transition observed for breast was also true for leg. Therefore, the results suggest that the relative contribution of various segments of the myosin/actomyosin molecule differs with pH, because pH affects differently the maximum degree of protein-protein interaction (transition peak heights) that can occur at any particular transition.

To summarize these findings, the aggregation pattern of duck breast was similar to that of duck leg at pH 6.00. As the pH was reduced to 5.50, the differences became larger as the aggregation pattern of the duck breast became more similar to that of chicken breast than to duck leg. This finding provides further evidence that, although the duck breast is known to have red fibers (Smith et al., 1990), it also has characteristics of white fibers which may become dominant as the pH is reduced to 5.50. Smith et al. (1991) reported in their study that the pH of duck *pectoralis* muscle decreased from 6.25 to 5.66 (0.25 to 24 hr postmortem). The final pH value of 5.66 is close to 5.50, suggesting that differences in the aggregation properties of duck breast muscles may also be a function of postmortem metabolism correlated with time.

Thermal transition temperatures of crude myofibrillar protein solutions or SSP preparations relate to the conformational changes occurring in the major proteins involved: actomyosin, actin and myosin. Wright et al. (1977) reported, using differential scanning calorimetry (DSC), that myosin displayed either one, two, or three transitions, depending on pH and ionic strength. For the myosin molecule in particular, different regions of the molecule (head, tail and hinge) differ in thermal stability, and thus have different transition temperatures. Using

light scattering techniques, Acton and Dick (1986) found two distinct thermal transitions for chicken breast and thigh actomyosin. The Tm's obtained were 49.2 and 60.2°C for breast and 52.6 and 57.9°C for leg (pH 6.00, 0.6M NaCl). The first transition (Tm1) was attributed to the association of the myosin heads of actomyosin and from the early stage of helix to coil transition of the myosin tail region.

The second transition (Tm2) was attributed to the aggregation of the myosin rod segments. Ziegler and Acton (1984), using light scattering techniques, found two distinct thermal transitions in bovine natural actomyosin: 43.0 and 56.0°C at pH 5.5, and 48.5 and 57.5°C at pH 6.0 (0.6M NaCl). The decrease in Tm with decreasing pH was attributed to a proton-coupling mechanism which required less energy to produce conformational transitions with increasing H^+ concentration. These observations on transition temperatures and peak height variations suggested that the aggregation behavior of different segments of the myosin/actomyosin molecule may differ depending on pH and muscle type. Probably, aggregation of one segment of the myosin/actomyosin molecule affects either the participation of other segments of the molecule or the aggregation pattern of the actomyosin complex, thus affecting the aggregation pattern of the entire extract.

The scope of our work did not allow the conclusive assignment of peaks to specific proteins or protein fragments. However, based on previous work on myosin, actomyosin and whole muscle protein transitions (Samejima et al., 1983; Wright and Wilding, 1984), the first and third transitions can reasonably be attributed to aggregation of the myosin tail, and the second to the myosin head region, in agreement with Samejima et al. (1983) and Wright and Wilding (1984). The transition assigned to actin occurs at higher temperatures (about 70°C and above), and thus was not observed.

Heat treatment of myosin results in dissociation of the light chains from the intact myosin core (Gershman et al., 1969), thus uncovering sites on the heavy chains that participate in aggregation. The charge distribution of the myosin molecule and subsequent release of light chains may well be altered by pH (Gershman et al., 1969; Weeds and Lowey, 1971). Because of differences in the light chain composition of white vs red myosins, these pH effects would be different for breast and leg, hence leading to different aggregation patterns.

An interesting observation was the formation of a fibrous web-like structure during aggregation of duck breast SSP at pH 6.00, whereas leg SSP formed a uniform non-structural cloudy solution on heating. This web-like structure was apparently formed after the first transition, because its presence was not detected below 50°C. Since the second and third transitions occured above 50°C, aggregation of either the head or hinge region, corresponding to the second and third transitions, respectively, aggregation was likely to contribute to the formation of this structure. As opposed to the cloudy solution formed by the leg-SSP, the web-like structure formed by the breast suggested more structured protein-protein interaction rather than protein-solvent interaction. The exact reason why this fibrous structure was present only in breast suspensions, and only at pH 6.00, is not known.

CONCLUSIONS

THE EFFECT of pH was different in the aggregation behavior of duck leg and breast proteins. In comparison to previous work on chicken, duck breast proteins exhibited a dual physicochemical behavior typical of both chicken leg (red fibers) and chicken breast (white fibers). Duck leg proteins, however, had properties typical of chicken leg, but not chicken breast. This information provides evidence that fiber type of the muscle is primarily responsible for differences in aggregation properties between muscles of different anatomical origin and function. The pattern of aggregation differed for duck breast and leg proteins, but differences were smaller than those reported for chicken breast and leg proteins. These differences between breast and leg muscles could have been due to differences in light chain composition which likely would affect aggregation patterns of their respective light meromyosin (LMM).

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Metallic Cations Affect Functional Performance of Spray-Dried Heat-Treated Egg White

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ABSTRACT

The functional performance of four metallic cations (Cu^{++}, Al^{+++}, Fe^{+++}, Zn^{++}) in spray-dried, heat-treated egg white were evaluated. Cu^{++} excelled in all functional categories involving foams and angel food cakes. Zn^{++} was least effective. These data suggest that Cu^{++} would be a suitable additive to spray-dried egg white systems.

Key Words: egg-white, albumin, copper, trace metals, minerals

INTRODUCTION

LIQUID EGG WHITE contains about 12% conalbumin which reacts with many polyvalent cations including aluminum, copper, iron and zinc. To a lesser extent, other proteins may be involved. These ions may affect egg white functional performance in foams by altering whip-time, volume, firmness and/or stability (MacDonnell et al., 1955; Nakamura and Sato, 1964; Cunningham and Lineweaver, 1965; Baldwin, 1986; Powrie and Nakai, 1986). Cunningham and Lineweaver (1965) added Al to liquid egg white to stabilize conalbumin during pasteurization. This complex disassociates above pH 8, hence, lactic acid was added to lower the pH to 6.5-7.0. The heat stability of the metal ion complexes with conalbumin increased in the following order: Zn^{++}, Cu^{++}, Al^{+++}, Fe^{+++} (Donovan et al., 1975).

The copper bowl has been preferred for centuries in the culinary art of making good egg white foams (McGee et al., 1984). A likely hypothesis explaining this effect is that Cu is absorbed from the bowl during whipping. Yet, the effects of this ion and others on functional performance have received little attention.

All of the previous research on metallic cations has involved either liquid egg white systems or protein isolates prepared therefrom. The role of these ions on the foaming properties of fermented (glucose-free) spray-dried egg white before and after heat treatment has not been reported. Another objective of this research was to quantify these effects by varying the concentration of each cation.

MATERIALS & METHODS

Egg white source

Commercial liquid egg white containing <0.015% yolk was obtained from Kraft, Inc., Marshall, MO, and held frozen in 1.8-L polyethylene tubs at 15°C. As needed, it was thawed overnight at 4°C, warmed to room temperature (≈25°C) and reblended without air incorporation. The pH was adjusted to 7.0 with 10% citric acid.

Fermentation

Sugar removal was accomplished with a culture of *Klebsiella oxytoca* B317 serially adapted in aseptically prepared liquid egg white. After fermentation at 25–30°C for 8–12 hr, or until negative according to a glucose test paper strip, the pH was adjusted to 6.5 with citric acid.

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Addition of cations

Various amounts of 0.01M $ZnCl_2$, $CuCl_2$, $FeCl_3 \cdot 6H_2O$, and $AlCl_3 \cdot 6H_2O$ solutions were added to the fermented liquid egg white to give 0.0, 0.05, 0.10, 0.50, and 1.00 mM concentrations. On a metal-conalbumin basis, those complex saturations would be about 0, 15, 150, and 300%, respectively.

Spray-drying

All fermented egg white samples were dehydrated in a pilot plant spray-dryer equipped with an internal mixing pneumatic nozzle (Spraying Systems Co., fluid # 2850, air # 67-2-20-70). It was operated at an air pressure of 40 psi and liquid egg white at 20 psi. A positive displacement gear pump (Zenith Products Company, West Newton, MA) was used to meter the liquid flow. The heat source was from a direct-fired natural gas burner supplied with a forced draft air-gas mixer. The samples were placed in polyethylene bags.

Heat treatment and storage

The samples were divided into equal portions and placed in polyethylene bags. One portion was heat-treated at 54°C for 10 days, then both portions were stored at room temperature. In this report, unheated spray-dried egg white is identified as USDEW and heated samples as HSDEW.

Foaming properties

Distilled water (90mL) and 10.8g SDEW were placed in a calibrated bowl of a Hobart Model K-4-B mixer and whipped at speed 10 for 90 sec. Volume was determined by measuring foam depth. Firmness was observed after transferring the foam to a 9.5 cm depth and 10.2 cm diameter SS back extrusion cell with care to avoid compression or air pockets. A Model 1132 Instron Universal Testing Machine (Instron Corp., Canton, MA) equipped with a 9.3-cm-diameter compression probe attached to a 2-kg load cell was used to determine firmness. Stability was evaluated by noting the volume of liquid drainage from the foam after holding in a 1.8-L covered container at room temperature for 30 min.

Angel food cake volume

Angel food cakes were prepared from the following formulations and procedures:

Mix A: 10.8g SDEW, 16.2g superfine sugar, 0.45g salt, 0.3g cream of tartar.

Mix B: 75.8g superfine sugar, 33.0g cake flour.

Both mixes were sifted separately to provide good mixing of ingredients. Distilled water (90 mL) was added to the mixing bowl. Mix A was sifted again over the water and stirred at speed 2 for 15 sec to wet the powder (K-4-B Hobart Mixer) followed by whipping at speed 10 until a slightly folded medium peak was formed. Mix B was sifted over the foam in three portions, then folded into the foam after each addition with a rubber spatula (ca 20 strokes). Batter (100g) was placed in each of two rectangular pans and baked in a preheated oven for 25 min at 200°C. After baking, the pans were cooled on a wire rack and the volume measured by the rapeseed displacement method. The average volume of the two cakes was considered a single observation.

Table 1—Foam volume from U- and H-SDEW with various concentrations of four added cations

Added cations (mM)	Foam volume (mL)							
	U-SDEW				H-SDEW			
	Zn^{++}	Cu^{++}	Fe^{+++}	Al^{+++}	Zn^{++}	Cu^{++}	Fe^{+++}	Al^{+++}
0.00	410[a]	410[d]	410[c]	410[b]	727[ab]	727[c]	727[c]	727[a]
0.05	385[a]	487[cd]	567[b]	657[a]	700[b]	943[b]	952[a]	753[a]
0.10	389[a]	583[c]	580[b]	673[a]	743[ab]	1020[ab]	880[abc]	770[a]
0.50	380[a]	753[b]	640[a]	683[a]	797[a]	1110[a]	842[abc]	780[a]
1.00	396[a]	942[a]	710[a]	710[a]	790[a]	1110[a]	780[bc]	807[a]

[a-d] Means (n = 3) with the same superscript letters are not significantly different at $p < 0.05$.

Table 2—Foam firmness from U- and H-SDEW within various concentrations of four added cations

Added cations (mM)	Foam firmness (g)							
	U-SDEW				H-SDEW			
	Zn^{++}	Cu^{++}	Fe^{+++}	Al^{+++}	Zn^{++}	Cu^{++}	Fe^{+++}	Al^{+++}
0.00	657[a]	657[c]	657[c]	657[c]	1213[a]	1213[c]	1213[a]	1213[b]
0.05	652[a]	939[a]	1163[b]	1472[a]	1208[a]	1779[b]	1567[a]	1542[a]
0.10	597[ab]	1243[bc]	1217[ab]	1487[a]	1193[a]	1832[ab]	1396[a]	1550[a]
0.50	473[c]	1837[ab]	1366[ab]	1427[a]	1355[a]	1871[ab]	1508[a]	1550[a]
1.00	540[b]	2118[a]	1407[a]	1482[a]	1370[a]	2075[a]	1527[a]	1544[a]

[a-c] Means (n = 3) with the same superscript letters are not significantly different at $P < 0.05$.

Table 3—Foam stability from U- and H-SDEW with various concentrations of four added cations

Added cations (mM)	Foam stability (mL)							
	U-SDEW				H-SDEW			
	Zn^{++}	Cu^{++}	Fe^{+++}	Al^{+++}	Zn^{++}	Cu^{++}	Fe^{+++}	Al^{+++}
0.00	47[a]	47[a]	47[a]	47[a]	20[a]	20[a]	20[a]	20[a]
0.05	40[ab]	41[ab]	42[ab]	34[b]	16[ab]	9[b]	10[b]	16[a]
0.10	36[bc]	35[b]	42[ab]	35[b]	11[abc]	2[c]	10[b]	16[a]
0.50	28[c]	23[c]	39[b]	34[b]	8[bc]	0[c]	11[ab]	20[a]
1.00	30[c]	17[c]	36[b]	30[b]	7[c]	0[c]	12[ab]	19[a]

[a-c] Means (n = 3) with the same superscript letters are not significantly different at $p < 0.05$.

Table 4—Angel food cake volume from U- and H-SDEW with various concentrations of four added cations

Added cations (mM)	Cake volume (mL)							
	U-SDEW				H-SDEW			
	Zn^{++}	Cu^{++}	Fe^{+++}	Al^{+++}	Zn^{++}	Cu^{++}	Fe^{+++}	Al^{+++}
0.00	320[a]	320[b]	320[b]	320[a]	313[a]	313[c]	313[c]	313[b]
0.05	337[a]	343[ab]	314[b]	335[a]	326[a]	336[abc]	324[bc]	333[ab]
0.10	345[a]	358[ab]	346[a]	334[a]	337[a]	352[ab]	347[a]	338[a]
0.50	331[a]	354[ab]	351[a]	322[a]	329[a]	320[bc]	337[ab]	327[ab]
1.00	329[a]	367[a]	347[a]	319[a]	320[a]	356[a]	333[b]	333[ab]

[a-c] Means (n = 3) with the same superscript letters are not significantly different at $p < 0.05$.

Table 5—Means for functional performance of U- and H-SDEW for all four added cations

Cation	Foam			Cake
	volume (mL)	stiffness (g)	drainage (mL)	volume (mL)
Control	568[c]	935[c]	33.2[a]	316[c]
Zn^{++}	573[c]	924[c]	22.1[bc]	332[b]
Cu^{++}	869[a]	1720[a]	15.8[c]	348[a]
Fe^{+++}	738[b]	1393[b]	25.4[ab]	337[b]
Al^{+++}	729[b]	1507[b]	25.4[ab]	330[b]

[a-c] Means (n = 24) with the same superscript letters are not significantly different at $p < 0.05$.

Statistical analysis

Analysis of Variance and Duncan's Multiple Range Test were used to determine significant differences between means in columns as indicated by different superscripts (Snedecor and Cochran, 1980). USDES and HSDEW data were treated separately.

RESULTS & DISCUSSION

Foam volume firmness and stability

Since three sub- and two supersaturated levels were tested, cation concentrations were not as effective as expected (Table 1). Yet, some significant differences occurred from both the U- and H-SDEW samples. Cu^{++} was the most effective. The generally higher foam volumes from H-SDEW may partially be explained by the protective effect of metallic cations on the heat denaturation of conalbumin. The addition of Cu^{++} at various levels consistently increased foam firmness (Table 2). Concentrations of the other cations were less effective but some significant differences occurred. The amount of Zn^{++} was least important in both systems.

The most dramatic effect on foam stability was caused by Cu^{++} in H-SDEW. At the two supersaturated ionic levels zero drainage occurred (Table 3). Also, this was the only system where Zn^{++} showed any consistent improvement as a function of cation concentration. Here again, heating the SDEW generally improved foam stability as it did with foam volume (Table 1). Note, all samples in the foam trials were whipped for one specified speed and time. Some of the results may have differed had a subjective condition been used, as with the angel food cake test.

Angel food cake volume

Angel food cake is a good indicator of both foaming (leavening) and coagulative properties of egg white. Varying the whip-time to form a medium peak may have reduced some differences reported above (Table 4). Cu^{++} and Fe^{++} addition indicated some effect of cation concentration. Heating did not improve performance of SDEW in angel food cakes.

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Mineral Cations Affect Microstructure of Egg Yolk Granules

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ABSTRACT

Granule dialysis caused partial release of calcium and breakage of the granular structure. In the presence of sodium chloride, sodium replaced calcium bound to the proteins and destabilized the structure of granules. By contrast, iron binding was not modified by the presence of sodium. EDTA bound all the cations, broke the phosphocalcic bridges and destabilized the structure. On the other hand, the addition of bivalent or trivalent cations at low concentration strengthened the structure which resisted changes in pH or ionic strength. All data showed the importance of ionic bridges (especially phosphocalcic bridges) in the association of LDL, lipovitellin and phosvitin in the form of spherical structures.

Key Words: Egg-yolk, minerals, cations, microstructure

INTRODUCTION

THE GRANULES are major components of yolk. They contain about 70% lipovitellins, 16% phosvitins and 12% low-density lipoproteins (Burley and Cook, 1961) and their size ranges from 0.2 to 2μm (Bellairs, 1961; Chang et al., 1977). Radomski and Cook (1964) suggested that a granular unit consists of a lipovitellin-phosvitin complex and lipoprotein. But this granular structure is dissociated under high saline conditions (1.71M NaCl) (Burley and Cook, 1961). The high concentration of bivalent (Ca^{++}, Mg^{++}) and trivalent (Fe^{+++}) cations in egg yolk, and the presence of phosphoproteins (mainly phosvitin), suggests the presence of ionic bridges between the phosphate groups of the phosphoseryl residues of the phosphoproteins. We have previously shown the importance of the modification of pH, ionic strength and nature of mineral cations on some physicochemical properties of the yolk, i.e., solubility and viscosity (Causeret et al., 1991). To gain further insight into the granular structure we further studied the role of the mineral cations.

Egg yolk contains about 1.1% mineral elements. The major element is phosphorus which is mostly found in the proteins and lipids. Calcium and potassium are the main cations (Table 1), but calcium, magnesium and iron all seem to be important in the microstructure of the granules. Work carried out with casein (Dickson and Perkins, 1971; Mesnier et al., 1989) and phosvitin (Allerton and Perlmann, 1965) showed that calcium and magnesium bind to the same sites, with the same affinity constant. Sites of high affinity are on the phosphoseryl groups of the proteins. Our objective was to determine the effect of calcium which is 17 times more concentrated than magnesium in the granules. We also studied iron which would probably combine differently which justified studying it, in spite of its low concentration.

MATERIALS & METHODS

Preparation of granules and pH adjustment

Eggs of Warren Isa hens, less than 48hr old were used. The yolks were separated from the albumen and rolled on absorbing-paper to remove adhering albumen and chalazas. The vitellin membrane was broken and the vitellus was immediately collected. Yolk was diluted with an equal volume of aqueous NaCl solution (1% w/v) and the resulting mixture was centrifuged at 10,000 x *g* for 45 min, according to the method of McBee and Cotterill (1979). The sedimented granules were washed with deionized water and centrifuged once more. The pH of each suspension was adjusted by adding 1M HCl or NaOH with continuous stirring.

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Analytical Methods

The protein content was determined by the Kjeldahl method. The contents of calcium and iron of yolk were obtained by atomic absorption spectrophotometry. For calcium, a solution of lanthanum was used as a spectrophotometric buffer (Pinta, 1979). The dilutions were prepared with deionized water.

Polyacrylamide gel electrophoresis

Proteins were separated by polyacrylamide gel electrophoresis in a vertical slab unit according to the method of Maurer (1971). In order to allow the migration of proteins in the polyacrylamide gel, it was necessary to dissociate the granules 0.6M aqueous NaCl solution (for a solution of granules at 2% proteins). Electrophoretic conditions were: stacking gel -4% polyacrylamide (T = 3.85%, C = 2.6%); running gel -7.5% polyacrylamide; electrophoresis buffer - Tris 0.05 M/Gly 0.04 M, pH 8.3. Bromophenol blue was the tracking dye used to monitor progress of the protein separation. The gels were fixed in a solution of trichloroacetic acid (12%) for 1 hr. The staining solution was 0.1% Coomassie blue in a solution of 0.1M aluminum nitrate/25% isopropanol/10% acetic acid/1% Triton X-100, prepared by the method of Hegenauer et al. (1977). The destaining solution was acetic acid/methanol/water (70:300:630, v/v/v).

Scanning electron microscopy

The microstructure of the yolk granules was observed in a Jeol-JSM 35 scanning electron microscope, with cryo-unit. All samples were frozen and fractured in liquid nitrogen, then immediately transferred into the vacuum space of the cryo-unit. The accelerating voltage was not allowed to exceed 8 kV to prevent local overheating of the sample.

Solubility index of protein

Granular protein suspension (200 mL) was centrifuged for 20 min at 4,000 x *g*, and the protein concentration of the resulting supernatant fluid was determined by the Kjeldahl method. The solubility index (SI) of protein was expressed as:

$$SI\ (\%) = \frac{\text{protein conc of supernatant fluid (mg/L)}}{\text{initial protein conc (mg/L)}} \times 100$$

Equilibrium dialysis

Protein dispersion (1 mL of 2% w/w) was placed in dialysis tubing (Visking cellulose tubing, 8/32 in) then dialyzed against 10 mL deionized water, at 4°C for 2 hr, with continuous stirring. The measure of mineral elements in the two-compartment dialysis allowed the quantity of mineral elements bound by the protein to be determined.

RESULTS & DISCUSSION

Effects of minerals on dissociation of granules

Addition of sodium chloride to a solution of granules brought about solubilization of the granular proteins, which implied the

Table 1—Mineral content of egg yolk (mg/100g of yolk).

	P	S	Cl	Ca	K	Na	Mg	Fe	Zn
Staron (1980)	500	170	140	130	120	50	15	6.1	3.6
Stadelman and Cotterill (1986)	599	165	176	148	100	52	13	6	3.8

Table 2—Release of minerals of granules during dialysis. Average of three replications.

	Released cations (%)		
Released	Dialysis of granules	Dialysis of granules + NaCl (0.58M)	Dialysis of granules + EDTA (0.025M)
Calcium (%)	17.4	35.4	99.6
Iron (%)	0.0	0.0	94.0

Table 3—Solubility of granular proteins after addition of mineral (calcium 36.6 mM, iron 46.6 mM, sodium chloride 0.58M) or EDTA (0.025M). Protein concentration = 2%. Average of three replications.

Granules	Solubility index (%)
alone; pH = 6.4	3.4 ± 0.1
+ Calcium; pH = 6.4	10.4 ± 0.6
+ Calcium; pH = 9	11.6 ± 0.1
+ Iron; pH = 6.4	7.9 ± 0.1
+ Iron; pH = 3	18.4 ± 0.2
+ Iron; pH = 9	2.9 ± 0.1
+ Iron + NaCl; pH = 6.4	8.9 ± 0.1
+ EDTA; pH = 6.4	20.8 ± 0.1

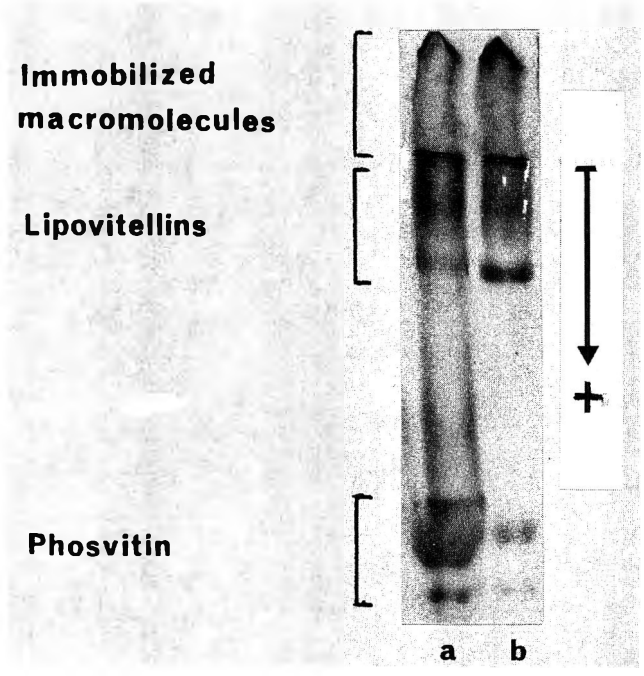

Fig. 1—Polyacrylamide gel electrophoretic patterns of (a) soluble and (b) insoluble granular fractions in the presence of EDTA (0.025M) (protein concentration = 2%).

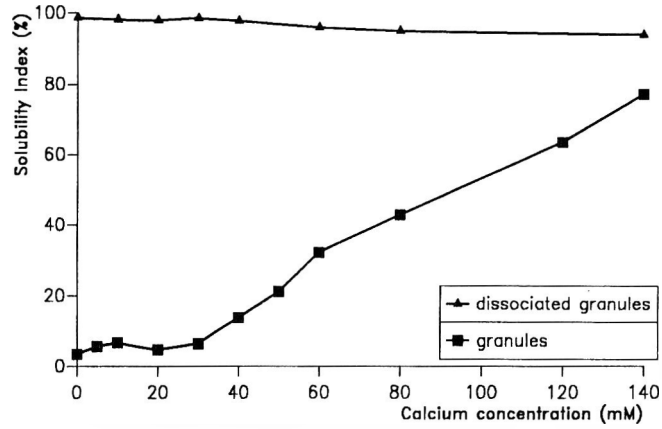

Fig. 2—Solubility of granular proteins as a function of calcium concentration (protein concentration = 2%). (Averages of three replicates).

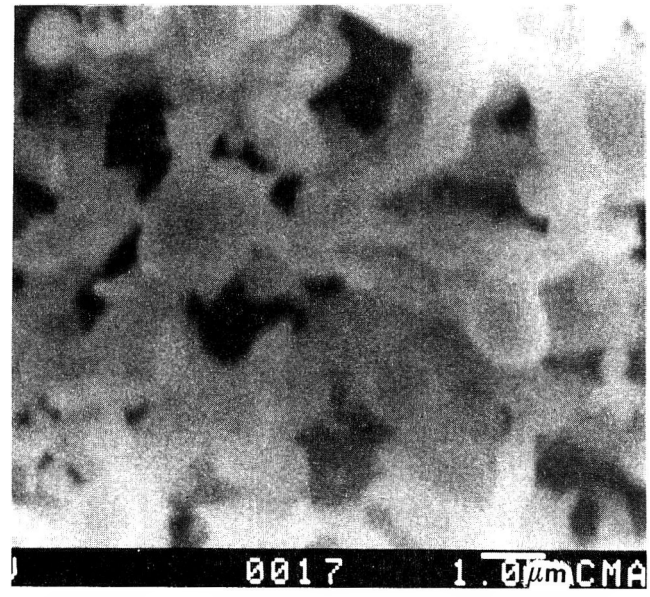

Fig. 3—Electron micrographs of granular fractions in the presence of calcium at pH 9 (protein concentration = 2%).

whole granule was destroyed (Causeret et al., 1991). After 18 hr dialysis of a solution of the granules vp water, 17.4% of the total granular calcium was found in the external compartment (Table 2). Some of the calcium was therefore in a free form, or linked only weakly to the protein.

Where sodium chloride was added to the granules, 35.4% of the calcium crossed the membrane during dialysis (Table 2). NaCl thus had the effect of liberating 35.4 − 17.4 = 18% of the bound calcium. Bivalent calcium was in fact replaced by monovalent sodium at much higher concentrations. As a consequence, the phosphocalcic bridges were broken, and this caused destruction of the granules.

In contrast to calcium, iron remained totally in the protein, with or without the presence of NaCl. This was explained by the fact that, at carboxylic and amino binding sites, iron could form complexes by co-ordinate bonding. These linkages had a very high affinity constant and were not easily broken. During the destruction of the granules by NaCl, iron was present in quantities too small (0.17 mg/g protein) to stabilize the structure. Therefore a minimal amount of iron was required to avoid solubilization of granules by NaCl.

Effect of EDTA

Addition of ethylenediaminetetraacetic acid (EDTA), which chelates the cations, to a solution of the granules, showed the importance of the phosphocalcic bridges in the granular structure. EDTA was seen to fix virtually all the calcium (Table 2) and iron of the granules. On the other hand, solubility measurements indicated that the addition of EDTA changed the solubility index of the granular proteins from 3.4% to 20.8%

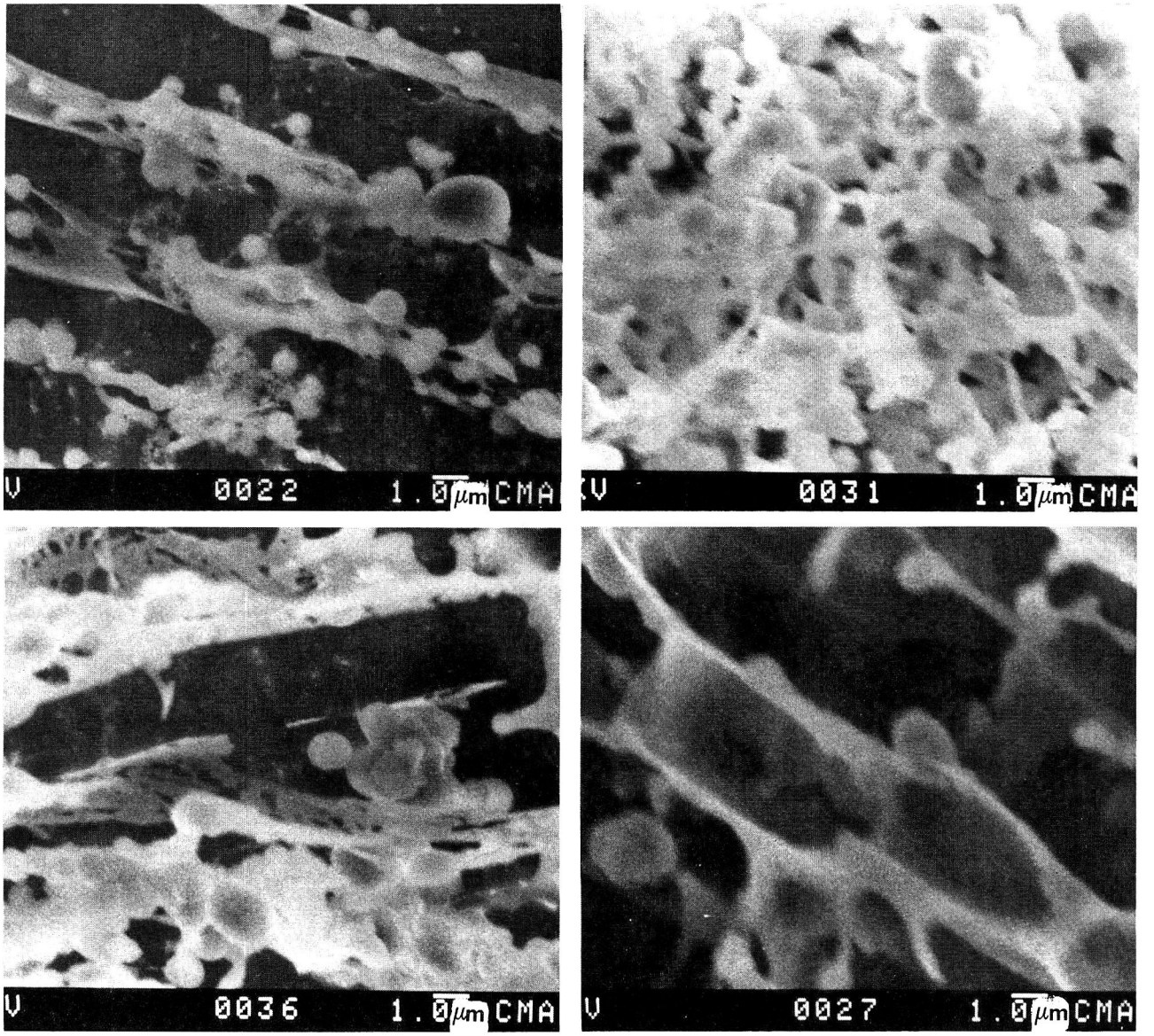

Fig. 4—Electron micrographs of granular fraction in the presence of iron (46 mM).Too left pH 6.4; top right pH 3.0; bottom left pH 9.0; bottom right in the presence of NaCl (0.58M) at pH 6.4.

(Table 3). This could be explained as partial destruction of the granules or due to total destruction followed by precipitation.

The results of electrophoresis of the soluble and insoluble fractions (Fig. 1) showed that phosvitin was not present in the insoluble fraction. The addition of EDTA had therefore caused a complete dissociation of the granules accompanied by a precipitation of the LDL and lipovitellins. The observed effect was the same as that involving addition of NaCl followed by dialysis.

During the addition of NaCl (0.58 M), the Ca^{++} ions in the granules were replaced by Na^{+} ions at a higher concentration. The result of this was a dissociation of the granules due to the rupture of the calcic bridges. Dialysis of this solution eliminated Na^{+}, Cl^{-} and Ca^{++} ions allowing at this pH the electric attractive forces and, therefore, the protein precipitation of LDL and lipovitellins. The highly charged phosvitin remained soluble (Causeret et al., 1991). On the addition of EDTA, similar results were found: EDTA irreversibly complexed all the mineral cations, and without their replacement, total dissociation of the granules resulted. Demineralized LDL and lipovitellins had a net charge of zero and were precipitated. The effect of NaCl on behavior of the cations and of EDTA on the microstructure confirmed the presence of ionic bridges within the granules. Phosphocalcic bridges were the main association between proteins.

Effect of the addition of minerals on granule structure

Calcium. We studied the behavior of the granules in the presence of calcium chloride. Figure 2 shows the solubility index of the granular proteins as a function of calcium concentration. Above 30mM, the granules progressively lost their structure. Solubilization was not sudden as when NaCl was added. The solubility index of the granules dissociated by NaCl was on the other hand, little changed by the presence of calcium. Even at 140 mM of calcium, the added sodium remained in excess in relation to calcium, and the calcium did not bind in sufficient quantity to the proteins to cause precipitation. Solubility measurements in the presence of calcium seemed to indicate that in low concentrations it had a cohesive effect on the granular proteins and in high concentrations a dispersive effect. To confirm the cohesive effect of the bivalent cations at low concentrations we employed scanning electron microscopy to observe the granules at different pH's in the presence of calcium.

To encourage the formation of phosphocalcic bridges, we

added calcium to the medium such that the molar ratio of Ca: P was 2 : 1. The pH was maintained at 6.4 during the addition of calcium to a final concentration of 36.6 mM. The solubility index of the proteins in the solution (10.4%) was little changed by the alkalization of the medium (Table 3). Microscopy (Fig. 3) confirmed that at pH 9 the presence of calcium allowed the maintenance of the microstructure of the granules, whereas in the absence of calcium they were destroyed (Causeret et al., 1991). The negative charges which appeared during alkalization were neutralized by the calcium ions present in the medium; electrostatic repulsions were minimized and the structure preserved. The granules observed at pH 9 had an appearance very similar to those observed at pH 6.4 in the absence of calcium.

Iron. As before, we studied the cohesive effect of iron at low concentrations by solubility measurements and microscopic observations at different pHs. Iron was also added to the solution of granules so that the molar ratio of Fe: P was 2 : 1 (46 mM). The concentration of mineral elements after dialysis showed that 82% of the total calcium was liberated in the presence of iron. It displaced most of the calcium bound to the phosphates due to its higher affinity constant for the binding sites and because of its excess concentration compared to calcium.

The results concerning the solubility at different pHs are shown in Table 3 and the corresponding photographs from microscopy in Fig. 4. At pH 9, the solubility index was less than at pH 6.4 (2.9% Vp 7.9%), but the microscopic observations (Fig. 4,) showed many similarities. Added iron prevented the destruction of the granules at alkaline pH and worked in the same manner as calcium. The increase of the density of the matrix at pH 9 could be explained by the increase in ionization and the number of ionic bridges in which the iron was involved. The slight lowering of the solubility could be due to the binding of iron on the initially soluble proteins, which resulted in their precipitation.

At pH 3, the solubility index was higher (18.4%), but microscopy showed that the granules remained (Fig. 4). During acidification, positive charges were generated and caused electrostatic repulsions which had a tendency to destroy the granules. The result of this was an increase in solubility of the proteins and in the density of the observed matrix. However, because of the low value of pK for the phosphates, the coordinate bonds caused by the iron would resist acidification and allow the maintenance of part of the structure in spite of the increase in charge of the proteins.

The addition of NaCl did not change the solubility of the granules in the presence of iron (Table 3); this demonstrated the protective role of iron on the structure. Mircroscopy (Fig. 4) confirmed that the granules were still present in the medium. The iron occupied all available sites and caused formation of co-ordinate bonds with a very high affinity constant. Moreover, added sodium did not have a high enough affinity constant, and was not present in sufficient quantity to displace the bound iron. That allowed the granules to remain whole.

CONCLUSIONS

THE MICROSTRUCTURE of the granules in egg yolk depended very much on the physicochemical characteristics of the medium such as pH, ionic strength and bivalent cations. Modification of one of these parameters was sufficient to irreversibly destructure the granules. The phosphocalcic bridges were the principal (and perhaps only) cause of protein association in the granules. These contain 80% of the calcium in egg yolk. Addition of NaCl, which destructures the granules, caused a liberation of calcium. On the other hand, dialysis of the granules, which results in a partial desalination, was accompanied by only a partial destabilization of the microstructure. Addition of a calcium-chelating agent (EDTA) caused a complete destruction of granules. Inversely, the addition of minerals at low concentrations could strengthen structure and prevent the degradation normally caused by changes in pH or ionic strength.

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Cholesterol Lowering Activity of Ropy Fermented Milk

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ABSTRACT

The effect of ropy fermented milk on serum cholesterol in rats was studied. Basic diets containing slime-forming *Lactococcus lactis* subsp. *cremoris* SBT 0495, its non slime-forming variant SBT 1275, and acidified reconstituted skim milk, with 0.5% cholesterol added, were fed to F-344 rats for 7 days. Serum cholesterol level of rats fed the ropy fermented milk were the lowest among the three treatments. The serum high-density lipoprotein (HDL) cholesterol/total cholesterol ratio of rats fed ropy fermented milk was the highest. Slime materials produced by *L. lactis* subsp. *cremoris* SBT 0495 had a beneficial effect on rat cholesterol metabolism.

Key Words: ropy sour milk, cholesterol, rat feeding, Lactococcus

INTRODUCTION

VIILI is a ropy fermented milk traditionally made in Finland. The ropy consistency is caused by products of slime-forming lactococci (Pettersson, 1988). The chemical composition of the product is complicated, and the nature of the ropiness has not been addressed (Sundman, 1953; Nilsson and Nilsson, 1958; Macura and Townsley, 1984). We recently reported that the ropy characteristic was caused by a mixture of a phosphorus-containing polysaccharide and a protein produced by the organism (Nakajima et al., 1990). Much interest has been given to the physiological effects of ropy milk. Forsén et al. (1987) demonstrated the immunopotentiating activity of slime-forming *Lactococcus lactis* subsp. *cremoris* T-5, and Kitazawa et al. (1988, 1990, 1991) have pointed out the antitumor activity of this type of organism.

Many reports have been published indicating fermented milks had serum cholesterol-lowering ability (Rao et al., 1981; Grunewald, 1982; Pulusani and Rao, 1983). We reported *Lactobacillus acidophilus* SBT 2056 had a cholesterol-lowering effect in rats fed hypercholesterolemic diet (Suzuki et al., 1991). Our objective was to determine the effect of ropy fermented milk, produced with a slime-forming lactococci, on the serum cholesterol level in rats fed high-cholesterol diets.

MATERIALS & METHODS

Materials

All reagents were reagent grade and obtained from Wako Pure Chemicals (Osaka, Japan). Vitamin mixture AIN-76, salt mixture AIN-76 (American Institute of Nutrition, 1977) and cellulose powder were purchased from Clea Japan Inc. (Tokyo, Japan), and cholic acid (sodium salt) from Sigma Chemical Company (St. Louis, USA).

Lactic acid bacteria and fermented milk preparation

Slime-forming *Lactococcus lactis* subsp. *cremoris* SBT 0495 and its nonslime-producing variant *L. lactis* subsp. *cremoris* SBT 1275 were maintained as described previously (Nakajima et al., 1990; Toba et al., 1990). A 12% reconstituted skim milk medium was heat-treated at 121°C for 10 min. *L. lactis* subsp. *cremoris* SBT 0495 and *L. lactis* subsp. *cremoris* SBT 1275 were individually inoculated at 5% into the medium and incubated at 20°C for 20 hr. The final pH of each culture was 4.7. Another similarly heat-treated skim milk sample was acidified to pH 4.7 with 10% lactic acid and used as the control. Fermented milks and acidified milk were freeze-dried prior to preparing the hypercholesterolemic diet.

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Animals

Male F-344/Jcl rats (11 wk old; Clea Japan Inc., Tokyo, Japan) were fed a commercial diet (Type SE-2, Oriental Yeast Co., Ltd., Tokyo, Japan) and housed individually in an air-conditioned room for 1 wk. Then three groups of six animals each were fed a hypercholesterolemic diet containing fermented milk of *L. lactis* subsp. *cremoris* SBT 0495 (A), fermented milk of *L. lactis* subsp. *cremoris* SBT 1275 (B), and acidified skim milk control (C), respectively. The composition of these diets, according to Nagata et al. (1981), is shown in Table 1. After 7 days feeding, the animals were sacrificed following an overnight fast. Ether-anesthetized rats were killed by cardiac puncture and blood was collected from each. The livers were immediately removed, weighed, and kept frozen for later lipid analysis.

Lipid analysis

Liver lipids were extracted by the method of Bligh-Dyer as described by Johnson (1971). Total serum cholesterol, HDL cholesterol, triglyceride, and liver lipid concentration were determined enzymatically using commercial analysis kits (Kyowa Medix Co., Ltd., Tokyo, Japan).

Statistical analysis

Statistical significance of the differences among diet groups were calculated according to Tukey-Kramer's multiple comparison (Kramer, 1956; Tukey, 1977).

RESULTS & DISCUSSION

THERE WERE no significant differences in body and liver weights and feed intake among (Table 2) the treatment groups throughout the study. Thus energy intake, while feeding the high-cholesterol diets, was not different among the three diet groups. The serum cholesterol levels (Fig. 1) of the test groups were: ropy fermented milk, 84.0 ± 11.3, nonropy fermented milk, 95.7 ± 9.8, and acidified milk with lactic acid, 102.0 ± 6.7 mg/dL. The difference in total serum cholesterol between groups fed ropy fermented milk and acidified milk was significant ($p < 0.05$). The HDL cholesterol/total cholesterol ratios between the group fed ropy fermented milk and the group fed acidified milk were also significantly different ($p < 0.01$). The average serum cholesterol level and HDL/total cholesterol ratio

Table 1—Composition of diet used in cholesterol feeding experiment

Component	(%)
Fermented or acidified milk	10
Casein	18
Lard	10
Soybean oil	1
Vitamin mixture A N-76	0.85
Salt mixture AIN-76	4
Cellulose	3
Choline chloride	0.2
Sucrose	52.325
Cholesterol	0.5
Cholic acid (sodium salt)	0.125
Total	100

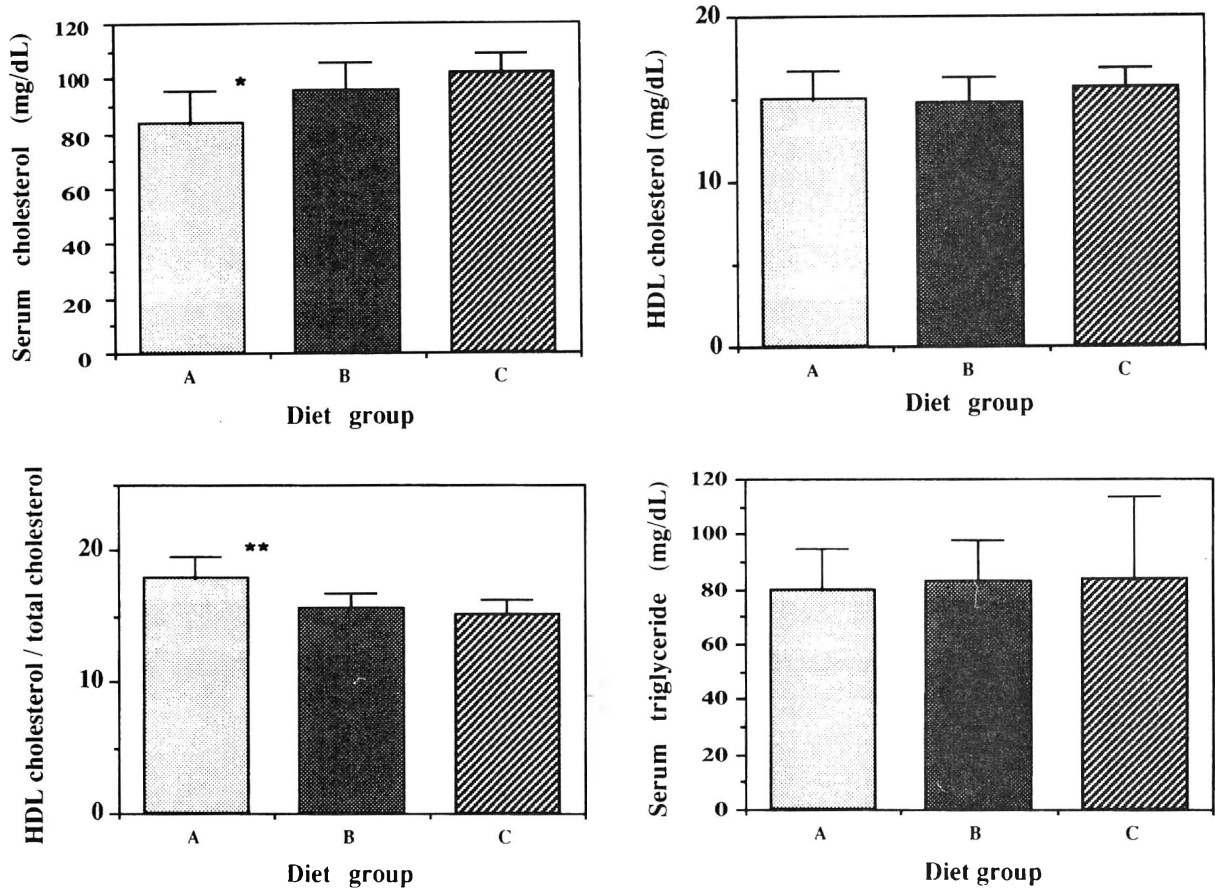

*Fig. 1—Effect of fermented milk on serum lipids in rats. Diet groups: (A) Ropy sour milk, (B) Nonropy sour milk, (C) Acidified milk. Values were expressed as mean ± SD. The differences of means were calculated by Tukey-Kramer's multiple comparison. *Significantly different from acidified milk (C), $p<0.05$. **Significantly different from acidified milk (C), $p<0.01$.*

Table 2—Food intake and rat body and liver weights during high-cholesterol diet feeding experiment[a]

	Diet groups		
	Ropy milk (A)	Nonropy milk (B)	Acidified milk (C)
Body weight (g)*	230.3 ± 7.9	231.2 ± 13.0	232.9 ± 8.0
Food intake (g/day)*	13.9 ± 1.0	14.2 ± 1.2	14.4 ± 0.8
Liver weight (g)*	7.6 ± 0.4	7.7 ± 0.7	7.7 ± 0.5

[a] (A) Ropy milk (*L. lactis* subsp. *cremoris* SBT 0495); (B) nonropy milk (*L. lactis* subsp. *cremoris* SBT 1275); (C) Acidified milk with lactic acid.
* Differences not significant.

Table 3—Effect of fermented milks on liver lipids of rats fed high-cholesterol diets

	Ropy milk (A)	Non-ropy milk (B)	Acidifed milk (C)
Total cholesterol (mg/dL)[a]	102.6 ± 25.1	115.0 ± 24.4	126.2 ± 13.0
Triglyceride (mg/dL)[a]	278.5 ± 91.6	265.0 ± 50.5	285.6 ± 61.7

[a] Differences among feeding groups are not statistically significant.

between ropy milk and non-ropy fed groups were not statistically significant. The amounts of triglyceride in the serum of groups (A), (B) and (C) were not significantly different.

The amount of liver cholesterol differences (Table 3) among the three groups were not statistically significant. Likewise, the liver triglyceride concentrations were not significantly different.

The nonslime-producing strain *L. lactis* subsp. *cremoris* SBT 1275 used was obtained as a spontaneous variant of the parent strain (*L. lactis* subsp. *cremoris* SBT 0495) with serial transfer at 30°C (Toba et al., 1990). The phenotypic difference between *L. lactis* subsp. *cremoris* SBT 0495 and SBT 1275 was slime productivity. We hypothesized that the extracellular slime material played an important role in cholesterol absorption from our feeding experiment. The slime material produced by *L. lactis* subsp. *cremoris* SBT 0495 was shown to be a mixture of polysaccharide and proteins (Nakajima et al., 1990). We believe that the water-soluble extracellular polysaccharide (Nakajima et al., 1992), named viilian by Adachi (1992) is resistant to digestive enzymes and acts like dietary fiber. The proposed mechanism of dietary fiber action is that it can adsorb cholesterol or bile acid, and has water-holding activity and anion-exchange activity (Story and Kritchevsky, 1978). As the polysaccharide produced by *L. lactis* subsp. *cremoris* SBT 0495 has a negative charge, the anion-exchange activity could not be effective. However, the slime material is water-soluble and has water-holding activity (Toba et al., 1990).

The reason why the difference between ropy fermented milk-fed group and nonropy fermented milk-fed group was not clear might be related to the low productivity of slime material in the ropy fermented milk. It was difficult to purify the extracellular polysaccharide that is one of the components of the slime material produced by the slime-forming lactococci, and therefore its precise concentration was not determined. However, we have shown the amount of slime material from whey permeate culture was ≈500 mg/L (Nakajima et al., 1990). It was estimated that the slime content in ropy fermented milk was nearly the same as that in whey permeate culture. This amount of slime materials might not be enough to adsorb cholesterol or inhibit cholesterol absorption *in vivo*.

Lactobacillus acidophilus was reported to have an inhibitory effect on increasing cholesterol level (Suzuki et al., 1991). The effect on humans has been reviewed by various authors (Thompson et al., 1982; Jaspers et al., 1984; Lin et al., 1989;

Gilliland and Walker, 1990). Gilliland (1989) pointed out that the strain specificity of *Lactobacillus acidophilus* was responsible for the differences in such results. There were also wide variations among the strains of *Lactobacillus acidophilus* in the ability to assimilate cholesterol (Gilliland et al., 1985). Being part of the normal intestinal flora, the strain was expected to affect cholesterol metabolism in the intestine.

There are also several reports in which milk, fermented by other than normal intestinal bacteria, lowered the serum cholesterol level in rats (Rao et al., 1981; Grunewald, 1982; Pulusani and Rao, 1983). Jaspers et al. (1984) suggested that organic acids could possibly be the cholesterol-lowering factor. Gurr (1989) pointed out the strong popular belief among many people regarding the serum cholesterol-lowering effect of fermented milks. However, scientific evidence to support this view has not been provided. We mentioned that the extracellular slime material which was produced by *L. lactis* subsp. *cremoris* SBT 0495 was beneficial to cholesterol metabolism. Most of the results concerning fermented milk indicated the cholesterol-lowering effect was brought about through feeding normal diets. We used high-cholesterol diets in order to test the possibility that fermented milk had an inhibitory effect on cholesterol absorption and to show the possibility that cholesterol-lowering activity of ropy-fermented milk acts like dietary fiber. There are no published reports mentioning the effect of these bacterial slime materials on lipid metabolism.

At present, it is difficult to explain the precise mechanism of cholesterol-lowering activity of ropy fermented milk. Organic acids could be responsible for the effect in this type of fermented milk. In order to elucidate the mechanism, more experiments must be conducted. Furthermore, if the concentration of the extracellular polysaccharide was higher than that of the strain we used, the ropy fermented milk could act strongly like dietary fiber. We should select the strains of high slime-producing capacity or genetically improve bacterial slime production. Unlike fermented milks made with intestinal flora bacteria, fermented milks produced by nonintestinal bacteria can not be expected to colonize or implant in the intestine and affect lipid metabolism. With regard to the cholesterol-lowering effect of fermented milk, which are made with nonintestinal flora bacteria, we should also consider strain specificity.

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Rapid *p*-Nitroaniline Test for Assessing Microbial Quality of Refrigerated Meat

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ABSTRACT

The *p*-nitroaniline test, a rapid method to estimate microbial quality of refrigerated meat, was applied to many retail meat samples from several animal species to confirm its applicability. This method allows determination of psychotroph levels between 10^4 and 10^8 CFU/cm^2 in about 2.5 hr. A good correlation was found ($r = 0.88$, $p < 0.001$) between this method and the standard plate count technique. Because of the yellow color of the *p*-nitroaniline released during reaction, bacterial loads higher than 10^5 CFU/cm^2 develop a color intense enough to be detected visually.

Key Words: meat, microbes, *p*-nitroaniline, rapid-test, bacterial quality

INTRODUCTION

ASSESSMENT of the bacteriological quality of refrigerated meat by direct methods based on colony formation are precise but time consuming, requiring 2–3 days to complete. It would be very useful, from a practical point of view, to have a rapid, inexpensive, sensitive and accurate method for counting bacteria on meat. The method should be, in addition, a measure of the degree of acceptability/freshness/spoilage and not simply assess rank spoilage, which in many cases could be detected by sensory tests. To achieve this goal, several approaches have been proposed, such as bioluminescence, impedance and DEFT. The *p*-nitroaniline test (Pérez de Castro et al., 1988) is based on the aminopeptidase activity of Gram-negative bacteria, which cleaves L-alanine-*p*-nitroanilide to yield *p*-nitroaniline which is easily determined spectrophotometrically at 390 nm.

If any technique is to gain acceptance the results must be similar to, or correlate with, those of accepted standard method(s). In the original study (Pérez de Castro et al., 1988) the *p*-nitroaniline test correlated well with the standard plate count in tests made in a laboratory environment. Our objective was to obtain further information and to confirm the applicability of the *p*-nitroaniline test in practice with trials with many retail meat samples.

MATERIALS & METHODS

MEAT SAMPLES were purchased in several local retail markets and consisted of different size meat pieces from various species (pork 37, beef 42, lamb 16 and poultry 9). Additionally, 2 samples of horse meat and fish were analyzed. Bacteria on the meat surface were collected by the swab method (Patterson, 1971) from a 16 cm^2 area in 0.75% (w/v) sterile saline solution (10 mL). Psychrotrophs were determined by the pour plate method on Plate Count Agar (OXOID) at 17°C for 17 hr plus 7°C for 72 h (Søgaard and Lund, 1981).

The aminopeptidase activity was measured by using L-alanine-*p*-nitroanilide (SIGMA) as substrate according to the scheme shown (Fig. 1). Readings at 390 nm were referred to a reference curve prepared with *p*-nitroaniline (SIGMA) in the range 0–16 μg *p*-nitroaniline/mL.

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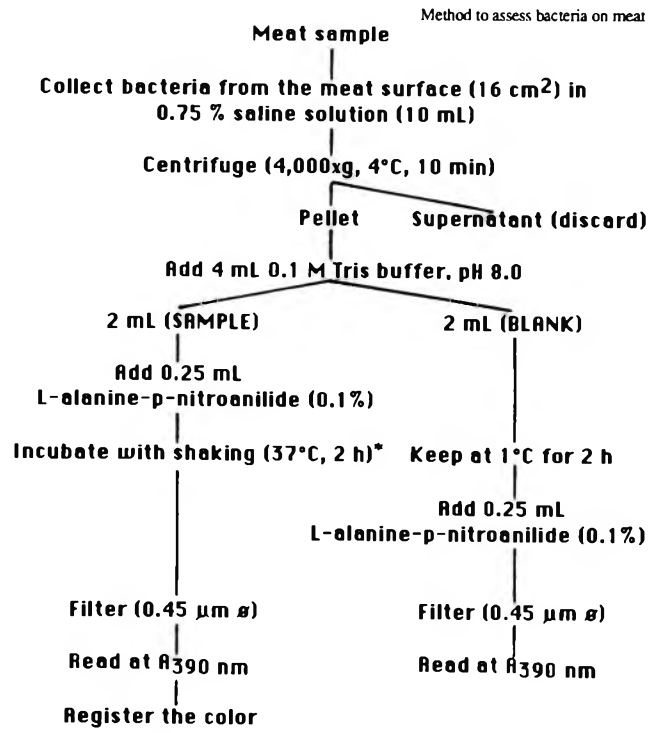
Statistical analysis was performed with BMDP statistical software in a CDC-CYBER mod 180–855 computer.

RESULTS & DISCUSSION

BY PLOTTING the $\log_{10}$ CFU/cm^2 of meat vs the $\log_{10}$ *p*-nitroaniline/2 hr/cm^2 released by psychrotrophic bacteria from meat, a linear relationship was observed over the range 3.5×10^3 CFU/cm^2 to 1×10^8 CFU/cm^2 (Fig. 2). Meat samples from different species were grouped for plotting and statistical analysis since the regression line slopes of beef, pork lamb and poultry samples were similar (2.15, 2.32, 2.10 and 2.24, respectively). A good correlation was obtained ($r = 0.88$, $p < 0.001$), being the regression line $y = 6.1053 + 2.1933x$ where x is the $\log_{10}$*p*-nitroaniline/2hr/cm^2 and y the $\log_{10}$ CFU/cm^2 of meat. The correlation coefficient was lower than that ($r = 0.96$) obtained previously (Pérez de Castro et al., 1988). This difference has been attributed to the sample handling. In the preliminary work, meat samples were stored at 4°C after sampling to mimic the growth of the microflora during normal storage. However, in our present study samples were immediately analyzed after sampling. Therefore, in this study greater flora heterogeneity was expected. The correlation coefficient obtained for the *p*-nitroaniline test was similar to those reported for other rapid methods, such as DEFT ($r = 0.88$ to 0.96)



*** It was shown that pH 8.0 and 37°C for 2 h were the most appropriate conditions for the aminopeptidase activity (Pérez de Castro et al., 1988).**

Fig. 1—Flow chart to perform the p-nitroaniline test for assessing the bacterial quality of refrigerated meat.

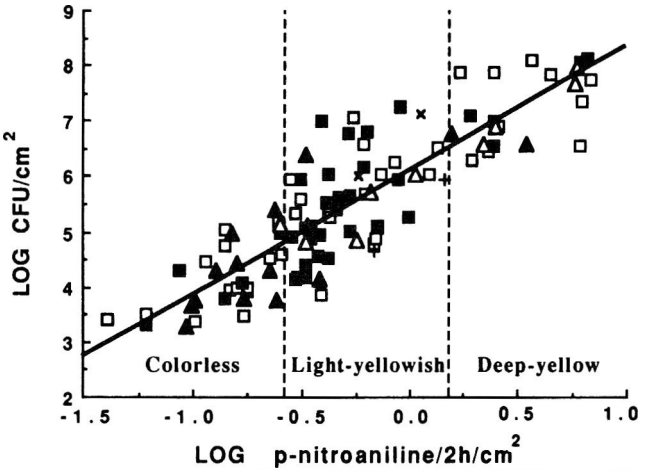


Fig. 2—Relationship (on logarithmic scale) between aminopeptidase activity of psychrotrophic bacteria and psychrotroph counts on retail refrigerated pork (■), beef (□), lamb (▲), poultry (△), horse (X) and fish (+); (n = 108).

(Shaw and Farr, 1989) and lower than those described for bioluminescence (r = 0.96) (La Rocco et al., 1986) or impedance (r = 0.97) (Firstenberg-Eden, 1983).

Most meat samples (70%) containing bacterial loads from about 5×10^3 to 5×10^4 CFU/cm² of meat presented sufficient aminopeptidase activity to release small but quantifiable amounts of *p*-nitroaniline (0.15–0.25 μg/cm² of meat) although the reaction medium remained colorless (Fig. 2). When bacterial numbers were between 1×10^5 to 5×10^6 CFU/cm², the amount of *p*-nitroaniline liberated from most samples (84%) was enough to develop a light-yellowish color, which was visually detected (0.3–1.3 μg/cm² of meat). A total of 19 of 26 samples (73%) with bacterial numbers higher than 5×10^6 CFU/cm² developed a deep-yellow color during incubation (> 1.3 μg *p*-nitroaniline/cm² of meat). The threshold at which off-odors of spoiled refrigerated meat are detected by sensory tests is about 5×10^7 CFU/cm² (Ayres, 1960; Ingram and Dainty, 1971). This may have important practical implications because the *p*-nitroaniline test reaction medium develops a deep-yellow color when the refrigerated meat is either spoiled (in this case the test would be unnecessary) or very close to be spoiled. On the other hand, a colorless reaction medium would indicate a very good microbial quality of meat. Samples yielding a light-yellowish color would be more difficult to classify. Nevertheless, they could be considered, from a practical point of view, as having acceptable microbial quality but relative short remaining shelf-life. Then, it would be still possible to take some decisions (e.g. offering the meat to the consumer at a lower price than normal) in order to avoid product condemnation.

The accuracy (95% confidence level) and the reproducibility of the *p*-nitroaniline test have been previously calculated (Pérez de Castro, 1989) in ±0.5 $\log_{10}$ units and ±0.37 $\log_{10}$ CFU/cm², respectively. The accuracy was slightly lower than those reported for other rapid methods, such as bioluminescence (±0.28) (La Rocco, 1986) or impedance (±0.30) (Firstenberg-Eden, 1983), while the reproducibility was similar to those reported for impedance (Firstenberg-Eden, 1983) and DEFT in milk (Pettipher et al., 1983).

The sensitivity of *p*-nitroaniline test (about 10^4 CFU/cm²; Pérez de Castro, 1989) and that of the other rapid methods (Firstenberg-Eden, 1983; La Rocco, 1986; Shaw and Farr, 1989) may be considered as appropriate for assessing the bacterial content of meat. All of them are able to detect bacterial counts lower than 1×10^5 CFU/cm² when samples may be still considered of a high microbial quality. Similarly, all methods are coarsely comparable in terms of accuracy, reproducibility and correlation degree with the colony count technique. Nevertheless, bioluminescence and impedance methods are costly since they require high cost equipment. In this sense, DEFT and *p*-nitroaniline tests are the most favorable. However, several important differences may be established between them. The elapsed time of the DEFT test is shorter than that for the *p*-nitroaniline (0.5 hr vs 2.5 hr), but the great advantage of the *p*-nitroaniline test over the DEFT, and also over the other mentioned methods, is the possibility to assess the microbial quality visually because of the yellow color developed.

Although the *p*-nitroaniline test is useful to estimate bacterial content from the surfaces of all kinds of refrigerated meat, the technique has a limitation. The test can not be applied to ground meat because, in that product, the organisms are not only on the surface. It would be necessary to develop an appropriate method to collect bacteria from ground meat samples for applying the *p*-nitroaniline test. In summary, the *p*-nitroaniline test seems to be a rapid and inexpensive method for routine assessment of the degree of acceptability/freshness/spoilage of refrigerated aerobically stored meat pieces.

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Processing and Frozen Storage Effects on the Iron Content of Cod and Mackerel

J. V. GOMEZ-BASAURI and J. M. REGENSTEIN

ABSTRACT

Processing and subsequent frozen storage affected the iron content of cod (*Gadus morhua*) and mackerel (*Scomber scombrus*) muscle tissue. Frame mince was obtained from the bone rack, without the head or viscera remaining, after filleting. Frame mince had significantly higher iron levels than intact fillets with or without skin or fillets that were subsequently minced. Skin-on fillets had more iron than skin-off fillets. Cod frame mince had about 50% heme iron, while mackerel frame mince ranged from 20–64%. Nonheme iron increased during frozen storage due to heme breakdown. Storage above −14°C was more deleterious to the heme molecule than lower temperatures (−20°C or −40°C).

Key Words: cod, mackerel, fish, iron, storage stability, heme iron, nonheme iron

INTRODUCTION

DIETARY IRON derived from beef, poultry, or fish is more easily assimilated than iron from plants (Layrisse et al., 1968; Cook and Monsen, 1976; Bjorn-Rasmussen and Hallberg, 1979). The contribution of fish iron to the daily diet is more important as decreased consumption of red meat and increased dietary fish becomes more widespread.

For total iron, Gordon and Roberts (1977) found values ranging from 0.2 to 1.0 mg/100g for 14 Pacific species. Atlantic mackerel (*Scomber scombrus*) (Leu et al., 1981) had 1.2 mg iron/100g of edible portion (fillets without belly flap), mostly from the dark meat. The percentage of dark muscle changed with season and, therefore, total iron. Teeny et al. (1984) found 0.2–0.9 mg iron/100g with northeast Pacific species, while Jhaveri et al. (1984) found 0.2 to 0.5 mg iron/100g for some southern New England marine species. Crawford et al. (1972) determined that mechanically separated flesh from different fish carcasses ranged from 3.6 to 21.9 total iron mg/100g wet weight.

Fischer and Deng (1977) compared the dark flesh of mullet (*Mugil cephalus*) with beef sternomandibularis muscle. The dark flesh of the lateral line of mullet had a total iron of 5.7 mg/100g, much higher than beef muscle at 2.6 mg/100g. The heme iron constituted 25–44% of the total iron for the fish compared to 71–89% in beef. Silberstein and Lillard (1978) determined that mechanical deboning increased the hemoglobin content of minced mullet. These results agreed with Froning and Johnson (1973) and Field (1976) who found that mechanical deboning of poultry and red meat increased hemoglobin levels. Fish nonheme iron could be readily absorbed when compared to other nonheme iron sources (Layrisse et al., 1968; Martines-Torres and Layrisse, 1971; Cook and Monsen, 1976; Bjorn-Rasmussen and Hallberg, 1979).

Our objective was to determine the effects of processing and frozen storage on the heme and nonheme iron in fillets, fillet minces and frame minces of cod (*Gadus morhua*), a commercial species; and Atlantic mackerel (*Scomber scombrus*), an "underutilized" species. (Frame minces are obtained from the bone rack left after filleting headed and gutted fish).

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MATERIALS & METHODS

Experimental design

The experimental design included the variables of mincing, storage temperature, and time. The experimental unit was one Nalgene polyethylene jar (30g capacity, Sybran Nalge Co., Rochester, NY). Each experiment was done in duplicate and subsequently replicated. Samples were randomized in all cases and heme iron, nonheme iron, and total iron were measured for each experimental unit.

Sample Preparation

Frozen cod. Gutted commercial cod (40 kg) were purchased from Connolly Seafood Co., Boston, MA. The fish were unpacked immediately and divided into two lots. Each lot was randomly subdivided into four groups, covered with ice and stored at 2°C for 2 hr. The fish were processed (headed and filleted) and the backbones or racks with adhering meat (frames) became the fifth group. The treatment groups were: (1) fillets skin-off; (2) fillets skin-on; (3) fillets skin-off, minced; (4) fillets skin-on, minced; and (5) frame mince. The fillets in the first two treatments were finely chopped and thoroughly hand-mixed. The fillets from the other treatments and frames were further processed through a Beehive RSTC-02BX-S05 deboning machine (Beehive Machinery, Sandy, UT) with a pressure setting of 0-7 for fillets, and S-5 for frames. The deboner head had 1.5 mm holes. Samples were packed in the Nalgene jars which were randomly stored at −7°C, −14°C, and −40°C (Kelvinator, Manitowoc, WI) for up to 150 days.

Frozen mackerel. Headed and gutted mackerel (40 kg) were treated like the cod except that they were left overnight in the cooler. An additional treatment was added: headed and gutted whole fish mince (H&G). Mincing was at a pressure setting of R-5 for fillets, and P-4 for H&G and frames. The Nalgene jars were stored overnight at 0°C and then at −7°C, −14°C, −20°C, and −40°C for up to 99 days.

Fresh mackerel. Headed and gutted mackerel (20 kg) were deboned at pressure settings of R-5 for fillets, and R-4 for H&G and frames and stored at 0°C for 14 days.

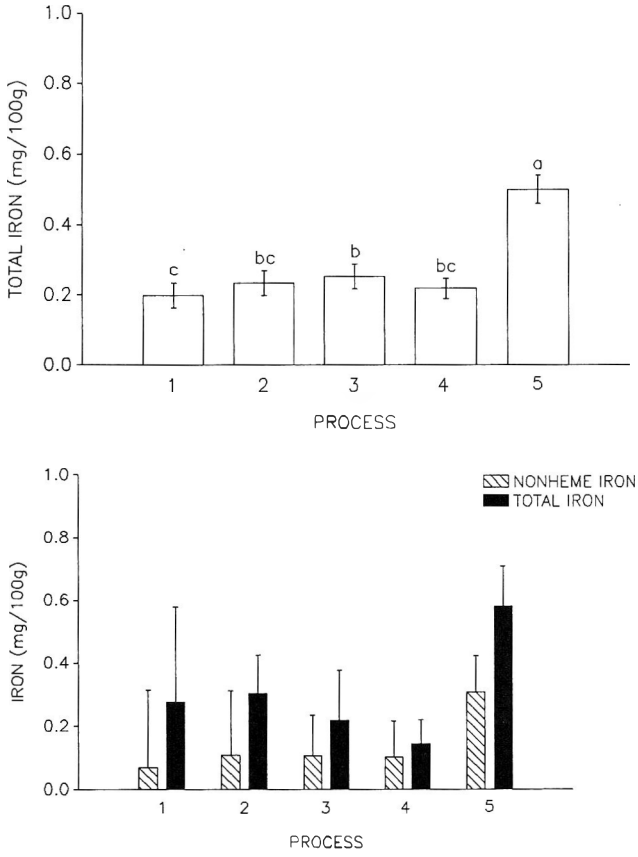
Dark muscle from fresh mackerel. Using the fish from experiment 3, mackerel dark muscle was obtained by carefully separating the muscle along the lateral line from the white muscle. The treatments used were all intact muscle: (1) fresh whole fillet skin-off; (2) fresh whole fillet skin-on; (3) light muscle skin-off; (4) dark muscle skin-off. The fish was stored for 14 days at 0°C.

Statistical analysis

Results were analyzed by the Statistical Analysis System (SAS Institute Inc., Cary, NC). The data were tested for normality and outliers by studentized residual plots and Cook's distance measure (Neter et al., 1985) and outliers removed. Then the General Linear Model (Proc GLM) was used to test hypotheses with the F statistic at $\alpha = 0.05$. Comparisons between treatment means were made using Duncan's multiple range test (Duncan, 1955) at the 5% confidence level.

Reagents

All chemicals used were ACS grade from Sigma (St. Louis, MO), unless otherwise stated. Distilled, doubled deionized (ddd) water (Ion-X-changer, Cole-Palmer, Chicago, IL) was used to prepare all solutions. All glassware was washed with tap water and Sparkleen (Fisher, Springfield, NJ), rinsed with distilled water, soaked overnight in an acid bath (1N HCl), rinsed with ddd water, and air dried.



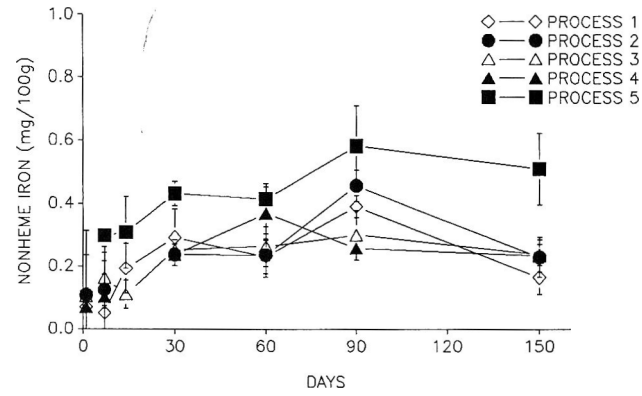


Fig. 1—Processing and time effects on the iron content of cod: (a) total iron; (b) nonheme and total iron at time zero; (c) nonheme iron. Error bars are 95% confidence intervals. Bars with different letters are significantly different at $p<0.05$. 1: Skin-off fillets, 2: Skin-on fillets, 3: Skin-off fillet mince, 4: Skin-on fillet mince, and 5: Frame mince.

Total heme pigments assay

Extraction of total heme pigments was by the method of Warris (1977) with a slight modification. Heme pigments were determined by the cyanomet procedure (Tentori and Salvati, 1981). Fish (10g) was homogenized with 20 mL of phosphate buffer (pH 6.8, 0.04 M) for 3 min at setting No. 4 in an Omni-Mixer homogenizer (Omni, Waterbury, CT). The homogenate was centrifuged at 7,649 × *g* (8,000 rpm) in a RC2-B high speed refrigerated centrifuge at 2–4°C with an SS-34 rotor (Sorvall, Norwalk, CT) and filtered through Whatman No. 3 filter paper (Whatman, Maidstone, England). The filtrate was brought to volume (50 mL) with the buffer and stored overnight at 0°C. Filtrate (3 mL) was reacted with 3 mL of Drabkin's solution (Sigma: Sodium bicarbonate, 100 parts; potassium ferricyanide, 20 parts; and potassium cyanide, 5 parts) in a borosilicate test tube (10 mm o.d. × 12 cm length). After 10 min the absorbance was read at 540 nm in a Hitachi 100-60 spectrophotometer (Hitachi, Tokyo, Japan). Heme pigment concentration was calculated using the cyanometmyoglobin mM extinction coefficient of 11.0 (L/mM) at 540 nm using a molecular weight of 16,110 for myoglobin (Drabkin, 1978). Besides the reagent blank, no other blank was used. The following formula was used to calculate heme pigment concentration:

$$C = (A \times DF \times V \times 16110) / (11000 \times S)$$

where: C: heme pigments concentration in mg/g sample; A: absorbance at 540 nm; DF: dilution factor; V: final volume of extract in mL; and S: weight of sample in g.

Nonheme iron assay

Nonheme iron was determined according to Schricker et al. (1982). Fish (4 g) was weighed into a screw cap test tube and 0.2 mL 0.39% (w/v) sodium nitrite added. Tubes were vortexed and 15 mL of the acid mixture were added (6N HCl (Fisher) and 40% trichloroacetic acid (TCA) (Fisher) were mixed in equal volumes and prepared fresh each day. The tightly capped, vortexed tubes were placed into an incubator at 65°C (Model R25, New Brunswick Scientific, New Brunswick, NJ). Samples were incubated for 20 hr with shaking (100 rpm) and then cooled at room temperature for 3 hr. The samples had a dark precipitate and a brownish supernatant. Supernatant (1 mL) was transferred to a borosilicate culture tube and 5 mL of the nonheme iron color reagent (prepared fresh daily) were added. After vortexing and standing for 10 min, the absorbance was read at 540 nm. The nonheme iron color reagent was prepared by mixing a 1:20:20 ratio of (1) bathophenanthroline solution (bathophenanthroline disulfonic acid, sodium salt, 0.162g, dissolved in 100 mL of ddd water with 2 mL thioglycolic acid (96–99%) stored in an amber bottle at 1°C); (2) ddd water; and 3) saturated sodium acetate solution.

Standard nonheme iron curve

The nonheme iron standard curve (0.00–0.08 mg/100 mL) prepared for each assay, was obtained from 1 mL of 1 to 5 ppm iron (diluted from an iron stock solution, 50 mg Fe/dL in 0.8N nitric acid, with 0.5N HCl) with 5 mL of the nonheme iron color reagent. Nonheme iron concentration is:

$$C = (I \times DF \times V)/S$$

where: C: nonheme iron concentration in mg/100g sample; I: mg of nonheme iron/100 mL of extract; DF: dilution factor; V: final volume of extract in mL (acid mixture + sodium nitrite + water content); and S: weight of sample in g.

Total iron assay

Total iron was determined by the method of Miller (1987). Fish (4g) were weighed into a Pyrex ignition test tube and oven dried for 24 hr at 95°C (Boekel Industries, Philadelphia, PA). Samples were cooled in a desiccator and moisture content determined by weight loss. The ignition tubes were placed into an aluminum block (8 × 6 × 3 in) in a muffle furnace (Thermocouple Barber-Colman Model 293-C) at 250°C for 1 hr, the temperature raised to 450°C and ashed overnight. After cooling to room temperature, the aluminum block was placed on a hot plate and 1.0 mL concentrated nitric acid (Fisher) added to each tube and heated at 80°C overnight. The block was again transferred to the furnace and the samples were ashed at 450°C for one more hr and cooled to room temperature. The ash had a white color. Concentrated HCl (0.5 mL) was added. The tubes were mixed on a vortex and left for 2 hr. Water (2 mL ddd) was added and the tubes were again mixed on a vortex. More ddd water was added to bring

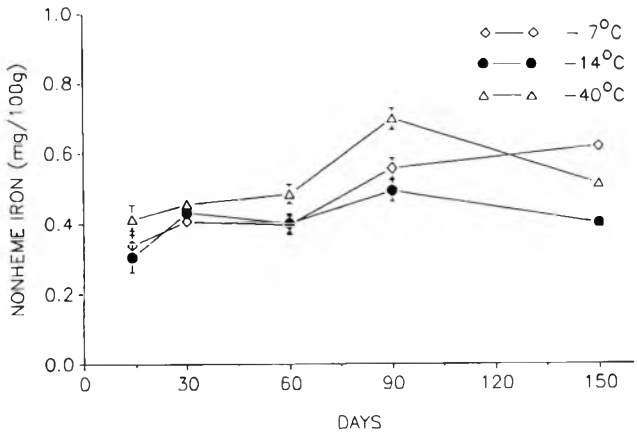


Fig. 2—Temperature and time effects on cod frame mince nonheme iron. Error bars are 95% confidence intervals.

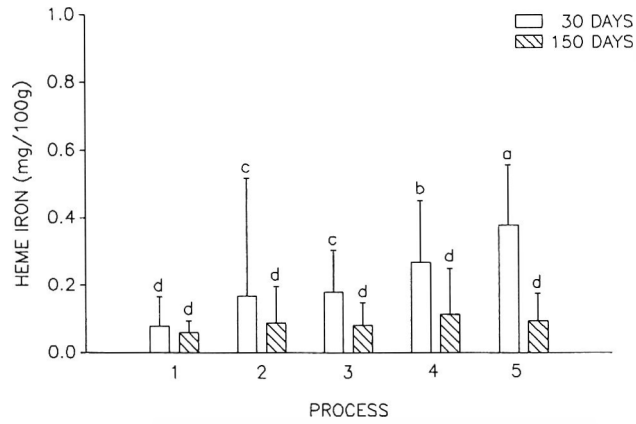


Fig. 3—Processing and time effects on cod heme iron. Values are the means of 3 temperatures. Error bars are 95% confidence intervals. 1: Skin-off fillets, 2: Skin-on fillets, 3: Skin-off fillet mince, 4: Skin-on fillet mince, and 5: Frame mince.

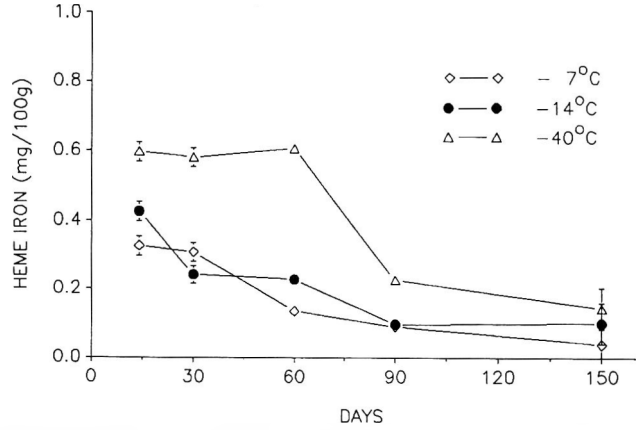


Fig. 4—Temperature and time effects on cod frame mince heme iron. Error bars are 95% confidence intervals.

the final weight to 10g and the tubes remixed. Digest (2 mL) was placed into borosilicate culture tubes followed by the addition of 1 mL of 3% (w/v) hydroxylamine hydrochloride solution and 1.5 mL of total iron color reagent. The tubes were mixed on a vortex and left to stand for 10 min after which the absorbance was read at 533 nm. Total iron color reagent consisted of bathophenanthroline disulfonic acid, sodium salt, 30 mg, dissolved in a small volume of ddd water and diluted to 100 mL with 3M sodium acetate solution and stored in an amber bottle at 1°C. A 95% recovery of a certified iron standard carried through the procedure was obtained (Standard reference material 1577a, bovine liver, 19.4 ± 2.0 mg Fe/100g, National Bureau of Standard Reference Materials).

Standard curve for total iron

The total iron standard curve (0.00–0.22 mg/100 mL) was constructed daily by reacting 2 mL of each working iron standard solution (1–5 ppm) with 1 mL of 3% hydroxylamine hydrochloride and 1.5 mL of total iron color reagent. Total iron concentration was:

$$C = (I \times DF \times V) / S$$

where: C: total iron concentration in mg/100g sample; I: mg of total iron/100 mL of digest; DF: dilution factor; V: final volume of digest; and S: weight of sample in g.

RESULTS & DISCUSSION

Iron content of frozen cod

Mince from cod frames had significantly higher iron (0.5 mg/100g) than fillets or fillet minces (0.2–0.3 mg/100g) (Fig. 1a). Previous studies (Froning and Johnson, 1973; Webb et al., 1976; Field, 1976; Sanchez et al., 1978; Field et al., 1979) reported that marrow increased the iron in minced products. Direct contact of fillets with the deboning machine or any metal to metal abrasion (Lee and Toledo, 1977) appeared to increase the iron in minced fillets as seen by the significant difference between skin-off fillet mince and its unminced counterpart. The similarity between minced and unminced skin-on fillets, may be due to the action of the mechanical deboner: most of the skin appeared to have been left intact in the mince waste stream. The values for Atlantic cod fillets agreed with those of Jhaveri et al. (1984) of 0.2 mg/100g, and were somewhat lower than the 0.38 mg/100g reported (Agricultural Handbook No. 8-15, USDA, 1987).

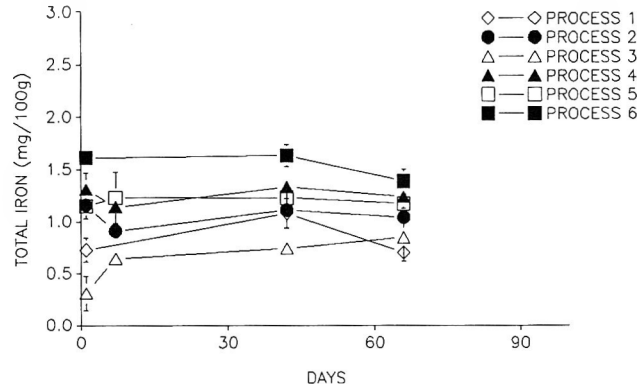
The percentage of nonheme iron (Fig. 1b) for the fillets seemed relatively low except for skin-on fillet mince which had 71% nonheme iron (mainly because of the lower total iron). Cook and Monsen (1976) found that in cod 30% of the total iron was heme iron. Martinez-Torres et al. (1975) found 80% nonheme iron in snapper (*Lutjanus* sp.). During frozen storage (Fig. 1c) nonheme iron increased with frame mince remaining higher than the other samples throughout. The nonheme iron increased during frozen storage presumably reflecting a decrease in heme iron. Interestingly, the nonheme iron after 60 days was significantly different as a function of storage temperature, with the −40°C sample having a higher value than the other samples at 60 and 90 days (Fig. 2).

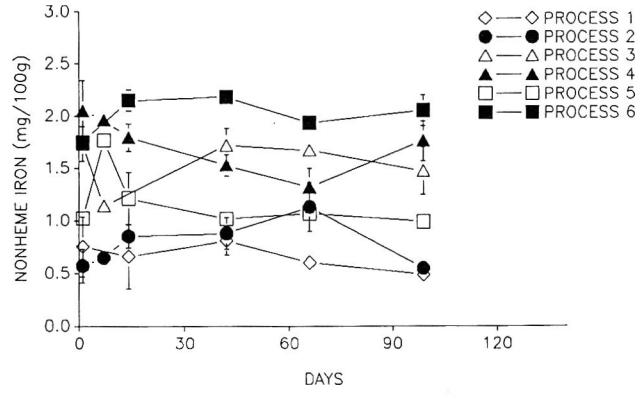
Heme iron appeared lower at 150 days than at 30 days for all samples even though not all were significant (Fig. 3); again suggesting heme breakdown. Frame mince had significantly more heme iron than minced and unminced fillets, probably due to marrow.

Frame mince stored at −7°C and −14°C (Fig. 4) showed a steady decrease in heme iron with time, while at −40°C the heme decrease was significant only after 60 days. This suggested that something during storage triggered a rapid breakdown of the heme molecule. This was consistent with the increase in nonheme iron at the same temperature (Fig. 2).

Iron content of frozen mackerel

Skin-off fillets had less iron than fillets with skin on, fillet minces with skin on had more iron than unminced fillets or minced skin-off fillets (Fig. 5a). Frame mince had a significantly higher iron content. The iron in H&G mince was similar to that of minced skin-on fillets and was lower than the frame minces, most probably due to a dilution effect, i.e., the presence of more flesh in H&G fish than in frames. The total iron content of mackerel fillets without skin, 0.7–0.9 mg/100g, and mackerel fillets with skin, 1.1–1.3 mg/100g, were near the 1.2 mg/100g found by Leu et al. (1981) and the 0.8-1.2 mg/100g





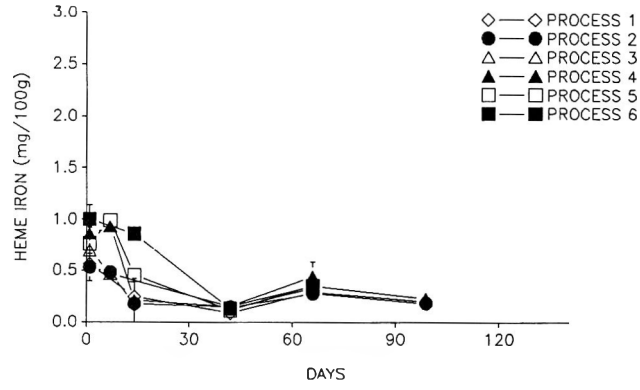


Fig. 5—Processing and time effects on the iron content of mackerel: (a) total iron; (b) nonheme iron; (c) heme iron. Values are the means of 4 temperatures. Error bars are standard errors. 1: Skin-off fillets, 2: Skin-on fillets, 3: Skin-off fillet mince, 4: Skin-on fillet mince, 5: H&G mince, and 6: Frame mince.

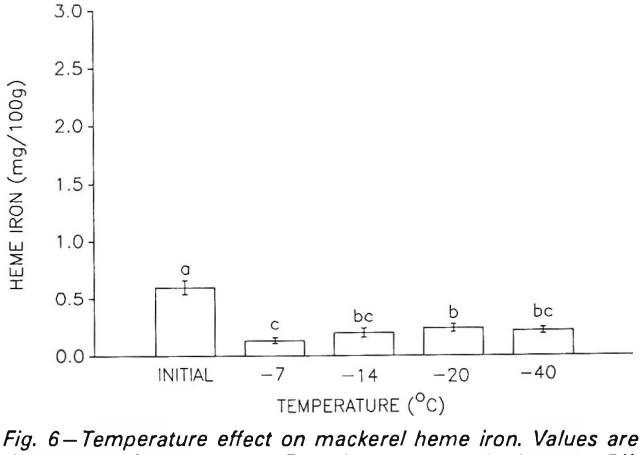


Fig. 6—Temperature effect on mackerel heme iron. Values are the means of 6 processes. Error bars are standard errors. Different letters are significant at $p < 0.05$.

reported by Sidwell (1981), but were somewhat lower than the 1.6 mg/100g in Agricultural Handbook No. 8-15 (USDA, 1987).

Nonheme iron in mackerel frame mince was significantly higher than any other treatment (Fig. 5b). Changes over time were widely scattered. Nonheme iron of mackerel ranged from 0.6–2.0 mg/100g which exceeded the total iron in some cases. The nonheme iron methodology is known to include 5–6% of the heme iron (Schricker et al., 1982) which may account for part of the error. Nevertheless, nonheme iron should not exceed total iron. These results suggest that the methodology was overestimating the actual nonheme iron present in mackerel. Rhee and Ziprin (1988) have suggested inclusion of a second blank to correct for the brownish color of the extracts, which they found interfered in the final determination of the chromophore.

The heme iron in fillet minces (0.7–0.9 mg/100g) were higher than those of unminced fillets (0.5 mg/100g) (Fig. 5c). Thus 46-65% of the iron in fillets is heme iron; while in H&G and frame minces heme iron is higher, 63–64% of the total iron. The decrease in heme iron occurred mainly during the first 30 days of frozen storage. Storage temperature seemed to affect heme iron depletion (Fig. 6) with −7°C significantly less than −20°C.

Iron content of fresh mackerel

The values for the total iron of fish stored for 2 wk on ice at 0°C (Fig. 7a) agreed quite well with those of Exp. 2. However, about 20% of the iron in frame mince was heme iron, much lower than the 63% found in Exp. 2. Mackerel fillets had 26–45% heme iron, again much lower than the previous 46–65%. Thus short-term storage seemed to decrease heme iron.

Iron content of dark muscle of fresh mackerel

In a separate experiment, dark mucsle had the highest total iron with the light muscle having the lowest (Fig. 7b). Dark muscle had more iron (both heme and nonheme iron) than light muscle in agreement with published values (Brown, 1962; Fischer and Deng, 1977; Love, 1970). The actual values were similar to those for mackerel fillets (Leu et al., 1981; Sidwell, 1981) which had 33–43% heme iron while dark muscle had 56%.

In summary, fillets with skin on generally had more iron than those without skin. Frame minces had significantly higher iron than fillets due to the marrow which contributes significant heme iron. The variation in mackerel heme iron may be associated with differences between lots of fish (May vs July), with the latter lower in heme iron. Changes in iron distribution were also observed in different batches of Atlantic cod (unpublished data). Heme iron decreased with time of frozen storage resulting in increased nonheme iron. Higher frozen storage temperatures may lead to slightly faster decrease in nonheme

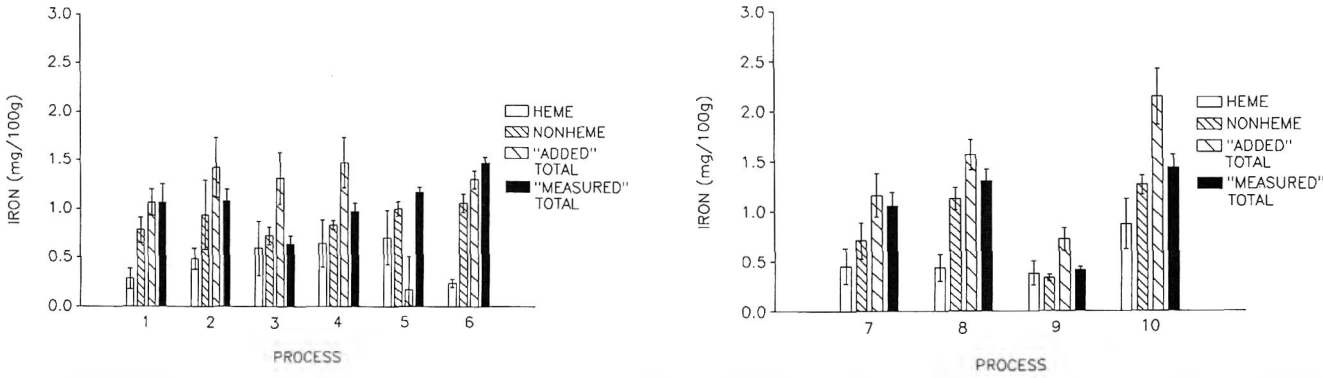


Fig. 7—Processing effect on the iron present in mackerel: (a) 1: Skin-off fillets, 2: Skin-on fillets, 3: Skin-off fillet mince, 4: Skin-on fillet mince, 5: H&G mince, and 6: Frame mince; (b) 7: Skin-off fillets, 8: Skin-on fillets, 9: Light muscle, and 10: Dark muscle. "Added" total (heme plus nonheme), "Measured" total (direct determination). Values are the means of 6 replicate analysis. Error bars are standard errors.

iron which may have been overestimated, especially in mackerel.

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Vacuum Packaging, Ascorbic Acid and Frozen Storage Effects on Heme and Nonheme Iron Content of Mackerel

J. V. GOMEZ-BASAURI and J. M. REGENSTEIN

ABSTRACT

Vacuum packaging and ascorbic acid were tested for their ability to prevent heme iron loss in mackerel (*Scomber scombrus*). Vacuum packed samples had less heme breakdown than air packed samples at frozen storage temperatures above −20°C. Ascorbic acid protected the heme at all temperatures when compared to air or vacuum packed samples.

Key Words: mackerel, heme iron, nonheme iron, ascorbic acid, storage stability

INTRODUCTION

THE TWO POOLS of iron in fish, heme and nonheme, were observed to change during frozen storage, nonheme iron content increased while heme iron decreased (Gomez-Basauri and Regenstein, 1992). Evidence suggests that the porphyrin ring is cleaved oxidatively with a subsequent release of iron (Schmid and McDonagh, 1979; Igene et al., 1979; Schricker et al., 1982). Our objective was to test whether the exclusion of oxygen (vacuum) and addition of ascorbic acid (an antioxidant) would protect the heme iron present in mackerel (*Scomber scombrus*).

MATERIALS & METHODS

Experimental design

A completely randomized factorial design was used to study packaging (air, vacuum, air + ascorbic acid), temperature (−14°C, −20°C, −30°C, and −40°C), and time (0, 30, 60, 120, and 180 days) for mackerel fillets and headed and gutted (H&G) mackerel mince. Heme, nonheme, and total iron content were measured. The experimental unit was one bag of 300g of fillets or mince (see sample preparation below). Samples were run in duplicate and the entire experiment replicated. The nonheme and total iron data were obtained from two subsamples each in duplicate. The heme iron data consisted of two subsamples for each experimental unit with a single determination. All samples were randomized and reported data were averages of all determinations.

Statistical analysis

Results were analyzed as in Gomez-Basauri and Regenstein (1992) at a 5% confidence level.

Sample preparation

Commercially obtained gutted mackerel (≈180 kg) were prepared as previously described (Gomez-Basauri and Regenstein, 1992). The fish were hand processed into skin-on fillets (not boneless, with belly flaps); and headed and gutted mince (H&G). The H&G fish were deboned with the Beehive RSTC-02BX-S05 (Beehive Machinery, Sandy, UT) with a pressure setting of G-3. The deboner head had 1.5 mm holes.

The packaging materials used were: (1) high barrier (B-bags), and (2) nonbarrier (L-bags) plastic bags (Cryovac, Simpsonville, SC) (Santos, 1988). Samples receiving air treatments were packed in L-bags (7″ × 20″); bags were folded but not sealed to allow air access. Samples receiving vacuum treatments were packed in B-bags (≈14 cm x 51 cm). Vacuum packaging was carried out using a heat sealer attached to a vacuum pump, set at 15 psi and sealed for 15 sec. After sealing, the sealed sample was inserted into another B-bag and the second bag vacuum was sealed.

Fillets were dipped into a 1% aqueous ascorbic acid (Pfizer, New York, NY) solution (0.77 kg fillets/L solution) for 1 min and then drained. H&G fish were sprinkled with ascorbic acid and manually mixed for a short time to give a 0.5% (w/w) mixture prior to deboning. Both fillets and H&G mince were packed in L-bags that were folded but not sealed. Each bag contained 3 mackerel fillets (≈300g) or 300g of mince. Following packaging, all samples were frozen in a −20°C high velocity walk-in freezer for 24 hr. Samples were then distributed at random to freezers with storage temperatures of −14°C (Kelvinator, Manitowoc, WI), −20°C, −30°C, and −40°C (Revco Scientific, Asheville, NC).

Reagents

All chemicals and glassware used were as described in Gomez-Basauri and Regenstein (1992).

Sampling

Samples were thawed (at 0, 30, 60, 120, and 180 days) for ≈24 hr in plastic bags in a cold room at 8°C. Samples were homogenized as described by Gomez-Basauri (1991). Samples (30g) were weighed into a 450 mL Mason jar. Distilled doubled deionized water (ddd), 150 mL, chilled to 2°C, was poured into the jar. Fillet samples were homogenized for 3 min at setting 8 in the Omni-Mixer homogenizer (Omni, Waterbury, CT) followed by homogenization with an Omni-Gen rotor generator assembly (Omni-Gen 201) for 3 min at setting 8. Mince samples were homogenized for 3 min at setting 8 using only the rotor generator assembly.

Heme iron assay

Fish slurry (25g) was centrifuged at 30,590 × *g* (16,000 rpm) for 40 min in a RC2-B Sorvall high speed refrigerated centrifuge (2–4°C) with a SS-34 rotor (Sorvall, Norwalk, CT) and the supernatant was filtered through Whatman No. 3 filter paper (Whatman, Maidstone, England). Total heme pigment was measured with this water extract by direct spectrophotometric measurement at 525 nm in a Hitachi 100-60 spectrophotometer (Hitachi, Tokyo, Japan). Calculations were made using a millimolar extinction coefficient of 7.6 (Bowen, 1949) and a molecular weight of 16,110 for myoglobin (Drabkin, 1978). Heme iron was calculated based on myoglobin having 0.35% iron (Drabkin, 1978).

Nonheme iron assay

The determination of nonheme iron was carried out according to Schricker et al. (1982), and the suggestions of Rhee and Ziprin (1987) for an "extract" blank with some modifications in the preparation of the incubated extract for iron determination. Procedures were also modified from Gomez-Basauri and Regenstein (1992). Fish slurry (15g) was weighed into a screw cap test tube and 0.2 mL 0.39% sodium nitrite added. The test tubes were vortexed, 15 mL of the acid mixture (6N HCl and 40% TCA (Fisher)) were added, the tubes tightly capped, vortexed, and placed into an incubator set at 65°C (Model R25, New Brunswick Scientific, New Brunswick, NJ). Samples were incubated for 20 hr with shaking (100 rpm), left to cool at room temperature for 3 hr, vortexed, and quantitatively transferred to 50

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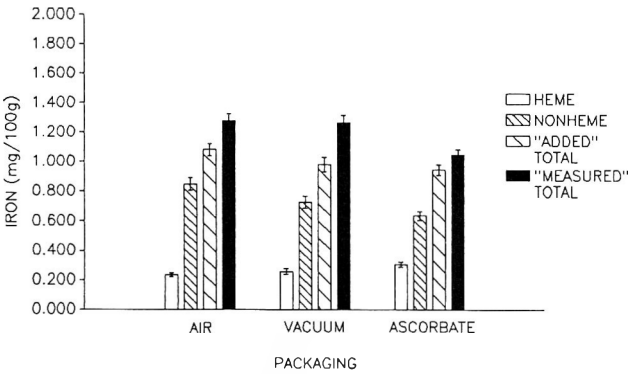

Fig. 1—Packaging effect on the iron content of mackerel fillets. "Added" total (heme plus nonheme), "Measured" total (direct determination). Error bars are standard errors.

mL polypropylene centrifuge tubes. After centrifugation at 3,615 × *g* (5,500 rpm) for 10 min (RC2-B centrifuge, SS-34 rotor), 1 mL of the supernatant was transferred to a borosilicate culture tube and 5 mL of the bathophenanthroline color reagent were added. The tube was mixed on a vortex and left to stand for 10 min after which the absorbance was read at 540 nm.

Extract and reagent blanks

The determination of the incubated "extract" blank was done according to Rhee and Ziprin (1987). Supernatant (1 mL) was transferred to a borosilicate tube and 5 mL of the "color" reagent were added (the part corresponding to the bathophenanthroline was replaced with ddd water). This absorbance was subtracted from the previous sample absorbance. A reagent blank (TCA-HCl, sodium nitrite, and ddd water instead of sample) was carried through all of the procedures and its absorbance was also subtracted from the sample readings. The nonheme iron content of the samples was determined using an iron standard curve (0.00–0.66 mg/100 mL). The iron standards were diluted with the reagent blank solution.

Total iron assay

Some modifications were introduced to the method described by Miller (1987) and Gomez-Basauri and Regenstein (1992). Fish slurry (15g) was weighed (to the nearest 0.01g) and placed in an ignition test tube. The samples were heated for 2 hr in the muffle furnace set at 250°C instead of 1 hr as previously described. After wet ashing the residue was too dark in some instances. It was visually observed that the final ash residues were whitish-gray when the acid residue had a light brown to yellowish color. More nitric acid was added to provide a better digestion. The samples were re-ashed at 450°C for 2 hr (an increase of 1 hr). The ash at this point usually had a white color; if not, the nitric acid digestion was repeated.

RESULTS & DISCUSSION

Iron content of mackerel fillets

Fish fillets dipped in ascorbic acid had a higher percentage of heme iron and less nonheme iron than vacuum and air packaged fillets (Fig. 1). The air pack had the highest nonheme iron and lowest heme iron content of all 3 packaging treatments. Fillets stored in air at −14°C had lower heme iron values than the other temperatures of storage for the same treatment (Fig. 2a). Fillets dipped in ascorbic acid had higher heme iron than air and vacuum treatments stored at −14°C, −20°C, and −40°C. No differences were observed between temperatures for the ascorbic acid treatment. Vacuum and air packed fillets showed no differences in heme iron content when stored below −14°C. Air samples had higher nonheme iron at all temperatures of storage when compared to vacuum or ascorbic acid treatments except at −30°C where the vacuum treatment was significantly higher than the ascorbic acid treatment (Fig. 2b).

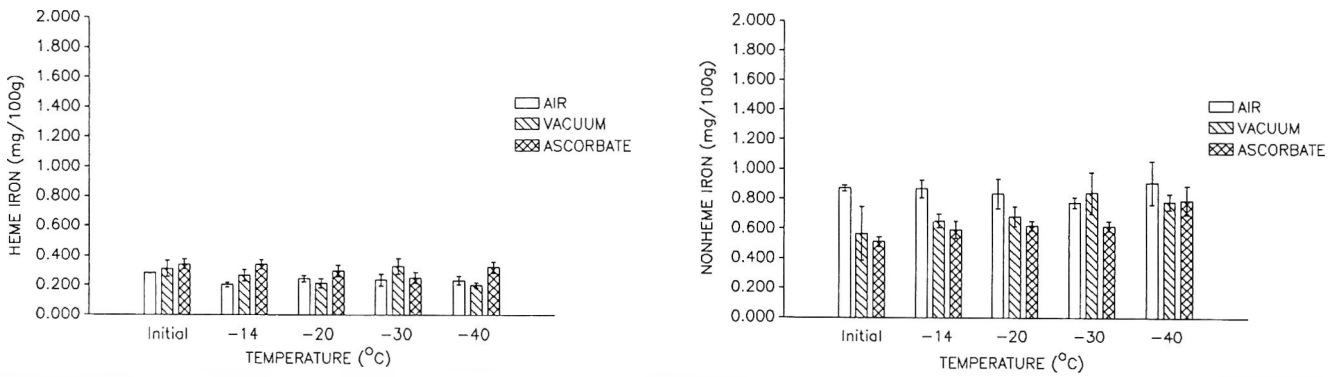

Fig. 2—Packaging and storage temperature effects on the iron content of mackerel fillets: (a) heme; (b) nonheme. Error bars are standard errors.

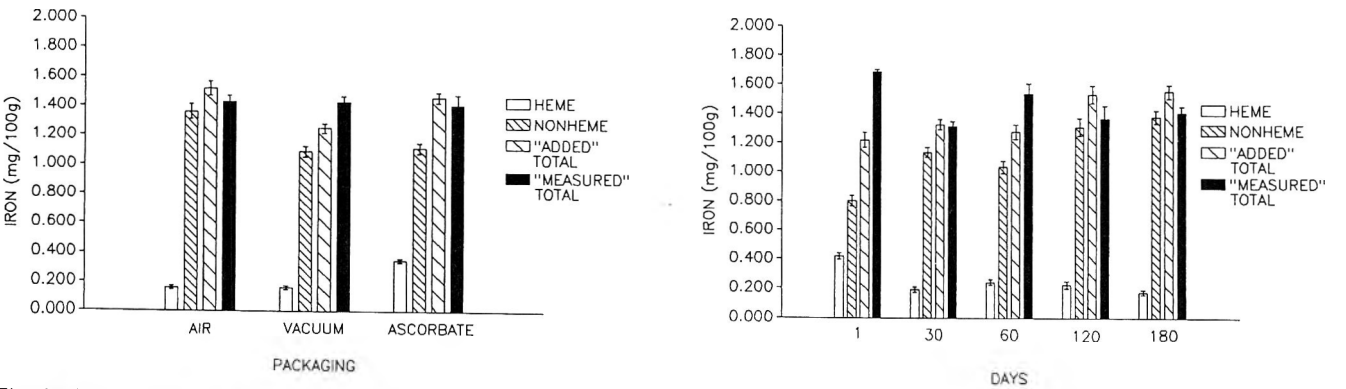

Fig. 3—Iron content of H&G mackerel mince: (a) packaging effect; (b) time effect. "Added" total (heme plus nonheme), "Measured" total (direct determination). Error bars are standard errors.

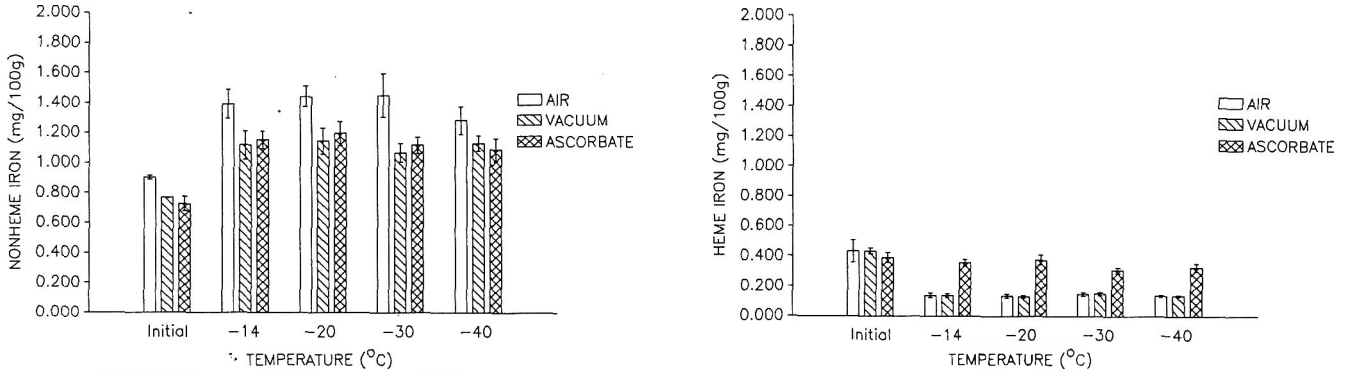

Fig. 4—Packaging and storage temperature effects on the iron content of H&G mackerel mince: (a) nonheme; (b) heme. Error bars are standard errors.

Iron content of H&G mackerel

Ascorbic acid treated H&G mackerel mince had more heme iron than air and vacuum packed H&G mince samples, while nonheme iron levels were lower in vacuum and ascorbic acid than in air (Fig. 3a). A significant decrease in heme iron of H&G mackerel mince after 30 days of frozen storage was observed (Fig. 3b). At the same time, nonheme iron levels increased significantly after 30 days of frozen storage. During the last stage of storage (120–180 days) nonheme iron was significantly higher than during the first stage (30 and 60 days).

Effects of different packaging and temperatures of storage on the nonheme iron content of H&G mackerel mince (Fig. 4a) were compared. Storage in air resulted in significantly higher levels of nonheme iron than in vacuum or ascorbic acid treatments regardless of temperature used except at −40°C, (where no significant difference with vacuum treatment was observed). Heme iron in minces treated with ascorbic acid was significantly higher than air and vacuum samples at all temperatures of storage (Fig. 4b). No significant differences in heme iron content between minces stored in air or vacuum were observed.

Our results suggest that vacuum packaging was not as effective in preventing heme iron breakdown as ascorbic acid, which has been suggested to work as an antioxidant with fatty fish (Hwang, 1988).

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Textural Changes of Canned Chum Salmon Related to Sexual Maturity

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ABSTRACT

Eating quality of chum salmon decreases as the fish migrate towards natal streams to spawn. Fish were assigned to maturity grades according to outward appearance and processed according to normal commercial practice. Texture of the canned product was assessed by nine trained sensory judges and by instrumental texture profile analysis (TPA). Both methods revealed significant differences between grades but the sensory method was more sensitive. Standardization of scores of each judge reduced error due to tendency of judges to use different portions of continuous scoring lines. Major textural changes in canned salmon were not evident before the transition from grade 3, dark to grade 4, spawning fish.

Key Words: chum salmon, flavor, texture, sexual maturity, fish

INTRODUCTION

CHUM SALMON (*Oncorhynchus keta*) are harvested commercially in north Pacific coastal and inland waters, as they migrate back to their natal streams to spawn. They stop feeding before entering fresh water and must survive on lipid and protein stored in the muscle and other tissues. Stresses associated with migration include acclimatization to fresh water, an often lengthy fresh water migration, elevated water temperatures, bacterial and fungal disease and the physiological changes accompanying sexual maturation (McBride et al., 1965). One result is a decrease in eating quality of the fish. Quality deterioration includes softer texture, development of characteristic late-run flavor (Durance and Collins, 1991) and color losses (Reid, 1991). Even so, for effective resource management (Holmes, 1992), a large portion of the commercial catch is required to be late-run. (In addition, aboriginal salmon fisheries in British Columbia are largely late-run by treaty arrangement).

Previous studies have reported changes in color and proximate composition of chum salmon (Hatano et al., 1983; Ando and Hatano, 1986). Bilinski et al. (1984) reported the impact of sexual maturation in captivity on quality of canned coho salmon (*O. kisutch*) The objectives of our study were to quantify textural changes in canned chum of increasing maturity grades and to compare sensory texture and instrumental texture profile analysis (TPA) as methods for evaluation of this product.

METHODS

Maturity grades

Fish were obtained from British Columbia Packers Ltd. (Richmond, B.C. Canada), except spawning fish which were collected from Chilliwack salmon Hatchery (Chilliwack, B.C.). Fish were graded, dressed, washed, packaged in double 6 mil polyethylene bags and stored at −30°C. Fish were graded with the aid of a published set of nine illustrations, A to I, of maturing chum (Anon., 1986), with a tenth grade, J, added to include spawning fish. Grade 1 corresponded to categories A to D; grade 2, E to G; grade 3, H to I; and grade 4 category J. Grade 1, called silver-brights, were blue-black in color with a silvery sheen and no noticeable color changes or water marks. Grade 2, intermediates, showed loss of uniform silver skin color and distinct changes in form of perpendicular color bars, light red to dark green or black, and the skin was thick and covered with a layer of slime. Grade 4, spawning fish had a very dark green, black or yellow skin color, very thick skin and a heavy slime layer.

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Processing and sample preparation

After storage, fish were thawed overnight at −4°C; skin and major bones were removed; fish were cut into 3.8 cm thick steaks, canned and retorted at 120°C for 65 min as previously described (Durance and Collins, 1991). Cans were held for 1 mo at room temperature (≈23°C) before being tested. From each fish 3 cans were opened, drained and flaked together with two forks (3-5mm flakes) to form one experimental unit. Half of each unit was used for sensory and half for instrumental analysis of texture. Eight grade 1, five grade 2, seven grade 3 and eight grade 4 fish were examined.

Sensory analysis of texture

Texture analysis was modeled after the General Foods Texture Profile method (Brandt et al., 1963; Szczesniak et al., 1963). Nine panelists participated in three training sessions to develop by consensus a list of texture attributes for canned salmon as previously described (Durance and Collins, 1991). Briefly, samples were examined in 10 sessions, with each judge evaluating not more than four samples/session. Panelists compared salmon samples to canned reference samples; commercial flaked ham, a soft, juicy non-fibrous food and commercial, solid albacore tuna, a firm, dry, fibrous food. Reference samples were from one production lot. Panelists scored samples with a vertical line across a horizontal scale of six arbitrary units length; anchor points represented reference samples. Sensory judges were in individual booths under red lights. Each sensory attribute was judged independently, on differently coded samples, so as to eliminate halo effect in which scores assigned for one attribute may influence estimates of other attributes.

Instrumental analysis of texture

The Texture Profile Analysis method of Bourne (1978), based on compression of samples twice with a Universal Testing Machine (Model 1122, Instron Corp., Canton MA) was used. Five samples of flaked salmon (10g each) from each experimental unit were reformed by gentle pressure between nylon disks, in a 50 mL disposable syringe which had been severed at the zero line, to yield 2.5 cm diameter by 2 cm thick disks (Durance and Collins, 1991). Digitized force/deformation curves were collected and analyzed as previously described.

Statistical analysis

Experiments were unbalanced designs {i.e. unequal numbers of fish (F) per grade (G)} in which fish were nested within grades. For sensory attributes, both raw scores and standardized scores, in which judge (J) effects were removed by z transformation of all scores within each J, were examined. Model 1; raw score = overall mean + G + J + F(G) + G*J + error: Model 2, standardized score = overall mean + G + F(G) + G*J + error. Instrumental data were square root transformed to reduce heterogeneity of variance {e.g. $(\text{cohesiveness})^{1/2}$ = overall mean + G + F(G) + error}. Analysis utilized SAS statistical software (SAS institute Inc., Cary, NC).

Table 1—Analysis of variance of raw sensory texture scores of canned chum salmon, graded according to sexual maturity

		Firmness	Dryness	Fibrousness	Chewiness
Grade	F-ratio	7.06	13.86	22.68	8.22
	P-value	0.0031	<0.0001	<0.0001	0.0015
Fish(Grade)	F-ratio	5.31	3.22	3.47	4.97
	P-value	<0.0001	<0.0001	<0.0001	<0.0001
Judge	F-ratio	3.14	0.77	7.95	3.27
	P-value	0.0020	0.6268	<0.0001	0.0014
Grade*Judge	F-ratio	1.67	0.97	1.54	1.63
	P-value	0.0275	0.5008	0.0546	0.0336

Table 2—Analysis of variance of standardized[a] sensory texture scores of canned chum salmon, graded according to sexual maturity

		Firmness	Dryness	Fibrousness	Chewiness
Grade	F-ratio	7.24	14.33	21.83	8.47
	P-value	0.0028	<0.0001	<0.0001	0.0013
Fish(Grade)	F-ratio	5.19	3.15	3.59	4.97
	P-value	<0.0001	<0.0001	<0.0001	<0.0001
Grade*Judge	F-ratio	1.44	0.83	1.01	1.48
	P-value	0.0874	0.6974	0.4529	0.0715

[a] Scores of individual attributes were standardized within each judge.

Table 3—Mean, raw sensory scores of texture of canned chum salmon of increasing maturity grades

Grade	Firmness	Dryness	Fibrousness	Chewiness
1	7.7[a](0.9)[c]	8.3[a](0.8)	8.6[a](0.8)	7.4[a](0.9)
2	6.8[a](1.2)	7.2[a](1.1)	7.8[a](1.0)	7.1[a](1.0)
3	6.5[a](1.2)	5.2[b](0.9)	7.0[a](0.9)	7.4[a](0.9)
4	3.1[b](0.8)	3.8[b](0.8)	2.8[b](0.5)	3.1[b](0.7)

[a,b] Different superscripts within a column indicate differences between adjacent grades, P<0.05.

[c] Standard error of mean score of nine judges.

RESULTS & DISCUSSION

Sensory evaluation of texture

Analysis of variance (ANOVA) of raw sensory scores indicated significant differences between grades for each sensory attribute tested; firmness, dryness, fibrousness and chewiness. Variation between fish within a grade was also highly significant for all attributes, indicating that texture of different fish was far from homogeneous, even within a grade.

Judge effects were significant ($P < 0.05$) in three of the four attributes and grade by judge interaction was significant for two attributes (Table 1), although fish(grade)*judge interaction was not significant. These, together with an examination of individual scoring patterns of judges, lead to the conclusion that despite training and references samples, judges tended to use different portions of the linear scale. For example, judge 'X' used only the right half while judge 'Y' used the full scale. Both means and variances for identical sets of samples were different between judges. Mean differences may be corrected by blocking for judges and nonhomogeneous variance is typically reduced by data transformation, (such as square roots). In this case however, standardization of scores within each judge was expedient. Standardization forced all judge scores into the same scale. ANOVA of standardized scores resulted in a slight increase in the power of the analysis. P values of grade effects were reduced and the grade × judge interaction was nonsignificant (Table 2). Perhaps a more substantial benefit was the improved clarity of data presentation (Fig. 1). Standardization removed the judge effect by calculation, and error bars were reduced, compared to raw score means. Mean standardized scores showed a clear trend towards decreased firmness, dryness, fibrousness and chewiness as maturity increased.

Single degree of freedom contrasts were employed to test for differences between means of adjacent grades (Table 3). The largest differences in texture occurred in the transition from grade 3 to grade 4. All possible comparisons were not tested (e.g. grade 2 vs grade 4) as this would reduce the power of the test. It is very possible however, that real differences did occur between, for example, firmness of grade 1 and grade 3.

Table 4—Mean instrumental TPA parameters of four grades of canned chum salmon

Parameter[c]	Grade 1		Grade 2		Grade 3		Grade 4	
Area1	202.	(25)[d]	132.	(32)	170.	(20)	139.	(29)
Area2	78.5	(9.6)	57.	(11.5)	70.9	(15.7)	48.7	(11.0)
*Hard13	8.3[a]	(1.0)	5.2	(1.2)	6.7	(0.8)	5.1[b]	(1.1)
Hard2	5.6	(0.7)	3.5	(0.8)	4.5	(0.6)	3.5	(0.6)
*Cohesiveness	1.06[a]	(0.04)	1.08[a]	(0.03)	1.04[a]	(0.02)	1.16[a]	(0.03)
Springiness	1.83	(0.13)	1.99	(0.07)	2.00	(0.11)	1.64	(0.18)
Slope1	36.3	(5.9)	19.2	(4.8)	28.8	(4.4)	20.4	(4.7)
*Slope2	38.2	(6.0)	20.6	(5.8)	30.2	(4.9)	20.5[b]	(6.1)
Gummi.	21.5	(2.7)	14.1	(3.4)	17.9	(2.4)	14.6	(3.1)
Chewi.	96.4	(14.1)	70.9	(17.6)	84.1	(12.3)	60.6	(16.0)

* Significant differences between grades, P<0.05.

[a,b] Different superscripts within a row indicate significant differences between grade means, P<0.05.

[c] Two outliers removed from data set.

[d] Standard errors of five replicate measures in brackets.

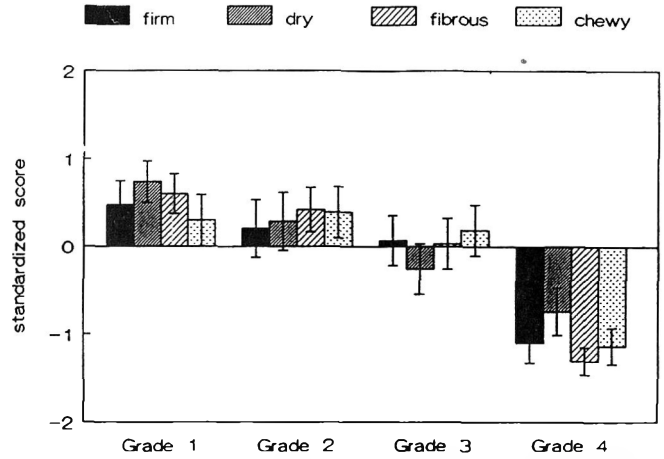

Fig. 1—Average standardized sensory scores, ± standard error, of four grades of chum salmon. Samples with greater firmness, dryness, chewiness or fibrousness than the grand mean for each attribute were scored greater than zero.

Instrumental TPA

Variance of raw TPA data increased with increasing texture parameters such as hardness and cohesiveness. These values tended to decrease with grade. Apparently texture variability was greatest between immature fish while the texture parameters of canned spawning fish were more homogeneous. Square root transformation of the data was performed to homogenize variances and avoid violation of the assumptions of ANOVA. Mean values of TPA parameters of flaked, reformed samples were determined (Table 4). Two samples were deleted from the data set and not replaced, because their standard deviations between replicate measures were unusually high. Only cohesiveness, slope2 and hardness1 were significantly different at $p < 0.05$.

Reproducibility of TPA parameters was affected by sample preparation. Johnston (1981) found variations in instrumental texture with position on the fish. Aksnes et al. (1986) reduced variation by using only the middle portion of fillets for sensory analysis. Borderias et al. (1983) found better correlations between instrumental-compression and sensory measures for minced fish than for fillets. Szczesniak (1987) stated that mincing fillets effectively eliminated heterogeneity within the sample. In our study, three cans from different locations on the

Table 5—Correlation coefficients (r) between instrumental TPA parameters and sensory attributes of canned chum salmon

Instrumental parameters	Sensory attributes[a]			
	Firmness	Dryness	Fibrousness	Chewiness
Area1	0.347	0.239	0.324	0.366
Area2	0.432	0.235	0.404	0.451
Hardness1	0.387	0.197	0.323	0.375
Hardness2	0.376	0.182	0.316	0.378
Cohesiveness	0.788*	0.521*	0.827*	0.815*
Springiness	0.411	0.325	0.538*	0.489
Slope1	0.388	0.176	0.292	0.379
Slope2	0.410	0.182	0.328	0.413
Gumminess	0.314	0.143	0.237	0.324
Chewiness	0.344	0.200	0.319	0.385

[a] n = 19

* Significant at $P<0.05$.

fish were sampled randomly and combined to make an experimental unit. Because three cans were flaked and mixed together, location effects were reduced in replicate, reformed samples and fibre orientation was random. Flaking and reforming was justified because canned salmon is not commonly consumed in chunk form and because both instrumental and sensory analysis were performed with flaked samples. Nonetheless, variation between replicates was relatively large with coefficients of variation typically 25–30% (data not shown). Variation between standardized sensory scores for the same samples was only slightly worse at 25–36% of means.

Correlation between mean sensory attributes and mean instrumental TPA parameters of identical experimental units was low (Table 5). Only cohesiveness correlated significantly ($P<0.05$). Cohesiveness increased as fibrousness, chewiness, firmness and dryness decreased. However, sensory judges were not asked to evaluate cohesiveness directly. Increased cohesiveness is consistent with known physiological changes in maturing salmon. Utilization of myofibrillar proteins for energy results in a relative increase in the concentration of connective tissue proteins. Upon retorting, connective tissue gelatinizes to a sticky, cohesive product.

Correlations between sensory attributes were high in spite of the fact that each attribute was evaluated separately, with judges unaware that they were evaluating the same experimental units.

CONCLUSIONS

MAJOR CHANGES in texture of canned chum salmon did not occur before the transition from grade 3 (dark) to grade 4 (spawning). At least in terms of texture, grade 3 fish may have greater marketability than is currently realized. Processing to reduce late-run flavor may be required however. Standardization within judges was an effective means of dealing with the tendency of different sensory judges to use different portions of the linear scale for scoring identical samples. As well as clarifying data presentation, standardization improved homogeneity of variance between judges and increased the power of ANOVA to distinguish between samples with small but real differences. Sensory evaluation was more efficient than instrumental TPA as a means of distinguishing between canned chum of different maturity grade. The four sensory attributes tested correlated highly with each other. Thus, evaluation of a single attribute, such as dryness or fibrousness would be sufficient for most studies of the type reported here.

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Sodium Asocorbate Affects Surimi Gel-forming Properties

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ABSTRACT

Effect of sodium-L-ascorbate (SA) on the gel-forming properties of Alaska pollock surimi was studied with respect to optimum level, surimi quality, gel setting, vacuum chopping, freeze-thaw and thermal stabilities, and salt and moisture levels. SA significantly improved gel cohesiveness and sensory firmness of fiberized products with maximum strengthening effect at a 0.2% level. Its effectiveness was directly related to surimi quality regardless of vacuum treatment indicating the unimportance of airborne oxygen. Freeze-syneresis promoted by ascorbate during frozen storage was moderated by the use of hydroxypropylated-modified starch.

Key Words: ascorbate, surimi, pH, freeze-thaw, thermal stability

INTRODUCTION

TEXTURAL PROPERTIES of surimi-based products are affected not only by the quality of surimi but also by the type and level of ingredients incorporated during preparation. In addition to ingredients that are used in surimi-based products, ascorbate is being considered as a potential gel-strengthening ingredient based on reports by Yoshinaka et al. (1972) and Nishimura et al. (1990).

Since L-ascorbic acid (L-AA) has an effect similar to an oxidizing agent in bread dough, first discovered by Jorgensen in 1939, it has been used widely as a dough improver in the baking industry (Cathcart and Edelmann, 1944; Tsen and Bushuk, 1963; Tsen, 1965; Carter and Pace, 1965; Meredith, 1965; Johnston and Mauseth, 1972; Elkassabany and Hoseney, 1980; Lillard, 1980; Lillard et al., 1982). The underlying mechanism for dough improvement was the formation of S-S bonds through oxidation of sulfhydryl (-SH) groups by reducing oxidized ascorbate. A similar effect was proposed in gelling of surimi (Yoshinaka et al., 1972; Nishimura et al., 1990), and the use of L-AA has been suggested for gel-strengthening in thermally induced surimi gels. The gel strength measured in those studies was based on the effect of L-AA only and the measurement made on molded products using the tension test (Shimizu et al., 1962) and the punch test with limited parameters. Because of its near neutral pH, sodium-L-ascorbate (SA) should have preferentially been considered over L-AA, along with erythorbic acid (ErA) for its wide availability. We thus decided to re-examine the gel-strengthening properties of SA and ErA in addition to L-AA on both molded and fiberized products with expanded parameters using compression and shear tests. Compared to Shimizu's tension and punch tests, the compression test measures the resistance of the gel sample to compressive failure which more correctly represents a cohesive and binding property (Lee and Chung, 1989).

The objectives of our present study were: (1) to investigate the effect of SA on the gelling potential of surimi of two different grades (regular and spawning); (2) to determine optimum conditions for the SA gel-strengthening effect in surimi gel; and (3) to determine the effect of SA on freeze-thaw stability of surimi-based products.

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MATERIALS & METHODS

Materials

Surimi of two different grades (regular and spawning), as well as from different lots, were used. The surimi manufactured during the spawning season was used only in comparing with regular surimi for optimum levels. Frozen surimi prepared from Alaska pollock (*Theragra chalcogramma*) was supplied by Profish International (regular) and Oceantrawl (spawning) (Seattle, WA). Both surimi contained 4% sorbitol, 4% sucrose and 0.2% sodium tripolyphosphate, and were stored frozen at -20°C. Use of surimi from the different lots reflected variations in gel strength among the control samples. To take this into consideration, each experiment always included a respective control. Sodium-L-ascorbate (SA) and L-ascorbic acid (L-AA) were provided by Takeda U.S.A., Inc. (Orangeburg, NY). Erythorbic acid (ErA) was purchased from Eastern Kodak Co. (Rochester, NY). All other chemical reagents were obtained from Sigma Chemical Co. (St. Louis, MO).

Preparation of surimi gel

Half-thawed surimi (-2°C) was vacuum-chopped at 1500 rpm in a Stephan chopper (model UM 5, capacity 2.5 kg, Stephan Machinery Corp., Columbus, OH) for a study of vacuum chopping effect or in a Hobart silent cutter for determination of the ascorbate effect on surimi gel properties, with salt, SA and chilled water. The Stephan chopper was equipped with vacuum and cooling units. Cooling was achieved by circulating ice chilled water through a jacket. The temperature of the surimi paste during chopping was kept at 10°C, to protect the protein functionality of surimi (Douglas-Schwarz and Lee, 1988). Calculated amounts of water were added to adjust the final moisture level of all formulations to 78%. Modified starch (Nustarr, Staley Mfg., Decatur, IL) was added to some batches to determine to what extent it could moderate the degree of freeze syneresis of surimi gels prepared with SA. In some batches, moisture levels were varied from 78 to 80% and salt from 0 to 1.5% to determine if ascorbate would increase moisture tolerance of the surimi gel and would decrease the salt level necessary for solubilization.

Molded samples were prepared by stuffing the paste into casings (Nojax cellulose, 25 mm diam), heating immediately 20 min at 90°C in a steam-cooker, except for those being subjected to a thermal stability test, and cooling 10 min in cold water (15°C). All gels were kept at 4°C for 24 hr prior to gel testing.

Fiberized gel samples were also prepared following the procedure described by Yoon and Lee (1990). The paste was extruded into a thin sheet through a rectangular nozzle with a narrow opening (1.5 mm × 25 mm). The extruded sheets were then partially heat-set 2 min at 110°C (air temperature) in the oven and scored into 1.5 mm-wide strips by a device similar to a noodle cutter. The resulting sheet of strips was folded manually into a rope, wrapped with a satin sheet and cooked 15 min at 90°C in a steam-cooker.

pH adjustment of SA, L-AA and ErA

When 0.2% (surmi weight basis) L-AA or ErA was added to surimi, the pH of the paste dropped somewhat (6.88 for L-AA; 6.82 for ErA) from that of the control (6.95), but considerably from that of the SA (7.10). In order to confirm the effect of pH alone, L-AA, ErA and SA were added separately to surimi after adjusting the pH of L-AA, (2.71) and ErA (2.75) to that of SA (6.99) by titrating with 0.1N NaOH or after adjusting the pH of SA and ErA to that of L-AA (2.71) with 0.1N HCl. The total water for 78% moisture accounted for that which had been used for titration.

Measurement of SA effect

SA was chosen in this study since its pH is near neutral. In order to determine the gel-strengthening effectiveness and optimum level of

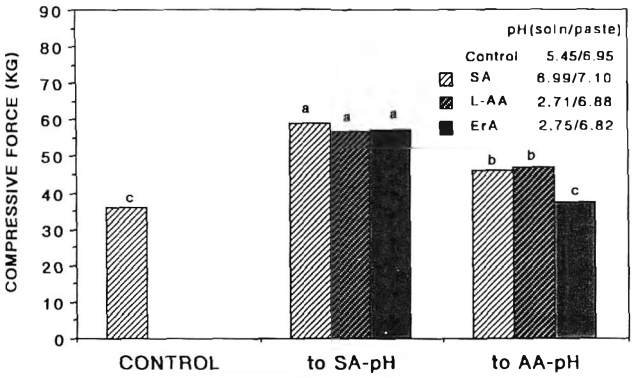

Fig. 1—Comparison of ascorbate, ascorbic acid and erythorbic acid for their gel-strengthening effect in surimi gels. The pH was adjusted to that of 0.2% SA-containing surimi paste (7.10) or 0.2% L-AA-containing surimi paste (6.88). Soln: solution (dist. water for control; SA, L-AA or ErA + dist. water, where each solute was dissolved in the predetermined amount of water to give 0.2% on a surimi weight basis at 78% moisture). SA = sodium ascorbate, L-AA = ascorbic acid, ErA = erythorbic acid. [a-c]Means (n = 5) with different letters are significantly different (p < 0.05).

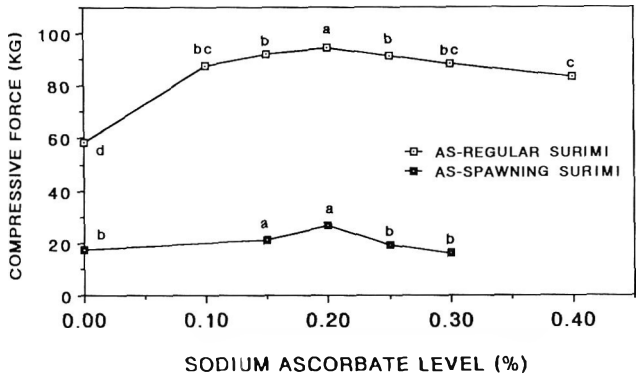

Fig. 2—Effect of ascorbate level on compressive force of surimi gel. Seven different levels of SA were incorporated into surimi of two different grades (regular and spawning). [a-c]Means (n = 5) with different letters within each source of surimi are significantly different (p < 0.05).

SA, 7 different levels of SA (0, 0.1, 0.15, 0.2, 0.25, 0.3 and 0.4%, on a surimi weight basis) were incorporated into surimi of 2 different grades (regular and spawning), where the spawning grade surimi was manufactured during the spawning season. Chopping was done in a Hobart silent cutter. The dose-response curves were prepared to determine the optimum level and the gel-strengthening effectiveness of SA.

Freeze-thaw and thermal stability

Samples with an optimum level of SA, both molded and fiberized samples, were subjected to two freeze-thaw cycles. Penetration force and expressible moisture were measured on molded samples. Shear test and sensory evaluation were conducted on fiberized samples to check the extent of rubbery texture development as a result of freezing. To determine whether the extent of heating affected the gel-strengthening property of ascorbate, products formulated with an optimum level of SA were subjected to 4 different heating times (10, 20, 30, and 40 min) at 90°C. Resulting products (both molded and fiberized) were evaluated for gel properties.

Vacuum chopping

To determine if vacuum chopping affected the gel-strengthening effect of ascorbate, surimi of 2 different grades were chopped with and without vacuum. Maximum vacuum was applied to exclude airborne oxygen from the surimi paste using a Stephan vacuum chopper operated at 65 mbar, the maximum vacuum achieved on the machine. This experiment was included to determine whether vacuum treatment may deplete the oxygen available for oxidation of ascorbate responsible for gel-strengthening of surimi. Gel samples were prepared at the optimum SA level and chopping time. Different times (2, 3, 4, 5 and 6 min) were tested to determine the optimum.

Instrumental measurement of gel properties

Testing parameters for gel properties included compressive force (cohesiveness), penetration force (firmness), expressible moisture (water binding ability) for molded products having a cylindrical geometry (25 mm length and 25 mm dia) and shear force for fiberized products. Compression and penetration tests were carried out following the procedures described by Lee and Chung (1989) and shear test by Yoon and Lee (1990) using an Instron testing machine (Model 1122, Instron Corp., Canton, MA).

Sensory evaluation

Sensory evaluation of the gel texture was conducted by an experienced trained panel of five people who had been routinely evaluating surimi-based products over the past several years. Samples were presented to panelists in the fiberized form at refrigerated temperature. They were asked to score 4 textural characteristics (firmness, chewiness, rubberiness, moistness) for intensity and desirability, as well as overall desirability using a structured 9-point scale (1:least, 9:highly) (Yoon and Lee, 1990).

Statistical analysis

Analysis of Variance was performed to determine the significance of variations among treatments. The Duncan's Multiple Range Test was used to determine the significance of differences between treatments. Correlation coefficients were also determined for degree of correlation between variables. Statistical analysis was carried out the Statical Analysis System (SAS Institute, Inc., 1982).

RESULTS & DISCUSSION

Effect of SA, L-AA and ErA

The pH adjustment of L-AA or ErA to SA (pH 7.10) increased gel strength significantly more ($p < 0.05$) than that to L-AA (pH 6.88) or ErA (pH 6.82, Fig. 1). In a previous study, Yoshinaka et al. (1972) also reported that both L-AA and ErA showed the same effect on gel strength at pH 6.4. However, this was in contrast to a bread-improving action in which L-AA is stereospecific and the other isomer (ErA) has a negligible activity (Lillard et al., 1982). No explanation has been given to such discrepancy in the activity of ErA between the surimi and the bread systems other than the difference in chemical nature of the samples.

Optimum SA level for gel strength

For both regular and spawning grade surimi, the gel strength (compressive force) of molded samples reached maximum compressive force at 0.2% SA with increases of 64% (regular) and 53% (spawning) over the control, respectively (Fig. 2). Thus, the SA's gel strengthening effect appears to be related to original surimi quality. Based on the current theory (Tsen and Bushuk, 1963; Tsen, 1965; Meredith, 1965; Lillard, 1980; Lillard et al., 1982), the gel-strengthening effect of SA can be explained by increased cross-linking through conversion of SH to S-S bonds as DHAA is reduced to SA. DHAA is formed as a result of oxidation of SA during the comminution process. The gel strength decreased steadily after the level reached 0.2%. This could be explained by the presence of ascorbate in the reduced form at an excessively high level (>0.2) which could hinder the formation of S-S bonds, resulting in reduction of gel strength.

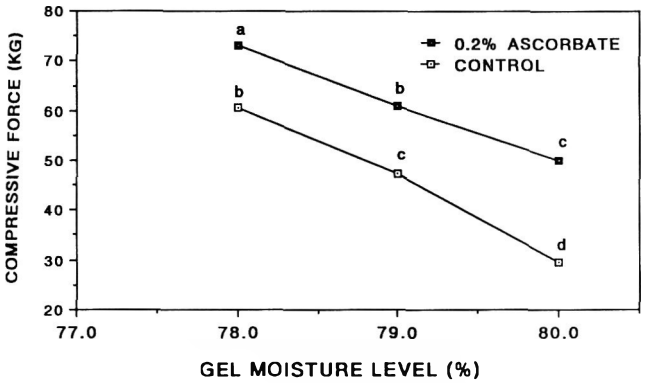

Fig. 3—Effect of ascorbate on moisture tolerance of surimi gel. The compressive force of the control at 78% moisture was equivalent to that of the 0.2% SA at 79.1% moisture. All gel samples were prepared with 6% modified starch (Nustar). [a-d]Means (n=5) with different letters are significantly different (p<0.05).

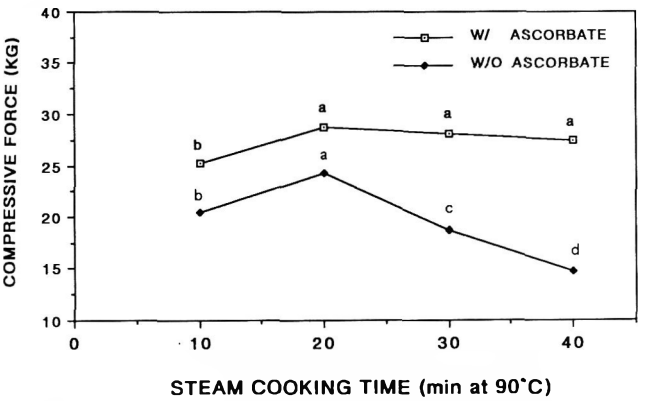

Fig. 4—Effect of cooking time on compressive force of surimi gel. [a-d] Means (n=5) with different letters within each group are significantly different (p<0.05).

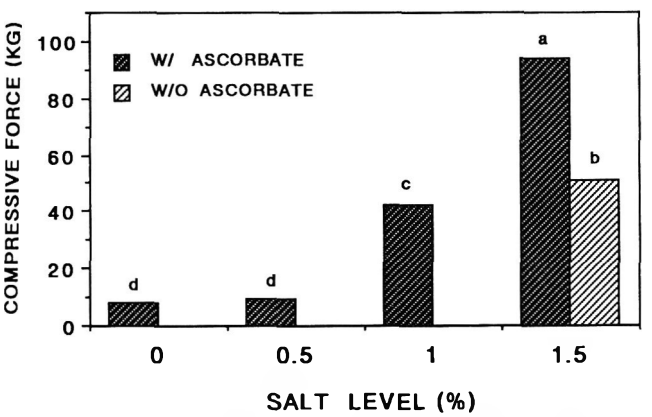

Fig. 5—Effect of ascorbate on compressive force of surimi gel at varying salt levels.[a-d] Means (n=5) with different letters are significantly different (p<0.05).

Moisture tolerance of surimi gel

The moisture tolerance of surimi gel was recorded with and without SA (Fig. 3). Gel-strengthening effect of SA was shown consistently throughout the moisture levels (78–80%). The gel strength with SA at 79.1% corresponded to that without SA at 78%. With addition of SA the moisture level could be increased at least 1% without reducing gel strength.

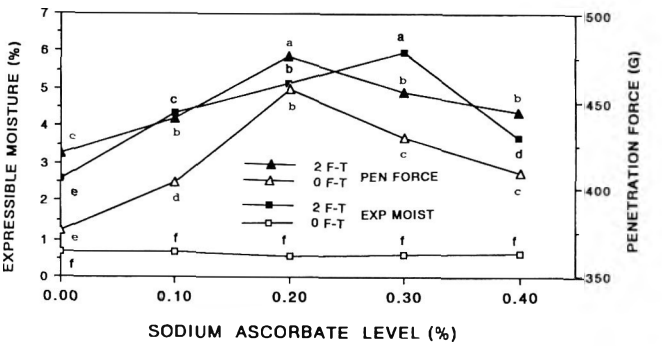

Fig. 6—Effect of ascorbate level on expressible moisture and penetration force of surimi gel after two freeze-thaw cycles. F-T: freeze-thaw cycles.[a-f] Means (n=5) with different letters are significantly different (p<0.05).

Thermal stability

The cohesiveness (compressive force) of both gels with and without SA treatment reached peak at 20 min and thereafter decreased with extended period of cooking at 90°C (Fig. 4). In gels without SA the decreases after 20 min cooking were significant, while in those with SA the decreases were insignificant ($p<0.05$). According to Douglas-Schwarz and Lee (1988), steam-cooking longer than 20 min may cause protein denaturation accompanied with extensive unfolding and aggregation of protein molecules leading to an unfavorable alteration in the network structure. Such a structural change in the gel network reduces water binding and gel cohesiveness.

It was likely that extensive heating at a high temperature of 90°C would affect unfavorably the gel-strengthening effect of SA in light of the thermally labile nature of SA. However, no significant reduction in gel strength was observed after SA treatment. This suggested no significant thermal degradation occurred to SA in the cooking period. This leads to a postulation that SA may have strengthened the protein gel network through crosslinking which resists a thermal breakdown of the gel network.

Salt level

Effect of SA on surimi gel property as affected by salt level was followed (Fig. 5). With addition of 0.2% ascorbate, 1% salt (on a surimi weight basis) produced about 80% of the gel strength that 1.5% salt had, without SA. That is, a reduced gel strength by less salt could be compensated by addition of SA.

Freeze-thaw stability

In molded products, after two freeze-thaw cycles designed for an accelerated frozen storage study, there were significant increases in expressible moisture and penetration force (Fig. 6). Those increases indicated an increase in moisture separation and rubberiness. This is a result of increased freeze syneresis which was promoted by S-S crosslinking in the presence of SA.

In fiberized products, maximum firmness was attained at 0.2% ascorbate when measured by shear force and sensory panel (Fig. 7 and 8), where the shear force values correlated moderately ($r=0.80$) with sensory firmness. This coincided with previous results of compressive force of molded products (Fig. 2). After two freeze-thaw cycles, however, shear force and sensory rubberiness markedly increased with maxima at 0.3% and 0.2% levels, respectively (Fig. 7 and 9), where shear force values correlated well with sensory rubberiness scores ($r=0.88$). This suggested that the shear test can be useful in determining the degree of rubbery texture of fiberized products

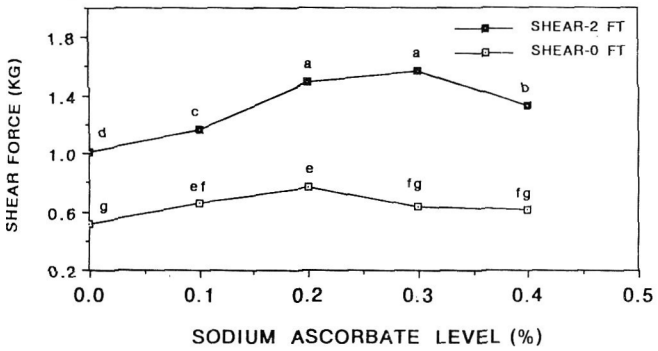

Fig. 7—Effect of ascorbate level on shear force of fiberized product. [a-g]Means (n=5) with different letters are significantly different (p<0.05).

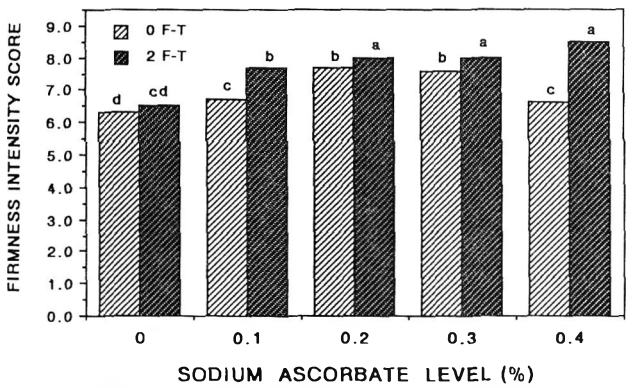

Fig. 8—Effect of ascorbate level on firmness of fiberized products. Sensory score ranges from 1 to 9 where 1=least, 5=moderately, 9=highly. [a-f]Means (n=5) with different letters are significantly different (p<0.05).

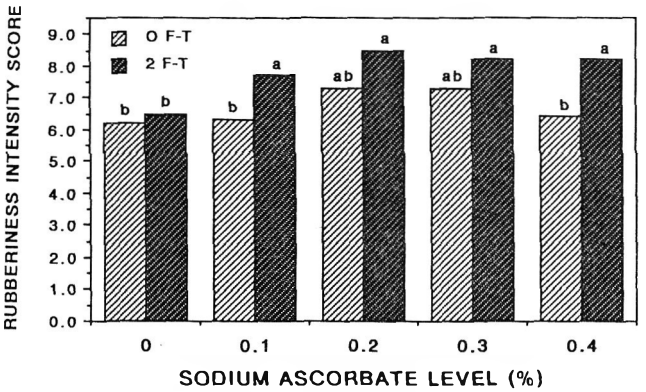

Fig. 9—Effect of ascorbate on rubberiness intensity score of fiberized products. [a-b]Means (n=5) with different letters are significantly different (p<0.05).

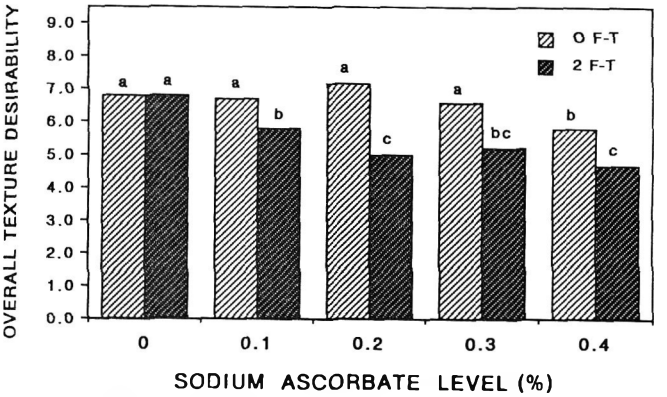

Fig. 10—Effect of ascorbate on overall desirability score of fiberized products before and after two freeze-thaw cycles. Sensory score range from 1 to 9 where 1=least, 5=moderately, 9=highly. [a-c]Means (n=5) with different letters are significantly different (p<0.05).

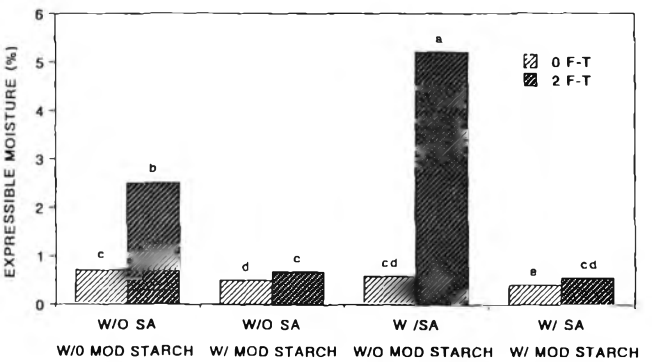

Fig. 11—Effect of ascorbate on expressible moisture of surimi gels with and without modified starch after two freeze-thaw cycles. SA: 0.2% sodium ascorbate. [a-e]Means (n=5) with different letters are significantly different (p<0.05).

Table 1—Comparison of vacuum chopping effect of SA on gel compressive force

Type of surimi	Compressive force (Kg)			
	w/o vacuum		w/ vacuum	
	0% SA	0.2% SA	0% SA	0.2% SA
spawning	9.3±0.62[d]	14.3±2.05[c]	9.7±0.47[d]	14.3±0.94[c]
regular	12.8±0.62[c]	24.2±2.09[b]	14.8±0.47[c]	27.7±0.47[a]

[a-d] Means (n=5) with different letters are significantly different (p<0.05).

after frozen storage. The marked increases in shear force and rubberiness after frozen storage clearly indicate that pronounced freeze syneresis occurred in the gel matrix, resulting in development of a hard, rubbery texture. The SA level for the maximum shear force (Fig. 7) coincided with that for the maximum expressible moisture (Fig. 6). The extent of freeze syneresis of a gel matrix (a ratio of shear force with SA to that without SA) after freeze-thaw cycles became steadily greater (1–1.55) with an increase in level of SA up to 0.3% (Fig. 7).

The overall texture desirability of fiberized surimi samples (Fig. 10) was in close agreement with sensory firmness with a peak at 0.2%, where a significant drop in the score was observed at 0.4%. The lower score was not related with any measurements. After 2 F-T cycles, the desirability score significantly decreased from 0.1% SA on with further drops but not continuously with an increase in SA level. The decrease in desirability score after two F-T cycles reflected increases in expressible moisture (Fig. 6) and sensory rubberiness due to freeze syneresis.

The freeze syneresis as determined by expressible moisture was, however, significantly ($p<0.05$) reduced by the use of hydroxypropylated-modified starch at 6% (Fig. 11). The use of 6% was in accordance with the level previously reported optimum (Lee and Kim, 1986). Without modified starch, expressible moisture markedly increased from 0.6% to 5.2% while a slight increase from 0.41% to 0.57% was noted with modified starch. This indicated that addition of modified starch would moderate increase in expressible moisture due to freeze syneresis.

Effect of vacuum chopping

When the Stephan vacuum chopper was used, the gel strength (compressive and penetration force) reached maximum at 3 or 4 min and gradually decreased afterwards. We decided to use 4 min as an optimum chopping time. Also, we assumed that vacuum could remove airborne oxygen for oxidation of ascorbate which is required for gel-strengthening effect of ascorbate. But, the results showed no significant differences in compressive force between gels prepared with and without vacuum (Table 1). We hypothesized that the oxygen in the aqueous phase from added water and surimi must be the source for the oxidation of ascorbate. Removal of oxygen in the aqueous phase by nitrogen gassing was thus considered as a next step in studying the role of oxidation of ascorbate in gel-strengthening effect. Otherwise, the present observation may implicate a negligible role of oxygen in the surimi gel-strengthening of ascorbate.

CONCLUSIONS

ALTHOUGH SA significantly improved gel cohesiveness and sensory firmness scores of both molded and fiberized products, it promoted freeze syneresis resulting in increased expressible moisture and rubbery texture development. Increased freeze syneresis after freeze-thaw cycles was moderated by addition of hydroxypropylated-modified starch. The gel-strengthening function of ascorbate in the spawning-season surimi was not as effective as in the regular-season surimi, suggesting that it was related to original surimi quality factors. Vacuum treatment did not affect gel-strengthening effect of SA indicating the unimportance of oxygen in activation of SA.

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*SPRAY-DRIED EGG WHITE. . .*From page 1322

CONCLUSIONS

TO MAKE ionic effects on functional performance more apparent, all data were pooled (Table 5). Cu^{++} excelled in all functional categories. These data suggest that trace amounts of copper would be a suitable added ingredient to both U- and H-SDEW. Fe^{+++} and Al^{+++} caused some improvement but the adverse red color of the Fe-conalbumin complex would negate its use. Zn^{++} offered little or no potential application in this regard. Cation concentration effects were not as effective as expected.

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Semi-continuous Hydrolysis of Sweet Potato Raw Starch by *Chalara paradoxa* Glucoamylase

TAKAHIRO NODA, TOSHIO OHTANI, TAKEO SHIINA, and YOSHIHIKO NAWA

ABSTRACT

Effect of preservatives was investigated on semi-continuous digestion of sweet potato raw starch by *Chalara paradoxa* glucoamylase which digests both raw and gelatinized starch. Potassium metabisulfite strongly inhibited raw starch digesting activity (RDA) although gelatinized starch digesting activity was only slightly inhibited. Potassium sorbate, among the preservatives examined, showed the weakest inhibition (below 10%) on RDA. In the presence of potassium sorbate, semi-continuous digestion of sweet potato raw starch in a 20L scale hollow fiber UF membrane pilot reactor proceeded efficiently at 30% (w/v) initial concentration of raw starch at pH 5.0 and 40°C. Glucose production was 5.7 to 6.0 g/hr/L.

Key Words: starch, sweet potato, hollow fiber reactor

INTRODUCTION

SWEET POTATO, a high starch content crop, is resistant to typhoons, drought, pests and diseases, and has high starch productivity (5.5 to 10 tons/year ha in Japan). Recent interest in energy conservation and in renewable energy sources has focused considerable attention to raw starch hydrolysis by amylase. Kainuma et al. (1985) reported a novel glucoamylase from *Chalara paradoxa* for digesting raw starch. This enzyme had, besides gelatinized starch digesting activity (GDA), stronger raw starch digesting activity (RDA) than that of well known glucoamylases from *Rhizopus* sp. or *Aspergillus niger* (Ishigami et al., 1985a). However, product (glucose) inhibition limits its commercial utilization (Mikuni et al., 1987). Digestibility of different types of raw starch from some varieties of sweet potato has also been reported (Noda et al., 1992).

Commercial utilization of this enzyme necessitates the use of highly efficient bioreactors. Digestion of raw starch by *Chalara* glucoamylase in a membrane reactor seems to be the best approach. In this process, free enzyme is added into the stirred tank reactor equipped with a UF membrane, and continuous removal of glucose through the membrane eliminates end-product inhibition. Membrane bioreactors have been used by many authors (Cheryan and Mehia, 1986; Gekas, 1986; Belfort, 1989; Wandrey and Wichmann, 1987). However, limited information exists on digestion of raw starch in a membrane reactor (Matsumura and Hirata, 1989).

Chalara paradoxa glucoamylase was reported to have maximum activity at pH 5.0 (Ishigami et al., 1985b). The optimal pH indicates possible concurrent fungi or yeast activity during the process. Matsumoto et al. (1982) noted a need for prevention of microbial contamination when working with uncooked raw materials. Sterilization on the other hand, requires energy. Potassium metabisulfite, a conventional preservative in the brewing industry, was reported to have little effect on raw starch digestion by *Rhizopus* sp. (Ueda, 1982) and *Endomycopsis fibuligera* (Saha and Ueda, 1983) glucoamylases. However in our preliminary experiments, *Chalara paradoxa* glucoamylase was found to be strongly inhibited by potassium metabisulfite.

Our objective was to determine the influence of preservatives on *Chalara* glucoamylase activity and semi-continuous digestion of sweet potato raw starch in a 20 L hollow fiber UF membrane reactor.

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MATERIALS & METHODS

Raw starch and glucoamylase

Sweet potato raw starch was donated by Shikine Sangyo Co. (Kagoshima, Japan). *Chalara paradoxa* glucoamylase was prepared on a large-scale, by Meiji Seika Co. (Japan) according to the method of Ishigami et al. (1985a).

Batchwise digestion

Small scale (70 mL) digestion of raw starch was achieved in a 100 mL beaker placed on magnetic stirrer in an incubator (40°C) to provide uniform suspension and heating. During the course of the digestion, 10 or 35 mL samples were withdrawn. To each 10 mL sample, NaOH (final 25mM) was added to inactivate enzymes, then glucose was determined after removal of undigested raw starch by centrifugation. The 35 mL samples were centrifuged directly at 5°C, and RDA and GDA were determined on the supernatant as described later.

Large scale (20L) digestion was done in a 30L pilot reactor. During the course of the digestion, 10–20 mL samples were withdrawn directly from the reactor by pipette, mixed with NaOH, and centrifuged for glucose determination.

Semi-continuous digestion in bench-top membrane reactor

The bench-top semi-continuous reactor consisted of a 350 mL vessel (Diaflow Cell 8400) with a UF membrane (YM5, Amicon Co. molecular weight cut-off 5000). The reactor was placed in an incubator at 40°C. Samples (10 mL each) were withdrawn for glucose determination during the course of the digestion. When glucose concentration reached about 7% (w/v) where end-product inhibition was not prevalent, the reaction mixture was ultrafiltered to yield about 50 mL of retentate. Raw starch, potassium sorbate and buffer solution were reintroduced to the retentate to obtain the initial concentrations and volume (300 mL), then digestion was continued. This procedure was repeated three times.

Semi-continuous digestion in pilot membrane reactor

A schematic diagram of the pilot reactor is given in Fig. 1. In a 30L tank (1) (Komatsugawa Chemical Engineering Co., Ltd., Tokyo, Japan), 6 kg of raw starch (30% w/v) suspended in 50 mM acetate buffer (pH 5.0) supplemented with 40g of glucoamylase (0.2% w/v) and 2g of potassium sorbate (100 ppm) to a final volume of 20 L was added. The enzyme reaction was performed at 40°C with stirring at 350 rpm. Glucose concentration was monitored and determined with a Glucose analyser (6) (TOA Electronics Ltd., Tokyo, Japan), and raw starch concentration was monitored with a Laser turbidimeter (6) (Komatsugawa Chemical Engineering Co., Ltd., Tokyo, Japan). When glucose concentration reached about 7% (w/v), the product was automatically separated from the reaction mixture by crossflow filtration with a hollow fiber UF membrane module (4) by operating a pressure

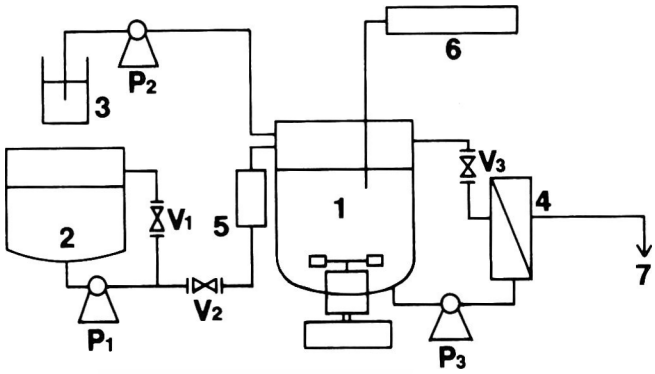

Fig. 1—Schematic diagram of the 20 L scale hollow fiber UF membrane pilot reactor: (1) Reactor; (2) Raw starch reservoir; (3) Buffer-Water tank; (4) Hollow fiber UF membrane (polyethersulfone); (5) Flow meter; (6) Sensors (for temperature, pH, level, glucose concentration and starch turbidity); (7) Balance (permeated glucose solution); (V1) and (V2) Shut off valves; (V3) Pressure control valve; (P1) Circulation pump; (P2) Feed pump; (P3) Pressure pump.

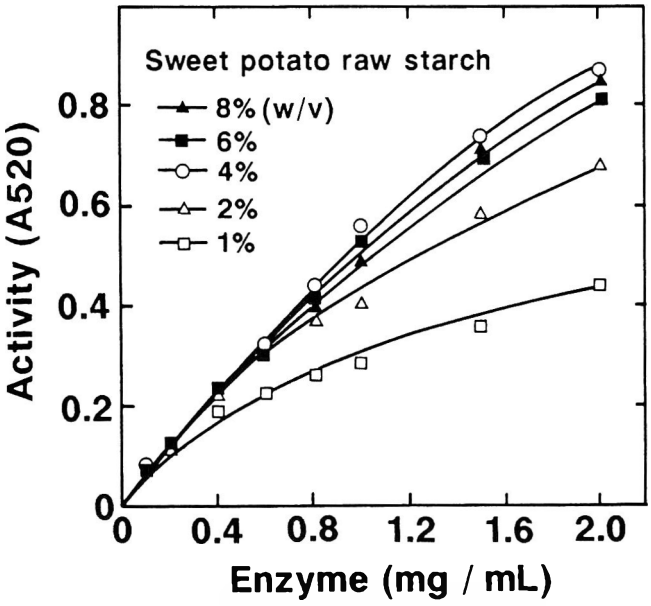

Fig. 2—Enzyme activity at different concentrations of raw starch and enzymes.

pump (P3) and opening a pressure control valve (V3). P3 was controlled by weighing the filtrate with an electronic balance. When raw starch concentration in the retentate reached 40%, operation of P3 was stopped and V3 was closed. Starch granules plugged the inlet of the hollow fiber membrane when concentration exceeded 40%. Make-up raw starch to replace that which was digested was added into the reactor from a reservoir tank (2) by closing valve V1 and opening valve V2. The buffer containing the preservative was fed from tank (3) by a feed pump (P2) to maintain the volume (level sensor, 6), and digestion was continued. The process was repeated. During digestion, raw starch in reservoir (2) was circulated by pump (P1) to prevent sedimentation of raw starch when V2 was closed and V1 was open. The hollow fiber UF membrane was a Polyether-sulfone (Dicel Chemical Co., with an effective membrane area 1.4 m^2 and a molecular weight cut-off 30000).

Measurement of enzyme activities

Prior to determination of RDA and GDA in samples from small scale (70 mL) batchwise digestions, the 30 mL supernatants were diafiltered at 5°C in a 50 mL Diaflow Cell (Amicon Co.,) equipped with a UF membrane (YM5, Amicon Co., molecular weight cut-off 5000) to a retentate volume of about 6 mL to remove glucose. Each

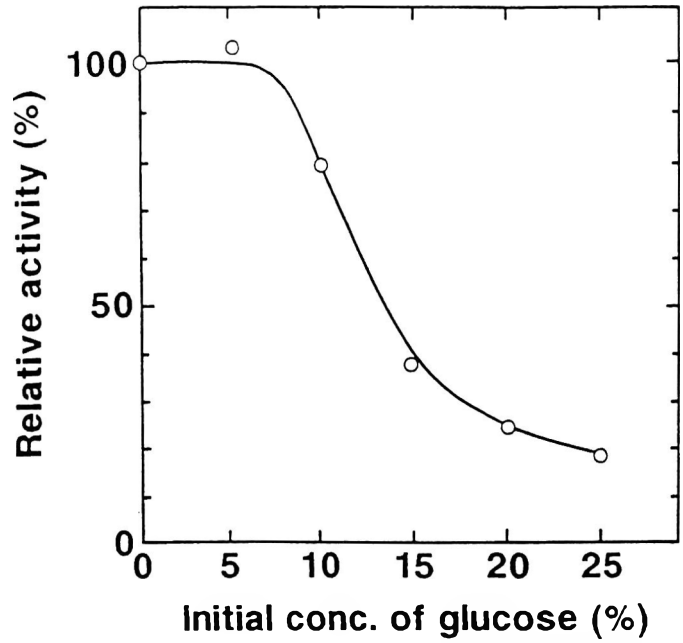

Fig. 3—Effect of initial concentration of glucose on digestion of raw starch by glucoamylase.

retentate was diluted with 24 mL of the buffer solution followed by diafiltration, and this procedure was repeated three times. No enzyme leakage or inactivation by the membrane was observed. The final retentate was used to assay for RDA and GDA.

RDA was determined by hydrolyzing raw starch (4% w/v, see Fig. 2 for initial concentration) with 1.0 mL of the final retentate at 40°C and pH 5.0 (50 mM acetate buffer to 10 mL total volume) with continuous shaking for 10 min. Enzyme reaction was stopped by adding NaOH. The mixture was centrifuged and glucose was analyzed in the supernatant.

GDA was determined by hydrolyzing cooked soluble starch (0.5% w/v, E. Merck, Darmstadt, Germany) with 0.2 mL of the final retentate at 40°C for 10 min at pH 5.0 (50 mM acetate buffer to a total volume of 2.0 mL). Enzyme reaction was stopped by adding NaOH, and glucose was determined.

Glucose determination

Glucose was determined according to the Somogyi-Nelson method (Nelson, 1944). In the semi-continuous digestion with the pilot reactor, a GLUCOSE ANALYSER (TOA Electronics Ltd., Tokyo, Japan) was used.

RESULTS & DISCUSSION

Stability of enzyme in presence of preservatives

Chalara glucoamylase is stable at 40°C and pH 5.0 (Ishigami et al., 1985b). The only reaction product is glucose (Kainuma et al., 1985). Strong end-product inhibition was reported for this enzyme in previous studies on alcohol fermentation of raw corn starch (Mikuni et al., 1987). Figure 3 illustrates end-product inhibition in the digestion of sweet potato raw starch. The inhibition was not observed below 7% of added glucose but was apparent at higher concentrations.

Figure 4 illustrates changes in RDA and GDA during the 70 mL batchwise digestion of sweet potato raw starch (10% w/v) in the presence of 350 ppm potassium metabisulfite. Glucose was produced at a high rate during the first 12 hr when glucose concentration reached 4%, but the rate decreased rapidly in the next 12 hr. Glucose concentration did not surpass 7.5% even after 144 hr of digestion. These results indicate inhibition of starch digestion. Also RDA decreased rapidly to 25% of the initial value during the first 12 hr (Fig. 4) followed by a gradual decrease. On the other hand, GDA decrease was much less than that of RDA.

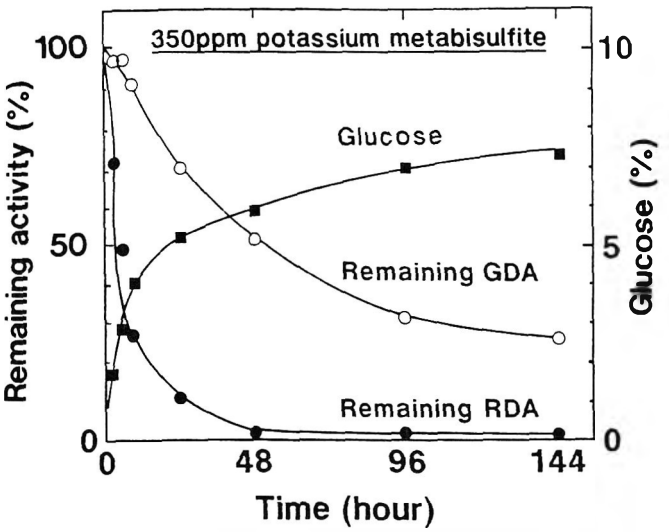

Fig. 4—Changes in RDA and GDA during batchwise digestion in the presence of potassium metabisulfite (350 ppm). The assay methods of RDA and GDA were described in MATERIALS & METHODS. Conditions for batchwise digestion: raw starch 10% (w/v); enzyme 0.2% (v/v); pH 5.0 (50 mM acetate buffer); temperature 40°C; total volume 70 mL.

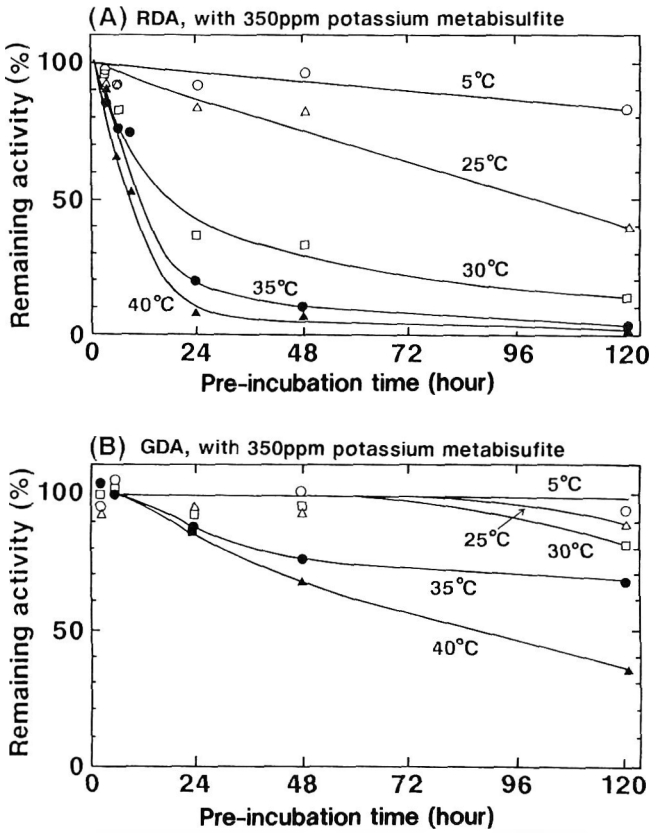

Fig. 5—Effect of pre-incubation temperature on RDA and GDA of glucoamylase in the presence of potassium metabisulfite (350 ppm). Glucoamylase 0.2% (w/v) was pre-incubated with potassium metabisulfite at 5, 25, 30, 35 or 40°C at pH 5.0 (50 mM acetate buffer), then remaining RDA and GDA were determined as described in MATERIALS & METHODS.

These results indicate that end-product inhibition was not the major factor responsible for the relatively low production of glucose, and suggest that inhibition of RDA by potassium metabisulfite was probably the predominant factor. This hypothesis was confirmed by pre-incubating the enzyme with potassium metabisulfite at different temperatures prior to digestion of raw starch.

Table 1—Influence of preservatives on digesting activities[a]

Preservatives	(ppm)	Remaining activity (%) RDA	GDA
Potassium sorbate	30	90	93
	100	91	100
	300	94	100
Sodium propionate	300	62	108
	1000	63	98
	3000	62	104
Benzoic acid	30	49	96
	100	44	96
	300	51	92
Potassium metabisulfite	50	90	102
	200	35	92
	350	7	95
None (control)		101	103

[a] After 72 hr pre-incubation of the enzyme (0.2%) with each preservative (24 hr as to potassium metabisulfite) at 40°C and pH 5.0, remaining enzyme activities were determined as described in MATERIALS & METHODS.

Figures 5(A) and 5(B) show effects of pre-incubation temperature on RDA and GDA, respectively, in the presence of 350 ppm potassium metabisulfite. At higher temperatures, RDA was very sensitive to potassium metabisulfite. RDA was only 20% or less of initial activity when pre-incubated at > 35°C for 24 hr, while it retained about 80% of its initial activity on pre-incubation at 5°C for 120 hr. On the other hand, GDA was less sensitive than RDA. Even after pre-incubation at 40°C for 24 hr, GDA was nearly 90% of the initial value. GDA decreased to less than 50% of the initial value only when pre-incubation was at 40°C for 120 hr. Potassium metabisulfite may have inhibited or denatured the enzyme.

Purified *Chalara* glucoamylase gave a single band on polyacrylamide disc gel electrophoresis, therefore both RDA and GDA were exhibited by the same protein (Ishigami et al., 1985b; Monma et al., 1989). The temperature dependent, selective inhibition by potassium metabisulfite on RDA suggests two possible sites in the enzyme for raw starch digestion as reported for glucoamylase from *Aspergillus awamori* (Hayashida et al., 1982). One is an active site for cleavage of the glucosidal bonds and the other is an affinity site for raw starch granules. The affinity site seems to be essential for initiation of raw starch digestion, and is sensitive to attack by potassium metabisulfite even at relatively low temperature.

It is clear that potassium metabisulfite cannot be used in the digestion of raw starch by *Chalara* glucoamylase. Therefore, other preservatives were examined. Table 1 shows the influences of four food preservatives on retention of RDA and GDA. Among them, potassium sorbate showed the weakest inhibition on RDA (less than 10% of the activity was lost). Sodium propionate and benzoic acid had little inhibitory effect on GDA, although they inhibited RDA considerably. Consequently, 100 ppm potassium sorbate instead of 350 ppm potassium metabisulfite was adopted in further experiments.

Figure 6 illustrates changes in remaining RDA and GDA during the 70 mL batchwise digestion of raw starch (10% w/v) in the presence of 100 ppm potassium sorbate. RDA was retained near its initial value (about 90%) for 72 hr. GDA changed little. Consequently, glucose concentration continued to increase even after prolonged digestion, in contrast to digestion in the presence of potassium metabisulfite. Sweet potato raw starch was almost completely hydrolyzed within 72 hr.

Semi-continuous digestion in bench-top membrane reactor

Glucose inhibition of enzyme activity was not exhibited until glucose exceeded 7% (Fig. 3). This indicates that digestion to produce < 7% glucose is feasible. Figure 7 illustrates data on semi-continuous digestion of raw starch (10% w/v) in the bench-

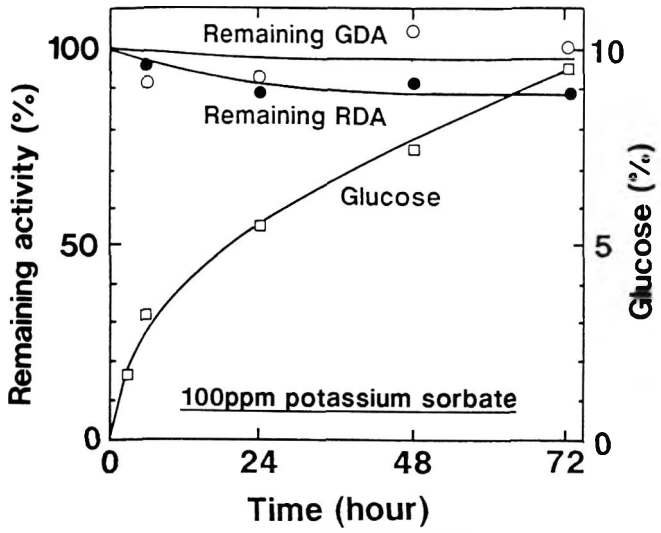

Fig. 6—Changes in RDA and GDA during batchwise digestion in the presence of potassium sorbate (100 ppm). The assay methods of RDA and GDA and the conditions for the batchwise digestion were the same as those in Fig. 4.

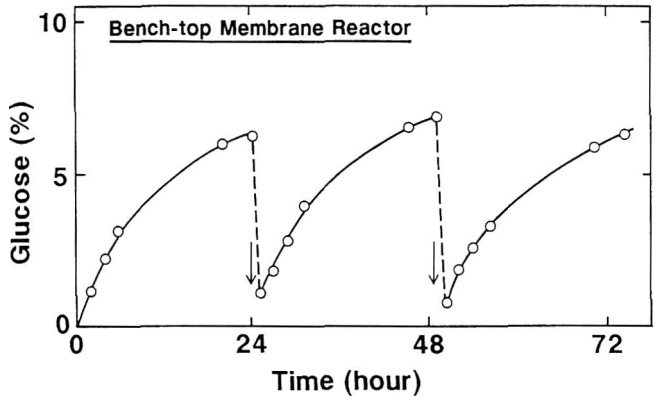

Fig. 7—Semi-continuous digestion of raw starch in the bench-top membrane reactor in the presence of potassium sorbate. The reactor vessel was partially emptied at the time indicated with arrows (↓) then re-filled. Conditions for digestion: raw starch 10% (w/v); enzyme 0.2% (w/v); potassium sorbate 100 ppm; pH 5.0 (50 mM acetate buffer); temperature 40°C; total volume 300 mL.

top UF membrane reactor in the presence of potassium sorbate. No decrease in production of glucose was observed in three cycles. Semi-continuous digestion of raw starch was efficiently carried out with the UF membrane reactor.

Semi-continuous digestion in pilot membrane reactor

High production rate of glucose and high substrate concentration are the key points for practical large scale digestion of raw starch. The influence of high concentration of raw starch on rate of glucose production was examined by batchwise digestion with the pilot membrane reactor (Fig. 8). Glucose production rate increased with an increase in the initial starch concentration from 10 to 50% (w/v). However, sweet potato raw starch granules plugged the inlet of the hollow fiber UF membrane of the pilot reactor when concentration exceeded 40%. Consequently, digestion was carried out at an initial 30% of undigested raw starch concentration.

Prior to operation of the pilot reactor system, influence of mechanical stress by pumping and passage through valves and the membrane on RDA was examined. In all cases (Fig. 9) RDA remained at more than 90% of the initial value. Fur-

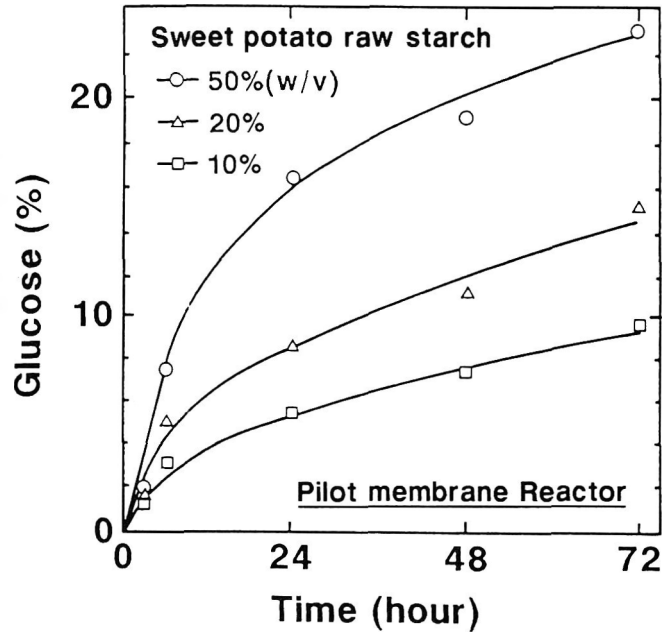

Fig. 8—Effect of initial concentration of raw starch on batchwise digestion in the 20 L scale membrane pilot reactor. Conditions for digestion: enzyme 0.2% (w/v); potassium sorbate 100 ppm; pH 5.0; temperature 40°C; total volume 20L.

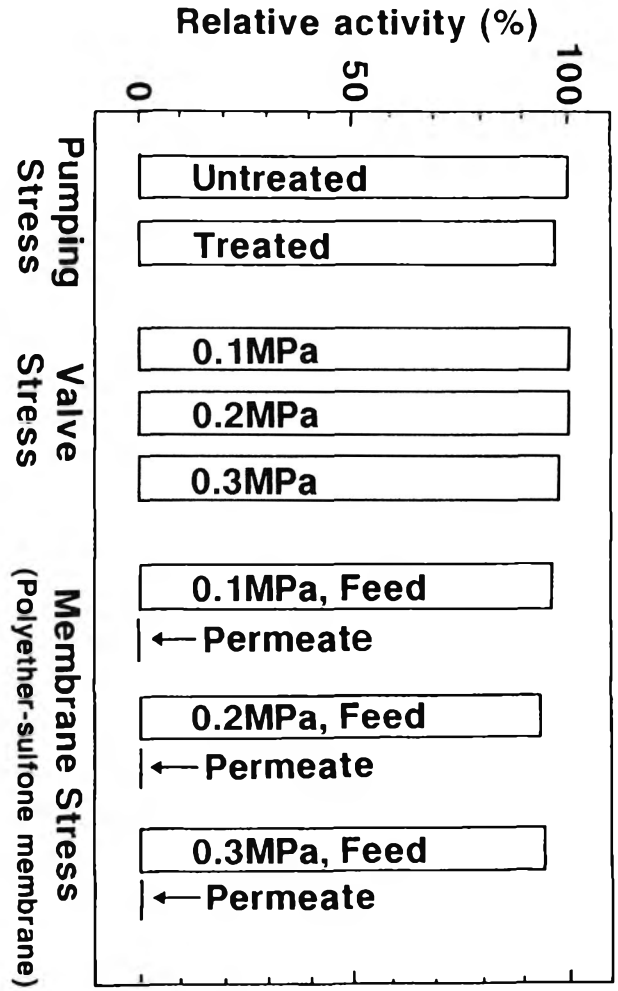

Fig. 9—Influence of various stresses on RDA in the 20L scale hollow fiber UF membrane pilot reactor.

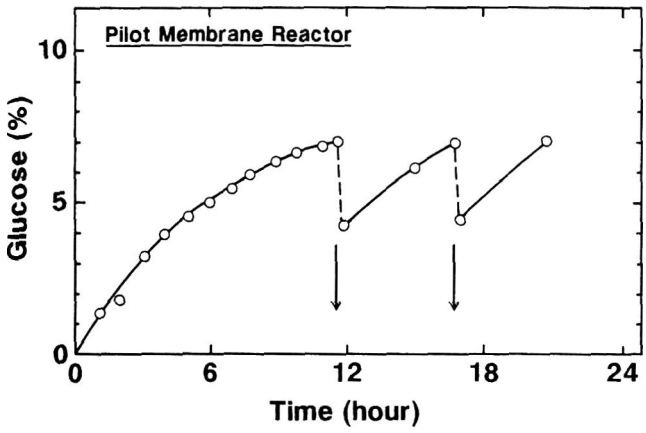

Fig. 10—Semi-continuous digestion of raw starch with the membrane pilot reactor in the presence of potassium sorbate. The reactor tank was partially emptied at the time indicated with arrows (↓) then re-filled. Reaction conditions: initial raw starch 30% (w/v); enzyme 0.2% (w/v); potassium sorbate 100 ppm; pH 5.0 (50 mM acetate buffer); temperature 40°C; total volume 20L (see MATERIALS & METHODS for operation of the reactor and Fig. 1 for schematic diagram).

thermore no enzyme activity was detected in the permeate suggesting that there was no enzyme leakage through the membrane.

Figure 10 illustrates repeated, semi-continuous digestion of sweet potato raw starch (6 kg/20 L) with the pilot reactor. The glucose production rate in runs 1, 2 and 3 was 5.84, 6.00 and 5.75 g/hr/L, respectively. Thus, even after the third filtration, no significant decrease in the glucose production rate was observed. A bioreactor which periodically removes glucose to avoid end-product inhibition, may be effective in enzyme recycling and indicates potential for industrial use.

CONCLUSIONS

POTASSIUM METABISULFITE was not suitable as a preservative in digestion of sweet potato raw starch by *Chalara paradoxa* glucoamylase because of its inhibitory effect on raw starch digesting activity. On the other hand, 100 ppm potassium sorbate prevented spoilage without inhibiting raw starch digesting activity. With potassium sorbate as preservative, semi-continuous digestion of sweet potato raw starch was efficiently carried out at pH 5.0 and 40°C resulting in glucose production of 5.7 to 6.0 g/hr/L.

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Cultivar, Specific Gravity and Location in Tuber Affect Puncture Force of Raw Potatoes

A. ANZALDÚA-MORALES, M.C. BOURNE, and I. SHOMER

ABSTRACT

Three potato cultivars were sorted into specific gravity groups. Slices of raw potato were subjected to a puncture test at six sites in the cortex tissue and eight sites in the pith tissue. The cortex tissue generally showed 25—65% higher puncture force than the pith tissue. With exception of pith tissue in Monona cultivar, puncture force increased with increasing specific gravity in cortex and pith for raw potato. Dry matter content increased with increasing specific gravity. The cortex tissue had 3—6% higher dry matter than pith tissue.

Key Words: potato, firmness, texture, specific gravity, tuber location

INTRODUCTION

THE POTATO is a major food crop with worldwide annual production exceeding 270 million tons (FAO, 1990). Factors affecting potato quality, continue to draw a high level of interest, especially texture. Many reports have been published on textural quality of potatoes (e.g., see reviews by Linehan and Hughes, 1969, and by Warren and Woodman, 1974).

The flesh of the potato tuber is comprised predominantly of two types of parenchyma tissue. The cortex is between the skin and the outer vascular tissue, and the pith is in the center inside the vascular ring of the tuber (Hayward, 1938). A thin layer of xylem and phloem cells (the vascular system) separates the cortex from the pith. This ring can be seen on the surface of a potato that has been cut transversely.

The potato appears to be mainly pith tissue and the cortex contributes only a small proportion of the parenchyma tissue. However, this perception is erroneous. If we assume a spherical shape for a small potato 50 mm diameter with cortex 4 mm thick, it can be calculated that the potato is comprised of 60% pith and 40% cortex tissue. Similarly, a large potato with a diameter of 130 mm and cortex 15mm thick would be comprised of 45% pith tissue and 55% cortex tissue. Therefore, the cortex tissue sometimes may exceed the pith in volume.

Hughes et al. (1975a, 1975b, 1975c) studied texture of cooked potatoes and their test material was taken from the vascular ring, avoiding the pith. Personius and Sharp (1938) and Sterling and Bettelheim (1955) compared tests on "predominantly inner tissue" vs. "predominantly outer tissue." Most reports on potato texture, however, do not specify whether cortex or pith tissue was used. In many cases it is likely that the test sample was a mixture of the two tissues in unspecified proportions. Puncture tests on intact potatoes measure the cortex tissue unless very deep penetrations are made.

Barmore (1937) indicated that a penetrometer could be used to test the degree of "doneness" of potatoes. He cited reports of puncture tests carried out at 10 sites within the pith to measure the degree of "doneness" of cooked potatoes for use in foodservice operations (Landreth, 1929; Sweetman and Lancaster, 1931; Lancaster and Sweetman, 1932; Thiessen, 1935). The cortex area was not considered in those studies. Nonaka (1980) found significant differences in extrusion force between slabs cut from the bud and stem end of cooked potatoes, but since the slabs were 16 mm thick, it is likely that the test material was a mixture of cortex and pith. Huff (1967) measured the tensile strength of Kennebec potatoes from tissue taken from the center, the skin and half way and found significant differences between the three regions. He found that strain energy decreased at the skin and increased in the center. Significant differences were found between the samples taken from the skin and the other parts of the tuber. However, all samples of the center and at half way between the skin and center belonged to pith tissue. Thus no indication was given about differences between cortex and pith tissues.

Sharma et al. (1959) performed puncture tests near the surface and in the internal area of cooked Katahdin potatoes and found significant differences between the two regions. However, only three tubers were used for each specific gravity range. This was the only study where the specific gravity of the potato was considered, although specific gravity is recognized as a quality factor in potatoes and is used for industrial sorting and grading (Brennan et al., 1976). Some studies have been reported where tests, mainly uniaxial compression, were carried out on samples taken from selected sites of the tuber, such as the pith (Diehl et al., 1979; Harada and Paulus, 1985) or the cortex (Davis et al., 1983). None of those reports measured both pith and cortex.

Our objective was to answer the question of whether there are variations in texture, measured by means of puncture (Bourne, 1979), between potato tissues, using large sample sizes. The effects of cultivar and specific gravity were also studied.

MATERIALS & METHODS

ONE TON LOTS of each of three potato cultivars (*Solanum tuberosum* L.) were obtained from nearby farms. They were washed, sorted into specific gravity groups by immersion in salt brines, washed again, dried and stored at 4°C and 90% RH until needed. Potatoes in the specific gravity range 1,040—1.049 were labeled as 1.045; for range 1.050—1.059 as 1.055 etc. The cultivars were Atlantic and Chieftain, grown on organic soil, and Monona, grown on mineral soil. Atlantic is used for baking, potato flakes and granules manufacture; Monona is recommended for chips; and Chieftain is for home use.

Puncture test

A flat-end cylindrical punch, 2.5 mm diameter was attached to the crosshead of a Lloyd universal testing machine (model M5K) with crosshead speed of 50 cm mi^{-1}. The punch diameter was selected after preliminary tests with punches of different diameters. Larger diameters were not suitable since sometimes they were wider than the cortex and smaller punches did not increase the discriminatory power of the test. The raw tubers were cut longitudinally into slabs 1.27 cm in thickness using a Hobart food slicer. Each slice was punctured 6 times around cortex and 8 times within the pith as shown in Fig. 1. The number of potatoes used was 15 or more, where possible. The peak force was recorded for each puncture and a computer program

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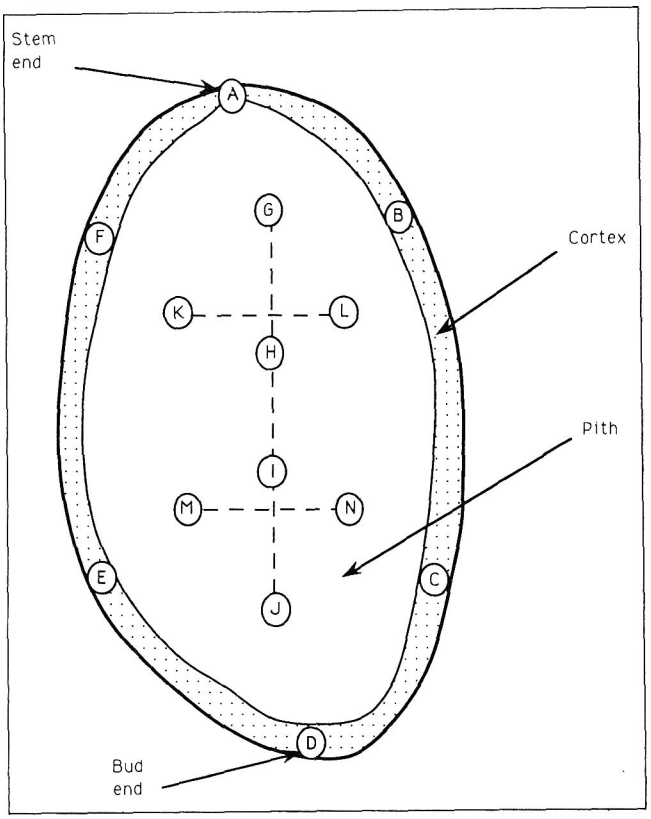

Fig. 1—Positions within the potato tuber for multiple puncture test.

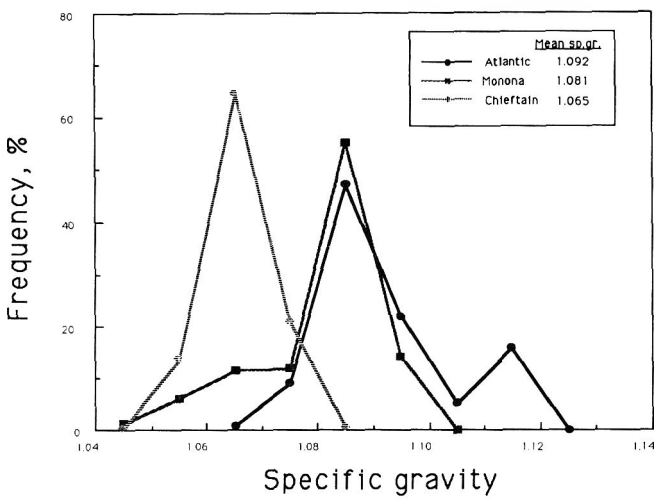

Fig. 2–Specific gravity distribution of raw potato.

was designed to read the charts with a digitizer connected to an Apple MacIntosh computer.

Moisture content

Potatoes from each variety and specific gravity were handpeeled. A slab was cut through the center as for the puncture measurements and tissue from cortex and pith was taken using a sharp knife. The samples were ground using a home food processor and aliquots were dried to constant weight in a vacuum oven at 70°C and 635 mm Hg of vacuum. Each moisture determination was made in triplicate.

RESULTS & DISCUSSION

Specific gravity distribution

The specific gravity distribution of the three cultivors indicated Atlantic has the highest average density (1.092), followed by Monona (1.081) and Chieftain (1.065) (Fig. 2). Although both Atlantic and Monona had the largest number of potatoes in the 1.085 range (47.3 and 55.2%, respectively), their distributions differed resulting in a lower average density for Monona.

Puncture of raw-potato

Cortex vs pith: Tables 1, 2, and 3 show the results of puncture tests on the three cultivars of raw potatoes. There was a consistent pattern of a higher force around the cortex than within the pith. This difference was found in all cultivars as well as for all specific gravities. With the exception of Chieftain, specific gravity 1.045, the differences between cortex and pith were significant. For Atlantic and Monona the cortex tissue was generally about 50% firmer than the pith tissue.

The higher puncture force for cortex tissue was probably related to the higher solids and starch contents that have been reported in this zone (Quarmby, 1981) since a firmer texture has been associated with those chemical parameters (Gould and Plimpton, 1985; Talburt et al., 1986). The difference in puncture force between cortex and pith generally increased as specific gravity increased. This may be due to immaturity of the low specific gravity potatoes. As the potatoes develop, the properties of the different zones become more strongly differentiated.

Stem end vs bud end

The puncture force at the stem end (site A) was significantly higher than for the bud end (site D) (if not significant they weren't different) only in a few cases (Atlantic 1,085, Monona 1.045, and Chieftain 1.075 and 1.085). This was confirmed through tests on smaller quantities of seven other cultivars (Anzaldúa-Morales and Bourne, 1992).

The mean puncture force for the three puncture sites closest to the stem end (A, B and F) were compared with the mean force for the three sites closest to the apical zone (C, D and E) using analysis of variance with the SAS package (SAS Institute, 1985). There was a higher force for the stem zone (ABF) than for the apical zone (CDE), in only two cases. Therefore, we concluded that the firmness of the stem end was usually not significantly higher than the bud end.

Effect of specific gravity

Specific gravity was associated with clear differences in puncture force (Tables 1,2,3). The puncture force for Atlantic differentiated all specific gravities with exception of 1.095 and 1.115. For Monona, all differences were significant except 1.055 and 1.065. For Chieftain all differences were significant, except 1.065 and 1.075.

Table 4 lists the slopes and correlation coefficients of the lines of best fit for the relationships between puncture force and specific gravity. The puncture force of the cortex increased steeply with increasing specific gravity for the three cultivars. For the pith it increased moderately for Atlantic and Chieftain and decreased slightly for Monona. Atlantic potatoes exhibited the highest slope of puncture force vs sp gr for the cortex (slope 98 N) and pith (slope 26 N). Experiments with smaller lots of other cultivars showed that the slope was generally higher for potatoes with higher specific gravity (Anzaldúa-Morales and Bourne, 1992)

The puncture test showed large variations between cultivars; the ranking for general firmness of the three varieties was: Atlantic > Monona > Chieftain.

The readings taken at the cortex were more suitable indexes of the texture of the tuber than the ones within the pith since they had lower coefficients of variance, yielded better correlations and the patterns observed were more consistent than those for the pith. In addition, in preliminary tests carried out

Table 1—Puncture force (N) of raw Atlantic potatoes measured at six specific gravity levels and 14 sites within the tuber

Puncture site	Specific gravity 1.065[A]	1.075[B]	1.085[C]	1.095[DE]	1.105[F]	1.115[DF]
Cortex Tissue						
A[BC]	9.3a	10.5ab	**10.3cd**	13.1a	14.0ab	13.4ab
B[AB]	9.2a	10.2abc	**12.7ab**	14.0a	15.0a	13.5ab
C[AB]	8.8ab	9.7abcde	**13.6a**	13.8a	14.4a	13.3ab
D[C]	9.4a	9.7abcde	**11.2bc**	12.5a	11.8b	11.6b
E[AB]	8.8abc	10.0abcd	**12.6abc**	14.4a	14.1ab	13.0ab
F[A]	8.5abc	11.8a	**12.2abc**	14.5a	15.7a	14.3a
Mean	9.0a	10.3a	**12.1a**	13.7a	14.2a	13.2a
Pith tissue						
G[D]	7.6abc	8.0cdef	**7.7e**	8.4b	8.6c	8.1c
H[D]	8.0abc	8.4bcdef	**7.8e**	8.0b	8.5c	7.8c
I[D]	8.0abc	7.8cdef	**7.6e**	8.0b	8.2c	8.0c
J[D]	7.3abc	7.7def	**8.0ed**	8.3b	8.8c	8.1c
K[D]	7.0bc	7.6ef	**7.6e**	8.2b	9.5c	8.6c
L[D]	6.7c	7.5ef	**7.8e**	8.5b	9.3c	8.1c
M[D]	6.8bc	7.0f	**7.5e**	8.1b	9.0c	9.0c
N[D]	7.4abc	7.6ef	**7.7e**	8.5b	9.0c	8.7c
Mean	7.3b	7.7b	**7.7b**	8.3b	8.9b	8.3b

Different lower case letters indicate significant differences according to Tukey's test ($p<0.05$) within each column.
Different capital letters indicate significant differences between locations and/or specific gravity levels. Boldface numbers are for the highest frequency specific gravity range.

Table 2—Puncture force (in Newtons) of raw Monona potatoes measured at five specific gravity levels and 14 sites within the tuber

Puncture site	Specific gravity 1.045[A]	1.055[B]	1.065[B]	1.075[C]	1.085[D]
Cortex tissue					
A[A]	11.3a	12.2a	11.8a	12.6ab	**11.8ab**
B[A]	10.6ab	12.1a	11.9a	12.9ab	**12.5a**
C[A]	9.7abcd	11.0a	11.1a	12.9ab	**11.8ab**
D[B]	9.3bcde	10.4a	10.7a	11.3b	**10.4b**
E[A]	9.7abcd	11.7a	11.7a	12.3ab	**12.0ab**
F[A]	9.8abc	11.2a	11.1a	13.7a	**12.2ab**
Mean	10.1a	11.4a	11.4a	12.6a	**11.8a**
Pith tissue					
G[C]	8.3cde	8.2b	8.2b	8.4c	**7.1c**
H[C]	8.0e	8.0b	8.3b	7.4c	**6.8c**
I[C]	8.1cde	8.2b	8.1b	7.6c	**6.6c**
J[C]	8.2cde	8.2b	8.0b	8.0c	**7.2c**
K[C]	8.0de	7.9b	8.2b	8.1c	**7.6c**
L[C]	8.1de	7.9b	8.1b	8.1c	**7.2c**
M[C]	7.9e	8.0b	8.2b	7.9c	**7.3c**
N[C]	8.1de	7.9b	8.2b	8.1	**7.0c**
Mean	8.1b	8.0b	8.2b	8.0b	**7.1b**

Comparisons significantly different at the 0.05 level within each column are indicated by different lower case letters.
Significantly different mean values of force for different specific gravities or puncture sites are indicated by means of different upper case letters. Boldface numbers are for the highest frequency specific gravity range.

Table 3—Puncture force (N) of raw Chieftain potatoes measured at five specific gravity levels and 14 sites within the tuber

Puncture site	Specific gravity 1.045[A]	1.055[B]	1.065[C]	1.075[C]	1.085[D]
Cortex tissue					
A[A]	9.7a	10.2a	**11.3a**	11.6a	13.0a
B[B]	8.3ab	9.8ab	**11.1a**	11.3ab	11.6ab
C[B]	8.1ab	9.1abcd	**11.1a**	11.3ab	11.9ab
D[BC]	9.2ab	9.1abcd	**10.abc**	9.8bc	10.9bc
E[B]	8.1b	9.0abcd	**10.3ab**	10.9ab	11.7ab
F[B]	8.0b	9.1abcd	**10.9a**	11.4ab	12.6ab
Mean	8.6a	9.4a	**10.8a**	11.1a	12.0a
Pith tissue					
G[CD]	9.1ab	9.4abc	**8.9bcd**	9.1cd	9.4cd
H[CD]	9.2ab	9.4abc	**8.9bcd**	9.1cd	8.9d
I[DE]	9.1ab	9.1abcd	**8.9bcd**	8.7cd	8.8d
J[DE]	9.0ab	9.2abcd	**8.8bcd**	9.0cd	8.9d
K[F]	7.8b	7.6d	**8.2d**	8.1d	8.3d
L[EF]	8.0b	8.1bcd	**8.5cd**	8.3cd	8.8d
M[F]	7.9b	7.8cd	**8.0c**	8.0d	8.2d
N[F]	7.7b	8.0cd	**8.3d**	8.2cd	8.4d
Mean	8.5a	8.6b	**8.6b**	8.6b	8.7b

Different lower case letters indicate significant differences according to Tukey's test ($p<0.05$) within each column. Different upper case letters indicate significant differences between locations and/or specific gravity levels. Boldface numbers are for the highest freqency specific gravity range.

on whole potatoes the force values obtained fell within the same range of readings for the cortex region. However, carrying out the multiple puncture around the 2 tissues is necessary in order to characterize fully the texture of the tuber.

Solids content

The solids content of the cortex tissue was consistently higher (Table 5) than for the pith. Generally it was 3 to 6% higher. This finding agreed with the report by Talburt et al. (1986). The results obtained in our study fell within the same range as other values reported (Reeve, 1970; Gould and Plimpton, 1985), and similarly, fit straight lines with high correlation coefficients ($R^2=0.92$ for the cortex and $R^2=0.81$ for the pith) This showed how well differentiated both tissues were with regard to dry matter content and indicated that the discrimination provided by the multiple puncture force was suitable for differentiating both tissues. The solids content increased as specific gravity increased. Gould and Plimpton (1985) reported that the solids content in potatoes was closely related to starch level. Therefore, it is possible that a relationship between puncture force and starch could be found, although our work did not include chemical tests.

Table 4—Slopes (m) and correlation coefficients (R^2) of lines of best fit to data of puncture force versus specific gravity of raw potatoes

		Atlantic	Monona	Chieftain
cortex	m (N)	97	46	85
	R^2	0.80*	0.65	0.97**
	df	5	4	4
pith	m (N)	25	−20	5
	R^2	0.73*	0.51	0.8*
	df	5	4	4

* $p\leq0.05$, ** $p<0.01$

The solids content generally correlated with the puncture force. Table 6 shows the slopes and correlation coefficients of the lines of best fit to data of puncture force vs dry matter content. The slope for the cortex was steeper than for the pith in all cases. All slopes were positive, with the exception of Monana pith. This behavior agreed with observations previously made and confirmed the close relationship between specific gravity, dry matter content and puncture force.

Table 5—Dry matter content (%) of raw Atlantic, Monona and Chieftain potatoes

	Atlantic		Monona		Chieftain	
Sp.gr.	Cortex	Pith	Cortex	Pith	Cortex	Pith
1.045	N.A.[a]	N.A.	16.3 (0.15)	11.2 (0.04)	15.0 (0.45)	12.3 (0.09)
1.055	N.A.	N.A.	20.1 (0.11)	12.3 (0.01)	18.5 (0.22)	14.2 (0.14)
1.065	20.4 (0.13)[b]	16.5 (0.31)	22.1 (0.38)	12.8 (0.54)	20.5 (0.09)	15.9 (0.32)
1.075	23.8 (0.15)	18.4 (0.11)	22.6 (0.27)	14.4 (0.21)	22.6 (0.08)	17.9 (0.32)
1.085	24.9 (0.13)	20.1 (0.49)	21.8 (0.08)	15.0 (0.32)	24.5 (0.24)	19.3 (0.23)
1.095	26.8 (0.33)	21.8 (0.36)	N.A.	N.A.	N.A.	N.A.
1.105	28.4 (0.21)	24.7 (0.48)	N.A.	N.A.	N.A.	N.A.
1.115	28.4 (0.16)	22.7 (0.20)	N.A.	N.A.	N.A.	N.A.

[a] N.A. = no potatoes available in this range.
[b] Values in parentheses are standard deviations.

Table 6—Slopes (m) and correlation coefficients (R^2) of lines of best fit to data of puncture force vs dry matter content of raw potatoes

		Atlantic	Monona	Chieftain
cortex	m (%)	0.63	0.32	0.36
	R^2	0.91**	0.83*	0.96**
	df	5	4	4
pith	m (%)	0.19	−0.20	2.23
	R^2	0.94***	0.49	0.78*
	df	5	4	4

* $p \leq 0.05$, ** $p < 0.01$, *** $p < 0.001$.

CONCLUSION

The designed puncture test detected differences in firmness in potatoes of three cultivars and several levels of specific gravity as well as in zones within the tubers. The maximum force for puncture differentiated between cortex and between cultivars. In general, the cortex tissue in raw potato was about 50% firmer than the pith. Within each cultivar the specific gravity and the puncture force correlated highly with a positive correlation in most cases. Future studies on physical properties of raw potato should recognize the large differences between cortex and pith tissues and should test each type tissue separately. Dry matter content values correlated highly with puncture force and showed a close relationship with specific gravity.

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Irradiation and Packaging Affect the Nitrate-Nitrogen Concentrations of Potatoes

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ABSTRACT

Russet Burbank and Kennebec potato cultivars were irradiated with dosages of 0.1 and 1.0 KGy and stored in polyethylene or paper bags for 1 or 3 mo at 5°C or 20°C. Tubers receiving irradiation showed a significant ($p<0.01$) increase in nitrate-nitrogen concentration as compared to controls. Those receiving the 1.0 KGy dose had the highest nitrate-nitrogen concentration. Tubers stored in polyethylene bags were significantly ($p<0.01$) higher in nitrate-nitrogen concentration than those stored in paper bags. Tubers stored at 20°C were significantly ($p<0.01$) higher in nitrate-nitrogen than those stored at 5°C. These trends were consistent for both cultivars. The cortex region was significantly ($p<0.01$) higher in nitrate-nitrogen concentration than the pith region. Kennebec tubers were significantly ($p<0.01$) higher in nitrate-nitrogen than the Russet Burbank tubers for all irradiation doses and storage temperatures.

Key Words: potatoes, nitrate, irradiation

INTRODUCTION

SPROUTING of potatoes during storage is highly undesirable since it can increase susceptibility of the tuber to bruising and shrivelling, lower the nutritive value, and result in higher levels of toxicants, glycoalkaloids and nitrates. Several methods are currently employed to lower the occurrence of sprouting. These include irradiation, chemical treatment, and low-temperature storage. A dose of 0.1 KGy was sufficient to prevent sprouting (Thomas, 1984). The use of irradiation doses up to 1.0 KGy for potatoes was approved by the FDA in 1964.

Previous work has shown that irradiation of potatoes can profoundly affect toxic constituents of the tubers. According to Wu and Salunkhe (1977) gamma-irradiation of tubers with 0.25-1.0 KGy inhibited synthesis of glycoalkaloids by 11-79%. Nair et al. (1981) reported 70% inhibition in glycoalkaloid synthesis using a dose of 0.1 KGy. Gosselin and Mondy (1989) found that gamma-irradiation of potatoes resulted in higher levels of glycoalkaloids when the tubers were stored in polyethylene bags as compared to those stored in mesh or paper bags. Mondy and Seetharaman (1990) found that the glycoalkaloid content of gamma-irradiated potatoes was higher when the tubers were stored in paper bags than when stored in polyethylene bags. Patil et al. (1971) and Bergers (1981) found no significant changes in glycoalkaloid content of potato tubers following irradiation.

Potatoes contribute up to 14% of the per capita ingestion of nitrates in the United States (White, 1975). The ingestion of high levels of nitrates and nitrites can result in methemoglobinemia, a condition characterized by the inhibition of oxygen transportation through the body (Hartman, 1982). Infants are particularly susceptible to this disorder (Hartman, 1982). Ingested nitrate can be reduced to nitrite, which can also react with secondary or tertiary amines to form carcinogenic and mutagenic N-nitroso compounds (Walters et al., 1979). Nitrosation reactions have been linked to cancers of the esophagus, stomach, large intestine, and bladder (Walters et al., 1979). The objective of our investigation was to study the effects of gamma-irradiation on the nitrate-nitrogen concentrations of 2 potato cultivars stored in 2 types of packaging materials.

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MATERIALS & METHODS

KENNEBEC and Russet Burbank potatoes grown at Cornell Vegetable Research Farm at Freeville, NY were used in this study. Planting and harvesting of potatoes were done mechanically. The potatoes were harvested 154 days (22 wk) following planting, washed and kept at room temperature for 7 days to allow the periderm to suberize. The tubers were then stored at 5°C and 95% RH in the dark prior to irradiation.

Potatoes were irradiated using a ^{60}Co source. The potatoes were first brought to room temperature overnight prior to irradiation . During irradiation the tubers were placed along the perimeter of a circle 70.9 cm from the center of the source. The tubers were irradiated at 0.00127 KGy/min for 79 min, or 788.5 min to receive a total dose of 0.1 or 1.0 KGy respectively. The dose rate of the source was measured using a Reuter Stokes ionization chamber calibrated with a National Bureau of Standards secondary source.

Tubers of size C (about 8 cm in diameter) were sliced longitudinally from the bud to stem end in order to facilitate uniform sampling at both the apical and basal ends. The slices were then separated along the vascular ring into the outer cortex (including the peel) and inner pith regions, frozen, lyophilized in a Stokes freeze dryer, ground in a Wiley mill through a 40-mesh screen and stored under nitrogen until analyzed. Both cortex and pith sections were analyzed for the nitrate-nitrogen concentrations. Sample preparation and analyses were performed immediately following irradiation, and after 4 and 12 wk storage.

Nitrate-Nitrogen (NO_3-N) analysis

The NO_3-N content was determined by the phenoldisulfonic acid method using an aqueous extract of the lyophilized powders (Ulrich et al., 1959). Analyses were performed in quadruplicate. Four tubers were used for each run.

Statistical analysis

Complete random design was employed, and statistical significance of the data was determined by analysis of variance with protected LSD test (Steel and Torrie, 1980). Statistical interaction between irradiation dosage, storage time and temperature, and packaging material were also calculated.

RESULTS & DISCUSSION

THE NITRATE-NITROGEN concentration was increased significantly ($p<0.01$) by irradiation as compared to untreated tubers of both cultivars stored either in paper or plastic (polyethylene) bags (Fig. 1). The irradiation dose of 1.0 KGy resulted in the greatest nitrate-nitrogen concentration of the tubers with some values 300% higher than controls. No significant ($p>0.05$) changes in nitrate-nitrogen due to storage were observed.

Nitrate-nitrogen concentration of tubers packed in plastic bags was significantly ($p<0.001$) higher than those stored in paper bags (Fig. 1). On an average, tubers stored in plastic bags showed nitrate-nitrogen concentrations 30% higher than

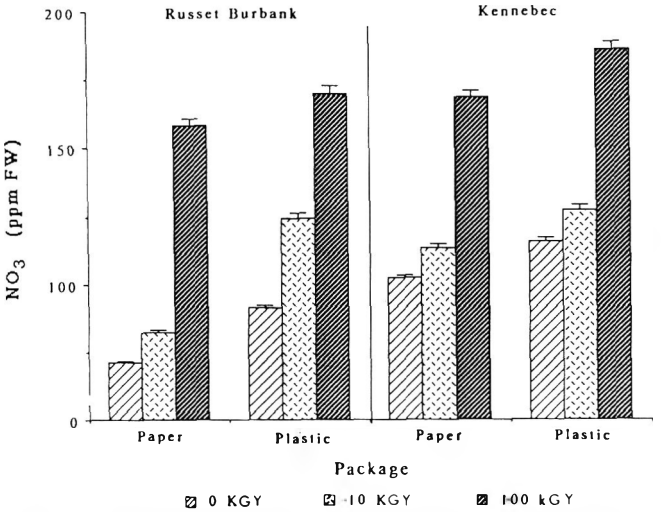

Fig. 1—Effect of irradiation on the nitrate-nitrogen concentration of the cortex section of Russet Burbank and Kennebec tubers stored at 5°C for 1 month. Similar trends were observed for the pith section as well as for tubers stored at 20°C.

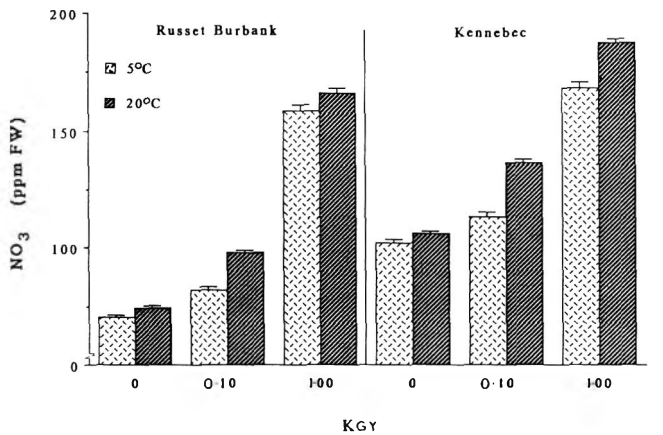

Fig. 2— Effect of storage temperature on the nitrate-nitrogen of the cortex section of irradiated tubers stored in paper bags. Similar trends were observed for the pith section as well as for tubers stored in plastic bags.

those stored in paper bags. This trend was consistent at all levels of irradiation as well as the two storage temperatures. Statistically significant interaction was observed between irradiation dosage and packaging material. Corrections for moisture losses over storage were made prior to obtaining the final nitrate-nitrogen concentrations.

Tubers stored at 20°C showed significantly ($p < 0.05$) higher concentrations of nitrate-nitrogen than those stored at 5°C (Fig. 2). This trend was consistent for all levels of irradiation as well as the two packaging materials. Nitrate-nitrogen concentration of the cortex region was significantly ($p < 0.01$) higher than that of the pith region. However, increases in the nitrate-nitrogen concentration due to irradiation were greater for the pith than the cortex region.

Significant ($p < 0.01$) differences due to the cultivar were also observed. The Kennebec tubers showed higher concentrations of nitrate-nitrogen than the Russet Burbank cultivar. However, the Russet Burbank cultivar showed greater increases in nitrate-nitrogen concentration following irradiation than the Kennebec cultivar.

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Gel-strength in Restructured Potato Products Affects Oil Uptake during Deep-fat frying

ELI J. PINTHUS, PNINA WEINBERG and ISRAEL SAM SAGUY

ABSTRACT

A controllable food system consisting of a restructured potato product was employed to study the effect of gel-strength on oil uptake. Water loss and oil uptake were affected markedly by gel-strength. Oil uptake decreased as gel-strength increased. The oil uptake ratio, U_R, (weight ratio of the oil uptake to the water evaporated during frying) was utilized to assess the effectiveness of reducing oil absorption during frying. This criterion was useful for comparison of restructured potato products with different initial or final moisture contents.

Key Words: potato, gel-strength, deep-fat frying, oil-uptake

INTRODUCTION

FATS and oils have unique properties, including flavor and a smooth mouthfeel which improve overall food palatability. Deep-fat frying is widely used by both the food industry and consumers. Frying is extremely complex and important in industrial or institutional preparation of foods (Varela, 1988). Despite the fact that vast amounts of foods are fried, the method is still considered to be more of an art than a science or technology (Blumenthal, 1991). Many factors have been reported to affect oil uptake, including oil quality, frying temperature and duration, product shape, its content (e.g., moisture, solids, fat, protein), porosity, pre-frying treatments (e.g., drying, blanching), coating and others (Selman and Hopkins, 1989).

An oil uptake criterion, U_R, expressing the weight ratio between oil uptake and moisture loss has been introduced:

$$U_r = \frac{\text{Oil uptake (g)}}{\text{Water removed (g)}}$$

The value of U_R is derived by considering the initial and momentary moisture and oil contents. The criterion was found to alleviate the dependency of oil uptake on water replacement and to be instrumental in assessing effectiveness of reducing oil uptake during deep-fat frying (Pinthus et al., 1992). Powdered cellulose and its derivatives have been shown to reduce oil uptake (Ang. 1989, 1990; Ang et al., 1990; Anon, 1987; Henderson, 1988; Pinthus et al., 1992). Recently, methyl cellulose was found to be significantly more effective than powdered cellulose in reducing the amount of oil uptake in donuts and falafel balls (Pinthus et al., 1992).

The main objective of our research was to elucidate and to quantify the effect of gel-strength (an integrated factor for compressibility and porosity) of a restructured potato product on oil uptake.

MATERIALS & METHODS

Restructured potato

The restructured potato product mass was prepared as follows: Potato flakes (Idaho), 230g; water, 753g; alginate (Manugel DMB, Kelco, San Diego, CA), 6g; NaCl, 5g; calcium sulphate dihydrate (Terra Alba F+P Grade U.S. Gypsum), 4.5g; and tetrasodium pyrophosphate (FMC, Philadelphia, PA), 1g. The levels of alginate, calcium sulphate dihydrate and tetrasodium pyrophosphate were modified to provide restructured potato products with different gel-strengths. The alginate solution was prepared in a mixer and added to the potato flakes, mixed for 1 min in a Hobart kitchen mixer at speed "1", and 1.5 min on speed "2." The remaining dry ingredients were added and mixing continued for another 1.5 min on speed "2." The mass was transferred to a bench scale "hot dog" 9L extruder (CGT Misanoa, Italy) and extruded into synthetic casing sleeves, (22 mm diam). The filled sleeves were frozen at a temperature of −20°C and kept frozen until evaluated.

Prior to frying, the sleeves were allowed to defrost at ambient temperature (≈23°C) and cut to cylindrical pieces, 35 mm length. The synthetic sleeve was carefully removed and the restructured cylindrical potato pieces were left at ambient temperature (≈23°C) for an additional 30 min. The average temperature of the samples prior to frying was 16±2°C. Samples were fried in a bench-top deep-fat fryer (12 cm depth) containing 2.5L soy oil at 170°C. The oil was preheated for 2 hr prior to frying (Blumenthal, 1991), and discarded after 6 hr. Samples fried for different periods of time ranging from 1 to 15 min, were removed from the frier and allowed to cool to room temperature on paper towels.

Gel-strength

Compression measurements were performed on a Brabender Structo-Graph (C. W. Brabender, South Hackensack, NJ) fitted with 2000-cmg spring and a 30-mm disk. Each sample was compressed individually. The moveable platen was operated at a speed of 1.5 mm/sec and chart speed was 1 cm/sec. Gel-strength is expressed as the maximum force at yield point/sample initial unit area/unit time. Results are expressed as Pa/sec. Data are the means of triplicates.

Moisture analysis

Ground samples were oven dried at 105°C overnight. Moisture content was calculated from the weight of the dry sample and expressed as percentage of original sample.

Fat analysis

A Soxhlet extraction (AOAC, 1984) of the dried samples was utilized.

Statistical analyses

Statistix© (Analytical Software, St. Paul, MN) software version 3.5 was utilized for regression analysis.

RESULTS & DISCUSSION

DUE TO THE COMPLEXITY of the deep-fat frying process a controllable food system should be employed to avoid natural variability normally encountered in agricultural raw materials. The initial oil concentration of the restructured potato product was negligible, hence, oil content and oil uptake were identical.

The gel-strength of samples produced according to the basic formula was 1700 Pa/sec. Samples produced with 125, 75 and 50% alginate, calcium sulphate and sequestrant, produced gel-strengths of 1860, 1530 and 850 Pa/sec, respectively. Samples with no alginate disintegrated to small particles at the onset of frying. Employing more than 125% of the basic amount of alginate created a very gummy mass inappropriate for extrusion.

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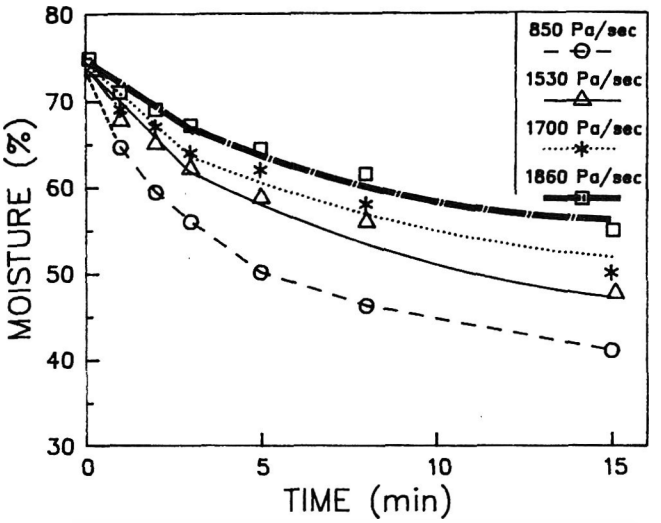

Fig. 1—Effect of gel-strength on moisture loss of a restructured potato product during deep-fat frying.

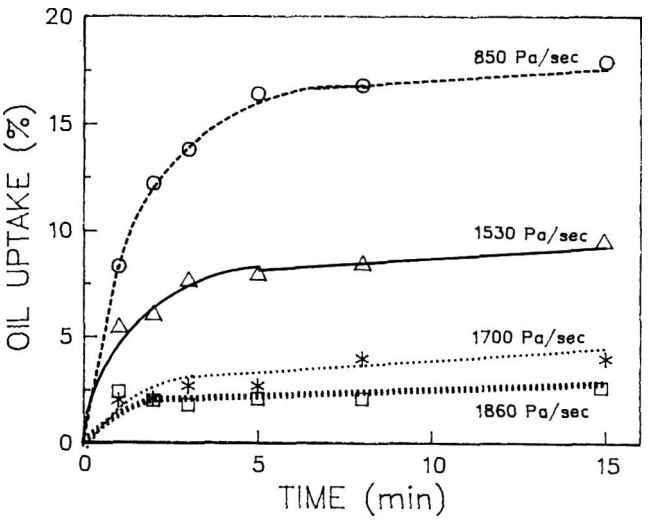

Fig. 2—Effect of gel-strength on oil uptake in a restructured potato product during deep-fat frying.

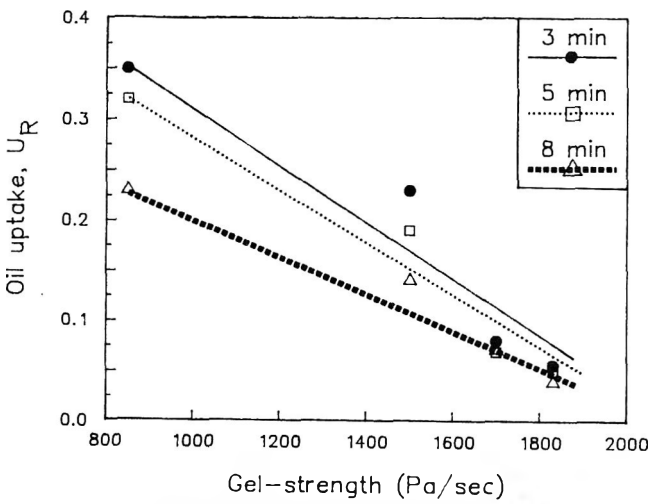

Fig. 3—Effect of gel-strength on oil "uptake ratio," U_R

Data indicated water loss during deep-fat frying was significantly lower (Fig. 1) at higher gel-strengths. Moisture losses occurred mainly during the first 5 min of frying, and increased with frying time in a nonlinear fashion. The data also indicate (Fig. 2) that increasing the gel-strength reduced markedly the amount of oil uptake. At high gel-strength, oil uptake leveled off after a relatively short frying time. For instance, at a gel-strength of 1860 Pa/sec, oil uptake leveled off after only 1 min and the sample adsorbed 0.5% oil. The value of U_R remained almost constant, indicating that moisture loss leveled off as well. At lower gel-strength (i.e., 850 Pa/sec), the oil uptake plateau was reached only after 5 min of frying.

The question, however, remains how much of this gel-strength effect on oil uptake can be attributed to water replacement (i.e. oil absorption occurs as moisture is removed from the food during frying; Varela, 1988). Studies claim that a higher initial moisture content results in a higher oil uptake (Gamble and Rice, 1988). In some cases, a linear relationship between oil uptake and water content of the final product was reported (Gamble et al., 1987).

Gel-strength reduced the oil uptake ratio, U_R, (Fig. 3) in all four gel-strengths tested. At typical frying times (i.e, 5 and 8 min), U_R decreased with gel-strength in a similar manner which appears to be linear ($r^2 = 0.948$ and 0.958, respectively). At shorter frying times (i.e., 3 min) data indicated that the system had not reached the quasi-equilibrium state. Nevertheless, a similar linear trend was observed ($r^2 = 0.913$).

The derived U_R values at a constant frying time further highlight the effect of gel-strength. For instance, at 5 min frying time, the values of U_R derived at gel-strengths of 850 and 1860 Pa/sec were 0.05 and 0.32, respectively. These values show a 6.4-fold difference in U_R. Comparison of oil uptake without considering the effect of water, resulted in a 7.8-fold difference (16.4 compared to 2.1%). The latter value is erroneous as it ignores the effect of different levels of moisture content remaining in the samples and the water replacement effect.

The effect of increased gel-strength cannot be explained merely as a creation of a barrier which reduces simultaneously water movement to the surface, rate of evaporation and oil uptake. These data provide additional evidence of the possible involvement of other product characteristics. Gel-strength is a general term which encompasses several basic product characteristics, such as cross-linking, compressibility, porosity and others. Further research is needed to elucidate the exact mechanism of oil uptake and the general applicability of the U_R criterion to other food systems and products.

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Stipe Trimming at Harvest Increases Shelf Life of Fresh Mushrooms (*Agaricus bisporus*)

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ABSTRACT

Trimming the stipe of cultivated mushrooms, *Agaricus bisporus*, from 35 mm to 5 mm from the cap immediately after harvest resulted in improved shelf life as indicated by reduced browning and slower cap opening. This effect was evident after 3 days storage at 12°C, and became more pronounced after 6 days. Trimming the stipes had no significant effect on postharvest respiration rate or bacterial growth; thus, the shelf life improvement was due to other factors. Stipe trimming reduced the weight (yield) of saleable mushrooms by about 10%; thus, shelf life improvement would need to offset any economic loss to growers to make this practice commercially feasible.

Key Words: mushrooms, *Agaricus bisporus*, shelf life, stipe trimming

INTRODUCTION

THE GENERAL TREND in mushroom marketing has shown a continous increase in fresh market sales from 1970 to 1991. In the 1971 growing season, 29% (66 million pounds) of the U.S. mushroom production was channeled to the fresh market, increasing to 68% (512 million pounds) by 1990 (USDA, 1991). Due to the increased level of fresh market sales, and because mushrooms are extremely perishable (shelf life after harvest 1–2 days at room temperature), several methods have been examined to improve quality and extend shelf life during marketing. Most such methods involved postharvest treatments such as refrigeration (Cameron and Chapell, 1970; Dredge, 1964), overwrapping packages, controlled atmosphere storage (Nichols and Hammond, 1973), and irradiation (Kramer, 1986; Kovacs and Vas, 1974). In our study, a new approach to improve quality and extend shelf life of fresh mushrooms has been investigated.

Results from a previous investigation (Ajlouni, 1991) revealed that mannitol content in the lower stipe of hybid mushrooms was high (28% of dry weight) compared to mannitol content in the gill (10%), and the upper stipe (19%). Due to that high level of mannitol in the lower stipe, and the fact that mannitol represents the main respiratory substrate (Hammond and Nichols, 1975) and it may be translocated between the various tissues of harvested mushroom (Schmidt, 1977). We hypothesized that trimming the lower stipe to 5 mm from the cap immediately after harvest would reduce the amount of respiratory substrate (mannitol), and indirectly improve the mushroom shelf life. Conventionally, growers leave as much stipe as possible on the fruiting bodies (about 35 mm) in order to maximize yield and consequent economic return.

Our objective was to examine the effect of stipe trimming (from 35 mm to 5 mm from the cap) at harvest on the shelf life of *Agaricus* mushrooms by measuring postharvest discoloration (browning), maturation (cap opening) and bacterial growth. Postharvest respiration rates of mushrooms with short and long stipes were determined. Also, effects of trimming the stipes on mushroom yield reduction, was evaluated.

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MATERIALS & METHODS

BOTH WHITE and off-white hybrids of *Agaricus bisporus* were used. Mushrooms were grown at different time intervals on traditional horse manure-based compost at the Mushroom Test and Demonstration Facility (MTDF) on The Pennsylvania State University campus. Fruiting bodies were harvested at the button stage of maturity (Hammond and Nichols, 1975). Within each crop, special care was taken to keep the stipe as long as possible during picking, and only the portion of the lower stipe that was in direct contact with the compost was removed. Harvested mushrooms were sorted on the basis of size and appearance within about 2 hr of harvest. Damaged, open-veiled and extremely large and small mushrooms (>35 mm and <25 mm in cap diameter) were discarded. The stipes of short stipe mushrooms were trimmed to 5 ±2 mm from the cap, and the stipes of long stipe mushrooms were left intact (35±2 mm). Mushrooms within each group were pooled, randomly selected and about 80 g were placed in 227-g linear polystyrene trays. Trays were overwrapped with polyvinyl chloride (PVC) film, ventilated with two 3-mm holes, and stored at 12°C.

Mushroom color, maturity, and total aerobic plate count were evaluated on days 0, 3, and 6. On each of these days, six trays were selected randomly to represent three replicates for color and maturity measurements, and three for total aerobic plate count. Color was measured using a Minolta Chromameter (Minolta CR-200, Dynamic Electronic Sales, Churchville, PA), and L, a, b values were recorded. Maturity was evaluated according to the method of Guthrie (1984), in which the degree of cap opening was judged based on an integer scale from 1 to 7. Total aerobic plate count was performed according to the method of Kramer (1986) as adapted from Witowski and Doores (1981). Respiration rate was examined daily by measuring the amount of $C0_2$ released by a given weight of mushrooms within 30 min at 12°C using a GLC procedure (Ajlouni, 1991).

The decrease in the weight (yield) of mushrooms as a result of stipe trimming from 35 mm to 5 mm from the cap was evaluated using the first, second, and third flushes of an off-white hybrid crop, and expressed as changes in the mushroom yield. All data were statistically analyzed using a personal computer and the StatView 512+ application (Brain Power, Inc., Calabasas, CA), and the mean values were compared by t-test.

RESULTS & DISCUSSION

STIPE TRIMMING is a process of cutting off the base of the stipe and the attached mycelium during harvest, which are usually in contact with the casing material. Normally, the stipes are left long (about 35 mm) in order to maximize yield and consequent economic return. Since the noticeable feature of harvested mushrooms during storage is the elongation of the stipes, mushrooms with short stipes would result in proportionally less elongated stipes after storage when compared to mushrooms with long stipes. Based on this consideration, Dredge (1964) suggested trimming the stipes to 13 mm instead of 25 mm or longer to avoid any notably change in appearance after harvest.

In our research, trimming the stipe immediately after harvest to 5 mm from the cap improved the shelf life of mushrooms as evidenced by the decrease in discoloration or browning (Fig. 1). Both long stipe (LS) and short stipe (SS) mushrooms showed similar degrees of whiteness on the day of harvest: L values were 84.2 and 84.8 for the LS and SS mushrooms, respectively. On days 3 and 6, discoloration (reduced L value) was significantly greater in the LS mushrooms. On the contrary, Minamide et al. (1985) found that trimming the stipe of na-

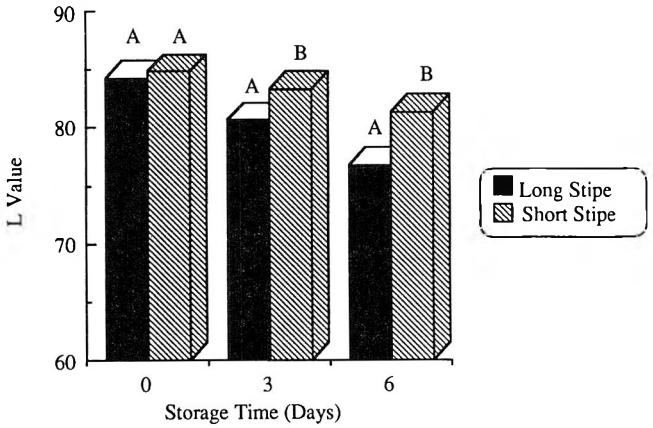

Fig. 1—Effect of stipe trimming at harvest on the color of mushrooms during storage at 12°C. Bars with the same letter within a day are not significantly different at the level of 0.05%.

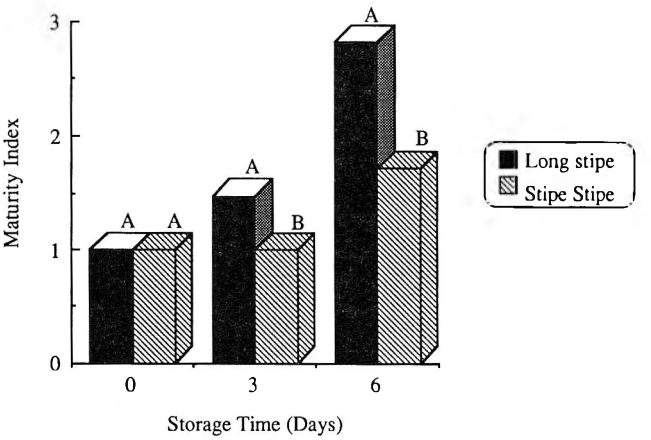

Fig. 2—Effect of stipe trimming at harvest on the rate of cap opening (Maturity Index) of mushrooms during storage at 12°C. Bars with the same letter within a day are not significantly different at the level of 0.05%.

meko (*Pholiota nameko*) accelerated postharvest browning. The discrepancy between studies might be due to differences ian mushroom species, storage conditions and additional postharvest treatment such as washing. After stipe trimming, the nameko mushrooms were washed and stored in polyethylene bags without ventilation holes at 20°C; these two practices were not used in our study.

Another indication of the postharvest senescence is cap opening during storage which can be measured by a maturity rating based on the stretching of the velum and exposure of the gill tissue (Guthrie, 1984). Caps of the LS mushrooms opened faster than the SS mushrooms, as evidenced by the differences in maturity index (MI) on days 3 and 6 (Fig. 2) and the visual appearance illustrated in photographs on day 6 (Fig. 3). The MI values were 1.0 and 1.5 for the LS and SS mushrooms, respectively, after 3-day storage, and increased to 1.7 (LS) and 2.9 (SS) after 6-day storage.

Previous research (Beelman et al., 1989) indicated that cultural or postharvest practices that decrease the browning of stored mushrooms were associated with reduced bacterial populations. However, no significant differences were observed in total aerobic bacterial plate counts between the LS and SS mushrooms (Fig. 4). The log CFU/g fresh mushrooms on day 3 were 7.83 and 7.84 for the LS and SS mushrooms, respectively, and continued to increase at the same rate. It seems that the longer shelf life of SS mushrooms was related to some factors other than bacterial population.

Reasons for the lower rate of cap development in the SS mushrooms as compared to the LS mushrooms are not clear. Since many higher plant hormones have been found in mushrooms, such as auxins (Konishi and Hagimoto, 1961) and ethylene (Turner et al., 1975), it is possible that the lower stipe contains or produces some growth-regulating components that can be translocated to other mushroom tissues and provide the biochemical signals necessary for cap opening and mushroom development. Trimming that stipe may eliminate those hormones and delay the deterioration and maturation of mushrooms. However, detectable quanitites of 1-amino-cyclopropane-1-carboxylic acid (ACC) were found in the lower stipe of *Agaricus bisporus* (Ajlouni et al., 1990). ACC is the precursor of the senescence hormone ethylene (Adams and Yang, 1979).

Recently, a compound with hormonal properties was isolated from mushrooms and identified as 10-oxo-*trans*-8-decenoic acid (ODA) (Mau, 1992). This compound is produced concurrently with the major mushroom aroma component, 1-octen-3-ol, especially when tissues of fruiting bodies or filaments of the mycelium are damaged or disrupted (Grosch and Wurzenberger, 1984; Schindler, 1989). ODA was found to stimulate the stipe elongation of mushroom fruiting bodies (Mau et al., 1992a). Interestingly, more 1-octen-3-ol was found in the upper stipe than in the lower stipe (Mau et al., 1992b), thus by inference it is apparent that ODA is produced more in the lower stipe when the tissue is subjected to cutting. In this research, at harvest the LS mushrooms were cut at the lower

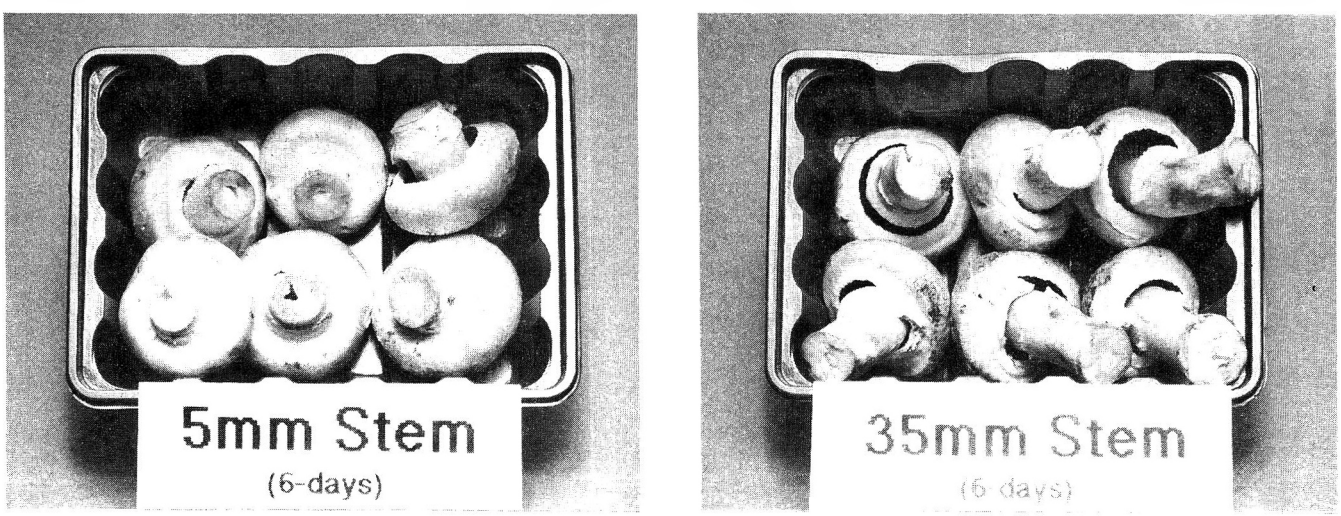

Fig. 3— Photographs of mushrooms with stipes trimmed to 5 mm from the cap and those with stipes left untrimmed (35 mm) after 6 days storage at 12°C.

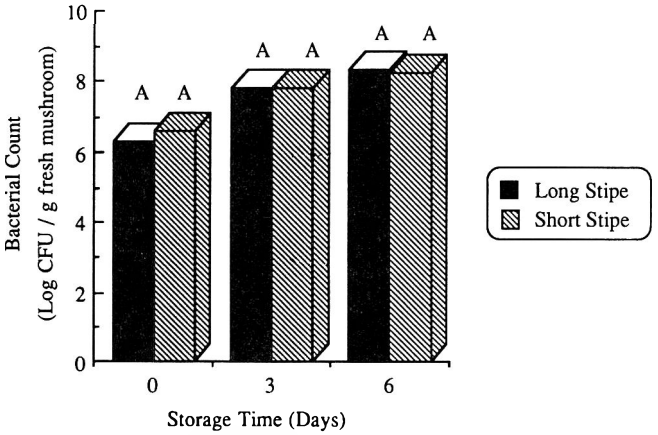

Fig. 4—Effect of stipe trimming at harvest on bacterial population of mushrooms during storage at 12°C. Bars with the same letter within a day are not significantly different at the level of 0.05.

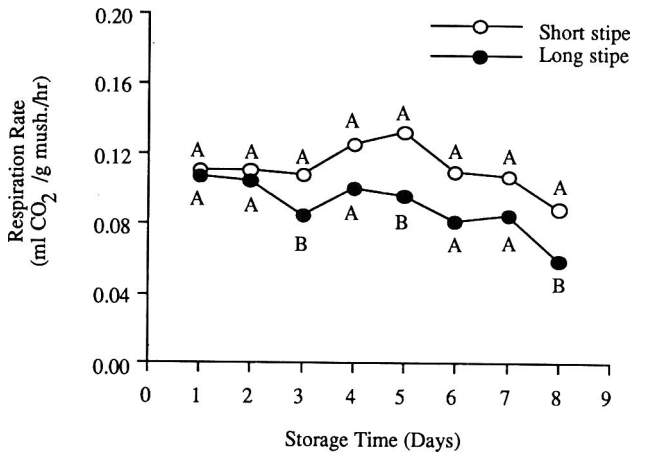

Fig. 5—Effect of stipe trimming at harvest on respiration rate of mushrooms during storage at 12°C. Values with the same letter within a day are not significantly different at the level of 0.05.

stipe, while the SS mushrooms were cut at the upper stipe. Therefore, more ODA formed in the SS mushrooms during stipe trimming might stimulate the faster postharvest development leading to senescence.

Another possible explanation for our observations could be related to mannitol translocation within tissues. The movement of dry matter from the lower stipe to the cap reportedly occurs even after harvest (Hammond and Nichols, 1975; Murr and Morris, 1975), and is apparently associated with mushroom postharvest development. The lower stipe has been found to be high in mannitol content (28% of the dry wt) as compared to mannitol in the gills (10%) and in the upper stipe (19%) (Ajlouni, 1991). Mannitol is considered to be the main respiratory substrate utilized by harvested mushrooms (Hammond, 1977), and it can be translocated between the various tissues. Moreover, Schmidt (1977) and Ajlouni (1991) demonstrated apparent translocation of mannitol from the lower to the upper stipes during postharvest storage of mushrooms. Because the process of upper stipe cell elongation is involved in cap opening, normal maturation would be slowed if substrate for elongation were not available. Translocation of mannitol from the lower to the upper stipes of mushrooms could possibly create high osmotic pressure in the upper stipe and force the movement of water in the same direction. The continuous withdrawal of water into the upper stipe of LS mushrooms would help the process of cell elongation and growth of the upper stipe that would, in turn, force the breakage of the veil and the opening of the cap.

Since the lower stipe contains high amounts of mannitol (Ajlouni, 1991), which is the main respiratory substrate in harvested mushrooms (Hammond, 1977), our original hypothesis suggested that the removal of the lower stipe would probaly eliminate a major source of manitol from the SS mushrooms and might reduce their respiration rate during postharvest storage. Contrary to this hypothesis, the respiration rate of the SS mushrooms was not found to be significantly higher than that of the LS mushrooms (Fig. 5). Average carbon dioxide produced during storage was 0.11 and 0.08 ml/g/hr for the SS and LS mushrooms, respectively. However, similar results were observed in the short stipe nameko mushrooms (Minamide et al., 1985).

Although the exact mechanism by which the shelf life of SS mushrooms is extended by stipe trimming, especially the reduced rate of browning, is not known, our results provide experimental evidence that the shelf life of fresh mushrooms could be possibly improved by this harvest practice. However, the yield of saleable mushrooms was decreased by about 10% as a result of trimming the lower stipe from 35 mm to 5 mm from the cap (Ajlouni, 1991). This reduction in the yield would decrease potential revenue for growers unless they receive a differential price for their product based on improvement in initial appearance due to shorter stipes or improved postharvest shelf life resulting in greater consumer demand or reduced loss of product in retail markets.

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Calcium Chloride and Potassium Sorbate Reduce Sodium Chloride used during Natural Cucumber Fermentation and Storage

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ABSTRACT

Cucumber fermentation characteristics and pickle quality were evaluated in brines containing equilibrium concentrations of 0–0.4% $CaCl_2$, 0–0.4% potassium sorbate and 0–10% NaCl. Changes in brine pH and acidity, cucumber texture and color, coliforms, lactic acid and total bacteria, yeasts and molds were followed over time. Results indicated that cucumber spoilage would eventually take place if NaCl or potassium sorbate were not present in the brine. The presence of $CaCl_2$ helped maintain cucumber firmness. A synergistic action between NaCl, $CaCl_2$ and potassium sorbate was seen, which allowed good quality pickles to be produced when moderate amounts of all three components were present in the brine (5% NaCl, 0.2% $CaCl_2$, 0.2% potassium sorbate).

Key Words: sodium chloride, calcium chloride, potassium sorbate, cucumbers, pickles, fermentation, brine

INTRODUCTION

CUCUMBERS harvested in the US are mainly preserved by fermentation and pickling for later use. Traditionally, cucumbers are fermented in brines containing 8–10% NaCl and stored in brines with up to 16–18% NaCl (Cruess, 1958; Etchells et al., 1975). The presence of salt in the brine creates a favorable environment for growth of lactic acid bacteria (LAB) during fermentation and suppresses growth of undesirable microorganisms during storage (Fleming et al., 1985, 1987). Furthermore, the presence of salt in the brine enhances firmness of the cucumbers (Bell and Etchells, 1961; Daeschel and Fleming, 1984). Brines are disposed of at the end of each storage period by dumping them into ground waters. Unfortunately, their high salt content may contribute to extensive water pollution. The chloride concentration in brine wastes exceeds the legal limit (230 mg/L) specified by the U.S. Environmental Protection Agency by as much as 100-fold (Humphries and Fleming, 1989). The high NaCl content of pickle processing wastes must be reduced and extensive research has been initiated to help achieve this.

Low salt brines containing calcium compounds have been successfully used in controlled (cultures such as *Lactobacillus plantarum* added) and anaerobic fermentations. Thompson et al. (1979) found that good quality pickles were obtained by a controlled fermentation process utilizing brines with 5.5% NaCl and 0.1% $CaCl_2$ and a storage temperature of 15.5°C. Fleming et al. (1987) reported that under controlled fermentation conditions, cucumbers could be effectively fermented in brines containing no NaCl and 0.2% $CaCl_2$, but some NaCl was necessary during storage to avoid extensive loss of firmness. They also found that excellent quality pickles could be produced by maintaining 2.6% NaCl and 0.2% $CaCl_2$ during fermentation and storage. Fleming et al. (1988) reached similar conclusions using an anaerobic fermentation process. They reported successful fermentation and storage in brines containing 2.7% or 4.6% NaCl and 0.07% Ca^{++}. However, both methods (controlled and anaerobic fermentation) are costly and complex and have limited appeal to the pickle industry. Pickle processing companies require simpler and more cost effective methods to ferment and store cucumbers in low salt brines.

A solution to the problem could be the addition to the brine of a firming agent ($CaCl_2$) in combination with an antimicrobial agent (potassium sorbate). Theoretically, such agents should protect the cucumbers from both spoilage and softening. The firming action of $CaCl_2$ (0.20–0.44%) has been shown by various researchers (Buescher et al., 1979; Hudson and Buescher, 1986; Fleming et al., 1987) and the antimicrobial properties of sorbic acid and its salts have been applied since the 1940's. Costilow et al. (1955, 1957) demonstrated that the addition of sorbic acid (0.01—0.1%) to the brine inhibited yeast activity during cucumber fermentation. Sofos and Busta (1981) reported that sorbates inhibited the growth of molds and many bacteria. Sorbic acid and potassium sorbate are both classified as GRAS substances (21 CFR 182.3640) and can be used commercially as antimicrobial agents. However, potassium sorbate at concentrations of 0.05–0.08% has been preferred for use by the pickling industry because of its higher water solubility (Sofos and Busta, 1981; Davidson and Juneja, 1990).

Although there has been research on the use of various salt concentrations, $CaCl_2$ and sorbate to improve cucumber fermentation, there has been no research that focused on the simultaneous use of these three components in natural cucumber fermentations. Therefore, the objective of our work was to study the effect of varying NaCl, $CaCl_2$, and potassium sorbate concentrations on the natural cucumber fermentation and on the subsequent quality of the pickles during storage.

MATERIALS & METHODS

Raw cucumbers

Pickling cucumbers (cv. Calypso) were obtained from a local pickling company a few hours after delivery to the plant. They were commercially washed, sorted for size (3.5–4.5 cm diameter) and microbial or mechanical damage, and placed into experimental brine solutions within one day.

Brine preparation, fermentation and storage procedures

Fifteen different brines (Table 1) with a volume of 50L each, were prepared in 200L open plastic barrels. Traditionally, brines are prepared with an amount of NaCl (desired for the fermentation), which is maintained at the initial concentration by regularly adding salt to the brine at the rate it is absorbed by the cucumbers (Cruess, 1958). However, a different method was used in our experiment in order to simplify and shorten the brining procedure. The initial brines contained NaCl, $CaCl_2$ and potassium sorbate concentrations double those desired for equilibrium. The addition of cucumbers to the brines, in a ratio of 50/50 (w/w), resulted in equilibrium concentrations within a few days (as indicated by the salt content of the brine which was measured regularly). This procedure presented the advantage of reduced handling time and involved the use of relatively high concentrations of NaCl in the brines at the beginning of fermentation. For example, the brines with an equilibrium NaCl concentration of 10% contained initially 20% NaCl. Cucumbers (50 kg) were added to each

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Table 1—Equilibrium composition of the brines prepared to study the combined effect of NaCl, $CaCl_2$ and potassium sorbate on the natural cucumber fermentation and storage characteristics

Treatment no.	Equilibrium concentration (%) NaCl	$CaCl_2$	K-sorbate	Acetic acid
1	0	0.0	0.2	0.16
2	0	0.2	0.0	0.16
3	0	0.2	0.4	0.16
4	0	0.4	0.2	0.16
5	5	0.0	0.4	0.16
6	5	0.0	0.0	0.16
7,8,9	5	0.2	0.2	0.16
10	5	0.4	0.0	0.16
11	5	0.4	0.4	0.16
12	10	0.0	0.2	0.16
13	10	0.2	0.0	0.16
14	10	0.2	0.4	0.16
15	10	0.4	0.2	0.16

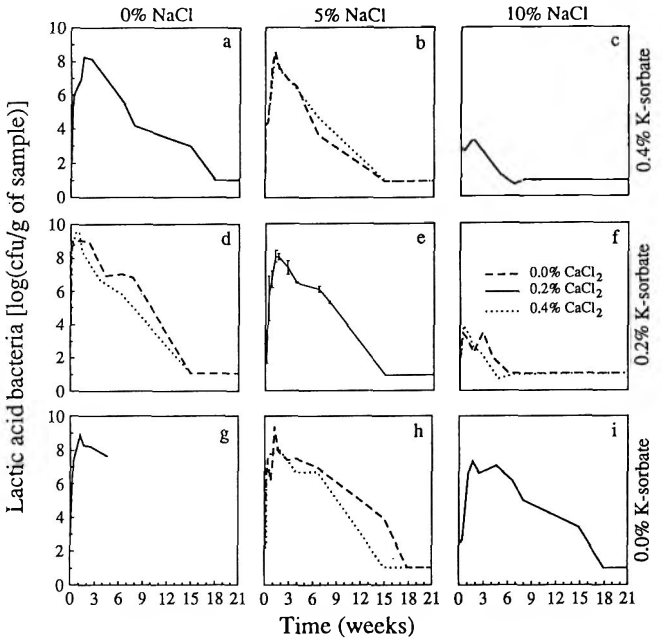

Fig. 1—Effect of NaCl, $CaCl_2$ and potassium sorbate concentration on the lactic acid bacterial growth during natural cucumber fermentation and storage (Bars of ± one standard error are given for replicated trials).

brine to reach the 50/50 (w/w) cucumbers/brine proportion. All brines were prepared with food-grade NaCl (Morton Thiokol Inc., Chicago, IL), $CaCl_2$ dihydrate (Mallinckrodt Inc., Paris, KY), potassium sorbate (Sigma Chemical Company, St Louis, MO) and glacial acetic acid (Fisher Scientific, Fair Lawn, NJ). After equilibrium, they contained 0.16% acetic acid; 0%, 5% or 10% NaCl; 0%, 0.2% or 0.4% $CaCl_2$ and 0%, 0.2% or 0.4% potassium sorbate (Table 1). The combinations of NaCl, $CaCl_2$ and potassium sorbate chosen for each brine were selected according to a three-level-three-factor experimental design (Box and Behnken, 1960). Two 254nm UV lights (Spectroline, Model XX-15F, Spectronics Corporation, Westbury, NY) were used to simulate the effect of sunlight, which inhibits the growth of molds, yeasts and other oxidative microorganisms on the brine surface (Lenigher, 1982; Humphries and Fleming, 1989). Furthermore, a plastic lid under constant force (weight) was used to submerge the cucumbers below the brine surface throughout fermentation and storage. All containers were stored in a closed room maintained at 21 ± 2°C.

Measurements

Brine pH, acidity and salt. The pH of the brines was measured with an accuracy of 0.01 units, using an Orion pH meter (Model SA720, Orion Research Inc, Boston, MA). From the beginning of the experiment until fermentation was complete, measurements were recorded daily. Afterwards, weekly or biweekly readings were taken. The acidity of the brines was determined weekly by titrating with standardized NaOH solution (Harris, 1987) using phenolphthalein (Mallinckrodt Inc., Paris, KY) as an indicator (Speck, 1984). The acid content of the brine was calculated as % lactic acid. The salt content of brines was measured once a week by titrating with 0.171N silver nitrate (Ricca Chemical Company, Arlington, TX) using dichlorofluorescein (Sigma Chemical Company, St Louis, MO) as an indicator (Speck, 1984). When deviations from the target salt content values (Table 1) were detected (usually up to +0.2% NaCl due to evaporation), corrections were made by adding the appropriate amount of water.

Cucumber texture. The cucumber texture was determined by the maximum penetration force method (Rodrigo and Alvarruiz, 1988) using an Instron UTM (Model 1132, Park Ridge, IL), at a crosshead speed of 10 cm min^{-1}. The cucumbers were cut lengthwise in half and three measurements/cucumber (one at the center and one at each side) were made with a cylindrical tip (diameter = 7.62mm). Once a week, measurements were performed on five cucumbers taken randomly from each batch, and the 15 values were averaged.

Microbial populations. Samples for microbial enumeration were taken twice a week during fermentation and once a week (or less often) after fermentation was complete. To provide an average, representative and more reproducible population, the samples were composed of 10 g of cucumber (mainly skin and some of the flesh immediately below the skin) and 10 mL of brine, mixed with 180 mL of sterilized 0.1% peptone water and stomached (Stomacher Lab-Blender 400, Seward Laboratory, London, England) for 30 sec.

Coliform bacteria were enumerated with Violet Red Bile Agar at 37°C (Difco Laboratories, Detroit, MI) and representative colonies were confirmed in Brilliant Green Bile Broth (Difco Laboratories, Detroit, MI) according to procedures given by Speck (1984). LAB were counted on acidified APT Agar (Difco Manual, 1984). Acidification was performed by adding 1 mL of 6M HCl in 100 ml of sterilized medium. The plates were incubated at 30°C for two days before counting the colonies (Speck, 1984). Total bacteria were enumerated with Plate Count Agar (Difco Laboratories, Detroit, MI). The plates were incubated at 30°C for three days before enumeration (Speck, 1984). Yeasts and molds were counted on Potato Dextrose Agar (Difco Laboratories, Detroit, MI) at 25°C. Two antibiotics, Chlortetracycline and Chloramphenicol (Sigma Chemical Company, St Louis, MO), were added to the medium just before plating (Speck, 1984).

Cucumber color. Interior and exterior L, a, b color values of 5 cucumbers were measured every week with a Hunterlab colorimeter (Model D25-PC2, Hunter Associates Laboratory, Reston, VA). Four exterior measurements were performed on every cucumber around its center and at ≈90° apart. The cucumbers were then cut lengthwise and 3 interior measurements were taken (one at the center and one at each side). Interior and exterior values of hue, chroma and ΔE were calculated as follows (Clydesdale, 1978):

$$\text{Hue} = \tan^{-1}(b/a) \quad [1]$$

$$\text{Chroma} = (a^2 + b^2)^{1/2} \quad [2]$$

$$\Delta E = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{1/2} \quad [3]$$

where, ΔL, Δa and Δb are the differences of the measured L, a and b values from the Hunterlab standard green color plate (#C2-30638) L, a and b values, respectively.

RESULTS & DISCUSSION

Microbial populations

Results of microbial enumeration (data not shown) indicated that coliform bacteria were present at the beginning of the experiment (initial populations were 30–1000 CFU/g of sample). However, they disappeared within 1 wk in all 15 batches.

LAB grew rapidly and produced a vigorous fermentation in brines containing 0% NaCl (Fig. 1a, d, g) or 5% NaCl (Fig. 1b, e, h). Within 1 or 2 wk of fermentation, the lactics increased from an initial population of 10^2–10^3 CFU/g of cucumber to ≈10^9 CFU/g of sample. However, brines containing 10% NaCl exhibited either slower fermentation characteristics (maximum population of 10^7 CFU/g when potassium sorbate was absent, Fig. 1i), or no fermentation (when potassium sorbate was present, Fig. 1c, f). The differences in LAB growth can be related to the method used for brine preparation. The

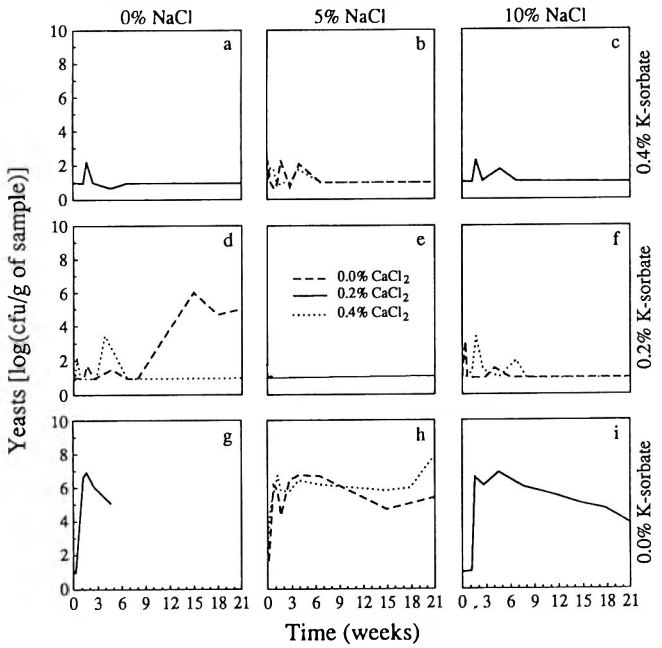

Fig. 2—Effect of NaCl, $CaCl_2$ and potassium sorbate concentration on the yeast growth during natural cucumber fermentation and storage (Bars of ± one standard error are given for replicated trials).

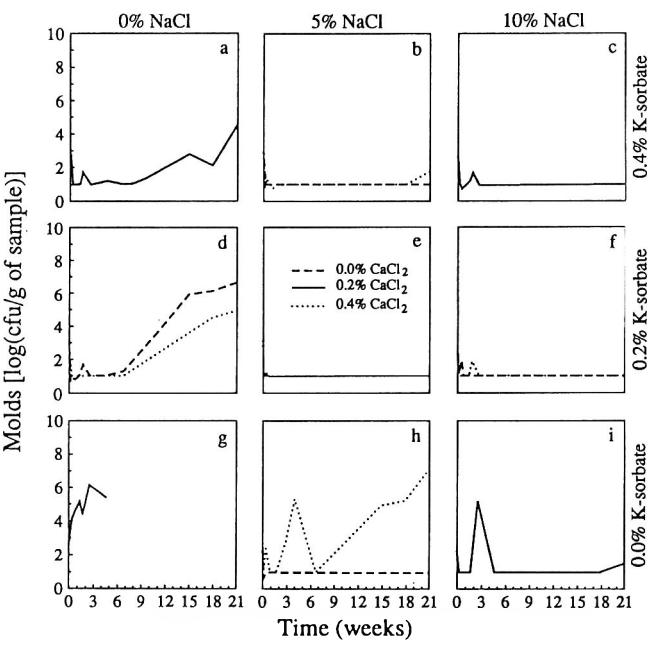

Fig. 3—Effect of NaCl, $CaCl_2$ and potassium sorbate concentration on the mold growth during natural cucumber fermentation and storage (Bars of ± one standard error are given for replicated trials).

brines containing initially 20% NaCl content, especially in the presence of potassium sorbate (an antimicrobial agent), inhibited growth of LAB (Costilow et al., 1957; Etchells et al., 1975).

Yeast enumeration results (Fig. 2) indicated that yeasts grew rapidly in brines containing no potassium sorbate (Fig. 2g, h, i). In the presence of high quantities of potassium sorbate (0.4%), however, yeast growth was completely inhibited (Fig. 2a, b, c). Moderate quantities of sorbate (0.2%) effectively suppressed yeast growth in brines containing either NaCl (Fig. 2e, f) or $CaCl_2$ (Fig. 2d), but only delayed yeast growth and contamination in brines containing no NaCl nor $CaCl_2$ (Fig. 2d). These results generally confirmed the inhibitory action of potassium sorbate against yeasts (Sofos and Busta, 1981), although 0.2% potassium sorbate in brines containing no NaCl nor $CaCl_2$ (Fig. 2d) was not sufficient to protect against yeast contamination. This concentration (0.2% of potassium sorbate) was higher than quantities generally considered as having an inhibitory effect (0.04%–0.1%)(Phillips and Mundt, 1950; Jones and Harper, 1952; Costilow et al., 1955). Therefore, to protect fermented cucumbers from microbial spoilage during the storage period, both NaCl and $CaCl_2$ were required in the brine when moderate amounts of potassium sorbate were used (synergistic action). When brines without NaCl or $CaCl_2$ were used, higher concentrations of potassium sorbate were necessary. This could lead to higher production costs, because potassium sorbate is a more costly raw material.

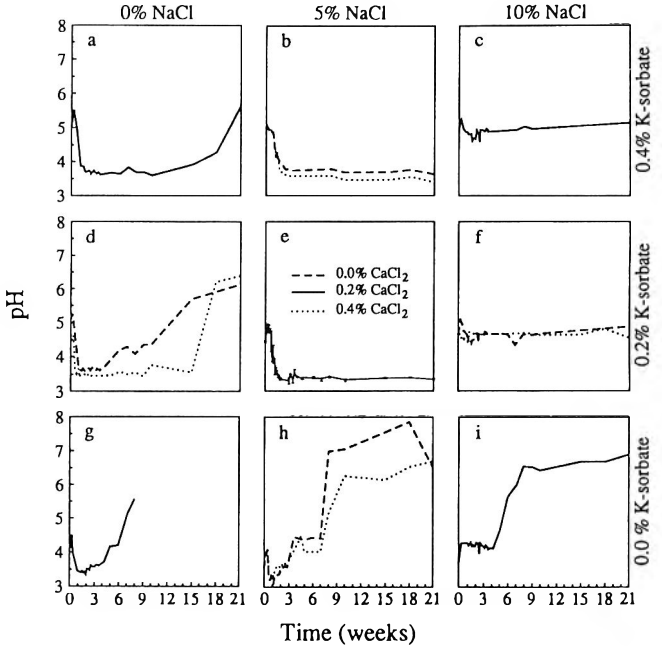

Fig. 4—Effect of NaCl, $CaCl_2$ and potassium sorbate concentration on the pH during natural cucumber fermentation and storage (Bars of ± one standard error are given for replicated trials).

Mold growth (Fig. 3) was completely inhibited in brines containing both NaCl and sorbate (Fig. 3b, c, e, f). When 1 of the 2 components was absent, however, mold growth varied despite the presence of UV light. For brines without NaCl (Fig. 3a, d, g) mold growth was rapid when sorbate was absent (Fig. 3g), delayed when sorbate was present at 0.2% (Fig. 3d), and very slow otherwise (Fig. 3a). Similar behavior could be seen in brines without sorbate and various NaCl concentrations (Fig. 3g, h, i). The growth of molds in the presence of UV light can be explained by the existence of shaded areas, where the UV light did not effectively penetrate. In such areas the intensity of the light was probably low enough to allow mold growth. In commercial applications with open tanks exposed to sunlight, mold growth is far less important. However, yeast and/or mold contamination may occasionally arise under certain conditions. These results confirmed that potassium sorbate is a mold inhibitor (Sofos and Busta, 1981) and showed that its action is proportional to the amount present in the brine. They also demonstrated that NaCl and potassium sorbate must both be present to avoid yeast and mold spoilage.

Brine pH and acidity

The pH (Fig. 4) of brines containing 0% NaCl (Fig. 4a, d, g) or 5% NaCl (Fig. 4b, e, h) decreased from an initial value of 3.5–5.0 to a value of 3.1–3.7 within the first 2 wk of

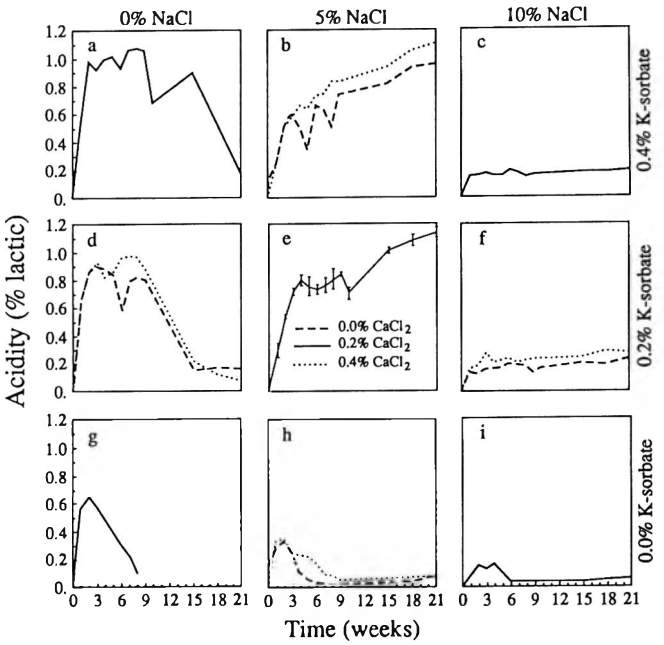

Fig. 5—Effect of NaCl, $CaCl_2$ and potassium sorbate concentration on the acidity during natural cucumber fermentation and storage (Bars of ± one standard error are given for replicated trials).

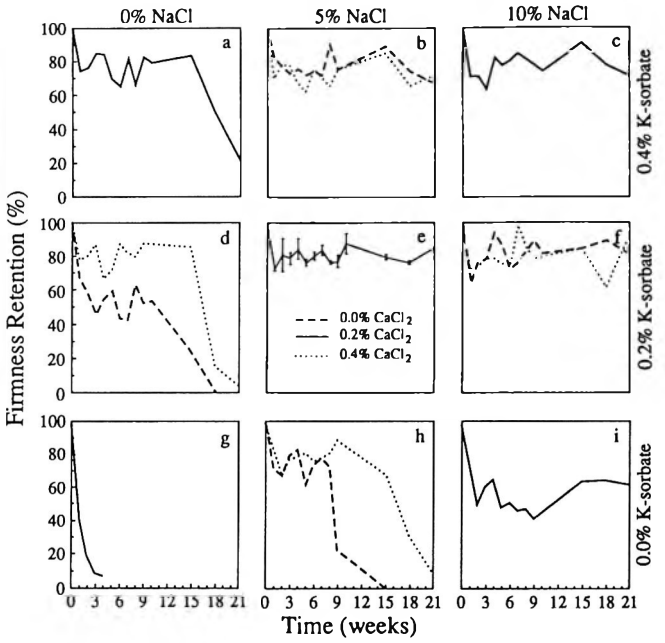

Fig. 6—Effect of NaCl, $CaCl_2$ and potassium sorbate concentration on the firmness retention of cucumbers during natural fermentation and storage (Bars of ± one standard error are given for replicated trials).

fermentation. During that time, the acidity (Fig. 5) of the same brines increased from 0% to 0.3–1% (Fig. 5a, b, d, e, g, h). These results agreed with the vigorous growth of LAB in brines containing 0% or 5% NaCl (Fig. 1). The LAB produced various acids (as end products of metabolism), which decreased pH of the brines and increased acidity. On the other hand, the pH of brines containing 10% NaCl was 4.2–4.9 1 wk after brining (Fig. 4c, f, i) and their acidity was ≈0.2% (Fig. 5c, f, i). This suggested that the cucumbers in 10% NaCl brines did not undergo regular fermentation (Ayres et al., 1980; Etchells et al., 1975).

During storage, the pH of brines containing both NaCl and potassium sorbate (Fig. 4b, c, e, f) remained constant at 3.3–3.8 or 4.6–5.0. When 1 of the 2 components was absent, however, the pH increased (Fig. 4a, d, g, h, i) and the acidity decreased (Fig. 5a, d, g, h, i) at various times. These changes (pH increase/acidity decrease) were the result of brine contamination by yeasts and/or molds and closely agreed with yeast/mold growth patterns (Fig. 2, 3). Yeasts and molds, by utilizing the acids produced during fermentation as a source of energy (Etchells et al., 1975), reduced the amount of acids present in the brine, and resulted in pH increase and acidity decrease.

Cucumber texture

Cucumbers fermented in brines containing both NaCl and potassium sorbate maintained good texture after 6 mo storage (Fig. 6b, c, e, f). They retained 70% to 90% of their initial firmness. On the other hand, cucumbers fermented in brines containing no NaCl nor potassium sorbate (Fig. 6g) soon became soft and mushy. They lost 90% of their initial firmness within 3 wks (Fig. 6g). When either NaCl or potassium sorbate was absent, the loss of cucumber texture was delayed (Fig. 6a, d, h, i), but not avoided. Cucumbers lost initial firmness faster in the absence of $CaCl_2$ than in presence of 0.4% $CaCl_2$ (Fig. 6d, h). These results showed that NaCl and potassium sorbate must both be added to the brine to obtain firm pickles and avoid yeast and mold contamination. They also confirmed the firming action of $CaCl_2$ (Buescher et al., 1979; Fleming et al., 1978; Hudson and Buescher, 1986).

Cucumber color

The color changes of the fermented cucumbers over time (hue, chroma, a/b ratio and ΔE values) showed similar trends for all 15 treatments. The variations in concentrations of NaCl, $CaCl_2$ and potassium sorbate did not significantly affect the cucumber color. Therefore, only the average chroma, hue, a/b ratio and ΔE values (interior and exterior) of the replicated treatments (5% NaCl, 0.2% $CaCl_2$ and 0.2% potassium sorbate) were presented (Fig. 7). The ratio a/b (exterior) and ΔE (interior) values increased while the hue (exterior) and chroma (interior and exterior) values decreased during the first few wks of fermentation. The decrease in the external hue values (from 120° to 90°), which occurred during the first week of fermentation, indicates that the external color of the cucumber became more yellow and less green. This color change was probably due to the conversion of chlorophyll into pheophytin (Francis, 1985) and it confirmed the visual impression that fermented cucumbers were more yellow than fresh cucumbers.

CONCLUSIONS

UNDER CONDITIONS simulating natural cucumber fermentation and storage, both NaCl and potassium sorbate were required in the brine to produce good quality pickles. When either was absent, spoilage occurred. In outdoor commercial fermentations exposed to sunlight, the problem of yeast and mold spoilage is less important. Thus, the use of sorbate may not be necessary with properly salted brines. With reduced salt natural fermentation and storage, however, sorbate may be needed to eliminate spoilage problems. Moreover, addition of $CaCl_2$ to the brine enhanced retention of firmness and reduced amount of potassium sorbate necessary to inhibit yeast and mold contamination. After 6 mo, cucumbers fermented and stored in brines containing 5% NaCl, 0.2% $CaCl_2$ and 0.2% potassium sorbate had excellent quality characteristics. This indicated that low salt concentration could be used with natural fermentation and commercial open tank storage, particularly when calcium and sorbate were added to the brine. However, a comprehensive optimization analysis should be performed to identify possible combinations of $CaCl_2$ and potassium sorbate

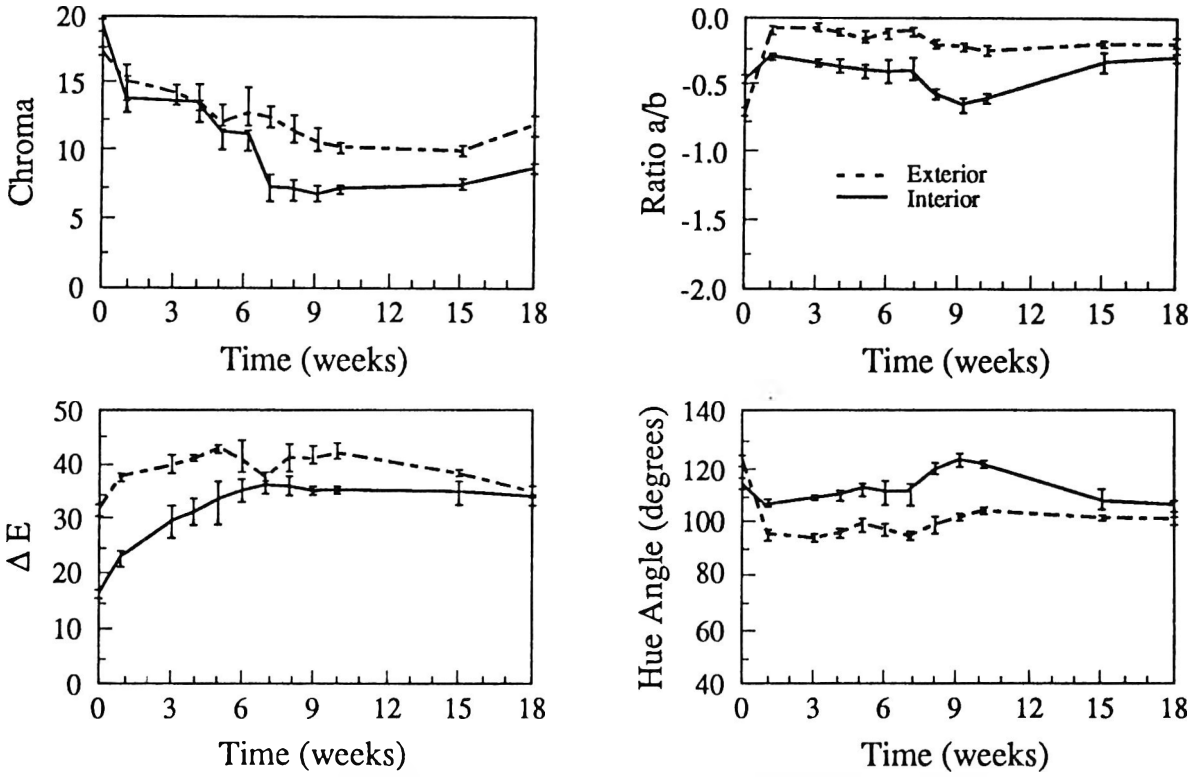

Fig. 7—Chroma, ratio a/b, ΔE and hue angle changes over time for cucumbers naturally fermented and stored in brines with 5% NaCl, 0.2% $CaCl_2$ and 0.2% potassium sorbate (Bars indicate ± one standard error).

concentrations which might allow lower NaCl concentrations to be used.

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Thermal Resistance of *Bacillus coagulans* in Double Concentrated Tomato Paste

A. J. SANDOVAL, J. A. BARREIRO, and S. MENDOZA

ABSTRACT

Thermal resistance was determined on a strain of *Bacillus coagulans* in double concentrated tomato paste (a_w = 0.95 at 23°C, pH = 4.0, 30.3°Brix, 70.1% moisture and acidity 1.30 g/100g citric acid. A microsyringe method was used with an inoculum of 1.3×10^4 spores/mL. Values of $D_{90°C} = 3.5$ min and $z = 9.5C°$ were obtained.

Key Words: tomato paste, Bacillus, microbes, thermal resistance

INTRODUCTION

VARIOUS MICROORGANISMS have been reported of practical importance in the deterioration of thermally processed tomato products, especially spore forming saccharolytic and butyric anaerobes such as *Clostridium pasteurianum* and *Clostridium butyricum* (Hersom and Hulland, 1964). Spore forming facultative anaerobes such as *Bacillus coagulans* have also caused spoilage of tomato products (NCA, 1968). Due to a higher thermal resistance compared with the saccharolytic and butyric spore forming anaerobes, *Bacillus coagulans* is normally used as an indicator in the design of thermal processes for sterilization of tomato products (NCA, 1968; York et al., 1975; Rodrigo et al., 1990).

The thermal resistance of *B. coagulans* in tomato products has been reported by various researchers. Youland and Stumbo (1953) studied the heat resistance of *B. coagulans* (ATCC 8038), subjected to moist heat. Knock et al. (1959) determined D values of *B. coagulans* in South African tomato juice. York et al. (1975) determined the heat resistance of *B. coagulans* (ATCC 8038) in tomato juice and Rodrigo et al. (1990) characterized the thermal resistance of strain ATCC 8038 in pureed and crushed tomatoes.

No published reports were found with regards to the thermal resistance of spore forming microorganisms in concentrated tomato paste. Thermal resistance values are essential for the design of the thermal processes involved in preservation of tomato paste, including continuous sterilization, hot filling, and aseptic methods. The objective of our study was to determine the thermal resistance of *B. coagulans* isolated from double concentrated tomato paste.

MATERIALS & METHODS

Sample collection and processing methods

Tomato paste samples were collected during industrial manufacture of double concentrated tomato paste in the 1991 tomato harvest season, in a commercial plant in Venezuela. The double concentrated tomato paste was processed according to normal industrial practice, employing a cold break process (Goose and Binsted, 1973; Gould, 1983).

Physical and chemical characteristics of tomato paste

To evaluate tomato paste characteristics that could affect thermal resistance of the microorganisms involved, the following analyses were performed: Water activity (a_w) at 23°C, employing psychrometric equipment (Decagon CX-1); paste pH (NCA, 1968) with a pH meter (Bantex-300A); soluble solids (°Brix), corrected for temperature and acidity (NCA, 1968; Goose and Binsted, 1973), employing an Abbé refractometer (Bausch and Lomb, model 3-E); moisture: utilizing a vacuum oven procedure at 70°C (AOAC, 1984); and acidity by electrometric titration (NCA, 1968; Goose and Binsted, 1973).

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Spore suspension and thermal resistance

The *Bacillus coagulans* strain was isolated and identified from double concentrated tomato paste as reported (Sandoval, 1991). This strain was isolated from samples from the industrial process after passing the heat exchanger and just before the hot fill-air cool process. Tomato juice agar (TJA) with pH adjusted to 5.0 was used for maintenance of the strain.

Sporulation of the selected strain was carried out in TJA at pH 5.0, incubating at 45°C for 3 days (Stumbo, 1973). The spores were collected when a level of about 90% sporulation was observed by microscope. They were washed three times and separated by centrifugation at 2600 rpm for 30 min and kept in M/15 phosphate buffer (pH 7.0) at 4°C until used. The spore concentration was determined by duplicate plate counts in GTY agar with pH 5.0, after incubation at 37°C for 24 hr.

Thermal resistance of the selected strain in double concentrated tomato paste was performed following the method developed by Kooiman and Geers (1975). This used vials (25 mm o.d. × 95 mm height) containing 5 mL. Tomato paste, sealed with a bakelite screw cap provided with a Neoprene septum and sterilized at 120°C for 20 min. The sterilized vials containing the tomato paste were introduced in a temperature controlled water bath (± 0.2°C) and stabilized at the required temperature. A copper-constantan thermocouple was introduced into one of the vials. Thermal resistance studies were conducted at 75, 80, 85, and 90°C. Spore suspension (50 μL) previously heated at 80°C for 20 min, were inoculated into each vial by means of a Hamilton microsyringe to obtain a final spore concentration of $\approx 1.3 \times 10^4$ spores/mL. Time was measured at regular intervals after inoculation of each vial. Time intervals depended on temperature studied ranging from 3 to 10 min. Five time intervals and a total of 15 vials (three of each time interval) were used for each temperature. In addition, three inoculated blanks not subjected to the thermal treatment were likewise prepared. After the designated time vials were removed from the heated water bath and introduced in an iced water bath at about 3°C to stop the thermal treatment. The surviving population was determined by plate counts in GTY agar, incubating at 37°C for 24 hr (York et al., 1975).

The thermal resistance parameters characterizing the microorganism studied were determined by plotting the survivor curves and the phantom thermal death time curve according to standard procedures (Hersom and Hulland, 1964; NCA, 1968; Stumbo, 1973). Data were fitted by means of a linear regression statistical program Statgraphics (Statistical Graphic Ssytem, version 2.6).

RESULTS & DISCUSSION

Physical and chemical characteristics

The physical-chemical characteristics obtained for double concentrated tomato paste, relevant to the thermal resistance of the microorganisms studied were (± standard deviation): a_w(23°C) = 0.953 ± 0.005; pH = 3.98 ± 0.11; °Brix (20°C) = 30.3 ± 0.5; Moisture (%) = 70.10 ± 0.20; acidity (g/100g citric acid) = 1.30 ± 0.45.

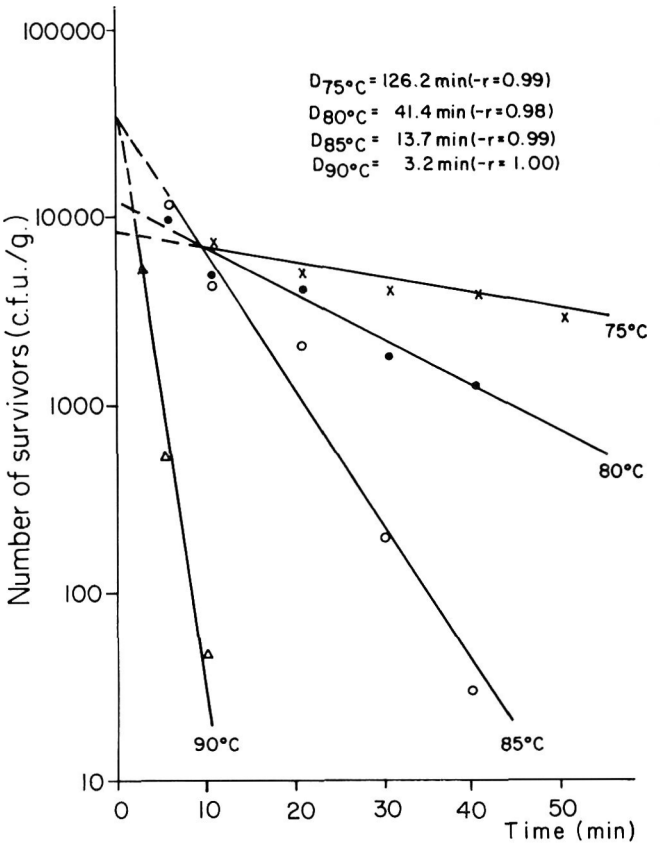

Fig. 1—Survivor curves for B. coagulans *in tomato paste.*

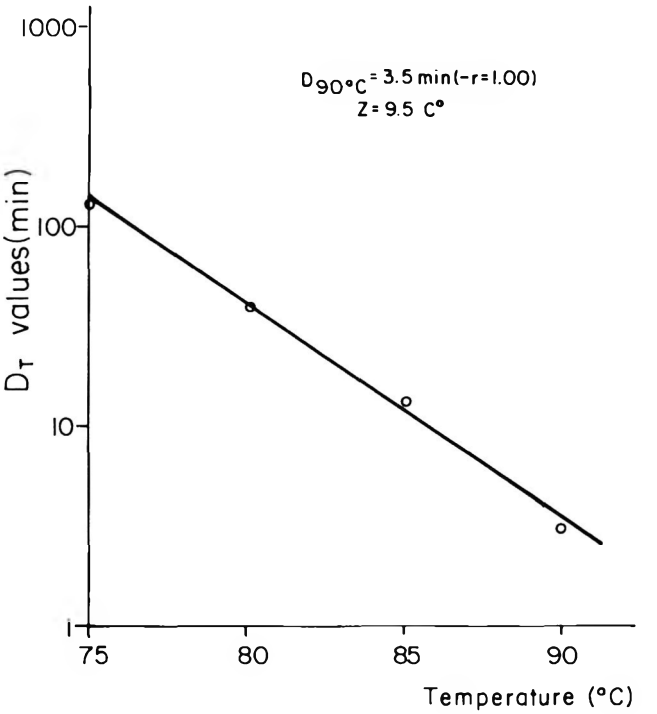

Fig. 2—Phantom TDT curve for B. coagulans *in tomato paste.*

Thermal resistance of Bacillus Coagulans

Values of $D_{75°C} = 126.2$ min, $D_{80°C} = 41.4$ min, $D_{85°C} = 13.7$ min, and $D_{90°C} = 3.2$ min were obtained (Fig. 1) after linear regression fitting of experimental data. High correlation coefficients were obtained in all cases ($r > 0.98$). The phantom thermal death parameters of $D_{90°C} = 3.5$ min and $z = 9.5C°$ were obtained (Fig. 2) for *B. coagulans* in tomato paste. Several authors have studied the thermal resistance of *B. coagulans* in tomato products. Youland and Stumbo (1953) reported values of $D_{104°C} = 1.72$ min and $D_{110°C} = 0.32$ min for *B. coagulans* (ATCC 8038). Knock et al. (1959) determined D values in South African tomato juice at 93.3, 96.1, 98.9, and 106°C of 13.4, 6.3, 3.1 and 0.51 min and a corresponding z value of 16.1C°. York et al. (1975) found values of $D_{100°C} = 0.53$ min. and $z = 15.6C°$ for the heat resistance of *B. coagulans* (ATCC 8038) in tomato juice with pH 4.2 employing an inoculum of 10000 spores/mL. Rodrigo et al. (1990) determined the thermal resistance of strain ATCC 8038 in pureed and crushed tomatoes at two different pH values, determining a heat resistance of $D_{90°C} = 12.3$ min and $z = 13.7C°$ for a pH of 4.5 and $D_{90°C} = 9.0$ min and $z = 13.9C°$ for a pH of 4.3.

Lower heat resistance values were obtained in our work for tomato paste, as compared with the reported values in tomato juice, crushed and pureed tomatoes indicated above. This could be due to the lower pH and higher soluble solids content and reduced a_w of tomato paste.

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Nutritional Quality of Hard-to-cook and Processed Cowpea

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ABSTRACT

Hard-to-cook (HTC) defect was induced in cowpea seeds by storing at 37°C and 85% relative humidity (RH) for 6 wk. HTC and control (stored at 7°C, 60% RH) seeds were boiled for 45 and 90 min or ground to flour. Flour was cooked as paste or extruded (20% H_2O, 150°C). Overall dietary quality was estimated as Feed Efficiency and Relative Response Ratio. Protein quality was determined as Nitrogen Efficiency and Relative Nutritive Value. Overall and protein nutritional quality were generally higher for control than for HTC cowpea diets. Processing affected control and HTC seeds differently. Boiling either control or HTC whole seeds improved both overall and protein quality compared to raw seeds, while extrusion substantially increased the quality of control seeds but reduced the quality of HTC seeds. Cooking as paste improved protein and overall quality of control seeds but not protein quality of HTC seeds.

Key Words: cowpeas, nutrients, hard-to-cook, protein

INTRODUCTION

COWPEA, *Vigna unguiculata* (L.) Walp., is the most important legume crop in West Africa, and provides more than half the plant protein in human diets in some areas of the semi-humid tropics (Dovlo et al., 1976). Hard-to-cook (HTC) defect has been demonstrated in various cultivars of *Phaseolus* and *Vigna* as well as other legume species. A recent survey of consumers in Nigeria revealed that HTC was identified as the leading constraint on cowpea consumption (Uwaegbute and Nnanyelugo, 1987). The populous coastal and savanna regions of West Africa experience an extended (4–6 mo) rainy season characterized by high relative humidities and temperatures which are conducive to development of the HTC defect. Hardening results in increased cooking time and a corresponding high energy cost to soften cowpea prior to consumption. HTC induced economic losses are high in developing countries where storage facilities are inadequate and high temperature and RH are prevalent.

Antunes and Sgarbieri (1979) reported that storage of dry beans under high temperature (37°C) and RH (76%) conditions had a severe effect on the rate of hardening and also lowered the protein quality and the availability of essential amino acids. The PER of the stored beans was reduced to about 40% of the original value after 6 mo. Molina et al. (1975) examined the interrelationship between storage, soaking time, cooking time and nutritive value of black beans. Storage (25°C, 3 and 6 mo) had a detrimental effect on the protein quality (PER) of beans subjected to any of the processes studied. A negative relationship was also observed between storage and protein digestibility. Uma Reddy and Pushpamma (1986) reported a 10–30% loss of lysine, methionine, and tryptophan in green gram, pigeon pea, and chickpea, and a decrease in net protein ratio (NPR) and protein digestibility during storage.

Though a relationship between HTC and protein quality has been described, the interaction of HTC and various cooking methods on overall nutritional quality has not been thoroughly characterized. In a previous study (Tuan and Phillips, 1991), we examined the effects of storage and processing on cowpea protein and starch digestibility. Protein digestibility was significantly lessened by high-temperature, high-relative humidity storage, especially for raw cowpea and cowpea flour which was cooked as a paste or extruded. Starch digestibility was not significantly affected. The objective of our present study was to evaluate the overall and protein nutritional quality of cowpeas which had been stored at control and accelerated storage conditions and then subjected to various processing treatments. Accelerated storage conditions were designed to develop the HTC defect.

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MATERIALS & METHODS

Storage conditions

Cowpea seeds (*Vigna unguiculata,* cultivar California Blackeye No. 5) were obtained from Kerman Warehouse, Kerman CA. Control seeds were stored at 7°C and 60% RH in tightly covered containers. Cowpeas for accelerated storage were dipped in a 70% ethanol solution containing 0.05% potassium sorbate/sorbic acid (pH 5.5) to retard mold growth and then air dried under cheesecloth. Beakers of saturated potassium chloride solution were placed into sealed plastic containers with seeds to control the inner environment at 85% RH (Pappas and Rao, 1987). The containers with the test samples inside were then stored at 37°C for 6 wk.

Processing treatments

Control (C) and test samples (T) were each divided into five subsamples of 4.54 kg for further processing (Tuan and Phillips, 1991): CB45-seeds were soaked overnight and boiled for 45 min; CB90-seeds were soaked overnight and boiled for 90 min; CF-seeds were ground into flours in a Retsch centrifugal mill with a 0.2 mm screen (model ZM1, Retsch GmbH, Haan, Germany); CP-part of the CF samples was slurried in water (1:4, flour to water ratio) and cooked to a paste at 100°C for 45 min; and CX-cowpea flour was adjusted to 20% moisture content and extruded at 150°C barrel temperature in a pilot scale extruder (Wayne Machine and Die Co., Totowa, NY) with the screw speed of 180 rpm as described by Phillips et al. (1984). HTC (test) samples were labeled respectively TB45, TB90, TF, TP, and TX.

Seed texture

The degree of hardness (shear-compression force) of control and HTC cowpea boiled for 45 and 90 min (50g cooked seed) were evaluated using an Instron Universal Testing Machine equipped with a Kramer test cell and a 500 kg load cell, with a crosshead speed and a chart speed of 50 mm/min each. The peak force required to shear the cooked seeds was expressed as N/g.

In vivo methods

Methods used to evaluate the nutritional quality of cowpeas via Weight Gain Coefficients and Relative Nutritive Values were those described by Canolty and Koong (1977) and Hegsted et al. (1968), respectively. A casein diet containing 0.015 g L-methionine/g casein and 42% corn starch to stimulate the starch content of cowpea served as a standard. Cowpea materials in the test diets served as protein and starch sources. The casein and cowpea diets were isonitrogenous (2.4% N), containing 1% vitamin mix, 3.5% mineral mix, and 5% corn oil, and were adjusted to 100% with sucrose (Tuan and Phillips, 1991).

Male weanling (21-day-old) Sprague Dawley rats (Charles River Laboratories, Wilmington, MA) were maintained on a stock diet until 24 days of age and randomly divided into 23 groups of five each.

Table 1—Effect of various diets on cumulative feed intake, body weight change and Feed Efficiency for rats fed diets for 21 days

Diet	Feed intake (g)		Body wt change (g)		Feed efficiency[h]	
	Group 1[g]	Group 2	Group 1	Group 2	Group 1	Group 2
C B 45[i]	151.68^{ab}	211.70^{a}	27.20^{a}	55.38^{bc}	0.18^{a}	0.26^{b}
T B 45	152.14^{a}	210.76^{ab}	24.20^{ab}	49.58^{cd}	0.16^{ab}	0.24^{bc}
C B 90	150.12^{abc}	214.34^{a}	26.70^{a}	56.66^{b}	0.18^{a}	0.26^{b}
T B 90	151.50^{ab}	210.16^{ab}	22.94^{ab}	48.68^{d}	0.15^{ab}	0.23^{c}
C P	150.52^{abc}	207.40^{ab}	22.84^{ab}	47.94^{d}	0.15^{ab}	0.23^{c}
T P	150.80^{abc}	202.60^{b}	19.34^{b}	40.02^{e}	0.13^{b}	0.20^{d}
CX	151.98^{a}	212.18^{a}	22.14^{ab}	51.32^{bcd}	0.15^{ab}	0.24^{bc}
TX	148.92^{c}	192.44^{c}	10.80^{c}	25.50^{g}	0.07^{c}	0.13^{f}
C F	150.22^{abc}	205.78^{ab}	18.68^{b}	36.86^{ef}	0.12^{b}	0.18^{de}
T F	149.58^{bc}	202.92^{b}	11.16^{c}	33.50^{f}	0.08^{c}	0.17^{e}
Casein	151.36^{ab}	211.84^{a}	26.76^{a}	66.20^{a}	0.18^{a}	0.31^{a}

[a-f] Means in the same column bearing different superscripts differ significantly at the 5% level.
[g] Group 1 was fed 7-8 g/day; Group 2 was fed 10-11 g/day.
[h] Feed Efficiency = body weight change/feed intake.
[i] C = control, T = HTC cowpeas, B = boiled 45 or 90 min, P = paste, X = extruded, F = flour.

Table 2—Regression equations relating body weight change and feed intake for rats fed various diets for 21 days

Diet	Regression equation[a]	R^2	WMC[b]	Relative response ratio	Diet difference[d]
C B 45	Y = 0.60(0.08)X − 416.01(90.96)	0.87	33.02	0.68	
T B 45	Y = 0.57(0.06)X − 415.80(68.14)	0.92	34.98	0.64	11
C B 90	Y = 0.59(0.04)X − 403.69(40.04)	0.97	32.42	0.67	8,9,11
T B 90	Y = 0.58(0.10)X − 442.72(117.79)	0.80	36.35	0.65	
C P	Y = 0.60(0.08)X − 457.81(93.87)	0.87	36.46	0.67	
T P	Y = 0.54(0.07)X − 431.75(85.67)	0.87	38.07	0.61	11
C X	Y = 0.66(0.10)X − 523.52(109.74)	0.85	37.83	0.74	8,9
T X	Y = 0.44(0.07)X − 389.84(76.13)	0.85	42.58	0.49	3,7,11
C F	Y = 0.41(0.10)X − 299.93(110.24)	0.70	34.50	0.47	3,7,11
T F	Y = 0.53(0.05)X − 477.70(59.26)	0.93	43.25	0.59	11
Casein	Y = 0.89(0.15)X − 664.94(160.78)	0.81	35.66	1.00	2,3,6,8,9,10

[a] Variables measured in grams/$kg^{0.75}$/21 days. Y represents change in body weight; X represents feed intake. Standard error in parentheses.
[b] Weight maintenance coefficient (dry matter/metabolic body size/day).
[c] Slope of the diet divided by the slope of casein diet.
[d] Indicates diets which have a significantly different regression slope from this equation at the 5% level.
See table 1 for diet codes.

Twenty groups were randomly assigned to the 10 cowpea diets, and two groups to the casein diet. One group was sacrificed at the start of the experiment for the purpose of estimating initial body moisture.

Animals were housed individually in wire cages in a controlled environment (22 ± 1°C, 50% RH), and water was provided *ad libitum*. For the first 14 days, one group receiving each diet was fed 7g of the diet/day, and the other group was fed 10g/day. These amounts were increased to 8 and 11 g/day, respectively, for the remaining 7 days to compensate for increasing animal size. Spilled food was collected and weighed daily. Animal weights were measured weekly and feed intakes were recorded daily. The experiment was terminated after 21 days.

Feed efficiency and relative response ratio

Feed Efficiencies were calculated by dividing the 21-day weight gains by the 21-day feed intakes. The mean body weight (in kg) for the experimental period, the average of the three weekly weights, was raised to the 0.75 power to determine each rat's metabolic body size (MBS). The slope of the regression line of feed intake vs body weight change (in MBS) obtained for a given diet was designated as the Weight Gain Coefficient (WGC) for that diet. The intake of diet that would result in neither a gain nor a loss of body weight, the Weight Maintenance Coefficient (WMC), was calculated from the regression equation by setting change in body weight equal to zero. Relative Response Ratio was obtained by dividing the slope of the cowpea diet by the slope of casein diet (Gillete et al., 1978; Canolty and Koong, 1977).

Determination of body water as an indicator of body nitrogen

The moisture content of carcass-minus-GI-tract was determined as an estimate of body nitrogen (Hegsted et al., 1968; Phillips, 1980). Rats killed on day 1 or day 22 were dissected, and GI tracts were removed. The fresh carcass-minus-GI-tract was weighed and body moisture was determined by oven drying for 72 hr at 100°C. The initial body water of each rat was determined by multiplying the weight of the rat by the mean moisture content of rats killed on day 1. Estimated body nitrogen change was calculated from body water change using the following equation:

$$Y = 0.027X + 2.86$$

Where Y equals (N% × 100)/H_2O%, in g; and X equals age in days (Pellett, 1967). Protein quality was assessed by calculating Nitrogen Efficiencies (NE = change in body nitrogen/nitrogen consumed) for each diet and intake level and by generating regression equations for body water vs. nitrogen intake for animals receiving each diet. Relative Nutritive Value was calculated as the ratio of slope of cowpea diet to that of the casein diet.

Statistical analysis

Results were analyzed by the Statistical Analysis System program (SAS, 1985) using one-way and two-way Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test. The various methods of assessing quality of cowpeas subjected to storage conditions and processing treatments were compared using the correlation procedure (Proc Corr) of SAS Institute, Inc. (1985).

RESULTS & DISCUSSION

Seed texture

The effects on cooked cowpea texture of all variables: storage conditions (7°C and 60% RH; and 37°C and 85% RH) and cooking time (45 and 90 min), as well as their interactions were significant at the 5% level. Mean shear/compression forces of seeds boiled for 45 and 90 min were 6.88 and 5.96 N/g, respectively, for control cowpeas and 42.78 and 29.39 N/g for HTC seeds. Several investigators have observed similar increases in seed hardness during high temperature/high RH stor-

Table 3—Effect of various diets on nitrogen intake, body water change and Nitrogen Efficiency for rats fed diets for 21 days at 2 feed intake levels

Diet	Nitrogen intake (g)		Body water change (g)		Nitrogen Efficiency[f]	
	Group 1[g]	Group 2	Group 1	Group 2	Group 1	Group 2
C B 45	3.59^{cde}	5.02^{b}	6.38^{abc}	25.66^{b}	0.072^{abc}	0.208^{b}
T B 45	3.54^{e}	4.91^{b}	5.49^{bc}	19.50^{cd}	0.063^{bc}	0.162^{c}
C B 90	3.68^{b}	5.25^{a}	8.01^{ab}	28.97^{b}	0.089^{ab}	0.225^{b}
T B 90	3.55^{e}	4.92^{b}	4.82^{bc}	18.94^{cd}	0.056^{bc}	0.157^{c}
C P	3.55^{e}	4.89^{b}	6.19^{abc}	19.24^{cd}	0.071^{bc}	0.160^{c}
T P	3.60^{cd}	4.84^{bc}	2.11^{c}	14.87^{de}	0.024^{cd}	0.125^{cd}
C X	3.89^{a}	5.43^{a}	5.71^{bc}	20.39^{c}	0.060^{bc}	0.153^{c}
T X	3.62^{c}	4.67^{c}	−3.68^{d}	3.47^{f}	−0.041^{e}	0.031^{e}
C F	3.56^{de}	4.88^{b}	2.54^{c}	11.80^{e}	0.029^{c}	0.099^{d}
T F	3.63^{bc}	4.93^{b}	−2.18^{c}	10.85^{e}	−0.024^{de}	0.089^{d}
Casein	3.56^{de}	4.98^{b}	10.56^{a}	39.48^{a}	0.121^{a}	0.314^{a}

[a-e] Means in the same column bearing different superscripts differ significantly at the 5% level.
[f] Nitrogen Efficiency = estimated body nitrogen change/nitrogen intake.
[g] Group 1 was fed 7-8 g/day; group 2 was fed 10-11 g/day.
See Table 1 for Diet Codes.

Table 4—Regression equations relating body water change and nitrogen intake for rats fed various diets for 21 days

Diet	Regression equation[a]	R^2	Relative nutritive value[b]	Diet difference[c]
C B 45	Y = 13.52(1.81)X − 42.19(7.89)	0.87	0.68	7,8,9,11
T B 45	Y = 10.21(1.54)X − 30.66(6.59)	0.85	0.51	3,11
C B 90	Y = 13.33(0.79)X − 41.03(3.57)	0.97	0.67	2, 5–11
T B 90	Y = 10.33(2.16)X − 31.83(9.26)	0.74	0.52	11
C P	Y = 9.66(1.71)X − 28.08(7.31)	0.80	0.48	3,11
T P	Y = 10.22(1.11)X − 34.68(4.74)	0.91	0.51	3,9,11
C X	Y = 9.52(1.07)X − 31.34(5.03)	0.91	0.48	1,3,11
T X	Y = 5.33(2.61)X − 22.19(10.93)	0.34	0.27	1,3,10,11
C F	Y = 6.77(1.39)X − 21.39(5.95)	0.75	0.34	1,3,6,10,11
T F	Y = 10.14(0.84)X − 39.08(3.65)	0.95	0.51	3,8,9,11
Casein	Y = 20.02(2.35)X − 60.44(10.15)	0.90	1.00	1–10

[a] Y represents change in body water, X represents nitrogen intake. Standard error in parentheses.
[b] Slope of the diet divided by the slope of casein diet.
[c] Indicates diets which have a significantly different regression slope from this equation at 5% level.
See Table 1 for diet codes.

age. For example, Sefa-Dedeh et al. (1979) observed that force to shear cooked cowpeas (100 min) increased from ~25 kg for seeds which had been stored at 0°C to ~100 kg for seeds which had been stored for 12 months at 29°C and 85% RH.

Overall nutritional quality

Canolty and Koong (1976, 1977) pointed out that the use of graded intakes of the same diet rather than graded levels of protein in *ad libitum* fed animals permits evaluation of overall dietary quality rather than protein quality alone. Feed efficiency can be calculated in an analogous way to PER. Weight gain coefficients are calculated from dose-response curves that correlated body weight gain and feed consumed in excess of maintenance requirements, providing a multipoint assay. All diets contained sufficient added vitamins and minerals to meet animal requirements and there was no design to test cowpea materials as a source of those nutrients.

The effects of various diets on cumulative feed intake, body weight change and feed efficiency for rats fed diets for 21 days were determined (Table 1). Rats fed various diets had body weight increases. The effects of experimental treatment on body weight change were less notable when feed intakes were severely restricted (7–8 g/day) than when feed intakes were mildly restricted (10–11 g/day). There was no significant difference in feed efficiency between casein diet and cowpea boiled for 45 and 90 min or control cowpea paste or extrudate when rats were fed 7–8 g/day. This indicated that these six diets were nutritionally comparable to casein diet in providing body basic requirements at this intake level. At the mildly restricted level, casein diet had the highest feed efficiency (0.313) and among processing treatments control cowpea diets were consistently higher than test cowpea diets. Significant differences between control and test diets were observed for cowpea which was boiled for 90 min, cooked as a paste or extruded. The difference was especially notable when rats were fed control extrudates compared to test extrudates (0.242 vs 0.133).

Linear regression equations were obtained for change in body weight as related to feed intake for rats fed various diets for 21 days (Table 2). The weight grain coefficients and thus the relative response ratios of rats fed control cowpea diets were higher than those fed HTC cowpea diets for all processing treatments except for uncooked flour. The WGCs of control cowpea boiled 45 min, cooked as paste, extruded and test cowpea boiled for 90 min were not significantly different from casein diet while the others were significantly lower. Among cowpea diets, WGCs of control cowpea boiled for 90 min or extruded were significantly higher than those of the two least effective diets, extruded test cowpea and control flour. Other diets were not significantly different. The ability of cowpea products to promote growth (relative response ratio) ranged from 46% to 74% of the reference. Weight maintenance coefficients of control cowpea diets were lower than those for test cowpea diets indicating less feed was needed to maintain body weight.

Protein nutritional quality

The amount of body water correlates very well with body nitrogen. Therefore, relating body water change to nitrogen intake can be used to estimate protein utilization. The results of nitrogen intake and body water change of rats fed various diets at 2 feed intake levels are shown in (Table 3). Since all 11 diets were isonitrogenous (2.4% N), the difference in nitrogen intake can be attributed to the amount of feed consumed. Loss in body water was observed in rats fed HTC cowpea extrudates and HTC raw cowpea flour when feed intake was severely resticted. Since the nitrogen intakes of rats

fed both diets were even higher than those of most of the other diets, the body water loss was due to the low protein quality of the two diets. There was body water gain for rats fed all 10 diets when feed intake was mildly restricted. In most cases, gain in body water was significantly higher for control than for HTC cowpea-fed groups. As was the case for feed efficiencies, nitrogen efficiencies at severely restricted intakes were low and only extrudates and flour were significantly different among processing treatment pairs (Table 3). At the mildly restricted levels, NEs were higher, although still low compared to studies when energy was not restricted (Miller, 1973). At this level, control boiled and extruded seeds had significantly higher NEs than their test cowpea counterparts.

Regression equations for nitrogen intake vs body water change were developed (Table 4). The casein diet had a significantly greater slope than all cowpea diets. Differences between regression slopes of control and test cowpeas subjected to the same processing treatments were not significant in most cases. However, the slope for test flour was significantly higher than for control flour. Relative nutritive values of cowpea diets ranged form 34% to 68% that of casein. In comparing results it appears that change in body water (nitrogen) was more sensitive to protein quality than change in body weight was to overall diet quality. That is, the less effective diets were better sources of energy than of protein.

Nutritional quality of HTC cowpeas was consistently lower than that of control peas. Previously reported (Tuan and Phillips, 1991) *in vitro* and *in vivo* protein digestibilities were highly and positively correlated to overall nutritional quality. Starch digestibility had little influence on overall nutritional quality. Cowpea starch contributed about 40% of non-protein calories in cowpea diets. Thus, while the *in vivo* method would be expected to be less sensitive to starch-calorie factors than protein factors, sizable reduction in starch digestibility would be expected to have an impact on overall quality. Whole boiled seeds generally had higher protein quality than cooked flour. The HTC defect is considered by most investigators to be principally a cell wall phenomenon in which the entry into cells of water needed for starch gelatinization and protein denaturation during cooking is restricted. This would be expected to reduce the digestibility of these macromolecules. For this reason, it was anticipated that grinding HTC seeds prior to cooking would help overcome the expected loss of nutritive quality due to the HTC defect. In fact the gap between HTC and control cowpea quality was wider for treatments in which the seeds were ground prior to cooking. It has been shown that mobilization and increase of phenolic acids occurs during development of the HTC defect (Srisuma et al., 1989) and binding of phenolics to protein has been shown to reduce digestibility (Hurrell et al., 1982). Further research to elucidate the mechanism of protein quality loss during storage is desirable. Present findings indicate that grinding hardened seed and cooking them as paste or by extrusion may result in nutritionally less inferior products. This needs to be confirmed by additional studies, and analysis of amino acid content and availability, phenolic acids and tannins, and other factors which may affect protein quality.

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Biochemical Characterization of a Low Trypsin Inhibitor Soybean

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ABSTRACT

A low trypsin inhibitor soybean (LTI) was characterized using electrophoresis, enzyme activity measurements, and gel exclusion chromatography. The protein profiles were similar to a control soybean. Gel exclusion chromatography resulted in two peaks of trypsin inhibitor activity in the control. The first peak, absent in LTI, proved to be Kunitz trypsin inhibitor and was electrophoretically isomorphic. The second inhibitor consisted of at least five isotypes and co-eluted with Bowman-Birk trypsin inhibitor. Shorter heating times were required to inactivate both trypsin and chymotrypsin inhibitor activity in LTI compared to control soybeans. The use of LTI may increase the economic viability of soybeans as protein supplements for humans.

Key Words: soybean, trypsin, inhibition, biochemistry, enzymes

INTRODUCTION

SOYBEANS (SB) are presently being widely used in North America as a protein source for animal feeds. A major disadvantage for using SB as a protein supplement for nonruminants is the presence of anti-nutritive factors which create the need for heat processing. This increases costs and reduces the economic viability of SB in animal feeding systems. Processing is required to denature trypsin inhibitors which otherwise impede protein digestion and normal growth of non-ruminants (Rackis et al., 1986).

Trypsin inhibitors in SB are comprised of two main types of water soluble proteins: Kunitz trypsin inhibitor (KTI), with a molecular weight of 21 Kd and Bowman-Birk trypsin inhibitor (BBTI) with a molecular weight of 8 Kd (Tan-Wilson and Wilson, 1986). Whereas KTI inhibits only trypsin, BBTI inhibits both trypsin and chymotrypsin (Ikena and Norioko, 1986; Seidl and Liener, 1972). A number of genetic varieties of SB exist, including a line which lacks the gene for KTI (Orf and Hymowitz 1979). A KTI-lacking isoline has been investigated for possible replacement of conventional SB in animal feeds (Han et al., 1990; Friedman et al., 1991). An early maturing low trypsin inhibitor soybean (LTI), designated X-2033-3-7-B, may also be of significance in helping to improve the nutritional value of SB (Chohan, 1991). This LTI line, developed at Agriculture Canada Research Branch (Ottawa), was derived by a F_5 selection from crossing McCall SB (maturity group 00 variety from Minnesota) with a near isogenic line of Williams 82 known as L81-4583 (an Illinois variety of maturity group IV). In each cycle progeny lacking KTI were selected and crossed again to Williams 82 to achieve an early maturing low trypsin inhibitor SB (Personal communication, Harvey Voldeng). Our study focused on establishing some of the biochemical characteristics of LTI and the effects of heat treatment on these characteristics as a basis for establishing its usefulness as a seed crop.

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Materials & Methods

Materials

Maple Isle (MI) and LTI soybeans were grown in Prince Edward Island by Agriculture Canada Research Branch, Charlottetown. LTI seeds were generously suppled by Agriculture Canada Plant Research Institute, Ottawa. Chemicals were purchased from Sigma Chemical Company (St. Louis Mo.), except where otherwise indicated.

Trypsin and chymotrypsin inhibitory activity

Trypsin inhibitory activity was measured by the method of Liu & Markakis (1989) which uses benzoyl-dl-arginine-p-nitroanilide (BAPA) as a substrate. For chymotrypsin inhibitor activity glutaryl-phenylalanine-p-nitroanilide (GPNA) was the substrate. Results were calculated in terms of Trypsin Inhibitor Units, TIU (1TIU = 1μmole of BAPA hydrolyzed min at 37°C), and Chymotrypsin Inhibitory Units, CTIU (1CTIU = 1μmole of GPNA hydrolyzed min at 37°C).

Extraction

Representative samples of SB were ground through 1 mm mesh (Brinkmann mill ZM1) using liquid nitrogen to prevent heating. For FPLC separations 0.5g samples were extracted with 3 mL of a solution containing 50 mM Tris, 10 mM $CaCl_2$ pH 8.2. Mixtures were homogenized for 5 sec with a Brinkmann Polytron Homogenizer, centrifuged for 15m at 1500 × *g* and filtered through 0.45 μm nylon syringe filters. For electrophoresis, without prior FPLC separation, the extraction used 0.1g samples in 3mL buffer. Proteins were measured by the method of Lowry (1959).

FPLC

Soybean extracts were further separated via liquid chromatography. A Pharmacia FPLC system was used with a gel exclusion (Superose 12HR 10/30) column. Injections were 3mg of protein in 100μL of extraction solvent. The flow rate was 0.2mL/min and 40-1 min fractions were collected after an initial delay of 50 min. The flow rate was increased to 0.5ml/min and the run continued until 200 min to clean the column before the next run. The collected fractions were put on ice and analyzed directly for trypsin inhibitor activity as described above. Fractions showing trypsin inhibitor activity were further analyzed by SDS-PAGE and native gel electrophoresis with trypsin inhibitor detection.

Electrophoresis

Electrophoresis was carried out on an LKB Midget electrophoretic system. SDS-PAGE was used for separating proteins on the basis of molecular weight (Laemmli, 1970). Native PAGE was used for separations on the basis of charge. In both cases 12.5% polyacrylamide gels were used; and either stained with coomassie blue (SDS-PAGE) or used to detect the presence of trypsin inhibitors by the method of Uriel and Berges (1968). Gels for trypsin inhibitor detection were immersed in 200 mg/mL trypsin in 100 mM sodium phosphate buffer, pH 7.5 for 20 min at 37°C, rinsed twice with distilled water and immersed in freshly made protease substrate for 10 min. Protease substrate contained 21 mg N-acetyl-dl-phenylalanine β-naphthyl ester (APNE) in 8.75 mL N,N-dimethylformamide freshly mixed with 42 mg o-dianisidine in 77 mL of 100 mM sodium phosphate buffer. Trypsin inhibitor zones were clear against a pink background.

Roasting

Whole soybeans were roasted on an electrical roaster designed for roasting SB with precise control of the temperature and residence time

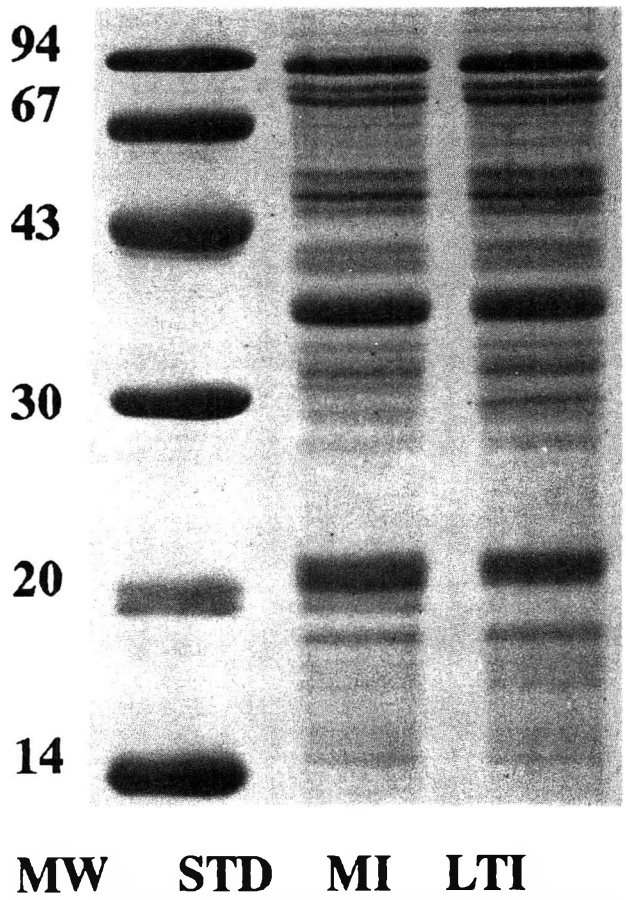

Fig. 1—SDS-PAGE of Maple Isle (MI) and Low Trypsin Inhibitor (LTI) soybeans. The arrow in the MI lane points to the Kunitz trypsin inhibitor which is missing in LTI. The second arrow points to the 30 KD protein which was found in LTI but not MI.

of the beans (Charlottetown Metal Products, Charlottetown, PEI). With this roaster the temperature could be selected. A metal conveyer belt moved the soybeans through the roaster at a controlled rate which determined residence time. Effects of roasting on chymotrypsin and trypsin inhibitory activity were tested in LTI and control SB for various roasting times at 250°C. Protein solubility was determined in 0.2% potassium hydroxide and used as an indicator of degree of roasting (Araba and Dale, 1988).

RESULTS

LTI contained 60% of the inhibitory activity of the control SB. Comparison of the protein profiles by SDS-Page revealed a missing protein band at 20 Kd in LTI (Fig. 1). A protein of 30 Kd was found in LTI which was not present in the control (Fig. 1). That band did not possess trypsin inhibitor activity. The remainder of the electrophoretic profile was similar for the two SB lines.

Gel exclusion FPLC resulted in 2 distinct peaks of trypsin inhibitory activity in the control and one in the LTI (Fig. 2 A & B). The first peak in the control contained 40% of the TIA and co-eluted with commercial KTI on FPLC, SDS-PAGE, and conventional PAGE with trypsin inhibitor detection (Fig. 1 and 2). The second peak had a similar retention time to the peak found in LTI and co-eluted with commercial BBTI. Trypsin inhibitor detection on electrophoresis gels showed five distinct bands of trypsin inhibitor activity from LTI extracts with none corresponding to KTI, while the control SB had a large KTI component (Fig. 2c). An electrophoretic analysis of the FPLC fractions showed that peak 1 possessed a single entity which corresponded to commercial KTI whereas peak 2 showed the subsequent appearance and disappearance of the other isotypes (Fig. 2D). Chymotrypsin and trypsin inhibitor activities

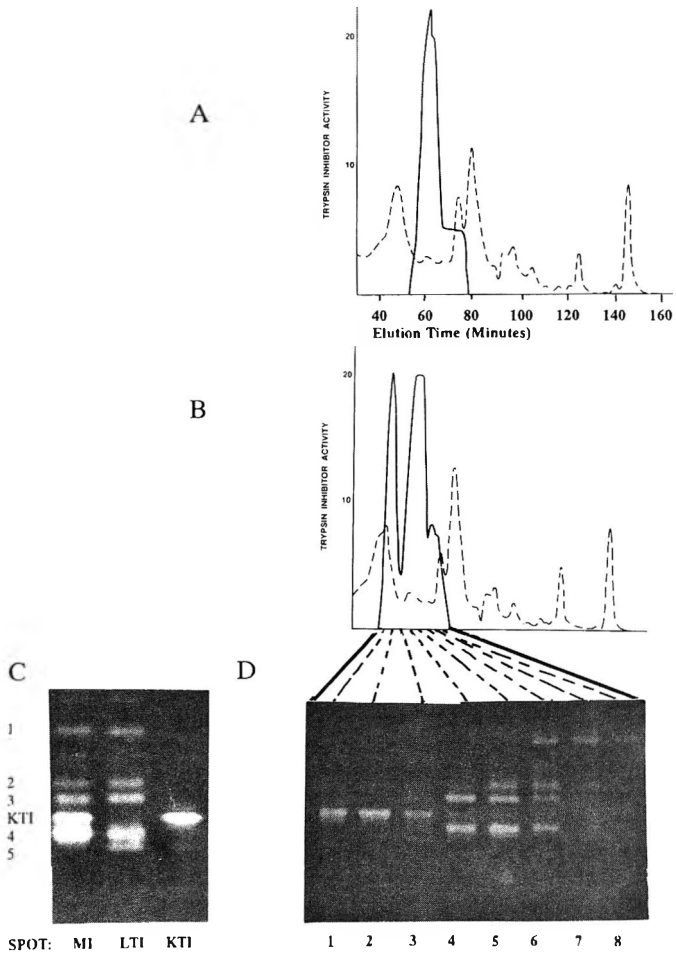

Fig. 2—FPLC chromatograms of soybean extracts LTI (A), and Control (B). The dark line is trypsin inhibitor activity per fraction. The protein profile, as indicated by absorption at 280nm, is shown as a dotted line. FPLC conditions are described in the text. Trypsin inhibitor detection on PAGE (C). MI=Maple Isle variety; LTI=Low Trypsin Inhibitor variety; KTI=Kunitz trypsin inhibitor standard. Trypsin inhibitor detection of FPLC fractions (D). The FPLC fractions analyzed on this gel were the fractions containing TIA. These fractions were collected at the times shown by the lines connecting the FPLC chromatogram to the gels.

were reduced faster in LTI SB upon roasting with an electric roaster (Fig. 3). LTI and control SB maintained a similar degree of potassium hydroxide solubility (Table 1).

Discussion

THE PROTEIN profile of LTI fits the general pattern of SB proteins found by Savoy (1977) in his studies on 21 different SB varieties. The major protein components found in the extracts of the SB had molecular weights of 84, 53, 37, and 21 KD. These proteins represent the α- and β-subunits of β-conglycinin and the acidic and basic subunits of glycinin, respectively, which comprise 65—80% of the total proteins in SB (Murphy and Adoracion, 1984; Sathe et al., 1987; Sato et al., 1986). The significance of a 30 KD protein found in LTI, but not MI extracts, is unknown. LTI proteins fit the general pattern found in SB, and by that criterion are unlikely to vary significantly in nutritional value from that of other SB varieties.

The Kunitz trypsin inhibitor appeared to be absent in LTI based on the SDS-PAGE profile. This was verified by FPLC and Trypsin Inhibitor detection on native electrophoretic gels. The difference in trypsin inhibitory activity in LTI and control SB corresponded to the amount of KTI in the control SB.

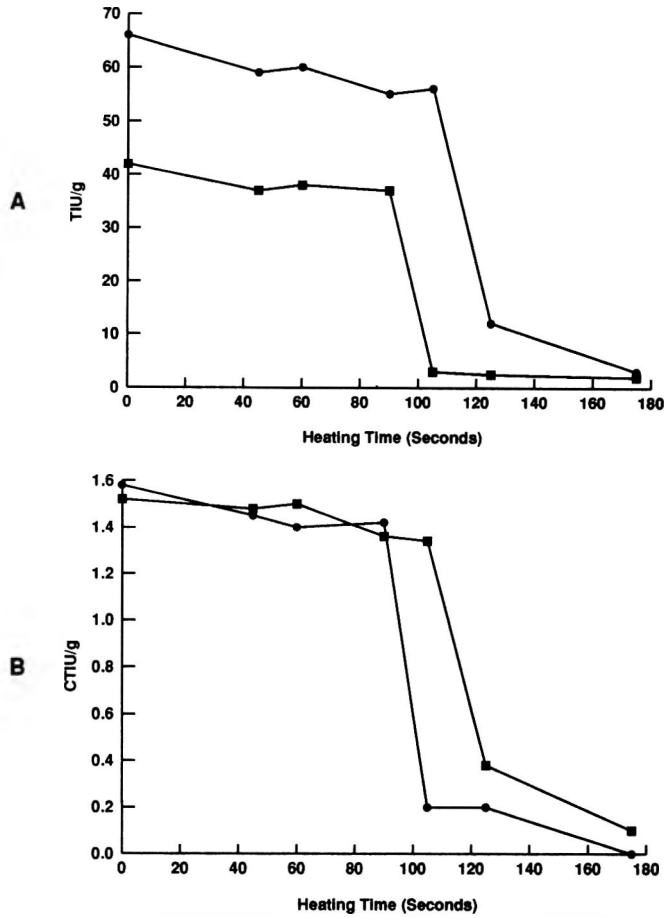

Fig. 3–Effect of roasting on trypsin inhibitor (A) and Chymotrypsin inhibitor (B) levels in Maple Isle (+), and Low Trypsin Inhibitor (□), Soybeans.

Table 1–Potassium hydroxide solubility[a] of LTI and MI soybeans after various roasting periods at 250°C. [Trypsin Inhibitor (TIU/gr) is in Brackets (mean ± sd, n = 2)]

Roasting time (sec)	Maple Isle	LTI
0	79 ± 5 (61 ± 4)	81 ± 7 (41 ± 3)
90	76 ± 2 (53 ± 6)	68 ± 5 (40 ± 5)
125	76 ± 3 (17 ± 4)	70 ± 5 (6 ± 3)
175	71 ± 2 (4 ± 1)	71 ± 3 (2 ± 1)
240	63 ± 5 (2 ± 1)	71 ± 4 (<1)

[a] Protein solubility in potassium hydroxide is expressed as the percentage of total proteins which are soluble in 0.2% KOH after shaking for 20 min. Results are expressed in terms of mean ± sd, n = 2.

Therefore, the absence of KTI sufficiently accounted for the lower amount of trypsin inhibitory activity in LTI.

Whereas the FPLC fractions from the first peak of trypsin inhibitor activity (corresponding to KTI) were electrophoretically isomorphic, fractions in the second peak showed electrophoretic polymorphism, indicating that BBTI had more isotypes than KTI in both types of SB (Fig. 2). Multiple forms of BBTI are known to exist in SB (Tan-Wilson and Wilson, 1988). Tan-Wilson et al. (1987) classified trypsin inhibitors in four major subgroups with multiple forms in each group. With that large number of molecular entities sharing a common function, it may be hypothesized that some of the proteins had other functions in the growth of SB, perhaps at different stages in seed development. If this were true, it would be very difficult to genetically remove the complete trypsin inhibitor array of proteins found in SB without seriously compromising the plant's ability to grow.

The Kunitz trypsin inhibitor was previously considered to be more heat labile than BBTI based on the activity loss of the purified inhibitors (Obara and Watanabe, 1971). However, DiPietro and Liener (1989) demonstrated that, in the SB matrix, BBTI was inactivated at a faster rate than KTI upon heating. In our investigation, LTI, which contains primarily BBTI isotypes, required less roasting than control SB in order to destroy both the chymotrypsin and the trypsin inhibitor activity (Fig. 3). This suggested that BBTI, and possibly KTI, were protected to a greater extent in the SB matrix which contains both inhibitors.

A SB isoline lacking KTI, characterized by Friedman et el. (1991), was found to require less steam heating time than standard SB to eliminate the trypsin inhibitor activity. In a related study, a KTI lacking isoline, designated LTS, showed significant antinutritional effects (Han et al., 1990). The unheated LTI line we investigated did not show improved growth performance in starter broiler chickens compared to unheated MI soybeans; however, it did show decreased effects on pancreatic hypertrophy (Chohan, 1991). Soybeans deficient in KTI still require some processing to eliminate the remaining antinutritional effects.

A shorter heating time at 250°C was required to inactivate the trypsin and chymotrypsin inhibitory activities in LTI compared to control SB. This allows for a more energy efficient processing of SB thereby reducing the cost of using SB in animal feeding systems. An additional problem, has been a common tendency for producers to roast SB beyond that degree necessary to destroy protease inhibitors, to the extent that the SB proteins become heat damaged and biologically unavailable. The LTI did not show greater sensitivity to heat damage than the control SB (Table 1).

The hypothesis that LTI soybeans require less heating compared to a conventional SB, in order to destroy trypsin inhibitors, is supported by our results. The increased use of LTI and similar lines of SB may improve the economic viability of SB as animal feed supplements.

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Physicochemical Properties of Isolated Soy Proteins from Normal, Broken or Damaged Seeds

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ABSTRACT

Soybean quality depends on growing, harvesting and storage conditions. The amount of broken or otherwise damaged seeds tends to increase during storage and may affect the quality of products. Protein isolates from altered seeds have physicochemical properties less useful than those of normal seeds. The effect is substantial on viscosity, gel forming ability and emulsion stability. Isolated proteins from damaged seeds mixed with those of normal seeds altered proportionally their gelation and water retention capacity of the gels. Viscosity or water absorbing capacity of protein isolates may be used as simple indicators to evaluate quality.

Key Words: soy proteins, broken seeds, soybeans, viscosity, gel-forming-ability, SPI

INTRODUCTION

TECHNOLOGICAL USES of soy protein isolates (SPI) depend largely on functional properties. However, commercial products may have diverse qualities representing different levels of protein denaturation and variable functionality (Wagner and Añon, 1990).

In Brazil the quality of soya beans is heterogeneous due to different growing, harvesting and storage conditions. Usually the amount of broken or otherwise damaged seeds can reach 10% of the total or higher and tends to increase with prolonged storage. Breakage of seeds occurs during harvesting mostly when moisture content is < 13%. Other damage such as rotting, shrivelling, purple, gray and brown discolorations of the seed coat, may be due to fungi attack (Silva et al., 1982; Yorinori, 1982). The effect of the degree of maturation, cultivar, storage and processing conditions, on the physicochemical properties of isolated soy proteins, have been well documented (Yao et al., 1988; Nash and Wolf, 1980; Nakamura et al., 1984; Hoshi et al., 1982a, b; Hoshi and Yamauchi, 1983a; Saio et al., 1982; Voutsinas et al., 1983; Matsudomi et al., 1985; Furukawa and Ohta, 1983).

Our objective was to determine the influence of seed quality on the yield and physicochemical properties of isolated soy proteins. We were also seeking simple indicators to evaluate quality of the isolates.

MATERIALS & METHODS

SOYA BEANS (Glycine max) were obtained from Sanbra S.A. (R.S.-Brazil) in August 1988 (end of the harvest season). They were separated by visual inspection into normal, broken, and damaged seeds. The flours were obtained by grinding the seeds (5 kg samples) to 60 mesh in a Marconi TE 345 mill and were defatted by hexane in a Soxhlet apparatus to final oil content <0.4%. The defatted flours, corresponding to each type of seed, were then used ot obtain acid precipitated proteins (APP).

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Acid precipitated proteins (APP)

APP were prepared according to the method of Furukawa and Ohta (1983) with slight modifications. The defatted flours from the different seed types (2 kg samples) were submitted to aqueous extraction at pH 7.0 and 50°C (ratio flakes to water 1:10), clarification of the extract by centrifugation and precipitation of the globulins by acidification to pH 4.5 with HCl (30%). The protein precipitate was washed with water and aqueous dispersions of the resultant APP, (protein concentration ≈13 w/w %) were prepared and the pH adjusted to 7.0 with NaOH. Protein concentration was determined by the Kjeldahl method (AOAC, 1985). We then determined solubility, apparent viscosity and color. Samples of the APP from the different seeds were also freeze-dried for further analysis.

Heat treatment of APP dispersions

After determination of solubility, apparent viscosity and color, the APP dispersions were heated to 85°C in a beaker by immersion in a water bath (90°C ≈30 min) and immediately cooled to 25°C (≈3 min). After standing for 2.5 hr at 20°C, we analyzed for solubility, color, emulsifying capacity, emulsion stability and gel texture.

Color and solubility

Color of the APP dispersions before or after heat treatment was measured using a Hunter-Lab Colorimeter (Optical Sensor D25-A). Solubility characteristics before and after heat treatment, in distilled water or 3% NaCl, were evaluated using the Nitrogen Solubility Index (NSI, AOCS, 1982). Results were expressed as percentage of soluble nitrogen (in H_2O or 3% NaCl) in relation to total nitrogen in the sample. Solubility characteristics of the APP after freeze-drying were evaluated after shaking 1 hr at 25°C or 50°C in distilled water, 0.2M NaCl or buffer (Tris-HCl 0.03M, pH 8.0, with 0.01M β-mercaptoethanol). Protein concentration was determined in the supernatant after centrifugation (16300 × *g*/20 min) by the method of Lowry et al. (1951). Determinations were made in triplicate.

Emulsifying capacity

The emulsifying capacity of the APP dispersions after heat treatment was determined according to Li-Chan et al. (1984) with some modifications. Protein solutions (50 mL of 0.5%) were titrated with soybean oil containing Sudan III 0.05% (to facilitate detection of the inversion point), at the rate of 20 mL/min. Determinations were made in a water bath at 20°C with a Janke & Kunkel homogenizer (RE 166 model) at 750 rpm for the first 3 min and 1400 rpm up to the inversion point. Emulsifying capacity was expressed as g oil/g protein. All determinations were in triplicate.

Emulsion stability

Emulsions were prepared at the ratio protein:water:vegetable fat 1:7:7 either in 2% NaCl or in water. The mixtures were homogenized in vacuum at 15°C. Samples (25 g) of the emulsions (in water or 2% NaCl) were exactly weighed and centrifuged at 860 × *g*/10 min for the mechanical resistance test. Emulsion heat stability was evaluated in two ways. Triplicate samples of each emulsion (after centrifugation) were kept at 5°C for 16 hr and then heated at 110°C for 1 hr or alternatively were frozen at −15°C for 16 hr and then heated at 100°C for 1 hr. The results were expressed as percentage of separated fat.

Table 1—Comparison of Protein, moisture and Nitrogen Solubility Index (NSI) of defatted soy flours from soybeans

	Seed condition		
	Normal	Damaged	Broken
Protein (%)[a]	51.2	50.4	50.4
Moisture (%)[a]	8.8	8.2	10.4
NSI in water (%)	75.4 ± 0.3	62.4 ± 0.3	77.2 ± 0.7
NSI in 3% NaCl (%)	68.9 ± 0.8	57.4 ± 0.7	70.7 ± 0.9

[a] Standard deviation (S.D.) < 0.1.

Table 2—Nitrogen solubility index (NSI), color and some physical properties of unheated acid precipitated protein dispersions from soybeans.

	Seed condition		
	Normal	Damaged	Broken
NSI in water (%)	93.6 ± 0.5	93.2 ± 0.3	94.9 ± 0.7
NSI in 3% NaCl (%)	83.0 ± 1.5	86.4 ± 0.7	98.4 ± 0.2
Color L	53.0	45.9	51.2
a	0.1	1.4	1.1
b	12.8	13.7	12.6
Apparent viscosity (cP)	68000	4000	16000
Concentration[a]			
moisture (%)	85.5	85.3	85.6
protein (%)	13.4	13.1	13.1

[a] S.D. < 0.1.

Textural parameters of the gel

For gels obtained by heating APP dispersions, texture was evaluated using a Fudoh rheometer model NRM-U002 A. To measure hardness (N) cylindrical gel samples (9.0 cm long and 6.3 cm diameter) were penetrated with a plunger (probe 1, 3.0 cm diam) to a depth set at 41 mm. To obtain breaking strength (N) and deformation (mm) gel slices 2 cm thick with 8.2 cm diam were penetrated by a spherical plunger (probe 4, 0.7 cm diam) until ruptured.

Apparent viscosity

Apparent viscosities of APP dispersions were determined with a Brookfield Synchro-Lectric Viscosimeter (RVT model), with spindle 6, at 2.5 rpm and at room temperature (≈23°C). The apparent viscosities of freeze-dried APP were measured for 15% w/w dispersions at 20°C in a Haake Rotavisco Model RV-2 rotational viscosimeter using a sensor system NV and a rotor speed varying from 0 to 128 rpm in 2 min. Apparent viscosity at 128 rpm was calculated as follows:

$$\eta \text{ ap} = \frac{\tau}{D} = \frac{G.S}{n} \text{ (mPascal·sec = centipoise)}$$

where G = instrument factor (mPa.sec/scale grade.min); S = scale value; n = rotor speed (rpm); τ = shear stress (mPa); D = shear rate (sec^{-1}).

Water absorbing capacity (WAC)

WAC was determined with the Baumman apparatus (Hermansson, 1972). This consisted of a glass filter placed in contact with a funnel connected to a horizontal graduated capillary filled with water. Freeze-dried APP (50 mg) was dusted onto a wet filter paper placed on top of the glass filter. The water absorbing capacity was expressed as uptake of water mL/g sample at equilibrium. Determinations were made at 25°C in duplicate.

Heat induced gel forming ability (GFA)

GFA was evaluated according to Utsumi et al. (1982) with some modifications. Degassed protein solutions (20 mg of freeze-dried APP dispersions in 100 µl of 35 mM phosphate buffer containing 0.4M NaCl) in hematocrit tubes were heated in a boiling water bath for 30 min, removed and immediately cooled in tap water. GFA was qualitatively evaluated by visual characterization of the final product using a scale from − − − − − (liquid, no gel) to + + + + + (firm gel). To evaluate water retention capacity the tubes containing the gel were centrifuged (3000 × *g*/45 min) and the volume of water separated was expressed as a percentage of the total gel volume. Measurements were made in duplicate.

RESULTS & DISCUSSION

Seed quality effect on yield of APP

The defatted flours from damaged seeds had a 13% reduction of the NSI in water and 11.5% reduction of the NSI in 3% NaCl, compared with normal seeds (Table 1). In obtaining APP, the amount of protein extracted in relation to the total present was 89.1% for normal seeds, 81.2% for damaged seeds and 90.7% for broken seeds. The amount of protein precipitated at pH 4.5 in relation to total extracted was 85.7% for normal seeds, 78.7% for damaged seeds and 85.4% for broken seeds. The amount of protein recovered in relation to the total present in flours was 76.4% for normal seeds, 63.9% for damaged seeds and 77.5% for broken seeds. Thus, there was a 7% reduction of the amount of protein precipitated at pH 4.5 (APP) and a 12.5% reduction of the overall yield of protein recovered from damaged seeds. One explanation could be the action of fungal peptidases producing, in damaged seeds, small molecular weight peptides that did not precipitate under these conditions. Broken seeds were not significantly altered in relation to normal seeds.

Physicochemical properties of APP dispersions before heating

Physicochemical properties of the three APP dispersions, without heating, were compared (Table 2). The NSI was similar for the three types of seeds except for the results for the broken ones, in 3% NaCl. In that case, the solubility of N in NaCl was considerably higher than the solubility in water. It was also higher than the solubility in 3% NaCl of the proteins isolated from normal and damaged seeds. The apparent viscosity of the APP dispersions from altered seeds was also significantly different from that of normal seeds. For broken and damaged seeds there was, respectively, a reduction of 76 and 94% of viscosity (Table 2), indicating the occurrence of structural alterations of the proteins. The color of dispersions was also altered for damaged as well as for broken seeds, as shown by the lower values of L (lightness) and higher values of *a* (red, Table 2).

Physicochemical properties of APP dispersions after heating

Heat treatment caused a decrease of solubility of the proteins of broken seeds in water or in 3% NaCl. For damaged seeds there was a reduction of solubility only in 3% NaCl. The solubility of the APP from normal seeds was not changed (Table 3). In spite of the reduction caused by heating, solubility in 3% NaCl of proteins isolated from broken seeds remained high when compared to others. Contrary to what we expected, it was also higher in 3% NaCl than in water (Table 3). There are no similar published results and at the moment it is difficult to explain. Furukawa and Ohta (1983) observed that heating APP dispersions (10% protein) at various temperatures (up to 90°C) for 30 sec reduced protein solubility in 3% NaCl from 91% at 60°C to 43% at 90°C, and was constant (>90%) when heated below 60°C. The solubility in water remained almost at the 100% level regardless of the heating conditions. Our results with damaged seeds were in accordance with their findings. On the other hand, the heat stability of proteins isolated from normal seeds was higher since the temperature used (85°C) was not enough to change solubility either in water or in 3% NaCl.

The color of the three dispersions became darker after heating (Table 3) from the values of lightness (L) and tendency to yellow (b). Damaged and broken seeds also produced dispersions darker than those of normal seeds (higher *a* and lower

Table 3—Nitrogen solubility index (NSI) and some physical and functional properties of acid precipitated protein dispersions (after heat treatment) from soybeans.

	Seed condition		
	Normal	Damaged	Broken
NSI in water (%)	92.8 ± 1.3	91.9 ± 1.2	82.7 ± 0.9
NSI in 3% NaCl (%)	81.1 ± 0.7	74.3 ± 0.1	93.8 ± 0.5
Color L	46.1	43.2	42.4
a	−0.6	1.7	1.6
b	11.1	11.5	9.8
Gel texture			
Hardness (N)	1.5	0.4	1.2
Breaking strength (N)	0.2	*	0.1
Deformation (mm)	10.9	*	10.6
Gel concentration			
protein (%)	14.1	14.3	14.2
Emulsifying Capacity			
(g oil/g protein)	1143	1491	1062
Emulsion Stability			
% fat separated			
After centrifugation			
without salt	0.0	traces	0.0
with salt	0.0	traces	0.0
After heat treatment (1h/110°C after 16h/5°C)			
without salt	0.0	81.7	0.0
with salt	0.0	85.9	0.0
After heat treatment (1h/100°C after 16h/−15°C)			
without salt	0.0	91.7	0.0
with salt	0.0	74.9	5.1

* No gel formation.

Table 4—Solubility of acid precipitated protein from different seeds after freeze-drying.

	Seed condition		
	Normal	Damaged	Broken
Soluble protein (%)[a]			
pH 8.0, 1:100			
Buffer[b] (25°C)	71.2	71.3	73.3
Water (50°C)	76.4	80.2	79.9
Buffer[b] (35°C)	80.4	83.0	81.4
0.2M NaCl (50°C)	50.4	62.7	54.1

[a] S.D. < 0.5.
[b] Tris-HCl 0.03M containing 0.01M β-mercaptoethanol.

Table 5—Water absorbing capacity (WAC) and apparent viscosity (η app) of freeze-dried isolated soy proteins from soybeans.

	Seed condition		
	Normal	Damaged	Broken
η app (cP)[a]	257	67	123
WAC (ml/g isolate)	4.0	2.7	2.9

[a] Values obtained for 15% w/w dispersions.

L). After heating, normal and broken seeds APP dispersions formed gels, but damaged seeds APP dispersions did not. Also, gels from APP from broken seeds were weaker than those formed by APP from normal seeds. This can be seen by hardness and breaking strength values (Table 3).

The emulsifying capacity followed a different pattern, being higher for proteins isolated from damaged seeds (25%) than for normal or broken seeds. However, the emulsions had a low stability to heat (Table 3). Emulsions formed by proteins isolated from normal or broken seeds showed high thermal stability. The APP emulsion from broken seeds for instance was only unstable in the presence of salt after 16 hr at −15°C and 1 hr at 100°C.

The results indicated a possible fungal contamination of damaged seeds suggesting proteolysis. This could have caused a lower precipitation yield, low viscosity of APP dispersions (Table 2), incapacity of gel formation, high emulsifying capacity and low thermal stability of emulsions (Table 3).

Aoki et al. (1981) reported increased emulsifying capacity and stability of emulsion by an HCl limited-hydrolysis of soy proteins. They emphasized that the effect of hydrolysis on emulsifying capacity and emulsion stability could be different depending on the kind and conditions of hydrolysis. Hamada and Marshal (1989) verified that 2 and 4% hydrolysis of soy proteins with alkalase increased emulsifying activity and decreased emulsion stability. Puski (1975), using fungal proteases, obtained similar results explained by the increase in the number of peptides at the interphase oil-water allowing a higher emulsification of oil. The protein layer on each oil droplet would also be thinner due to the nonglobular nature of small peptides resulting in emulsions with lower stability. In relation to gel formation, Puski (1975) reported with 10% w/w isolated soy protein dispersions in water, that a mild hydrolysis was adequate to abolish gelification after heat treatment (80°C/30min).

Results agreed with data we obtained for damaged seeds. Broken seeds probably suffered alterations different from proteolysis causing the observed change of behavior in solution, low viscosity, weak gels and small alterations of emulsifying properties. If proteolysis occurred to some extent either it was milder or it was caused by different enzymes.

Physicochemical properties of APP after freeze-drying

The solubility of APP (freeze-dried, no heat) in different solvents was compared (Table 4). Dissolution of proteins in buffer was slightly higher than in distilled water, probably due to the presence of β-mercaptoethanol (Clatterbuck et al, 1980; Nash et al., 1979; Nash & Wolf, 1980). As expected, it was higher at 50°C than at room temperature (50°C is used industrially to obtain protein isolate from defatted flour). The more significant difference observed among the isolates was the higher solubility of proteins from damaged seeds in 0.2M NaCl (Table 4). Comparing the results (Table 2, Table 4) we can see that freeze-drying altered protein behavior in solution mainly reducing solubilities. Catsimpoolas et al. (1969) reported that freeze-drying of glycinin (11S globulin) caused dissociation in two half-molecules (7S) and aggregation (15S). Formation of higher polymers (15S and >15S) was also demonstrated by Wolf et al. (1962) and Badley et al. (1975). Hoshi and Yamauchi (1983b) observed a reduction of -SH groups, an increase of -S-S-bridges and partial dissociation of glycinin caused by freeze-drying.

The effect of drying (especially spray-drying), as used commercially, could magnify the lessened effectiveness due to presence of altered seeds in low quality soya, on the functional properties of the protein isolates. There is a correlation between solubility in 0.2M NaCl and the extent of soy proteins denaturation. The correlation does not apply for solubility in water (Hermansson, 1986). Soy proteins completely denatured may have solubility in water similar to native ones, but they show low solubility (<20%) in 0.2M NaCl, regardless of pH. This is due to the aggregation tendency caused by the presence of salt. Wagner and Añon (1990) detected, for 13 commercial isolates, NSI in water from 24 to 84% while in 0.2M NaCl it varied only from 10 to 49%. They reported that the lower the solubility in NaCl the more denatured the proteins. In our case, the relatively high solubility in 0.2M NaCl for the three types of isolated proteins indicated that in all cases the proteins were probably in their native state (Table 4).

The values of WAC and apparent viscosity of the three isolates were compared (Table 5). Shear stress was related to shear rate (Fig. 1). As observed before (APP without freeze-drying), the isolated proteins from damaged and broken seeds had viscosities of dispersions lower than those from normal seeds (respectively 74 and 52% reduction, Table 5, Fig. 1). Water aborbing capacity was also lower for altered seeds (32% reduction for damaged seeds and 26% reduction for broken

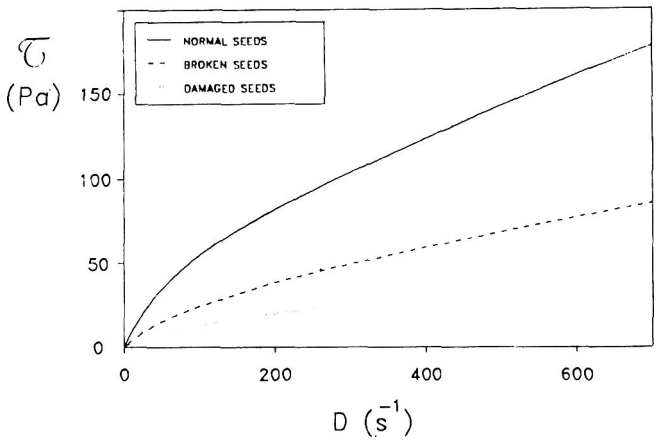

Fig. 1—Relationship between shear stress and shear rate for isolated proteins (15% w/w dispersions) from soybeans.

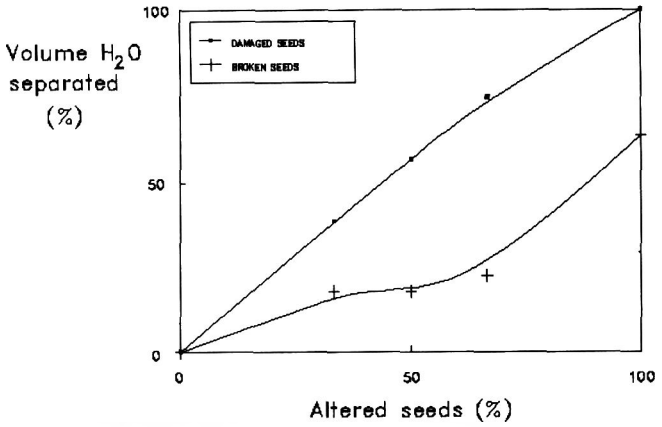

Fig. 2—Relationship between water retention capacity of gels and isolate composition.

Table 6—Gel forming ability of isolated soy proteins from soybeans and of their mixtures.

Isolated proteins from	% Isolate	Gel forming ability[a]	Vol H_2O separated / Total vol of gel	Volume H_2O separated (%)
Normal seeds	100.00	+ + + + +	0.0	0.0
Damaged seeds	100.00	− − − − −	11.1	100.0
Broken seeds	100.00	− − − − +	7.1	64.0
Normal seeds	66.66	− − + + +	4.3	38.7
Damaged seeds	33.33			
Normal seeds	66.66	− + + + +	2.0	18.0
Broken seeds	33.33			
Normal seeds	50.00	− − − + +	6.3	56.8
Damaged seeds	50.00			
Normal seeds	50.00	− − + + +	2.0	18.0
Broken seeds	50.00			
Normal seeds	33.33	− − − − +	8.3	74.8
Damaged seeds	66.66			
Normal seeds	33.33	− − − + +	2.5	22.5
Broken seeds	66.66			

[a] + + + + + = best gels, − − − − − = poorest gels.

seeds). WAC correlated with viscosity but not with solubility of isolated proteins, in accordance with Kinsella (1979).

GFA of the isolated proteins and of their mixtures (Table 6) showed the influence of the mixtures in water retention capacity of gels. This can also be seen in Fig. 2. The dispersions (20% w/w) of isolated proteins from normal seeds formed gels after heating at 100°C/30 min while dispersions from damaged or broken seeds did not. However, gels from broken seeds became more viscous than did those from the damaged ones, in accordance to results for APP dispersions (without freeze-drying, Table 3).

The isolated proteins from damaged seeds when mixed with isolated proteins from normal seeds, altered proportionally gelation and water retention capacity of gels. This influence also was found for broken seeds but to a lesser extent (Table 6, Fig. 2).

We could speculate that protein isolates from altered soybean seeds had less effective functional properties than those of normal seeds and that the extent of lost effectiveness would depend on the amount of altered seeds. There could also be a reduction of total yield. However, a final conclusion could only be derived experimentally by mixing different proportions of normal, broken or damaged seeds, preparing isolates (APP), and evaluating their respective physicochemical properties. There could be complex unpredictable interactions in the mixture.

At the end of harvesting the amount of broken and damaged seeds is respectively about 10 and 1% of the total. That could be enough to reduce the functional properties of the proteins isolated (Fig. 2). Also during prolonged storage and in some parts of silos there may be a higher concentration of altered seeds due to localized effects of temperature and humidity.

Viscosity of the dispersions was a simple indicator to predict quality of protein isolates. It allowed visualization of changes not detectable by emulsification or solubility measurements. GFA was also a good indicator though much more complex to evaluate. In the absence of a viscosimeter, the Baumman apparatus was also a simple, effective device since water absorbing capacity was well correlated with viscosity.

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Maturity and Storage Affect Freeze Damage in Peanuts

J. A. SINGLETON and H. E. PATTEE

ABSTRACT

Maturity and length of storage times were studied as factors influencing chemical properties of peanuts harvested at six different dates and artificially freeze damaged for 10 hr at −4°C. Conductivity of water extracts from freeze damaged peanuts generally decreased with maturity as did the amount of potassium found in the leachates. Two freeze-damage products, acetaldehyde and ethanol, decreased with maturity, indicating that maturity affected the extent of freeze damage. Acetaldehyde and ethanol concentrations also decreased during storage of freeze-damaged peanuts.

Key Words: freeze damage, peanuts, maturity, storage, conductivity

INTRODUCTION

PEANUTS (*Arachis hypogaea* L.), like most subtropical plants, are susceptible to freeze damage at harvest time when temperatures drop below 1–2°C (Singleton and Pattee, 1989). Some of the factors that influence the extent of freeze damage in peanuts are exposure temperature, length of exposure, seed moisture level, variety, seed mass, and seed maturity (Wampler, 1983; Singleton and Pattee, 1987; 1991). Peanut plants flower and peg throughout the growing season, therefore, bulk harvested peanuts represent a cross section of different maturity stages. Effect of maturity and seed size on carbohydrate composition was studied by Pattee et al. (1981). They reported seed size had a significant effect on carbohydrate content on a per seed basis and peanut butter made from small seed was of lower quality. Woodroof (1973) found that large, mature seed had a better flavor and texture, and Wampler (1983) found that peanut seed weighing 0.8 g or less had a higher moisture content and were more susceptible to freeze damage.

When peanut seed are exposed to cold or heat stress, the respiration process changes from aerobic to anaerobic producing increased concentrations of acetaldehyde and ethanol (Dickens, 1957; Whitaker and Dickens, 1964; Pattee et al., 1965). These anaerobic respiration products contribute to an off-flavor development in raw peanuts which can best be described as a fermented odor and taste. The increased production of these anaerobic products in peanuts can be monitored by headspace analysis and gas liquid chromatography (Singleton and Pattee, 1987). The increase in headspace volatiles concentration has been related to off-flavor intensity (Pattee et al., 1989; 1990).

After peanuts are harvested they are normally stored in warehouses for a period of time before processing. During this storage period, chemical changes occur. Storage time and seed size affect carbohydrates, amino acids, volatiles, and roast seed blanchability (Pattee et al., 1981; 1982). Therefore, peanut crop maturity and quality following storage are important to peanut processors.

This research was undertaken to determine the effect of maturity on the extent of freeze damage in peanuts (in the shell) and to monitor the concentration of anaerobic products during storage.

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MATERIALS & METHODS

Materials

Peanuts (cv. NC 6) were grown at the Peanut Research Station, Lewiston, NC, using recommended cultural practices (Sturkie and Buchannan, 1973). The recommended planting time for peanuts in NC is May 10. Harvesting began on September 17 and continued through October 21, 1986, at weakly intervals to insure a wide range of maturity levels. Peanuts were dug, combined, and brought to the laboratory. Samples from each harvest date were divided, and one portion was dried to 7% moisture. This sample was not exposed to freezing temperatures and was used as the control. A separate control was used for each harvest date. The remaining portion of the sample was placed in shallow metal pans and exposed to −4°C for 10 hr (in the shell). Due to the mass of peanuts used the time required to reach −4°C was ≈3 hr. The average fruit moisture content was 40% at harvest (wet basis).

Maturity index

The average peanut maturity level at each harvest date was estimated using the seed-hull ratio method (Pattee et al., 1977). A 150g subsample was taken from each harvest date to determine the ratio. The peanuts were hand shelled, and the hulls and the seeds were weighed separately. The seed hull ratio was derived for each harvest date by dividing the weight of the hulls into the weight of the seeds. As peanuts matured the weight of the seeds increased and the weight of the hulls decreased. Peanuts from the control sample were used for this determination. Triplicate determinations were made for each harvest date.

Freezing of peanuts

Peanuts (≈14 kg) were placed in shallow metal pans and exposed to −4°C for 10 hr in a low temperature incubator (precision, GCA Corporation, Chicago, IL). Temperature was monitored by placing a thermocouple in the center of an average-size-peanut and recording the temperature every 10 min during the freezing period using a data logger (Omnidata International Inc., Logan, UT). After freezing the peanuts were dried to ≈7% moisture using forced air (23–26°C) and then stored at 5°C and 60% relative humidity until analyzed. Stored peanuts were analyzed periodically on the dates shown (Table 3).

Conductivity measurements

A 50-g sample from each harvest date was placed in an Erlenmeyer flask with 250 mL distilled, deionized water and shaken for 2 hr on a wrist shaker. The extract was decanted and conductivity was measured using a conductivity meter (Amber Science, San Diego, CA) equipped with a dip cell. Triplicate analyses were run for both control and treated samples from each harvest date.

Table 1—Relationship between maturity index and harvest date

Harvest date	Days from planting	Maturity index[a]	SD
Sept. 17	130	1.51	0.11
Sept. 23	136	1.73	0.07
Sept. 30	143	2.22	0.08
Oct. 7	150	2.32	0.07
Oct. 15	158	2.57	0.01
Oct. 21	164	2.75	0.19

[a] Average of three replications.

Table 2—Effect of maturity on acetaldehyde and ethanol production in freeze damaged peanuts[a]

Maturity index damage	Control difference[b]	Freeze
	Acetaldehyde	
1.51	11.08	20.43
1.73	5.62	17.78
2.22	1.85	4.62
2.32	1.5	2.87
2.57	.95	3.05
2.75	.95	1.84
	Ethanol	
1.51	4.4	47.73
1.73	0	66.59
2.22	0	10.73
2.32	1.33	5.76
2.57	0	10.71
2.75	0	14.21
Average Acetaldehyde	3.66	8.43
Ethanol	0.96	21.63
	T Values	Prob>|T|
Acetaldehyde	4.41	.0004
Ethanol	5.2	.0001

[a] Average of three replications.
[b] Concentration in ppm

Table 3—Effect of storage on anaerobic products produced in freeze damaged peanuts.[1]

Maturity index	Dec	March	May	June
		Acetaldehyde[b]		
1.51	20.43	16.73	11.49	14.92
1.73	17.78	12.19	13.34	13.84
2.22	4.62	5.48	6.94	7.02
2.32	2.87	2.9	3.96	4.04
2.57	3.05	2.07	2.89	4.05
2.75	1.84	1.64	1.84	3.85
		Ethanol[b]		
1.51	44.75	17.73	13.01	6.36
1.73	45.52	29.73	19.49	11.85
2.22	10.73	6.89	6.17	1.72
2.32	5.76	6.65	0.77	0.76
2.57	8.75	1.3	0.13	18.82
2.75	14.21	8.68	4.89	15.46

	DF	F	Prob>F
Maturity index	5	2.66	.0001
Sample date	3	7.62	.0003
Matindx * Sampdate	15	6.98	.0001

[a] Average of three replications
[b] Concentration in ppm

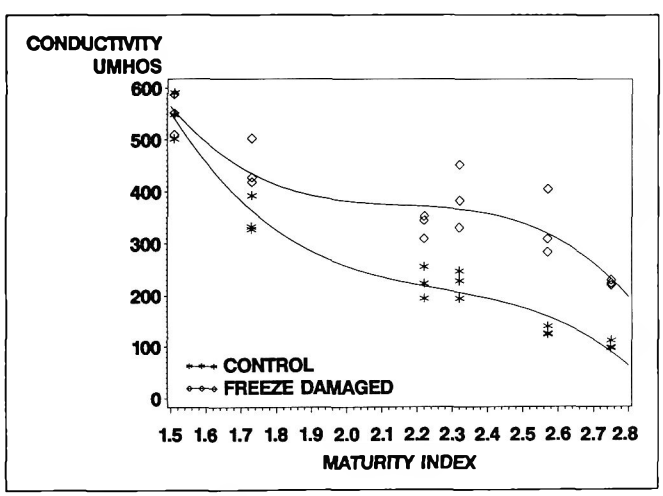

Fig. 1—Effect of maturity on the conductivity in the water extract from freeze damaged peanuts.

Cation analysis

Cation composition (Ca,K) of the extract from freeze-damaged seeds was determined by ion chromatography using a DIONEX 4000i ion chromatograph equipped with a cation micromembrane suppressor and a conductivity detector. A 20 µL sample of leachate was injected onto the column via an air actuated valve. Cations were separated on a hipc-cs3 column and eluted isocratically with 27.5 HCl/2.25 mM DL-2,3-diaminopropionic acid monohydrochloride (DAP)/2.25 mM L-histine monohydrochloride monohydrate solution at a flow rate of 1 mL/min. The regenerated solution was 70 mM tetrabutylammonium-hydroxide (TBAOH) flowing through the micromembrane suppressor at 5 mL/min. Eluted cations were detected with a conductivity detector at 300 uS. A potassium standard curve was used to establish linearity, and concentrations of the samples were calculated with an external chromatographic calculation method via a Spectra-Physics Winner, San Jose, CA data collection system.

Isolation of volatiles

Volatiles (acetaldehyde and ethanol) were removed from homogenized peanut samples using a dynamic headspace and porous polymer trapping technique (Singleton and Pattee, 1980). A 100 g sample was blended with 300 mL of distilled, deionized water in an Omni blender for 1 min. An internal standard (2-propanol) was added to the sample mixture prior to blending. The homogenate was placed in a 1 L round bottom flask fitted with a porous polymer trap. Valves were closed and the sample equilibrated for 15 min with constant agitation using a magnetic stirring bar. Volatiles were removed from the homogenate by aspirating for 5 min under vacuum (760 mm), and adsorbed on a chromosorb 102 (60–80 mesh) polymer trap. The polymer trap was removed and placed in the injector port of a Varian 3700 gas liquid chromatograph, and volatiles thermally desorbed at 200°C. Trapped volatiles were separated by gas liquid chromatography and concentrations calculated by the following procedure. Volatiles were separated on a Chromosorb 102 (80–100 mesh, 0.31 cm × 182.9 cm) column with the oven programmed from 100°C to 200°C at 2°C/min. Resolved peaks were integrated, concentrations calculated, and data sorted using a Spectra-Physics Winner Data collection system. A standard mixture of selected volatiles including the internal standard (2-propanol) was used for calibration and calculation of response factors.

Statistical analysis

Regression techniques were used to estimate the functional relationship between conductivity, potassium concentration in the extract, and maturity using regression analysis procedure (SAS Institute, Inc., 1985).

RESULTS & DISCUSSION

Maturity

Peanut seed at any given harvest date will span a broad range of physiological maturity development, and the average maturity level will differ across harvest dates. The relationship between estimated maturity and harvest date (for this study) is given in Table 1. Maturity index increased with each successive harvest date. The relationship shown was probably affected by heavy rainfall during the middle of the season. Increased rainfall as well as other environmental factors will delay maturity and this was reflected in the closeness of the maturity index values for harvest dates September 30 and October 7. The peanuts used for the maturity index were planted on May 10. The standard deviation values suggest this method gives a repeatable estimation of maturity.

Ion leakage

When seeds are damaged ion leakage occurs, and leakage of cellular constituents is generally considered as an indicator of membrane damage (Murata and Tatsumi, 1979). Conductivity measurements were made on water extracts collected from peanut seeds of different maturity levels to assess the extent of cellular damage caused by freeze-damage and the relationship of conductivity to maturity. Ion leakage from freeze-damaged peanuts as well as the control seeds was found to be affected by maturity (Fig. 1). The first harvest date showed very little difference between control seeds and freeze-damaged seeds. This was probably attributable to the immaturity

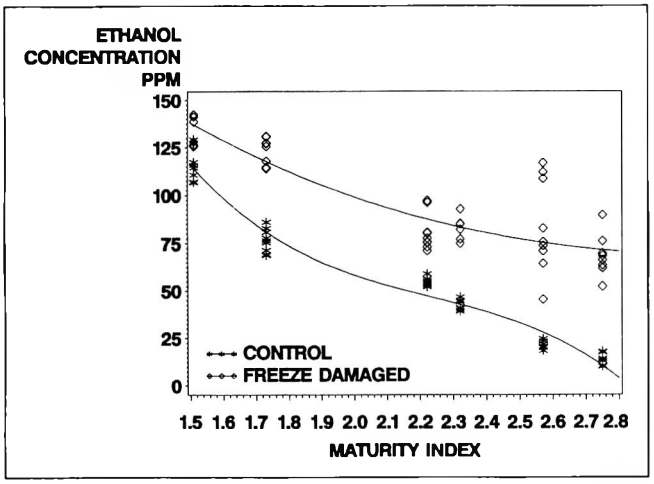

Fig. 2—Effect of maturity on potassium concentration in the water extract from freeze damaged peanuts.

of the samples since immature seeds leak ions to a greater extent than mature seeds. Greater differences between extract conductivity of control seeds and damaged seeds were found for the succeeding harvest dates. The coefficient of determination (R) for the functional relationship between conductivity of the extract and maturity for the control seeds was 0.98, and 0.89 for the damaged seeds. Although high R values were observed, conductivity values leveled off during the middle of the harvest season which indicated delayed maturity.

Cations, organic ions, and amino acids are the major contributors to conductivity in water extracts from plant material. Conductivity of freeze-damaged peanuts decreased as maturity level of the peanut seeds increased. Thus, a higher percentage of immature seeds at any given harvest date would result in greater freeze damage to the crop.

Potassium, the most abundant cation in peanuts (Wampler, 1983), controls the osmotic pressure of plant cells, participates in enzymatic reactions, and other metabolic functions. Figure 2 shows the relationship between maturity, freeze-damage, and potassium concentration found in the water extracts. Potassium efflux in the extract decreased as maturity increased. Coefficients of determination (R) for the relationship of potassium concentration in the extract to maturity was 0.97 for the control seeds and 0.96 for the damaged seeds. During the middle of the harvest season, however, the potassium concentration in the extract from the freeze damaged samples was similar for the third and fourth harvest date. A small amount of calcium ($\approx$3% of the total cations in the extract) was also found in the extract. The loss of this cation may be important because of its possible involvement in maintaining cell membrane integrity (Palata et al., 1977a; 1977b; Palata and Li, 1980). The loss of calcium from the cell membrane may also result in a passive influx of extracellular potassium followed by water uptake within the cells (Palata and Li, 1980).

Anaerobic product concentration

Upon incipient freezing of plant material the respiration process changes from aerobic to anaerobic, thereby producing higher concentrations of acetaldehyde and ethanol in freeze-damaged samples (Table 2). These compounds are volatile, easily analyzed, and serve as excellent markers for detection of both cold and heat stress to peanut seeds. A paired data analysis (SAS) was used to determine if there was a significant difference for both acetaldehyde and ethanol between the control and freeze damage treatment across all maturity indexes. The t values for acetaldehyde and ethanol were highly significant (4.41 and 5.20) indicating that the null hypothesis could be rejected with 99% confidence. A notable decrease occurred in the third harvest date suggesting that attainment of a certain maturity level minimized the effect of freeze-damage. Metabolic activity decreased and the mass of the seeds increased with maturity, both of which lessen the extent of freeze-damage to the seeds (Ashworth et al., 1985).

Peanuts are normally stored for a period of time before they are processed. During this time metabolic activity is ongoing. Anaerobic products tend to decrease upon storage (Table 3) which makes it difficult to determine the type of damage using marker compounds such as acetaldehyde and ethanol. An analysis of variance (SAS) was run to determine if the production of acetaldehyde and ethanol differed significantly with maturity index and storage time. The F values from the analysis indicated that the null hypothesis was rejected with 99% confidence for both acetaldehyde and ethanol. The significance of the cross product indicated that maturity index behaved differently in December than in June. At the beginning of storage peanuts that had been freeze damaged had a fermented odor and taste, and at the end of the storage period, a rancid odor was prevalent.

CONCLUSIONS

POTASSIUM concentration found in the water extract and conductivity of the extract from freeze damaged peanuts decreased as peanuts matured. Excessive rainfall during harvest season may have some effect on overall maturity, especially during the middle of the harvest season. Maturity affected extent of freeze-damage to peanuts, and anaerobic products produced by freeze damage generally decreased during storage. Differences in ethanol concentration from the beginning of storage to the end of storage were less for succeeding harvest dates.

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Physicochemical and Sensory Characteristics of Peanut Paste Stored at Different Temperatures

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ABSTRACT

When peanut paste was stored at 30, 40 and 50°C for up to 1 yr, that held at 30°C for 161 days had minimal deteriorative changes. At 50°C, the primary deterioration was increased browning, oxidized and cardboard flavors, and oil separation . Regression analysis indicated that ratings for chroma, cooked peanut, cardboard, and oxidized flavors were responsible for > 50% of the variation in response. When a detectable oxidized flavor was used as the indicator of storage deterioration, peanut paste had a predicted shelf life of 152 days at 30°C; 98 days at 40°C; and 96 days at 50°C.

Key Words: peanut paste, sensory, adhesiveness

INTRODUCTION

PEANUT KERNELS contain about 50% fat, 80% of which is unsaturated fatty acids, making them prone to oxidative and hydrolytic changes affecting quality and flavor (St. Angelo et al., 1977). Factors influencing stability of peanut-based products include genetic, cultural, handling, processing and composition variations in raw peanuts as well as environmental conditions during distribution (Shewfelt and Young, 1977).

Peanut paste is a nontraditional product that may serve as a base for different flavored spreads (Santos et al., 1989; Santos and Resurreccion, 1989a, 1989b). It is light in color, bland in flavor and spreadable. There is a need to study the behavior of peanut paste during storage to provide information on conditions and methods for maintaining quality during distribution (IFT Expert Panel and CPI, 1974). Peanut paste is to be microbiologically stable at ambient temperatures because of its low moisture content (< 4%). The water activity (a_w) of peanut paste is 0.44, a level at which microbial growth should not occur (Jay, 1986).

Our objective was to determine physicochemical and sensory characteristics of peanut paste stored at three temperatures. Specific objectives were to evaluate color, oil separation, texture measures, and sensory attributes of peanut paste stored at 30, 40, and 50°C, to determine significant indicators of deterioration in peanut paste, develop prediction equations for changes, and to determine correlations between objective and sensory measures of quality in peanut paste.

MATERIALS & METHODS

Preparation of peanut paste

One part shelled Florunner (cv.) peanuts (1988 crop) was added to six parts water and heated for 10 min at 90°C. The mixture was stirred continuously throughout this water extraction process. Peanuts were drained, rinsed with cold tap water, and the testa were manually removed. Water extraction was repeated two times on the blanched kernels. After water extraction, peanuts were dried ≈2 hr at 100°C to a moisture content of about 8% and then ≈42 hr at 40°C to a moisture content of ≤ 4%. Dried peanuts were passed through a Morehouse mill (Morehouse Industries, Fullerton, CA) set at a stone clearance of 0.2 mm and maintained at 77°C using a mixture of steam and water. Stabilizer (Fix X, Procter & Gamble, Cincinnati, OH; 2% by wt of paste) was mixed into the paste and the mixture was again passed through the mill. Paste (ca 250 g) was placed in wide-mouth ≈226 mL glass jars which were sealed with screw cap metal lids. Paste was allowed to set at ambient temperature (about 24°C) overnight. Two replicate batches were prepared.

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Storage and sampling

Samples from each replication of freshly prepared paste were stored at 30, 40 and 50°C in the dark. Two jars/replication/temperature were removed from storage and the paste evaluated for color, gas chromatographic profile of headspace volatile compounds, oil separation, flavor and textural characteristics at day 0 and after 14, 28, 56, 84, 112, 132, and 161 days of storage. The contents of each jar were mixed before sampling. Evaluations were terminated after strong oxidized flavors developed in pastes stored at 40 and 50°C for 189 days. Pastes that did not have objectionable flavors were evaluated after 189 and 366 days of storage.

Color evaluation

A Gardner XL-800 series tristimulus colorimeter with an XL-845 circumferential sensor (Pacific Scientific, Bethesda, MD) set with a yellow reference standard tile (L = 79.56, a = −2.17, b = 22.98) was used to measure the lightness (L), a, and b values of the paste. Peanut paste was evenly spread on the sample cup to a depth of about 10 mm. Four sets of readings were obtained per sample while rotating the cup 90° clockwise each time. Chroma ($[a^2+b^2]^{1/2}$) and hue angle ($\tan^{-1}$ b/a) were calculated (Little, 1975).

Instrumental texture evaluation

The textural properties of the paste were evaluated using a modified Texture Profile Analysis (TPA) previously described by Muego et al. (1990). Signals from the load cell were collected directly onto a floppy disk and the digital values were converted into texture measures of hardness, adhesive force, adhesiveness and cohesiveness using a program for a personal computer developed specifically for this study.

Oil separation

A portion of freshly prepared paste was filled into ground glass stoppered 50-mL graduated cylinders and stored at 30, 40, and 50°C. The volume of "free oil" was read directly from the scale on the graduated cylinders.

Sensory evaluation

Descriptive flavor analysis, using a 0 (none) to 15 (strong) intensity scale (Meilgaard et al., 1987), and descriptive texture analysis, using a 0 (not detectable) and 7 (strong) intensity scale (Muego et al., 1990), were used to evaluate two replications of four peanut paste samples (pastes from three storage temperatures plus a control stored at 4°C). The panel consisted of 12 judges from the Dept. of Food Science & Technology, Univ. of Georgia recruited from a pool of individuals trained in descriptive analysis as described by Meilgaard et al. (1987). Evaluations were conducted in a sensory laboratory in partitioned booths illuminated by incandescent lighting (two 50W spotlights). Judges were presented with peanut paste (ca 2.5g) in plastic ≈30 mL sample cups labeled with random three-digit numbers, on trays lined with white paper. Spectrum references for intensity (Meilgaard et al., 1987), English water biscuits (Peppridge Farm, Norwalk, CT), water, and cups for expectoration were provided in each booth. Judges were

Table 1—Definitions of sensory characteristics used in the evaluation of peanut paste by a trained panel

Descriptor	Definition
Flavor[a]	
Raw	Aromatic associated with raw peanut.
Cook	Aromatic associated with peanuts boiled in water for one hour.
Roasted	Aromatic associated with medium-roasted peanuts.
Oxidized	Aromatic associated with stale peanuts.
Cardboard	Aromatic associated with a flour paper sack.
Sweet	Taste on the tongue associated with sugars.
Bitter	Taste on the tongue associated with caffeine.
Texture[b]	
Oiliness	Degree to which free oil is perceived in the mouth.
Adhesiveness	Force required to remove the material that adheres to the palate during the normal eating process.
Gumminess	Amount of energy needed to disintegrate a semi-solid food to a state ready for swallowing.
Graininess	Degree to which grains or granules are perceived in the mouth.

[a] The descriptors and their corresponding definitions for raw peanut, roasted peanut, sweet and bitter were adapted from Johnsen et al. (1988); cooked peanut, from Muego et al. (1990); and, oxidized and cardboard flavors were defined by the trained panel.

[b] The texture descriptors and their corresponding definitions were adapted from Civille and Szczesniak (1973).

instructed to evaluate the samples for 7 flavor and 4 texture attributes (Table 1).

Panelists were instructed to familiarize themselves with the Spectrum™ references for intensity, place a half-teaspoon of peanut paste in their mouth, evaluate for flavor, expectorate, rinse their mouth with water, place a fresh sample in their mouth, and evaluate for texture. They were further instructed to evaluate the samples in the order designated on the ballot and eat a water biscuit between samples. Four peanut paste samples were evaluated by each judge in 2 separate sensory sessions.

Panelist performance was assessed by conducting a one-way analysis of variance (ANOVA), comparing peanut paste samples stored at 4 temperatures, for each panelist and descriptor as recommended by Powers (1988). Responses of panelists whose scores for oxidized flavor had probabilities ≤ 0.50, were used for subsequent statistical analysis of sensory data. Scores of three panelists that did not meet this criterion were dropped from subsequent analysis.

Analysis of data

Regression analysis was performed on the data collected up to 161 days for color, objective texture measures and sensory characteristics of peanut paste to determine their statistical relationship with storage temperature and time. Eight different regression models were considered, from the first order model with two independent variables:

$$Y_i = \beta_0 + \beta_1 X_{i1} + \beta_2 X_{i2} + e_i$$

and, six different combinations of one or both squared and cross product terms, to the full second order model with two independent variables and a cross product:

$$Y_i = \beta_0 + \beta_1 X_{i1} + \beta_{11} X^2_{i1} + \beta_2 X_{i2} + \beta_{22} X^2_{i2} + \beta_{12} X_{i1} X_{i2} + e_i$$

where: Y_i is the value of the response variable in the ith trial; β_0 is the intercept when Y equals zero; β_1, β_{11}, β_2, β_{22}, and β_{12} are parameters; X_{i1} is the number of days in the ith trial; X_{i2} is the temperature in the ith trial; e_i is a random error; and, $i = 1, \ldots, 48$.

The regression procedure (PROC REG) of the Statistical Analysis System, Inc. (Cary, NC) was used to generate significant probability ($P > F$), adjusted coefficient of determination (R^2) and predicted error sum of squares (PRESS) statistic for each of the eight different models. Dependent variables which could be explained by at least one model using the criterion of an adjusted R^2 greater than or equal to 0.50 were examined as possible predictors of deterioration in peanut paste. The partial F statistic between a proposed reduced model and the full second-order model was calculated as follows:

$$F = \frac{[SSE_{(reduced)} - SSE_{(full)}]/[df_{(reduced)} - df_{(full)}]}{MSE_{(full)}}$$

where: SSE is the Sum of Squares of Error; MSE is the Mean Square Error; and, df is degrees of freedom. Either the full model, or the simplest reduced model that was not significantly different from the full model at the 5% level, was selected as the final model for each possible predictor of storage changes in peanut paste.

Pearson correlation coefficients (r) between objective and sensory texture values were calculated to determine if associations between these response variables existed.

RESULTS & DISCUSSION

Physicochemical and sensory changes during storage

Peanut paste stored at the accelerated conditions of 40 and 50°C had strong oxidized flavors after 189 days and consequently those samples were not evaluated. Pastes held at 30°C did not have objectionable flavors and were evaluated after storage for 189 and 366 days.

Color. Response surfaces for L, chroma and hue angle of peanut paste stored for up to 161 days (Fig. 1) represent observed values and thus should not be used to predict color responses during storage. Regression analysis indicated that the relationship between L and storage variables was not linear. No apparent trend could be observed for the L data except that lowest L values were recorded at day 0 (Fig. 1). The change in peanut paste chroma was more prominent, expecially at 50°C where it reached an observed maximum value of 18.84 after 161 days (Fig. 1). This indicated that color saturation intensified during storage. Hue angle of paste stored at 50°C decreased with time (Fig. 1), indicating progressive browning, reaching an observed minimum of 88.74. Regression analysis

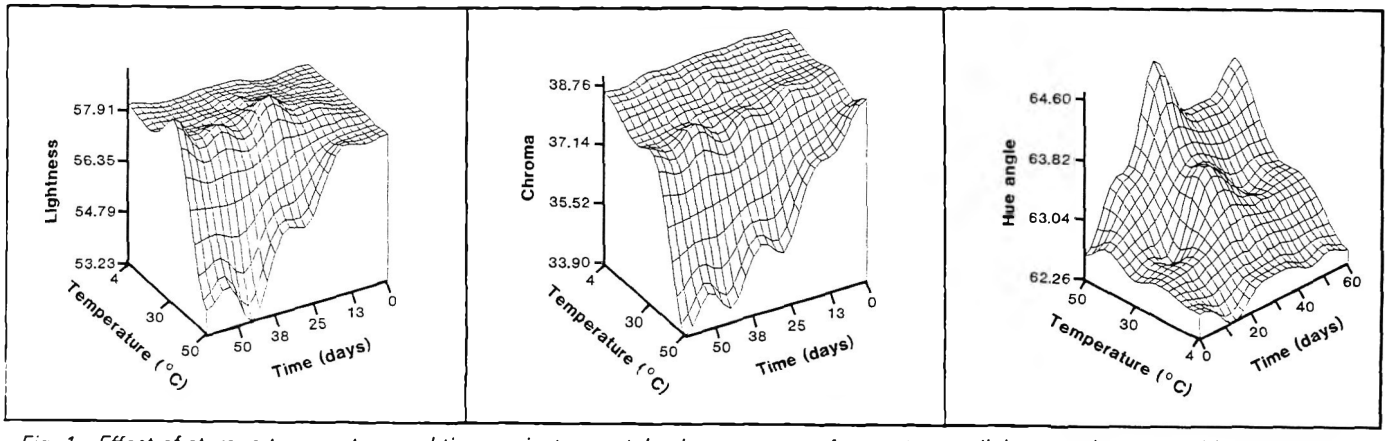

Fig. 1—Effect of storage temperature and time on instrumental color measures of peanut paste lightness, chroma, and hue angle.

indicated significant linear relationships between chroma and hue angle and the storage variables ($Pr < 0.05$).

The undesirable changes in color of peanut paste, particularly at 50°C, were probably due to nonenzymic reactions since the paste contains the reactants required for Maillard browning: an amino-bearing compound (protein), a reducing sugar, and water (Whistler and Daniel, 1985). Woodroof (1983) reported that darkening due to a reaction between protein and sugar was one of the four main types of deterioration that might affect the quality of peanut butter during storage. The basic groups in proteins may also catalyze aldol condensation of carbonyls produced from lipid oxidation resulting in brown pigments (Nawar, 1985).

L, chroma and hue angle of peanut paste stored at 30°C for 189 and 366 days were not significantly different from their values at day 161. Paste had mean L values of 68.59 and 69.50 after 189 and 366 days, respectively. Mean chroma values were less than the observed maximum at 50°C, i.e., 16.93 and 17.39 after 189 and 366 days, respectively, indicating that paste color did not intensify after 1 yr at 30°C. After 189 and 366 days at 30°C, paste had mean hue angles of 92.50 and 92.37, respectively, indicating that it did not exhibit browning.

Table 2—Oil separation observed in peanut paste during storage at three temperatures[a]

Storage time (days)	Storage temperature (°C)		
	30	40	50
0	−	−	−
14	−	−	+ + +
28	−	−	+ + +
56	−	−	+ + +
84	−	+	+ + + +
112	−	+	+ + + +
132	−	+ +	+ + + +
161	+	+ +	+ + + +
189	+		
366	+		

[a] (−) none; (+) trace; (+ +) a drop of oil; (+ + +) ca 0.5 mL; (+ + + +) ca 1 mL/50 mL paste; observations were based on duplicate samples for each 2 replications, at each temperature.

Oil separation. Observations on the appearance of free oil on the surface of peanut paste are summarized in Table 2. Separation of oil from peanut paste is undesirable. In peanut butter, oil separation may be an indication that the product is not fresh since free oil is more susceptible to oxidation than

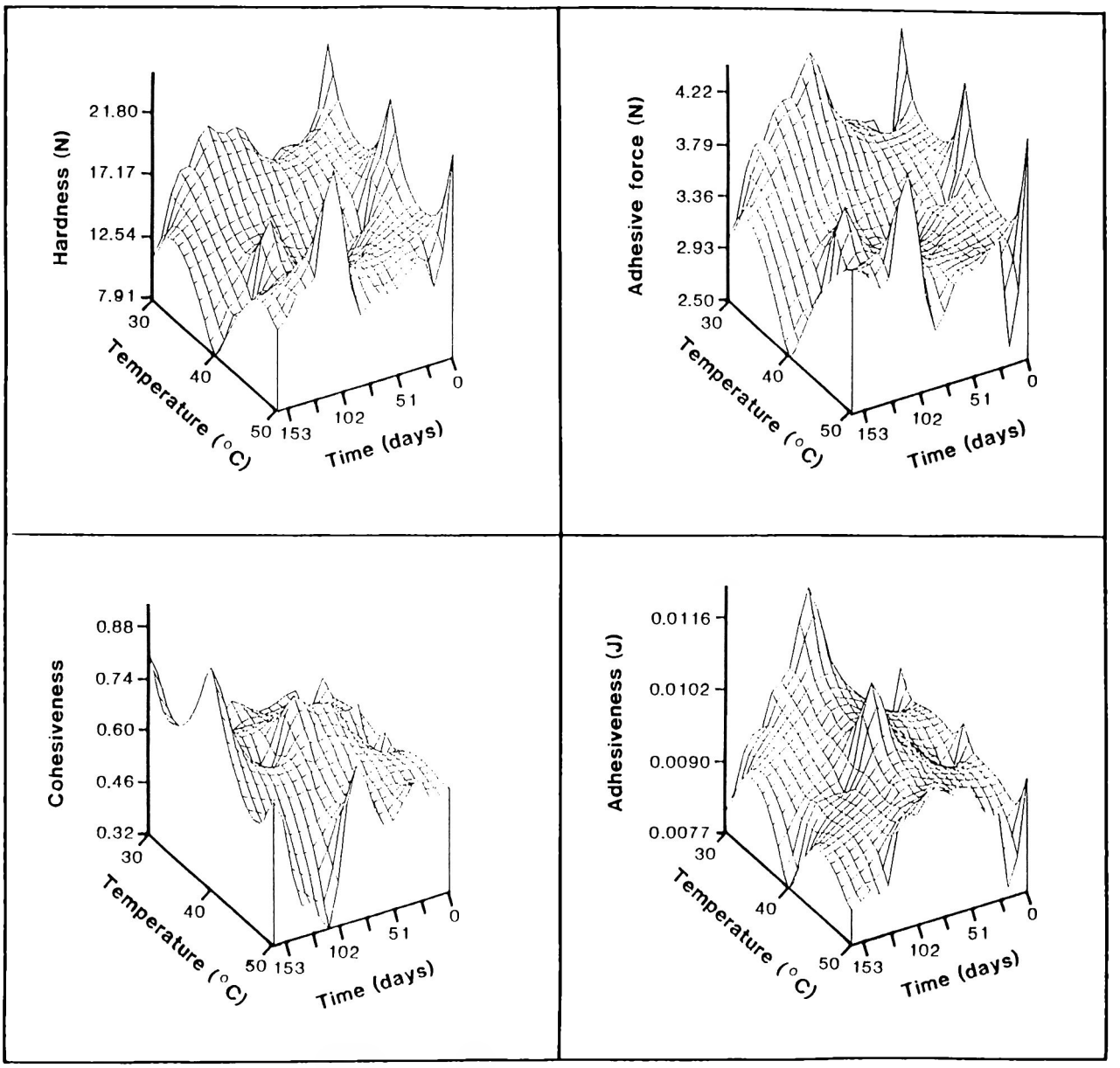

Fig. 2—Effect of storage temperature and time on instrumental texture measures of peanut paste hardness, adhesive force, cohesiveness, and adhesiveness.

Table 3—Mean ratings for the textural properties of peanut paste during storage at different temperature and time conditions as evaluated by a trained panel[a]

Storage conditions		Textural properties			
Temp (°C)	Time (days)	Ciliness	Adhesive -ness	Gummi -ness	Graini -ness
30	0	2.0	5.3	4.4	1.2
	14	1.7	5.5	4.6	1.1
	28	1.7	4.9	4.2	1.1
	56	1.7	4.8	4.4	1.2
	84	1.6	4.7	4.7	1.3
	112	1.5	4.8	4.5	1.1
	132	1.5	4.7	4.6	1.5
	161	1.6	4.6	4.4	1.2
	189	1.4	4.1	4.2	2.0
	366	1.9	4.6	4.9	1.7
40	0	2.0	5.3	4.4	1.2
	14	1.7	5.3	4.9	1.3
	28	1.2	4.9	4.3	1.1
	56	1.7	4.9	4.4	1.3
	84	2.1	4.7	4.5	1.6
	112	1.5	4.7	4.6	1.3
	132	1.8	4.9	4.4	1.6
	161	2.1	4.5	4.1	1.9
50	0	2.0	5.3	4.4	1.2
	14	1.8	5.4	4.9	1.4
	28	1.6	4.7	4.2	0.9
	56	1.9	4.5	4.7	1.6
	84	2.6	5.1	4.5	1.3
	112	1.5	4.7	4.5	1.9
	132	1.7	4.6	4.5	1.9
	161	1.7	4.5	4.1	1.8

[a] Values reported are means of 18 observations (duplicate evaluations from 9 panelists) using an intensity scale of 0 (none) to 7 (strong).

Table 4—Mean ratings for the flavor characteristics of peanut paste during storage at different temperature and time conditions as evaluated by a trained panel[a]

Storage conditions		Flavor attributes[b]						
Temp (°C)	Time (days)	RBN	CPT	RPT	OXD	CBD	SWT	BIT
30	0	2.9	2.5	1.0	0.0	0.7	2.4	0.8
	14	1.8	1.6	0.9	0.2	1.3	2.3	0.5
	28	2.1	2.1	1.1	0.2	1.4	2.2	0.6
	56	1.7	1.9	0.9	0.2	1.1	2.4	0.4
	84	2.2	1.5	0.9	0.9	1.8	2.4	0.8
	112	1.6	1.3	1.0	1.1	2.1	2.3	0.9
	132	1.4	1.4	0.5	1.0	2.1	2.7	0.8
	161	2.7	1.7	0.6	2.0	2.2	2.2	0.5
	189	2.2	1.7	1.1	3.3	2.2	2.2	1.2
	366	2.6	1.4	0.7	3.7	2.5	2.3	0.8
40	0	2.9	2.5	1.0	0.0	0.7	2.4	0.8
	14	2.0	1.8	0.8	0.4	1.6	2.2	0.4
	28	1.4	1.8	1.3	0.3	1.2	2.2	0.7
	56	2.2	1.7	1.0	0.9	1.5	2.5	0.6
	84	1.9	1.4	1.1	2.8	2.3	2.4	1.1
	112	2.0	1.5	0.7	3.3	2.4	2.2	1.0
	132	1.7	1.2	0.4	2.0	2.2	2.6	0.8
	161	1.9	1.1	0.6	3.0	2.3	2.0	0.9
50	0	2.9	2.5	1.0	0.0	0.7	2.4	0.8
	14	2.4	1.9	0.9	0.8	1.6	2.3	0.5
	28	2.1	1.5	1.1	0.5	1.6	2.0	0.8
	56	1.4	1.3	1.1	1.4	1.8	2.3	0.8
	84	1.6	1.2	0.8	2.4	2.4	2.3	0.6
	112	1.1	1.1	1.0	2.0	2.5	2.1	1.1
	132	1.3	1.2	0.9	3.5	2.3	2.4	1.0
	161	1.8	1.1	0.8	2.4	3.0	1.9	1.2

[a] Values reported are means of 18 observations (duplicate evaluations from 9 panelists) using an intensity scale of 0 (none) to 15 (strong).
[b] Raw/beany = RBN, cooked peanut = CPT, roasted peanutty = RPT, oxidized = OXD, cardboard = CBD, sweet = SWT, and bitter = BIT.

that in contact with ground peanuts (Woodroff and Thompson, 1945). Differences in specific gravity of the solid particles and oil in peanut paste could have resulted in gravitational separation of these components (Freeman and Singleton, 1952). Two percent hydrogenated vegetable oil (a blend of rapeseed and soybean oils) in granular form was added during milling to stabilize the paste. The results indicate that its addition was effective for samples stored at 30°C as there were only trace amounts of free oil after 366 days. Oil separation was evident in samples stored at 50°C probably because melted crystals of stabilizer and peanut oil rose to the surface. Woodroof and Thompson (1945) reported that there was a normal tendency for free oil to rise to the surface of peanut butter on standing for a few weeks at room temperature. Possibly temperatures of 40°C may be encountered in some warmer areas of the world but storage at 50°C is unlikely.

Texture. Response surfaces for the textural characteristics of peanut paste stored up to 161 days as evaluated by modified TPA (Fig. 2) are experimental values and thus interpolation between data points should not be done. Hardness was highest (23.14 N) in freshly prepared samples and appeared to decrease with storage time (Fig. 2). Response surfaces for adhesive force were similar to those for hardness (Fig. 2). Paste cohesiveness was variable and appeared to fluctuate between 0.50 and 0.70 (Fig. 2). Adhesiveness seemed to increase after 84 days from an initial of 9.7×10^{-3} J to 1.6×10^{-2} (Fig. 2). Regression analysis indicated that the variation in the observed texture values could not be explained by storage time or temperature or an interaction of both variables.

Hardness, adhesive force, and adhesiveness of peanut pastes stored at 30°C for 366 days were lower when compared to the freshly prepared samples. Paste hardness decreased to 13.10N and was 43% less hard; similarly, adhesive force decreased from 6.83 to 2.50N. Adhesiveness was at its lowest, 7.5×10^{-3}J. Paste held at 30°C for up to 366 days had a cohesiveness value of 0.54.

Mean intensity ratings for the textural characteristics of stored peanut paste as evaluated by the trained panel were summarized (Table 3). Oiliness was rated between 1.2 at 40°C and 28 days, and 2.6 at 50°C and 84 days, indicating that free oil in the samples was rated by panelists to be just detectable to slight (0 = none, 7 = strong), when the samples were observed to have a trace to visible (1 mL/50 g paste) oil separation. Gumminess in the stored peanut paste was rated as moderate. Ratings ranged from 4.1 to 4.9 during the entire storage period. Regression analysis indicated no statistical relationship between oiliness or gumminess and storage temperature and time conditions. Adhesiveness appeared to decrease slightly with storage time at all three temperatures but graininess increased slightly (Table 3).

Flavor. Raw and roasted peanut, sweet, and bitter intensities did not change significantly during storage (Table 4). However, cooked peanut intensity decreased with storage time while oxidized and cardboard flavor intensities, increased appreciably. Pearson correlation coefficient indicated that the cooked peanut flavor significantly correlated with oxidized ($r = -0.65$) and cardboard ($r = -0.75$) flavors. This might indicate that oxidized and cardboard flavors masked cooked peanut flavor. The ability of panelists to detect cooked peanut flavor would then decline as the paste aged.

The fat content of peanut paste was high, ≈50%, rendering the paste highly susceptible to off-flavor development associated with lipid oxidation. Oxidized and cardboard flavors are associated with this chemical change (Johnsen et al., 1988). In a high oil/protein product like peanut paste in which the peanuts had been heated, lipid degradation is more likely catalyzed by nonenzymic agents such as hemoproteins and metals than by lipoxygenase (Ory and St. Angelo, 1982). Total inactivation of lipoxygenase has been reported after the first water extraction (90°C for 10 min) in the preparation of peanut paste (Muego, 1991). Peanuts contain the hemoproteins, catalase and perioxidase (Cherry et al., 1973) and linoleic acid. When heat-treated, these hemoproteins have been reported to lose their enzyme activity but have been observed to catalyze linoleic acid oxidation to a greater degree than when unheated (Eriksson et al., 1970). Those researchers speculated that heat

Table 5—Selected regression equations for the prediction of four quality measures by storage time (X_1) and temperature (X_2)

Attribute	Prediction equation	Adjusted R^2
Color		
Chroma	$22.82 - 0.01393X_1 - 0.2807X_2 + 0.003495X_2^2 + 0.0004596X_1X_2$	0.75
Flavor		
Cooked peanut	$2.8 - 0.01655X_1 + 0.00006713X_1^2 - 0.01450X_2$	0.62
Cardboard	$0.08 + 0.01742X_1 - 0.00004857X_1^2 + 0.02075X_2$	0.76
Oxidized	$-8.26 + 0.01634X_1 + 0.3863X_2 - 0.004253X_2^2$	0.69

treatment promoted the unfolding of the hemoprotein, causing greater exposure of the heme groups to the substrate, or the shifting of ferric iron toward a higher spin state. Thus, even if after 10 min at 90°C enzyme activity decreased, the rate of oxidation of linoleic acid increased. St. Angelo et al. (1972) reported that the addition of 0.1% boiled peroxidase in peanut butter caused an increase in peroxidation, probably due to the metal-protein complex, which could be overcome by adding ethylenediamine-tetraacetic acid (EDTA). St. Angelo and Ory (1973) reported that salts and metalloproteins containing iron or copper were primary catalysts of fatty acid perioxidation in peanut butter during storage. In view of this, oxidation of peanut paste could have been catalyzed by salts and heat-denatured proteins containing iron or copper.

Prediction of shelf life

Each of the eight regression models evaluated contained two variables, temperature and time of storage, so that a more responsive far-reaching final model would be selected. Regression analysis indicated that at least 50% of the variation of only four quality measures could be explained (adjusted $R^2 > 0.50$) by at least one model. These were chroma and sensory ratings for cooked peanut, oxidized, and cardboard flavors. The final regressional model for each of these variables is presented in Table 5.

Oxidized flavor score was chosen as the indicator of peanut paste storage stability because it was the only attribute absent in the fresh sample and appeared only after storage. The fitted regression equation for oxidized flavor scores could be mathematically manipulated to predict number of days for peanut paste to attain a given oxidized flavor score at any storage temperature between 30 and 50°C. This equation is as follows:

$$E(X_1) = \frac{Y + 8.26 - 0.3863\ X_2 + 0.004253\ X_2^2}{0.01634}$$

where: $E(X_1)$ is the predicted number of storage days at a specified temperature; Y is the oxidized flavor intensity score; and, X_2 is a storage temperature between 30 and 50°C.

In our investigation, peanut paste that had an oxidized flavor score of 2 (just recognizable) or greater was considered unacceptable. Based on experimental data, peanut paste attained an oxidized flavor score of ≥ 2 after 161, 84, and 84 days at 30, 40, and 50°C, respectively. However, using the above prediction equation, peanut paste is expected to obtain an oxidized flavor score of 2 after 152 days at 30°C, 98 days at 40°C and 96 days at 50°C. Thus the predicted shelf life of peanut paste was overestimated at 40 and 50°C but underestimated at 30°C. The difference between the observed and predicted values can be attributed to random error inherent to regression models. It is expected that peanut paste would not be stored at temperatures above 30°C.

Results indicated that peanut paste is shelf stable and can withstand warm ambient temperatures. This property is highly desirable specially when peanut paste is used in food systems that are stored at ambient temperatures. Shelf life of peanut paste may be extended by suppressing lipid oxidation; this could be achieved through degassing, vacuum packaging and addition of an antioxidant or chelating agent in an aqueous solution during preparation of paste (Woodroof, 1983; St. Angelo et al., 1972).

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Kiwifruit Lipoxygenase: Preparation and Characteristics

STEWART BOYES, CONRAD PERERA, and HARRY YOUNG

ABSTRACT

Kiwifruit were analyzed for the presence of lipoxygenase (LOX) after mass-spectrophotometric analysis of kiwifruit pulp showed the presence of hexanal and hex-2-enal, considered to be break-down products of the reaction initiated by LOX. A spectrophotometric assay showed high levels of LOX when compared to other fruits and vegetables. Kiwifruit have at least one isoenzyme belonging to the class II group of LOX iso-enzymes, similar to those identified in asparagus, cucumber, tomato and apple. The LOX required detergent-solubilization and was partially purified. Relative to other known lipoxygenases kiwifruit LOX is heat-stable. It catalysed the hydroperoxidation of free fatty acids but not trilinolein.

Key Words: kiwifruit, enzyme, *Actinidia*, lipoxygenase, protein, heat stability

INTRODUCTION

LIPOXYGENASE (linoleate:oxygen oxidoreductase - EC.1.13.11.12) (LOX) in the presence of dissolved oxygen, catalyses the formation of fatty acid hydroperoxides from the Z,Z-1,4-pentadiene portion of polyunsaturated fatty acids; the most common substrates being linoleic and linolenic acids. Some LOX isoenzymes (class II and III), in the presence of fatty acids and dissolved oxygen, also co-oxidize carotenoids (Arens et al., 1973; Ben Aziz et al., 1971); and chlorophylls (Abbas et al., 1989). The hydroperoxides are broken down enzymatically and non-enzymatically to a variety of short chain carbonyl compounds. These breakdown products such as hexanal and E-hex-2-enal have been implicated in aroma and taste changes in many processed foods.

Williams et al. (1986) demonstrated, with a trained taste panel, that the addition of the enzymes lipase, peroxidase, and lipoxygenase to green bean purée caused flavor and aroma changes that were largely specific for each enzyme. Lipoxygenase was responsible for aroma changes defined as unripe banana, grassy, straw, ammonia, and partly responsible for the sour component. Lipoxygenase was also responsible for the overall aroma changes more so than the other two enzymes.

Micro-quantities of the hydroperoxide breakdown products, such as C_6 aldehydes, can cause aroma and flavor changes. In freshly processed kiwifruit such as pulp and juice, the compounds that contribute to aroma have been shown by "sniff analysis" to include hexanal, an intense grassy aroma, and E-hex-2-enal, grassy to almond-like depending on concentration (Young et al. 1983). A further compound, E-hex-3-enal has been identified in kiwifruit juice as having an intense grassy/hay-like aroma (Young et al. 1992). Thus, the "grassy hay-like" aroma of kiwifruit juice and the sour-rancid aroma and taste which develops in stored pulp (less than 12 months at −18°C; data not published) could be due to breakdown products of the reaction initiated by LOX.

The generally accepted nomenclature for the isoenzymes is LOX I (alkaline pH for maximal activity, 9.0–9.5, low carotene co-oxidizing ability), LOX II (acid pH 5.5–6.5, activated by calcium, and high carotene co-oxidizing ability) and LOX III (wide neutral pH maxima, inhibited by calcium and high carotene co-oxidizing ability) and these terms are used throughout this report. The general properties of the three classes including the amino-acid sequences have been well defined (Siedow 1991). Our objective was to develop a preparative technique and partial purification for the quantitative measurement of LOX in kiwifruit and determine some enzyme properties in order to classify the type of iso-enzyme(s) present.

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MATERIALS & METHODS

KIWIFRUIT (*Actinidia* (A Chev) Liang C.F. et Ferguson A.R. var. *deliciosa* cv. Hayward) were obtained from the DSIR Kumeu research orchard, picked at Brix 6.8 and stored at 1°C. Kiwifruit were also picked at monthly intervals from 90% petal fall in order to measure LOX during fruit development. French beans (*Phaseolus vulgaris* cv. Labrador) were grown in Hawkes Bay, central N.Z., transported overnight to Auckland and stored at −20°C.

Kiwifruit (k/f) lipoxygenase preparation

In all experiments, 10g of pericarp from three fruits were used, excluding all seeds and skin tissue. The pericarp was ground in liquid nitrogen by mortar and pestle to a fine paste. This procedure limits the activity of the endogenous protease, actinidin and the formation of polyphenolic-protein complexes. The paste was added to 90mL of ice cold preparation buffer. The standard buffer was 50mM Tris pH 7, containing 10mM diethyldithiocarbamate (DIECA), 1%(v/v) Triton X-100, 5g polyvinylpolypyrrolidone (PVPP) and 5g Dowex 1 anion-exchange resin. The mixture was stirred for 20 minutes, filtered through muslin cloth and the filtrate spun in a refrigerated Beckman J2-21 centrifuge at 6000 × *g* for 60 min. The supernatant was filtered (0.45 μm) and passed through a C_{18} reverse-phase cartridge (Waters Assoc. Seppak) to remove the excess detergent.

French bean lipoxygenase preparation

Pod and bean tissue (10g) were added to 90 mL of phosphate buffer (50 mM pH 7), mixed using a Turrax blender, filtered through muslin cloth and centrifuged at 6000 × *g* for 60 min. The supernatant contained the LOX. Triton solubilization was not required (Abbas et al., 1989).

LOX spectrophotometric assay

The Surrey assay (Surrey, 1964) was modified, so that a continual change in the absorption at 234 nm could be monitored on a Philips SP8-100 spectrophotometer, similar to the method described by Al-Obaidy and Siddiqi (1981). The stock linoleic acid substrate was dispersed in an alkaline solution containing 160μg/mL (0.28% v/v) Triton X-100 and the assay carried out at 25°C. Results of LOX activity can vary according to the micelle size and concentration in the substrate. The substrate was prepared in an identical fashion to ensure reproducible results from day to day, generally within 0–5% error of LOX activity. The sample cuvette contained Tris buffer (50 mM pH 7), 200μL of the stock substrate and 10–100μL of the enzyme preparation in a total volume of 3mL. The initial velocity of reaction was read over the linear change in absorbance which generally varied from 20–60 sec. One unit of activity was defined as the change in absorbance of 0.001/min.

In the experiments involving different substrates, linolenic acid was dispersed in Triton in an identical manner as used for linoleic acid. Linoleic and linolenic acids were also ethanol-dispersed, with an assay concentration of 1.6% ethanol. Trilinolein was dispersed in a combination of aqueous ethanol and acetone, with assay concentrations of

1.6% ethanol and 1.02% acetone. Reactions were generally enzyme initiated. The substrate concentrations used in the standard LOX assays were 0.6mM.

For the determination of LOX kinetics two detergents were used to disperse the linoleic acid substrate; Triton X-100 and Brij-35 (polyoxyethylene lauryl ether), both at 0.28% v/v concentration. When experiments were carried out at high substrate concentrations, the detergent concentration of the stock linoleic acid was reduced in order that the assay cuvette contained less than the critical micelle concentration. Results were taken from three to five preparations.

Protein assay

Protein concentration was determined by the Coomassie blue assay (Bradford, 1976) with BSA as the protein standard and read at 595 nm. Triton interferes in this assay and appropriate standards were used. Triton concentration was monitored in all stages of preparation by the absorbance peak at 223 nm. The absorbance of a 0.005% (v/v) Triton solution is 1.230 and therefore the contribution of proteins and other compounds of the kiwifruit preparation is small. A reading at 223nm of the 1% Triton-solubilized kiwifruit preparation read against a 1% Triton solution gave the approximate contribution of the Triton-solubilized proteins and other compounds of the kiwifruit preparation; a maximum of 2% of the total absorbance.

RESULTS & DISCUSSION

LOX recovery

Experiments were carried out in order to optimize the k/f LOX recovery (Table 1). In the control experiment (C) the paste was added to the 90 mL of buffer containing only PVPP and the Dowex 1 resin, both used to remove phenolics (Loomis et al., 1979; Gray, 1977). This procedure was then modified from the control experiment. The addition of Triton X-100 (TX) at an optimal concentration of 1% v/v substantially increased the recovery of LOX, suggesting that k/f LOX was largely membrane bound. A number of detergents were tested to solubilize LOX; Triton X-100 and X-114, Brij-35 and n-octyl β-D-glucopyranoside. Triton X-100 was the most efficient but glucopyranoside will be a useful detergent because of the high critical micelle concentration and low absorbance at 234 nm. A number of reaction buffers were also tested. At pH 7 and 50 mM, the most efficient buffer was Tris which resulted in LOX activity 10–15% greater than phosphate buffer, similar to results obtained with green bean LOX (Zhang et al., 1991). The most efficient buffer at pH 6.20 was piperazine. Citric and tartaric acid buffers gave less than 50% LOX activity in comparison to piperazine. High ionic concentrations (> 0.2M) inhibited k/f LOX activity. Kiwifruit contained four catecholase enzymes that were efficiently inhibited by DIECA at a concentration of 10mM (Park and Luh, 1985). The use of DIECA significantly increased the recovery of k/f LOX activity (from 0.030 to 0.037 Δ Abs/min).

The control/TX/DIECA preparation was centrifuged and the supernatant was passed through a C_{18} reverse-phase cartridge (RPC). The RPC reduced the Triton concentration from 1% to a maximum of 0.05%. The RPC also removed many nonpolar compounds including chlorophyll and carotenoids. When the RPC was used in conjunction with DIECA, an 8.5-fold increase in specific activity over the control-TX preparation was observed. The protein concentration of the final preparation varied from 65 to 125μg/mL and the k/f LOX activity varied 2.5-fold. Each of these preparations was stored for 10 days at 4°C; the C/TX preparation retained 36% of its original activity; C/TX/ DIECA (48%); C/TX/RPC (73%); and C/TX/DIECA/RPC (95%). If samples were frozen they lost all activity on thawing.

To test for self-destruction of the enzyme the reaction was allowed to run to completion for periods up to 180 min. An equal amount of the stock substrate was added to the reaction cuvette again. The initial velocity of reaction and total change in absorbance were almost identical to the original, showing that k/f LOX did not self-destruct in the presence of fatty acids and oxygen, as has been reported for soybeans (Smith and Lands, 1970). A number of detergents were tested for their efficiency as substrate dispersants. The two with the largest substrate turnover both have low critical micelle concentrations (CMC); Triton X-100 and Brij-35, 0.24 mM and 0.091 mM, respectively. At neutral and alkaline pH Brij-35 was a more efficient dispersant than Triton but was not suitable at acid pH. Triton was more efficient at acid pH and was the only detergent suitable for LOX analysis at pH 6.2.

Table 1—Kiwifruit lipoxygenase preparation[a]

	Δ Abs/ min.	Protein mg/mL	S.A.
C	0.008	0.132	1.21
C + 0.5% TX	0.028	0.228	2.45
C + 1.0% TX	0.030	0.250	2.40
C + 1.0% TX + DIECA	0.037	0.300	2.47
C + 1.0% TX + RPC	0.068	0.100	13.60
C + 1.0% TX + DIECA + RPC	0.104	0.100	20.80

[a] Column 1—change in absorbance/minute/50μL sample; Column 3—specific activity; change in absorbance/minute/mg protein; Abbreviations are C = control, TX = Triton X-100, DIECA = diethyldithiocarbamic acid and RPC = reverse-phase cartridge.

Table 2—Lipoxygenase levels during kiwifruit growth[a]

Month	Fruit weight[c] (g)	LOX activity[d] Δ a/min/g	Specific activity[d] Δ a/min/mg
January (30)[b]	47.4	11.3 ± 1.8	15.66 ± 1.64
February (61)	80.0	7.9 ± 1.5	8.20 ± 1.10
March (91)	82.7	8.8 ± 0.5	8.56 ± 0.74
April (127)	105.8	8.7 ± 1.3	7.62 ± 2.25
Total Feb–Apr	89.5	8.47 ± 1.27	8.15 ± 1.53

[a] Kiwifruit were picked at monthly intervals, following 90% petal fall.
[b] Days after petal fall.
[c] Average.
[d] Change in absorbance ± standard deviation.

LOX levels during fruit development

Some fruit such as muskmelon have more LOX specific activity during cell division and cell enlargement periods of development than at maturity (Lester, 1990). To determine the LOX levels during development k/f were picked at monthly intervals following 90% petal fall (12/3/90), which occurs 3–5 days postanthesis (Table 2). The flowering spread is 8–10 days and therefore the fruit picked at 30 days would range in age from 25 to 35 days. This is shown in the spread of the k/f weights: January, 36.5g–55.3g; February, 60.1g–86.6g; March, 60.6g–119.0g; and April, 79.4g–137g. For this reason fruit in the outer 10% weights were not used in this study. The average weight at harvest (5/21/91, 164 days after petal fall) was 112g. Twelve fruit were analyzed in January (30 days after petal fall; fruit shape at this stage was spherical), 21 in February (61 days; characteristic oval shape), 24 in March (91 days) and 21 in April (127 days). There was no statistically significant difference of the values for LOX activity per gram weight of fruit and specific activity between fruit harvested in February, March and April. There was a significant difference between the fruit harvested in January and the total samples from February to April; LOX activity/g pericarp ($p < 0.05$) and specific activity ($p < 0.005$).

The k/f harvested in May were analyzed over a time period which included more than 40 days of storage at 1°C. When storage time exceeded 40 days there appeared to be an increase in LOX activity (data not published). For the fruit harvested in May and analyzed within the 40 day storage time, the LOX activity/g (8.5 Δ abs./min./g pericarp and S.A. (8.0 Δ abs./min./g protein) were similar to the results from the period, February to April.

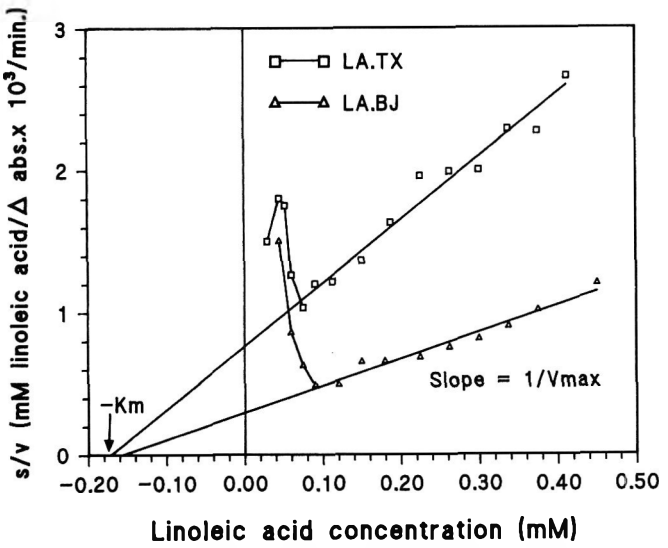

Fig. 1—Kinetics of kiwifruit lipoxygenase. Two detergents were used to disperse the linoleic acid (LA); Triton X-100 (TX) and Brij-35 (BJ). LA.TX gave linear results over a substrate concentration range of 0.4125 to 0.075mM (r^2=0.967) and LA.BJ from 0.450 mM to 0.09 mM (r^2=0.984). One unit of activity is defined as 0.001 change in absorbance/minute and is used to calculate s/v. The reaction was carried out at pH 7.0. Results below substrate concentrations that gave linear kinetics are included to show the sharp decline in LOX activity. LA.TX and LA.BJ gave K_m values of 0.176 mM and 0.157 mM, and V_{max} of 0.225 Δ abs/min and 0.531 Δ abs/min, respectively.

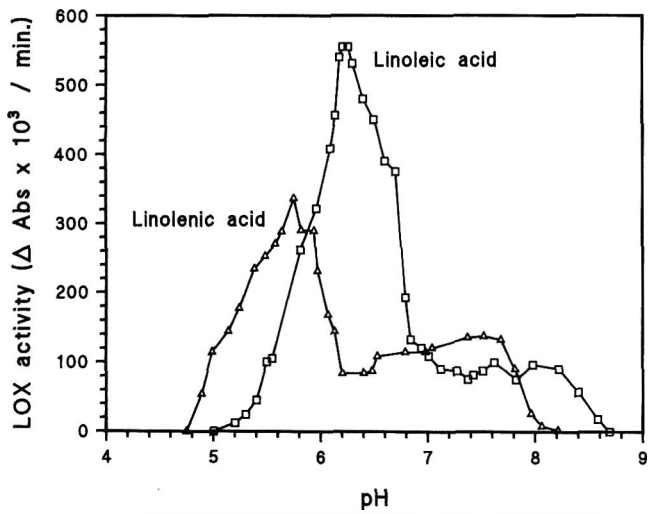

Fig. 2—pH profiles of kiwifruit LOX. The change in absorbance was read over a linear period which varied from 20–60 sec. Substrate concentration was 0.6 mM, dispersed in Triton X-100 and assayed in 50 mM piperazine and Tris buffers.

Kinetics

An Eadie plot (Fig. 1) was used to determine the K_m and the V_{max} since it gives a more even distribution of the substrate values than a Lineweaver-Burk plot. Lipoxygenase gave linear results over a narrow concentration range of 0.45 mM to 0.075mM linoleic acid at pH 7. The Triton and Brij dispersed substrates gave K_m values of 0.175 mM and 0.157 mM linoleic acid and V_{max} values of 0.225 and 0.531 Δ absorbance/minute respectively. The substrate concentration range was limited by the low critical micelle concentration of Triton of 0.24 mM. Concentrations above the CMC sharply inhibit the k/f LOX reaction. This has been reported with other lipoxygenases (Allen, 1968; Galpin and Allen, 1977). Substrate inhibition, at concentrations above 0.6mM, appears to occur with k/f LOX, but may also be attributed to the lowered ratio of detergent to linoleic acid. Substrate inhibition has been shown with other plant lipoxygenases; soybean LOX I (Reynolds and Klein, 1982b; Smith and Lands, 1972), pea LOX II and III (Reynolds and Klein 1982a) and tomato LOX II (Zamora et al., 1987).

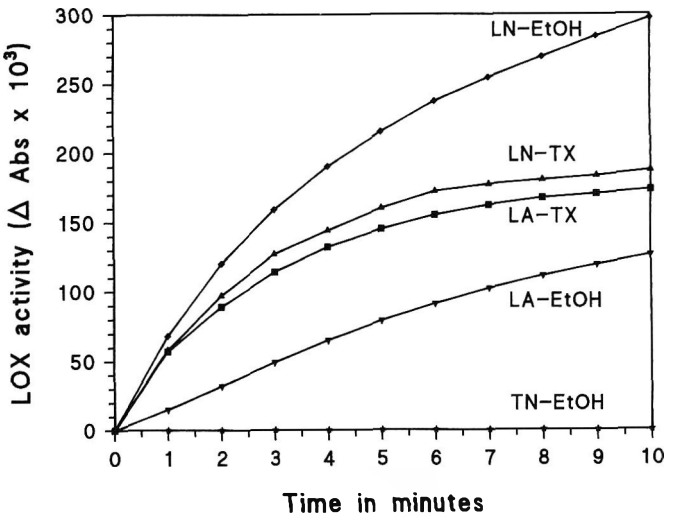

Fig. 3—Kiwifruit LOX activity using different substrates and dispersing agents. The reaction was carried out at 25°C and in Tris buffer pH 7 at a substrate concentration of 0.60 mM. There was no measurable activity with Trilinolein as the substrate. LA-TX, Triton-dispersed linoleic acid; LN-TX, Triton-dispersed linolenic acid; LA-EtOH, ethanol-dispersed linoleic acid; LN-EtOH, ethanol-dispersed linolenic acid; and TN-EtOH, ethanol/acetone-dispersed trilinolein. The LA.TX and LN.TX reactions were also carried out at pH 6.20 (see text).

pH profile

The pH profile (Fig. 2) was measured in piperazine and Tris buffers (50mM). Linoleic acid has two pH maxima, 6.20 and 7.6–8.0. Using linolenic acid as the substrate, the profile moved significantly towards the acid end of the scale with peak activities of 5.75 and 7.4–7.7. Similar pH profiles were observed for LOX II that is present in most fruits and vegetables studied to date.

Substrates and dispersing agents

Comparison of the activity of k/f LOX using substrates linoleic acid (LA) and linolenic acid (LN) dispersed in Triton (TX) and ethanol (EtOH), and trilinolein (TN) dispersed in ethanol/acetone were all carried out at concentrations of 0.6 mM and pH 7 (Fig. 3). The initial rates of activity for the LA-TX, LN-TX, and LN-EtOH substrates were similar when measured at 1 min, but the LN-EtOH reaction had a higher substrate turnover over 5 min. The LA.TX and LN.TX reactions did not run to completion; there was no increase in activity when oxygen was bubbled through the reaction buffer or more LOX added to the cuvette at 5 min. The difference in total substrate turnover could be explained by an accelerated breakdown of the fatty acid hydroperoxides when the substrate was dispersed in Triton, or not all of the substrate was available to the k/f LOX when dispersed in detergent. Some studies have shown that LOX reacts with the non-micellar substrate and the micelles act as reservoirs (Galpin and Allen, 1977), thus possibly limiting the substrate turnover.

Triton-dispersed linoleic and linolenic acids behaved differently at pH 6.20, with initial velocities of 0.321 and 0.195 Δ abs/min, respectively. The LA.TX reaction reached 0.500 Δ absorbance at 5 min and a maximum at 20 min whereas the

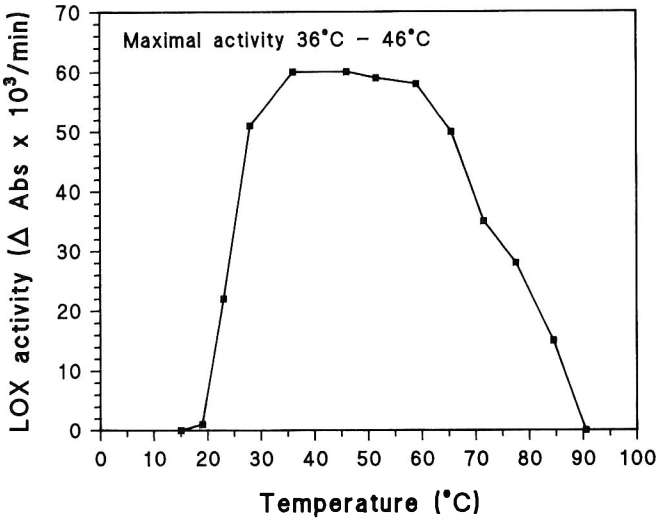

Fig. 4—Temperature stability of kiwifruit LOX. Lipoxygenase was measured at pH 7 using 0.3–0.6 mM Triton-dispersed linoleic acid. Samples were held at each temperature for 2 min and the LOX activity read immediately before cooling took place.

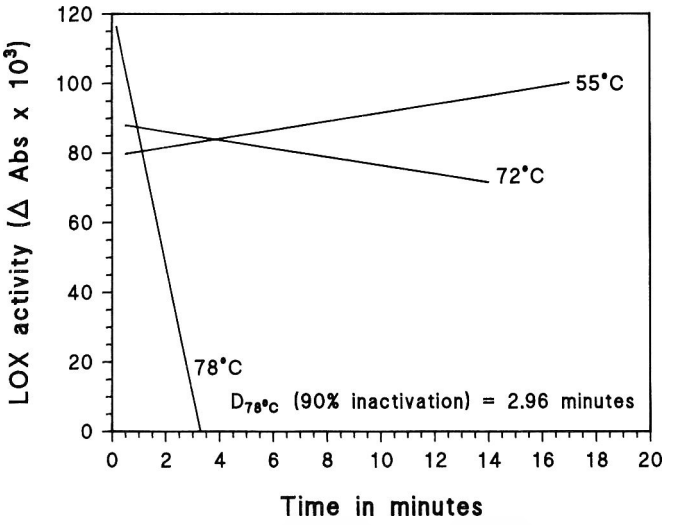

Fig. 5—Stability of LOX at high temperature. Lipoxygenase was measured at pH 7 using 0.3–0.6 mM Triton-dispersed linoleic acid. The control reading by standard assay, (at 25°C) was 0.054 absorbance units per minute.

LN.TX reaction reached a maximum of 0.149 at 1–2 min and then decreased to 0.075 at 5 min. From scans of the linoleic and linolenic reaction mixtures, a peak at 280nm diminished as the peak at 234nm increased. The linolenic hydroperoxide product appeared to break down at a higher rate at pH 6.20 than at pH 7.0.

Trilinolein, at pH 6.2 and 7.0 does not serve as a substrate for k/f LOX. In general LOX II has a similar reactivity with linoleic acid and linolenic acid when measured at pH 7. It has a wide range of activity with trilinolein in comparison to linoleic acid, as demonstrated in cowpea (Van Den and Mendoza, 1982), apple (Insook and Grosch, 1979), asparagus (Gavanthorn and Powers, 1989), broad bean (Al-Obaidy and Siddiqi, 1981), and pea (Reynolds and Klein, 1982a; 1982b).

Comparison of LOX activities

The Leguminosae family have high LOX activities. We have found that on a fresh weight basis, kiwifruit have about the same LOX activity as French beans. Annual plants generally have a much higher LOX activity than perennials (Pinsky et al., 1971; Rhee and Watts, 1966a). On this comparative basis kiwifruit have a high LOX activity.

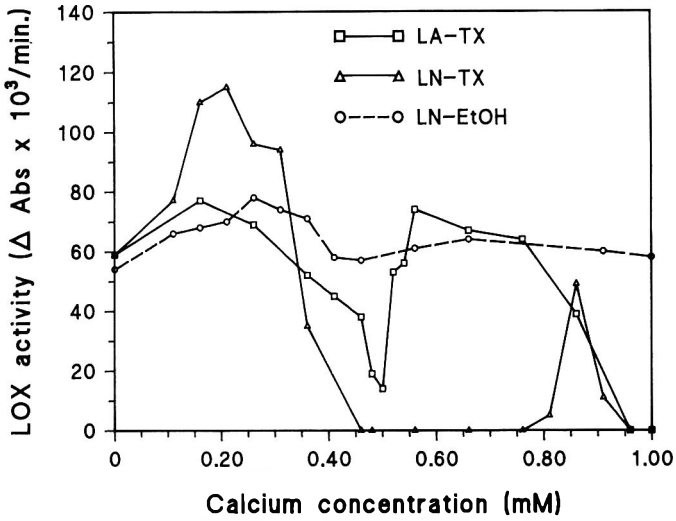

Fig. 6—Effect of calcium on LOX and LOX substrates. Calcium acetate was added to substrate (0.60 mM) plus reaction buffer (pH 7), shaken and the reaction immediately initiated by the addition of LOX. Abbreviations are LN-TX Triton-dispersed linolenic acid; LA-TX Triton-dispersed linoleic acid; and LN-EtOH ethanol-dispersed linolenic acid. Results are from five preparations.

Temperature stability

Samples were held at each temperature for 2 min (Fig. 4). The LOX activity was measured immediately after removal from the waterbath so that samples did not appreciably cool before the activity could be measured. Kiwifruit LOX had maximal activity at 36-46°C, similar to the 40°C maximum for tomato LOX (Bonnet and Crouzet, 1977). Activity could not be measured below 15°C, but if the reaction mixture was left in the cuvette, the LOX reaction quickly recovered activity as it warmed to room temperature (20–25°C). When samples held at temperatures from 70°C to 75°C were left to cool to room temperature, they partially recovered the activity that was lost on heating at the respective temperatures. The sample held at 90°C did not recover any activity on cooling. Kiwifruit LOX at high temperature (Fig. 5) gave a D value (90% inactivation) of 2.96 min at 78°C, but there was no loss of activity at 55°C after 20 min incubation. At 72°C there was only a slight loss of activity after prolonged heating. Kiwifruit LOX was very stable in comparison to other fruit and vegetable lipoxygenases; apple, 100% loss of LOX activity at 50°C for 2 min (In-Sook and Grosch, 1977); cucumber, 100% loss at 70°C for 2 min (Wardale and Lambert, 1980); and broad bean, 100% loss at 75°C for 10 sec (Al-Obaidy and Siddiqi, 1981).

Effect of calcium

Kiwifruit have a high calcium content; in cv Hayward, 25 mg per 100 g fresh weight (Visser and Burrows, 1983). About half of this is stored as the needle-shaped crystals of calcium oxalate (Perera et al., 1990) and this portion would be excluded by the standard preparation. The contribution of soluble calcium from a 50μL k/f LOX sample would be equivalent to 0.06 mM in the reaction cuvette. Kiwifruit LOX does not require calcium for activity. In the presence of the metal chelator EDTA or the more specific calcium chelator EGTA there was no change in k/f LOX activity from the original preparation.

To test the effect of calcium on k/f LOX, calcium from a stock solution of 12mM calcium acetate was added to the re-

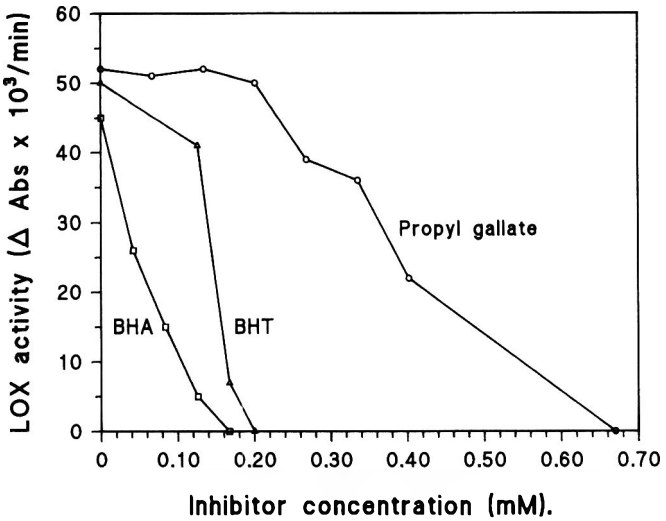

Fig. 7—Action of anti-oxidants on LOX activity. The reaction was carried out in Tris buffer at pH 7 with Triton-dispersed linoleic acid (0.60 mM). The inhibitors were added to the buffer plus-substrate, shaken and the reaction immediately initiated by the addition of LOX. Abbreviations are: BHA, butylated hydroxy-anisole; BHT, butylated hydroxytoluene.

action buffer and substrate, mixed and the reaction was immediately initiated by the addition of 50μL of the k/f LOX preparation (Fig. 6). Calcium appeared to affect primarily the substrate rather than the enzyme. When linoleic acid was dispersed in ethanol the effect of calcium was small. There was a small activation of the LOX reaction up to 0.30 mM calcium (144% of the control activity) but at higher concentrations, up to 5 mM calcium, the LOX activity was similar to the activity in the absence of calcium. The Triton-dispersed linoleic acid showed a complex reaction with calcium. The formation of calcium mono- and di-linoleate may reduce the effect of substrate inhibition of LOX in some fruits and vegetables and result in an activation at low calcium concentrations. We observed a similar reaction activation with k/f LOX (132% of the control activity at 0.16 mM calcium). However, calcium linoleate in a detergent medium did not serve as a good substrate for k/f LOX. At a calcium concentration of 0.50 mM and 0.06mM contributed from the k/f sample there would be little free linoleic acid and this resulted in an 80% reduction in activity. This effect (equimolar calcium to Triton-dispersed linoleic acid inactivating the reaction) has not been observed in other known lipoxygenases. When the calcium concentration was increased above equimolar levels to 0.9 mM the LOX activity returned but we know no explanation for this observance.

At calcium concentrations greater than 1 mM and Triton-dispersed substrate, LOX activity cannot be detected, possibly due to the effect of the calcium on the detergent micelle formation (Galpin and Allen, 1977). This pattern of the changes of activity with calcium was also observed with Triton-dispersed linolenic acid. The initial activation of the LOX reaction changed in line with the substrate concentration; at double the substrate concentration twice the amount of calcium was required to reach peak activity. With no change in substrate concentration and double the enzyme concentration, the same amount of calcium was required to reach peak activity. These results suggest that the calcium acted primarily on the detergent-solubilized substrate and not directly on the k/f LOX. Calcium interaction in the LOX reaction has been studied using soybean LOX (Zimmerman and Snyder, 1974; Restrepo et al., 1973) and pea LOX II (Reynolds and Klein, 1982a). Their results showed different interactions with the different LOX isoenzymes but the general conclusion was that calcium interacts primarily with the substrate.

Inhibitors

The K_i for the antioxidants were: BHA, 80 μM; BHT, 160 μM; and propyl gallate, 380 μM (Fig. 7). SHAM (Salicylhydroxamic acid) inhibited k/f LOX but because of its high absorbance at 234 nm an accurate result was not possible and another assay will be used to assess this. The order of efficiency of inhibition of LOX is generally BHA > BHT > Propyl gallate (Rhee and Watts, 1966b; Bonnet and Crouzet, 1977).

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Texturization of Sweetened Mango Pulp: Optimization using Response Surface Methodology

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ABSTRACT

Sugar added mango pulp was texturized with alginate. To optimize both the mechanical properties and thermostability of the products, an experimental design was used combined with response surface methodology. Alginate concentration was the major factor affecting mechanical properties of texturized pieces with higher strength as the gelling agent concentration increased. Furthermore, gel strength and thermostability generally increased as calcium and glucono-δ-lactone concentrations, and/or heat treatment duration increased. As large quantities of sweetened pulp were used, texturized pieces with high dry matter content and good flavor qualities were obtained.

Key Words: gelation, fruit analog, mechanical properties, texturization, thermostability

INTRODUCTION

THE TECHNOLOGY of fruit analog preparation using alginates or, in some cases, low methoxyl pectins has been described (e.g., Bradshaw et al., 1976, 1978; Barwick and Sneath, 1978; Wiggett et al., 1982; Hannigan, 1983). Sodium alginates are usually selected as gelling agents because of the thermostability of the products obtained. High thermostability constitutes an appreciable property in texturized fruits as they can be stabilized by pasteurization for use in dairy and ice-cream industries, or undergo cooking operations to be used in confectionery and baking industries.

Ample information has been given on the mechanical properties of pure gels obtained with these gelling agents with or without other polysaccharides (e.g., Luh et al., 1977; Pelaez and Karel, 1981). Nevertheless, for texturizing pulp or concentrated fruit juice, the conditions of gel preparation and properties of gels are modified: the pulp pH is often too low (pH < 3.5). Therefore, alginate may develop into alginic acid which forms a discontinuous gel with rheological properties different from those of calcium-alginate gels (Wood, 1975; Glicksman, 1983).

Adding large quantities of pulp can also cause notable weakening of resistance to deformation and the resulting texture qualities of the final products are not as effective (Kaletunc et al., 1990; Nussinovitch and Peleg, 1989). Furthermore, the homogeneous dispersion of the gelling agent in highly concentrated -and consequently highly viscous- pulps can result in problems during implementation. For these reasons, most of the patents and publications describing different procedures of fruit analog preparation from alginate gels refer to formulations with a low pulp percentage (in the range of 10–41% of the product weight). Kaletunc et al. (1990) tried to increase the fruit content percentage on apple juice and concentrated grapefruit juice. Note that, to obtain convenient products, the soluble dry matter content of the juices needed to be adjusted to 10°Brix by addition of distilled water.

Our objective was to modify the standard procedures of texturization to prepare fruit analogs containing 90% fruit pulp, either concentrated or sugared at 50°Brix. The advantage would be that the products so achieved afford excellent aromatic qualities and a high soluble dry matter content (50°Brix), which could reduce the cost of a final drying stage if required. We also studied the development of a method for evaluating thermostability of texturized fruits. The formulation and processing method to prepare texturized mango from pulp at 50°Brix were optimized in relation to qualities of final products (especially texture and thermostability) using an experimental design in association with response surface methodology.

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MATERIALS & METHODS

A COMMERCIAL high viscosity alginate powder (Louis François) was used to prepare the fruit gels. Dibasic Calcium Phosphate (Merck) and glucono-δ-lactone (Roquette) were used for alginate gelation. Pasteurized pulp of Indian mangoes (pH = 4.05; 18°Brix) was used as raw material. The total soluble solids content of the pulp was adjusted to 50°Brix with sucrose (pulp 61%, sugar 39%, wt). The quantity of added sugar was 30–40% (wt) for which the highest strength could be observed (Nussinovitch et al., 1991).

Texturization

Sodium alginate (0.6–1.4%, wt), dispersed in 5 mL of a distilled water and ethanol (1:1) mixture, was added (with vigorous stirring) to mango pulp (90%, wt) previously heated in a thermostated bath at 60°C. Preliminary studies showed that without heat treatment, gelation of the mixture could not occur. Several temperatures were tested: 50, 60, 70 and 80°C, during 10 min. As gelation starts at 60°C, the thermostated bath temperature was set at 60°C. After variable durations of heat treatment at 60°C (0 to 20 min), a suspension of insoluble calcium salt in 2 mL of distilled water (0.05–0.65%, wt) was added just before the glucono-δ-lactone (0–2%, wt). The ingredients were then vigorously mixed for about 10 sec before gelation occurred.

Mechanical tests

To measure the compressive stress and viscoelastic properties of the products, cylindrical texturized pulp specimens 3.00 cm^2 in section and 1 cm in height were molded in plastic rings in the 30 sec following the mixture preparation. Force at failure of mango gels was evaluated by penetration measurements in a 25 mL beaker filled with the mixture. All specimens so prepared were allowed to age 1 hr at 20°C, and kept at 6°C for 24 hr to equilibrate. Each gel was then kept at 20°C for 1 hr before measurements. According to Pelaez and Karel (1980) and Nussinovitch and Peleg (1990) the full equilibration of an alginate texturized product can take from 7 to more than 48 hr. Nevertheless Nussinovitch and Peleg (1990) also showed that most of the mechanical features of gels can be observed after 24 hr. All measurements were obtained using a Stevens LFRA Texture Analyser (model TA/1000), with a cell of 10 Newton capacity interfaced with a graphic recorder. The data were also recorded through a 21X Micrologger (Campbell) and transferred to a Goupil PC AT computer for analysis.

For compressive stress and viscoelastic properties measurement, the product specimens were compressed to a deformation of 3mm (30%) between parallel lubricated plates, at a deformation rate of 0.2 mm/s. The deformation of 3mm was then maintained constant and the fruit gels were allowed to relax. This test allowed for the direct measurement of the maximal compressive force for the deformation of 3 mm called F_o(N). The parameter S_o(N.cm^{-2}) was then calculated as follows:

$$S_o = F_o/A$$

where S_o represents the maximal compressive stress for the defor-

mation of 3 mm, and A, the specimen area (cm^2). The force-time relaxation curves were recorded for 2 min following deformation.

Having checked that the evolution of these curves was not affected by the manufacturing conditions of texturized mango pieces and in conformity with previous studies (Peleg, 1979), the Y(1 min) parameter, dimensionless ratio, was used to represent decay of the force. The parameter Y(1 min) was calculated as follows:

$$Y(1\ min) = (F_o - F_i)/F_o$$

where F_i is the force recorded after 1 min of relaxation.

For penetration measurements, each specimen was compressed to failure under a cylindrical probe 0.126 cm^2 in section, at a deformation rate of 0.5 mm/sec. The force-deformation curves were recorded and the force at failure F_F value (N) was used to characterize the texture of each specimen. All mechanical tests were performed in quadruplicate and the reported values are the mean values.

Thermostability measurements

Thermostability measurements were made on texturized mango samples aged 1 wk at 6°C, and cut into cubes with 1 cm sides. Each block of texturized mango was put on a filter-paper disc of known mass and covered with a 15 mL beaker. To test the resistance of the product at high temperature, the experimental set was then put in an oven at 130°C for 30 min. The temperature applied was > 100°C and the treatment duration chosen rather long so as to be as near as possible to the cooking conditions experienced in the baking and confectionery (pies and cakes) industries. The consistency of blocks was visually evaluated after the oven stage. The percentage of syrup at 50°Brix exuded during the thermostability test, as compared with the initial mass of gel was estimated as follows:

$$Ex\% = [(m_s \times 2)/m_g] \times 100$$

where m_s is the dry matter (g) of the exuded syrup retained by the filter paper; $m_s \times 2$ is the total mass of the 50°Brix syrup exuded during the thermostability test, and m_g is the initial mass of texturized mango pulp. The lowest exuded syrup percentage corresponded to the best resistance at high temperature and thus to the product with highest thermostability.

Experimental design and modeling

Based on preliminary experiments, the four independent variables considered as most important factors affecting mechanical properties and thermostability of the texturized mango pieces were alginate concentration (X_1), heat treatment duration (X_2), calcium phosphate concentration (X_3) and glucono-δ-lactone concentration (X_4). The other important variables were fixed: the total soluble solids content (50° Brix), the initial pH (4.05) of the pulp, and the temperature of the heat treatment (60°C). The experimental design adopted was 4 factors, 5 levels central composite (Hinchen, 1968). For each independent variable studied, the central value and interval between the levels were chosen according to preliminary studies. The coded values of the independent variables (−2 (lowest level), −1, 0 (medium level), 1, 2 (highest level)) were calculated for a rotatable design (Gacula and Singh, 1984) having the same predictive power in all directions from the center point with the best precision. Correspondence between these coded values and actual values are given in Table 1. The complete design consisted of 36 experimental points which included 12 replications of the center point (Table 1). Thus 36 texturized mango pulp products were prepared in a random order. The five dependent Y variables (or responses) to evaluate the texturized products were: pH (Y_1), compressive stress for a 3 mm deformation (Y_2), force decay parameter Y(1 min)(Y_3), force at failure (Y_4) and exuded syrup percentage (Y_5).

Statistical analysis

Data were analyzed by multiple regression to fit the following second order equation to all dependent Y variables:

$$Y = B_0 + \sum_{i=1}^{4} B_i X_i + \sum_{i=1}^{4} B_{ii} X_i^2 + \sum_{i=1}^{4} B_{ij} X_i X_j \qquad (1)$$

where B_o, B_i, B_{ii}, B_{ij}, are constant and regression coefficients of the model, and X_i the independent variables in coded values. The CStat program (CIRAD, Montpellier, FR) was used for the multiple regression involved in the modelization. Several response surfaces using the Statistical graphics system (STSC, Rockville, MD) were drawn to point out the main effects of independent variables on texturization. These three-dimensional representations show the effect of two independent variables selected on a given response, by imposing a constant value equal to zero (medium level) on the other two factors.

RESULTS & DISCUSSION

CONDITIONS of the formation of texturized mango pieces were compared with data on corresponding product properties (Table 1). The °Brix had been controlled and was about 50 ± 1 for most of the samples studied. All texturized mango samples had about the same color (orange-yellow) corresponding to the initial color of the mango pulp. They also had a satisfactory visual appearance and touch, except for the product corresponding to design point 31 which was pasty and granulous, and the one corresponding to design point 29 which was weak. For each experiment, gelation occurred, but the final products had considerably different mechanical properties depending on conditions. All gels showed a syneresis evaluated at about 10% by weight after 48 hrs. This phenomenon, which results from gel shrinkage, was also observed by Kaletunc et al. (1990) with the same order of magnitude. In our case, the higher syneresis was observed for the products containing a higher glucono-δ-lactone concentration (1.5g or 2g/100g).

Statistical analysis and response surfaces

Equation (1) was fitted to the experimental data (Table 2). Six equations were obtained and tested for adequacy and the quality of the fit was evaluated. The main results of this multiple regression analysis were developed for each of the dependent variables, with corresponding coefficients of determination (R^2). The models for force decay parameter Y_3(R^2 = 0.755), for the percentage of exuded syrup Y_5 (R^2 = 0.776) during the thermostability test and for compressive stress Y_2 (R^2 = 0.842) were considered approximate considering the low percentage of variability explained ($0.75 < R^2 < 0.85$)(Henika, 1982). These 3 models were used only for trend analysis. In the model for force decay parameter, R^2 was the lowest. According to Henika (1982), $R^2 < 0.75$ means a significant lack of fit. A careful inspection of residual errors was made (differences between predicted and experimental response values). This indicated that a higher order model would be required to better describe the effects of independent variables, and particularly alginate concentration. The models developed for pH and force at failure were considered adequate with satisfactory R^2 values (>0.85) and significant F values (probability of F < 1%).

From the analysis of the coefficient estimates for the five regression models, the four variables appeared to influence the texturized mango properties. As regards all response variables, the main effect of the formulation variables (alginate concentration, calcium salt concentration and glucono-δ-lactone concentration) was linear (first order). The response increased or decreased continuously within the experimental field. In contrast, regarding the heat treatment duration process variable, the significant effect was generally quadratic (second order). This indicated the existence of an optimum level within the duration range tested (0–20 min). According to significance tests on estimated coefficients, alginate concentration was the most important factor influencing mechanical properties (compressive stress, Y(1 min) parameter, and force at failure) and thermostability (exuded syrup percentage). The factor with the main effect on final pH was obviously the glucono-δ-lactone concentration. The relationships between independent and dependent variables are represented by the three-dimensional response surfaces (Fig. 1 through 8) generated by model equations developed from the statistical analysis.

Table 1—Responses of dependent variables to the gelation conditions

	Independent Variables*					Dependent Variables				
n°	alginate concentration (g/100g)	heat treatment duration at 60°C (min)	calcium salt concentration (g/100g)	Glucono-d-lactone concentration (g/100g)	Brix °Bx	pH	compressive stress (N/cm²)	force decay parameter Y(1min) (%)	force at failure (N)	exuded syrup percentage (%)	n°
1	0.8 (−1)	5 (−1)	0.2 (−1)	0.5 (−1)	51	3.70	0.72	38.66	0.36	4.28	1
2	1.2 (+1)	5 (−1)	0.2 (−1)	0.5 (−1)	50	3.91	1.11	32.52	1.26	1.64	2
3	0.8 (−1)	15 (+1)	0.2 (−1)	0.5 (−1)	50	3.82	0.92	39.41	0.52	1.58	3
4	1.2 (+1)	15 (+1)	0.2 (−1)	0.5 (−1)	49	3.95	0.98	31.51	1.05	1.49	4
5	0.8 (−1)	5 (−1)	0.5 (+1)	0.5 (−1)	50	4.17	0.93	39.75	0.71	3.10	5
6	1.2 (+1)	5 (−1)	0.5 (+1)	0.5 (−1)	46	3.92	1.31	35.21	1.23	2.03	6
7	0.8 (−1)	15 (+1)	0.5 (+1)	0.5 (−1)	51	3.92	1.05	42.75	0.52	2.35	7
8	1.2 (+1)	15 (+1)	0.5 (+1)	0.5 (−1)	49	4.13	1.42	37.41	1.55	1.81	8
9	0.8 (−1)	5 (−1)	0.2 (−1)	1.5 (+1)	50	3.45	0.97	40.91	0.60	4.61	9
10	1.2 (+1)	5 (−1)	0.2 (−1)	1.5 (+1)	51	3.42	1.23	34.61	1.69	2.02	10
11	0.8 (−1)	15 (+1)	0.2 (−1)	1.5 (+1)	50	3.49	1.04	42.55	0.74	3.43	11
12	1.2 (+1)	15 (+1)	0.2 (−1)	1.5 (+1)	50	3.54	1.52	35.44	1.31	1.58	12
13	0.8 (−1)	5 (−1)	0.5 (+1)	1.5 (+1)	48	3.49	1.06	42.12	0.73	5.54	13
14	1.2 (+1)	5 (−1)	0.5 (+1)	1.5 (+1)	51	3.55	1.78	41.16	2.23	2.36	14
15	0.8 (−1)	15 (+1)	0.5 (+1)	1.5 (+1)	50	3.58	1.41	49.14	0.59	3.16	15
16	1.2 (+1)	15 (+1)	0.5 (+1)	1.5 (+1)	50	3.67	1.74	42.80	1.71	1.59	16
17	1 (0)	10 (0)	0.35 (0)	1 (0)	49	3.74	1.41	41.60	1.17	2.47	17
18	1 (0)	10 (0)	0.35 (0)	1 (0)	51	3.75	1.41	42.04	1.01	1.80	18
19	1 (0)	10 (0)	0.35 (0)	1 (0)	50	3.69	1.05	41.22	0.80	2.12	19
20	1 (0)	10 (0)	0.35 (0)	1 (0)	52	3.83	1.26	40.63	1.22	2.15	20
21	1 (0)	10 (0)	0.35 (0)	1 (0)	52	3.73	1.36	40.53	1.24	1.91	21
22	1 (0)	10 (0)	0.35 (0)	1 (0)	50	3.84	1.53	43.76	1.14	1.88	22
23	1 (0)	10 (0)	0.35 (0)	1 (0)	50	3.64	1.30	39.10	1.27	2.49	23
24	1 (0)	10 (0)	0.35 (0)	1 (0)	51	3.67	1.51	41.27	1.27	1.77	24
25	1 (0)	10 (0)	0.35 (0)	1 (0)	49	3.84	1.42	40.52	0.90	2.60	25
26	1 (0)	10 (0)	0.35 (0)	1 (0)	52	3.65	1.19	39.71	1.40	2.81	26
27	1 (0)	10 (0)	0.35 (0)	1 (0)	51	3.75	1.36	41.33	1.40	2.58	27
28	1 (0)	10 (0)	0.35 (0)	1 (0)	52	3.77	1.40	41.30	0.95	2.47	28
29	0.6 (−2)	10 (0)	0.35 (0)	1 (0)	50	3.68	0.81	47.50	0.29	5.65	29
30	1.4 (+2)	10 (0)	0.35 (0)	1 (0)	51	3.77	1.86	38.72	2.23	1.54	30
31	1 (0)	0 (−2)	0.35 (0)	1 (0)	50	3.81	0.60	60.90	0.29	15.32	31
32	1 (0)	20 (+2)	0.35 (0)	1 (0)	50	3.96	1.65	44.11	1.26	4.91	32
33	1 (0)	10 (0)	0.05 (−2)	1 (0)	49	3.57	0.90	33.44	0.65	2.41	33
34	1 (0)	10 (0)	0.65 (+2)	1 (0)	51	3.81	1.53	44.05	1.29	2.30	34
35	1 (0)	10 (0)	0.35 (0)	0 (−2)	52	4.53	0.98	32.38	0.42	1.71	35
36	1 (0)	10 (0)	0.35 (0)	2 (+2)	48	3.20	1.54	41.10	1.64	2.45	36

* Values in parentheses are the coded levels of independent variables.

Table 2—Regression coefficients and analysis of variance of the second order polynomials[a] for five response variables

	RESPONSE VARIABLES				
Coefficients	final pH Y_1	Compressive stress Y_2	Force decay parameter Y_3	Force at failure Y_4	Exuded syrup percentage Y_5
B_0	3.742	404.867	41.084	114.739	2.254
Linear					
B_1	0.027	63.330****	-2.593****	46.527****	−0.905****
B_2	0.033*	38.341****	−0.728	4.683	−1.224****
B_3	0.068****	43.287****	2.332****	12.479***	0.048
B_4	−0.250****	42.796****	2.042****	20.166****	0.313
Quadratic					
B_{11}	−0.023	−11.763	−0.102	−3.794	−0.288
B_{22}	0.026	−18.569***	2.247****	−8.628**	1.652****
B_{33}	−0.014	−2.864	−1.193**	3.474	0.022
B_{44}	−0.021	−8.330	−1.694****	−2.131	−0.356
Interactions					
B_{12}	0.031	−9.779	−0.549	−4.728	0.337
B_{13}	−0.016	11.925	0.640	6.763	0.049
B_{14}	−0.008	10.515	0.197	8.102	−0.305
B_{23}	−0.009	2.207	0.731	−1.472	0.020
B_{24}	0.016	7.235	0.389	−6.041	−0.061
B_{34}	−0.023	4.537	0.544	0.601	−0.042
Variability explained =					
R^2	0.888	0.842	0.755	0.851	0.776
F	11.94	7.99	4.61	8.55	5.20
Probability of F =	0.00	0.00	0.09	0.00	0.04

[a] Model on which X_1 = alginate concentration, X_2 = heat treatment duration, X_3 = calcium salt concentration, X_4 = glucono-δ-lactone concentration is

$$Y = B_0 + \sum_{i=1}^{4} B_i X_i + \sum_{i=1}^{4} B_{ii} X_i^2 + \sum_{i=1}^{4} B_{ij} X_i X_j \qquad \text{Eq. (1)}$$

**** significant at 0.01 level
*** significant at 0.05 level
** significant at 0.10 level
* significant at 0.15 level

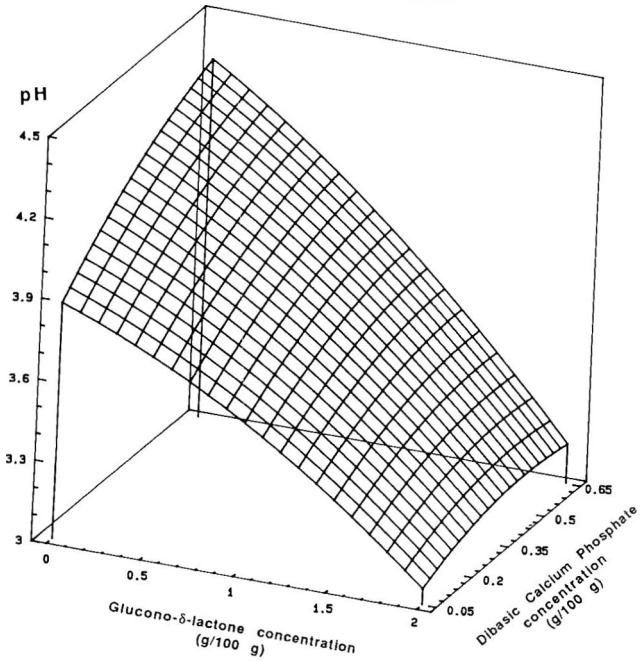

Fig. 1—Response surface for the effect of glucono-δ-lactone concentration and calcium salt concentration on the pH of texturized products at constant alginate concentration of 1% (wt) and constant heat treatment duration of 10 min.

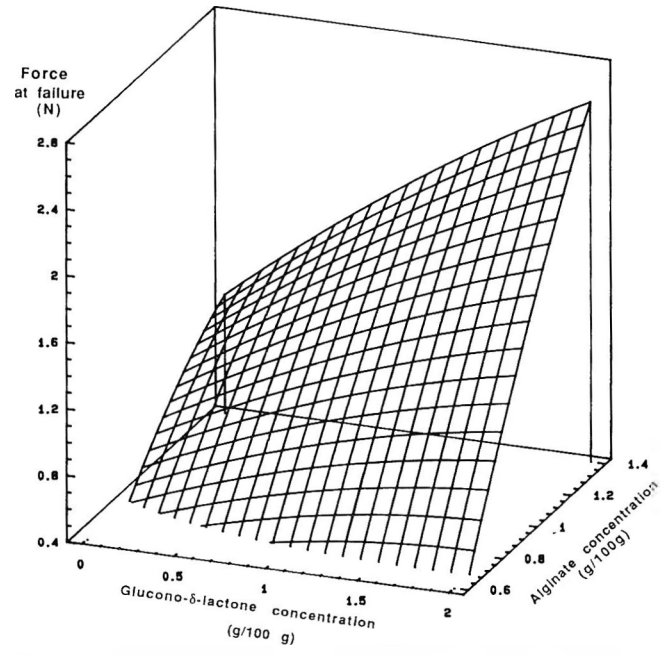

Fig. 3—Response surface for the effect of glucono-δ-lactone concentration and alginate concentration on the force at failure to texturized products at constant calcium salt concentration of 0.35% (wt) and constant heat treatment duration of 10 min (part of the surface for force at failure above 0.4N).

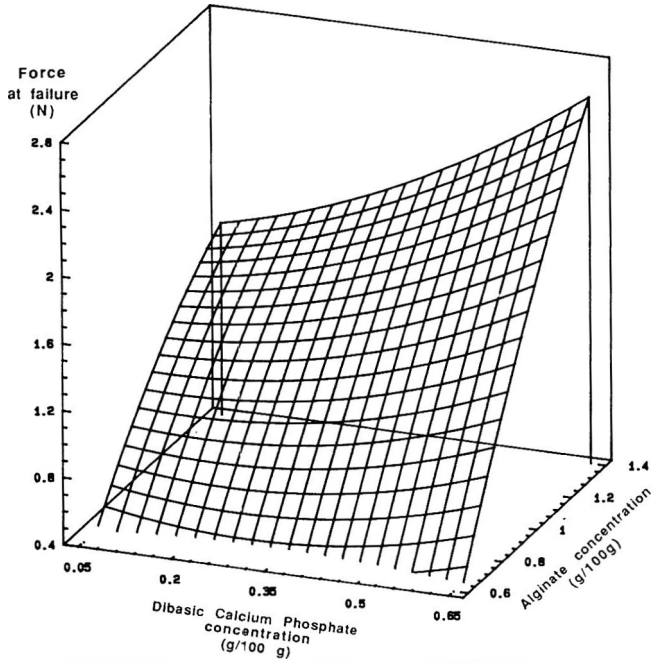

Fig. 2—Response surface for the effect of calcium salt concentration and alginate concentration on the force at failure of texturized products at constant glucono-δ-lactone concentration of 1% (wt) and constant heat treatment duration of 10 min (part of the surface for force at failure above 0.4N).

pH

In conformity with Glicksman (1983), preliminary studies showed that gels formed with pulp at pH below 3.5 proved unsuitable for making texturized products. The acidity constitutes a barrier to a homogeneous dispersion of the alginate within the fruit pulp. Consequently, the pH needs to be increased up to about 4.00 (e.g. with 2M sodium hydroxide solution)(Kaletunc et al., 1990) in order to work with acid fruit pulps. For this reason we decided to use mango pulp with an initial pH around 4. Nevertheless, when using insoluble calcium salt, it is necessary to acidify the product during the texturization process so as to release the calcium required for gelation and obtain final products with pH < 4.00. An acid pH such as this improves product firmness and its microbiological stability, and allows for better sensory qualities. For these reasons, the same as Pelaez and Karel (1980) and Kaletunc et al. (1990), we decided to use glucono-δ-lactone, which affords the property of being slowly hydrolysed in gluconic acid and gradually reduces pH. In our experiments, the final pH of the texturized mango pieces was in the range 4.53-3.2 respectively for 0 and 2% (wt) of glucono-δ-lactone corresponding to design points 35 and 36. The combined influence of glucono-δ-lactone and dibasic calcium phosphate concentrations is represented in Fig. 1. It appears that calcium salt concentration can reduce the acidifying effect of glucono-δ-lactone. Such an effect could be explained by the formation of different calcium chelates. Some chelates are formed with the gluconic acid released by the glucono-δ-lactone (Sipes, 1969). Nevertheless, the resulting pH increase was moderated by the calcium chelation caused by phosphate ions produced by the calcium salt dissociation. The final pH of the texturized product was the result of these complex and multiple equilibria. The effect of the calcium salt concentration was more visible at low glucono-δ-lactone concentration values, but remained relatively low. To obtain a product with a final pH < 4.0 irrespective of levels of the other factors, particularly calcium salt concentration, at least 1% (wt) of glucono-δ-lactone should preferably be added.

Mechanical properties

From a general viewpoint, the products presented highly different characteristics in texture, particularly as regards their firmness. The regression coefficients of the models regarding the compressive stress and force at failure of the final products showed that the effect of the four factors studied on those response variables was about the same. Generally, the two texture parameters S_O and F_F varied linearly in relation to the

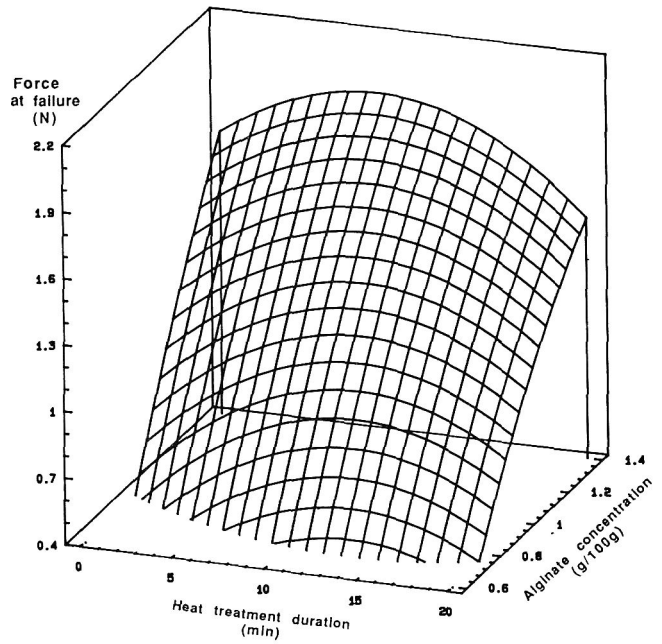

Fig. 4—Response surface for the effect of heat treatment duration and alginate concentration on the force at failure of texturized products at constant calcium salt concentration of 0.35% (wt) and constant glucono-δ-lactone concentration of 1% (wt)(part of the surface for force at failure above 0.4N).

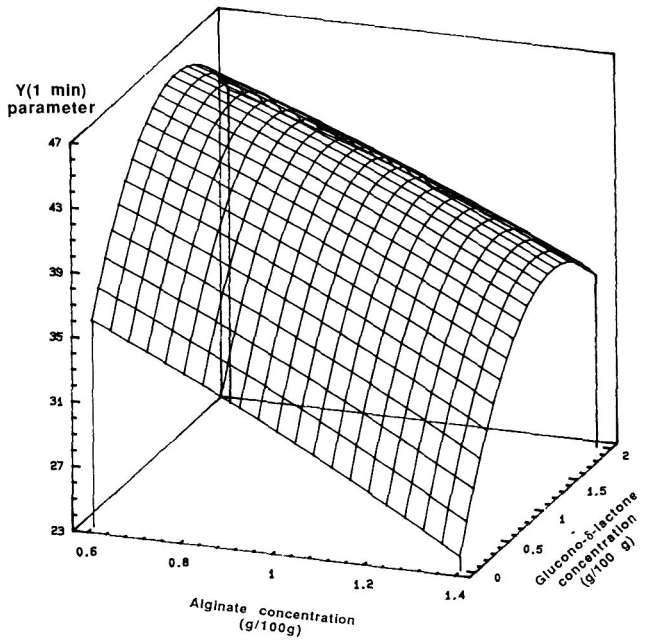

Fig. 5—Response surface for the effect of glucono-δ-lactone concentration and alginate concentration on the Y(1 min) parameter of texturized products at constant calcium salt concentration of 0.35% (wt) and constant heat treatment duration of 10 min.

alginate, calcium salt and glucono-δ-lactone concentrations, but in a quadratic way in relation to heat treatment duration. As the response surfaces for each of the two texture parameters presented nearly the same shape, the example of the force at failure evolution is given (Fig. 2 to 4). The increase in alginate, calcium salt and glucono-δ-lactone concentrations (Fig. 2 and 3) caused a simultaneous increase in firmness of texturized products. Concerning heat treatment duration (Fig. 4), the response surfaces gave an optimum for values between 10–15 min. Moreover, threshold-values for those response variables could be determined empirically. Below such values, the gels obtained were too weak, with a pasty texture and could not be sliced with a knife. These values were 2.5 Newton for the compressive stress at 3 mm and 0.4 Newton for force at failure. The texturization conditions allowing adequate gelled final products could be determined approximately through diagrams of response surfaces (Fig. 2, 3 and 4). Only the part of the surfaces above 0.4N is represented and it shows that the following conditions should preferably be applied to obtain a gel with suitable firmness: alginate concentration > 0.7%, glucono-δ-lactone concentration > 0.9%, heat treatment duration of 9 to 17 min and calcium salt concentration > 0.56%.

As regards portions of response surfaces above threshold values, gels could be also obtained but with quite different texture characteristics and dependent on formulation parameters and texturization conditions. Concerning the viscoelastic properties of the products, a study of the regression coefficients of the Y(1 min) parameter model showed that the 4 factors had a highly significant effect, whether it was linear and/or quadratic. These effects are shown in Fig. 5 to 7; Fig. 5 shows that the force decay parameter Y(1 min) of the products decreased with the alginate concentration, especially for low glucono-δ-lactone concentrations. This indicates an increase in solid properties of the products. From Fig. 6, an increased calcium salt concentration resulted in an increased force decay parameter, i.e., a loss of solid properties. Finally, Fig. 7 shows that minimal force decay parameter could be obtained with a heat treatment of about 12 min. For a better utilization of these results on the texturization conditions required to manufacture optimum products, it would be necessary to continue this study

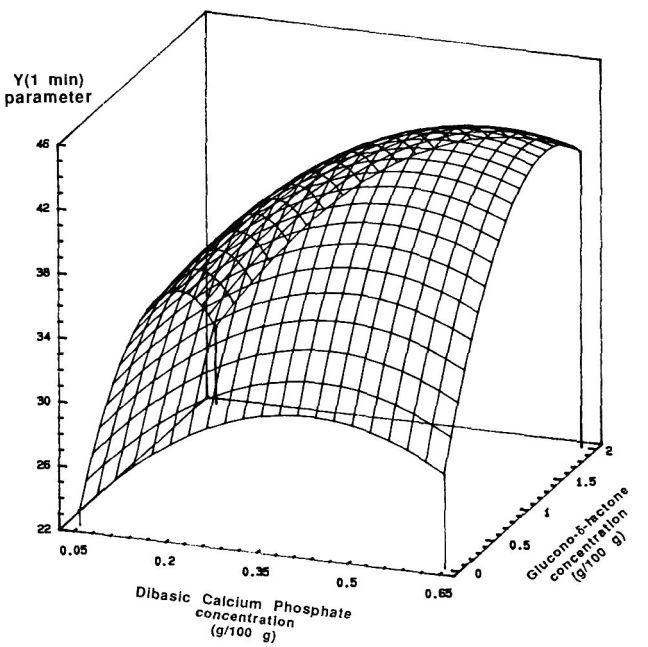

Fig. 6—Response surface for the effect of calcium salt concentration and glucono-δ-lactone concentration on the Y(1 min) parameter of texturized products at constant alginate concentration of 1% (wt) and constant heat treatment duration of 10 min.

with a correlation between texture measurements (especially regarding viscoelastic properties) and sensory evaluation.

Thermostability

Check samples texturized with pectin were tested under the same conditions as alginate-texturized mango cubes. After 30 min. at 130°C, all samples were examined. While pectin gels were completely melted down, alginate-texturized samples main-

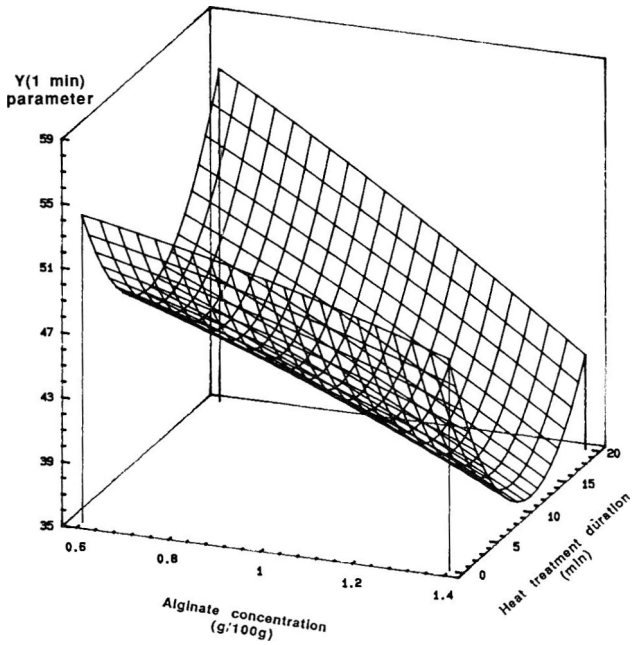

Fig. 7—Response surface for the effect of alginate concentration and heat treatment duration on the Y(1 min) parameter of texturized products at constant calcium salt concentration of 0.35% (wt) and constant glucono-δ-lactone concentration of 1% (wt).

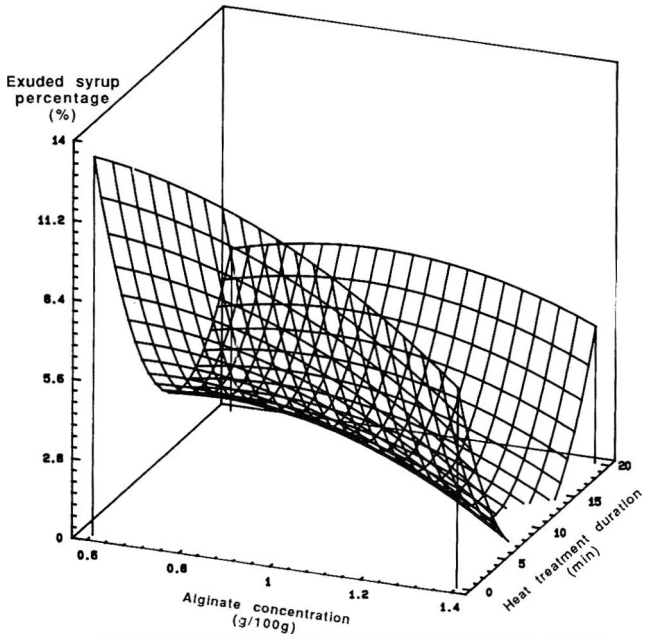

Fig. 8—Response surface for the effect of alginate concentration and heat treatment duration on the exuded syrup percentage of texturized products at constant calcium salt concentration of 0.35% (wt) and constant glucono-δ-lactone concentration of 1% (wt).

tained their cubic shape and still offered a good appearance, except for three. The sample corresponding to design point 31 did not undergo heat treatment at 60°C (0 min.). The sample (design point 29) prepared with the lowest alginate concentration (0.6% wt) and the sample (design point 13) prepared with both a low alginate concentration (0.8% wt) and a short heat treatment (5 min) were not completely melted but showed high deformation. These three texturized mango products had the highest exuded syrup percentages, (Ex%), respectively 15.32%, 5.65%, and 5.54% (Table 1). The values of regression coefficient (Table 2) confirm that the most important factor affecting the exuded syrup percentage was the heat treatment duration. Its first order effect and its second order effect were both highly significant. The second important factor with a highly significant first order effect was alginate concentration. The surface response representing the effects of these 2 important factors on the exuded syrup percentage is shown in Fig. 8. The effect of alginate appears relatively low as compared to the effect of heat treatment duration. It appears that the Ex% decreased when alginate concentration increased, particularly for short heat treatment durations. Both the alginate concentration and the heat treatment duration affected the gel network density and therefore the thermostability of the final texturized product. The lowest Ex% was obtained for medium heat treatment duration (8-9 min). Indeed, since the aqueous-ethanolic solution used for dispersing the alginate in the fruit pulp could not be totally eliminated by a heat treatment duration < 8 min, and since the alginate-pulp contact time was too short, the alginate chains could not expand to allow a conveniently dense and regular gel network to be formed. After more than 9 min at 60°C, the alginate chains under acid conditions seemed to initiate hydrolysis and formed a weaker network.

CONCLUSION

TEXTURIZED mango pieces with high soluble dry matter content (50°Brix) and maximum percentage content of fruit pulp could be produced. A glucono-δ-lactone concentration ranging from 1 to 1.5% and a heat treatment duration of about 8 min were optimal for the procedure to make mango analogs. Concerning the final texture of texturized mango pieces, all 4 factors studied had a major effect. Threshold values could be determined for those factors. Above such values, some products presenting quite different texture could be obtained but studies correspondence between mechanical properties and sensory evaluation are still needed.

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Relationship of Quality Characteristics of Peaches (cv. Loring) to Maturity

J.A. ROBERTSON, F.I. MEREDITH, W.R. FORBUS, JR., and B.G. LYON

ABSTRACT

The effect of maturity and ripening on the quality characteristics of Loring peaches was determined. Size, weight, Minolta *a* values, sucrose, total sugar, soluble solids (SS) and SS/TA ratio of cv. Loring peaches increased significantly with increased degree of maturity of fruit. Firmness, titratable acidity (TA) and delayed light emission (DLE) decreased significantly with increased maturity grade. The fruit of grades 1 and 2 did not attain sensory characteristics similar to tree ripe fruit (grades 6 and 7). The fruit of those grades were less sweet and more sour than grades 3–7. The optimum maturity for harvesting Loring peaches for optimum quality was maturity grade 5.

Key Words: peaches, sweetness, acidity, firmness, quality, flavor grade

INTRODUCTION

QUALITY of peaches is highly dependent on the degree of maturity. Peaches that are picked immature do not ripen to dessert quality, whereas those left too long on the tree become soft, do not ship well, and have reduced shelf-life (Fisher et al., 1943). To attain optimum quality, fruit must attain physiological maturity (i.e., minimum maturity for ripening) before they are harvested. Dull (1971) suggested that optimum quality was a compositional range within which 75% of consumers would consider the fruit as ripe and acceptable. A critical aspect is the determination of how many characteristics (i.e., physical, chemical and sensory) are necessary to adequately define quality.

Many of the physiochemical characteristics of maturing peaches have been studied in order to determine a suitable index to be used to harvest peaches of optimum quality. Rood (1957) in a study of five peach cultivars reported that firmness was the best single indicator of fruit maturity. Deshpande and Salunkhe (1964) suggested the ratio of soluble solids (SS) to acids could serve as a reliable index to peach maturity. Chapman and Horvat (1990) reported that the maximum malic/citric acid ratio could also be used as an index of maturity. However, these methods for estimating maturity are slow, subjective or the sample is destroyed during analysis. Delwiche and Baumgardner (1985) reported that ground color was a better at-harvest indicator of edible quality after ripening than flesh firmness. They developed a series of 6 standard color references in uniform color increments to match ground colors from immature to mature peaches. Color reference 3, which corresponds to "threshold maturity", was recommended as the minimum maturity for harvesting fruit of most cultivars (Delwiche, 1987). A tristimulus colorimeter is used to measure the ground color of peaches. The results are variable for chip three to six peaches because of increasing peach blush.

Forbus and Dull (1990) recommended using delayed light emission (DLE) as a nondestructive technique for measuring peach maturity. They evaluated 3 peach cultivars (cvs. Keystone, Loring and Nectar) using DLE, a low-intensity light emitted from a chlorophyll-containing plant material for several seconds after illumination. DLE highly correlated with maturity ($r = 0.96$) for all three peach cultivars. However, an attempt to predict physiological maturity by DLE was not made.

Several studies on changes in quality characteristics of peaches during maturity have been reported (Kader et al., 1982; Shewfelt et al., 1987; Meredith et al., 1989; Robertson et al., 1991). These provided only partial results on the relationship between physical, chemical and sensory characteristics and the optimum time for harvesting fruit. Our objective was to determine the physical, chemical and sensory characteristics of peaches (cv. Loring) of different maturities and to determine the optimum maturity for harvest.

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MATERIALS & METHODS

Samples

Peaches (cv. Loring) were obtained from the University of Georgia Horticulture Farm, Watkinsville, GA in July 1991. The fruit were hand picked on three dates, immediately transported to the laboratory and sorted into 7 maturity grades. The first harvest was made at 110 days after flowering (DAF) and the fruit were sorted into grades 1–4 using visual appearance and Clemson University color chips (Delwiche and Baumgardner, 1985). Grades 5 and 6 were harvested at 113 DAF and sorted by visual appearance. Fruit at 115 DAF and soft to the touch were harvested as tree-ripe and designated grade 7. Fruit ($\approx$100) of each grade were selected for physical, chemical and sensory analyses using DLE (Forbus and Dull, 1990). Peaches from grades 1 to 6 were artificially ripened at 20°C and 85–90% relative humidity for 1 to 5 days, depending on the grade, to a firmness of <10 newtons (N).

Physical measurement

Peaches (15) from each maturity grade, as harvested and after ripening, were measured for size, weight, skin color, DLE and firmness. The size of the fruit was determined by measuring the diameter midway between the stem and blossom end with vernier calipers. Ground color was measured on the greenest area of each fruit using a tristimulus colorimeter (Robertson et al., 1988). DLE was determined as described by Forbus and Dull (1990). Firmness was determined an opposite pared cheeks of 15 peaches using the Magness-Taylor pressure tester with an 8-mm tip. The mean of the 2 measurements was expressed in N.

Chemical analyses

Chemical analyses were conducted on the same 15 fruit of each maturity grade used for physical measurements. Individual peaches were sliced, pitted, and pureed in a Waring Blendor. SS and titratable acidity (TA) were determined on the puree (Robertson et al., 1988). Individual sugars were determined by HPLC as described by Meredith et al. (1988). Moisture contents were determined on $\approx$5 gm of peach mesocarp by drying in a vacuum oven at 60°C and $\approx$700 mm Hg vacuum for about 16 hr. Samples were removed from the oven and placed in a desiccator, allowed to equilibrate to room temperature and weighed. Moisture content was recorded based on weight loss.

Sensory evaluation

After ripening, peaches of all maturity grades were evaluated in duplicate for sensory characteristics by 15 panelists who had received general training for sensory analysis of fruits. Peaches were placed into two groups for sensory evaluation because panelists could not

Table 1—Effect of maturity and ripening on physical characteristics[a] of peaches (cv. Loring)

Maturity grade	Size (mm)		Weight (g)		Firmness (N)	
	As harvested[e]	Ripened[f]	As harvested	Ripened	As harvested	Ripened
1	56[a]	58[a]	103[a]	113[a]	73[a]	9[a]
2	61[b]	60[b]	126[b]	120[ab]	69[a]	9[a]
3	61[b]	60[b]	126[b]	121[b]	63[ab]	6[b]
4	63[b]	62[b]	142[b]	136[c]	61[b]	5[c]
5	69[c]	68[c]	170[c]	167[d]	28[c]	5[c]
6	69[c]	70[c]	171[c]	180[d]	13[d]	9[a]
7	70[c]	–	176[c]	--	7[e]	--

[a-d] Mean of 15 fruit; mean va ues within a column followed by different letter superscripts are significantly different ($P < 0.05$).
[e] Characteristics determined on fruit as harvested.
[f] Characteristics determined on fruit after ripening at 20°C and 85–90% RH for 1 to 5 days to a firmness of less than 10N.

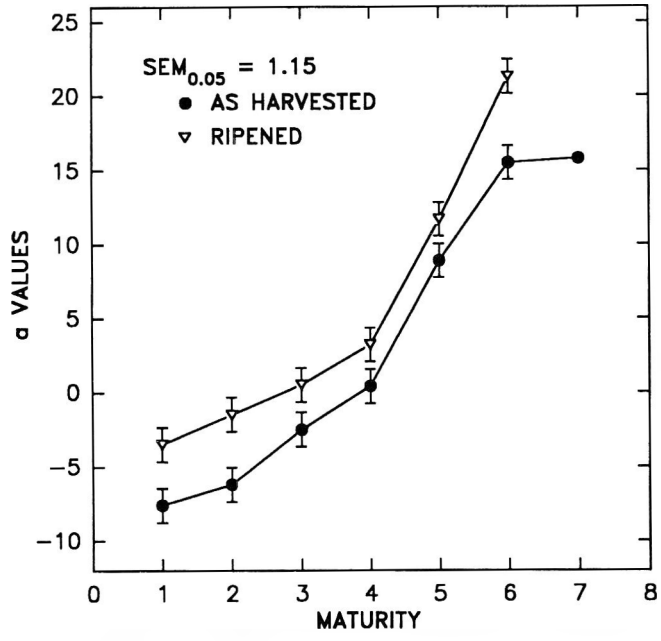

Fig. 1—Effect of maturity on ground color a values of Loring peaches as harvested and after ripening at 20°C.

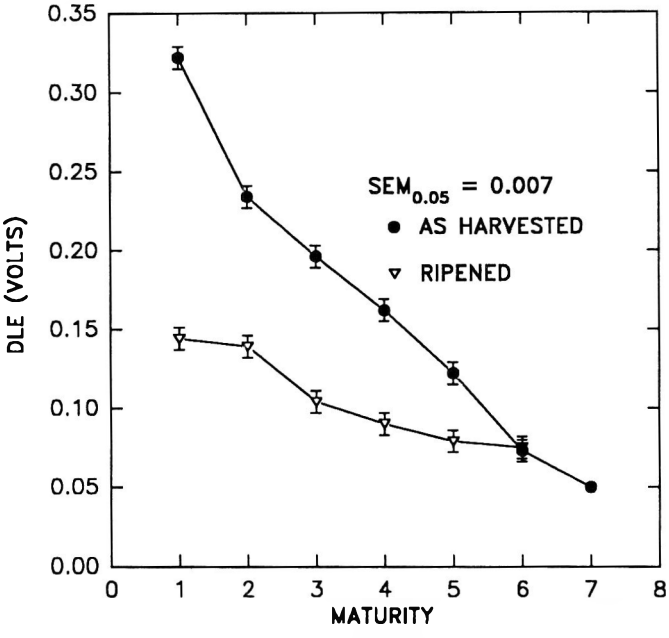

Fig. 2—Effect of maturity on delayed light emission (DLE) of Loring peaches as harvested and after ripening at 20°C.

evaluate all 7 classes at one time. Maturity grades 2, 4 and 6 were evaluated one day and grades 1, 3, 5 and 7 were evaluated the next day. Samples were washed, dried and peeled. Wedge-shaped slices were cut from the peaches. Wedges (2 or 3) were placed in cups coded with 3-digit random numbers. Cups were capped to allow headspace development. Fruits were evaluated for fruitiness, peach complex, sweet, sour, juiciness, firmness and overall acceptability. Responses for intensity of the attributes were recorded on the computer screen with a light pen as an input device. A 0 to 15 line scale was used with 0 being the lowest intensity and 15 the highest intensity of the attributes.

Samples were evaluated in individual sensory testing stations equipped with a computerized system (CSA version 4.1) for test presentation, data input, summation and storage as described by Findlay et al. (1986). Sample colors were masked with low pressure sodium vapor lamps.

Statistical analysis

Physical and chemical data were analyzed using the Analysis of Variance program of the Statistical Analysis System (SAS) for personal computers (SAS Institute, Inc., 1987). Main effects tested were maturity grade, as harvested and ripened. Means separation was by Tukey's test ($P < 0.05$). Sensory data were analyzed by the General Linear Models program of SAS. The main effects tested were maturity grade and panelist, as well as the maturity grade by panelist interaction. Maturity grade was also tested using the interaction term as the error. Mean sensory scores were separated by Tukey's.

RESULTS & DISCUSSION

THE EFFECTS of maturity and ripening on the physical characteristics of Loring peaches were compared (Table 1; Figs. 1 and 2). Size, weight and Minolta *a* values increased significantly ($P < 0.05$) with increased degree of maturity whereas, firmness and DLE decreased significantly with increased maturity grade. As expected, the most notable changes due to artificial ripening at 20°C were an increase in ground color *a* value (Fig. 1) and softening of fruit as indicated by firmness values (Table 1). Maturity grade 1 peaches required 5 days to soften from a firmness of 73N to < 10N. Grade 3, which is approximately the maturity that commercial growers attempt to harvest their fruit for shipping (Shewfelt et al., 1987) required 4 days to soften to < 10 N.

The rate of decrease of DLE values (Fig. 2), which highly correlated with chlorophyll content of peaches (Forbus and Dull, 1990), was greater for the fruit as harvested than when ripened. Statistical differences ($P < 0.05$) were found between freshly harvested and ripened peaches until maturity grade 6. DLE values for the as harvested and ripened maturity grade 6 peaches were not significantly different. The mean moisture content of the fruit (as harvested) of all maturities (87.6%) was not significantly different from that of the fruit when subsequently artificially ripened at 20°C (86.9%).

The effects of maturity and ripening on the chemical characteristics of Loring peaches are shown in Table 2 and Fig. 3 and 4. Sucrose and total sugar contents (Table 2), SS (Fig. 3) and SS/TA ratio increased significantly ($P < 0.05$) with increased maturity grade. TA (Fig. 4) decreased significantly ($P < 0.05$) with increased degree of maturity.

Differences in chemical characteristics of Loring peaches as harvested and after artificially ripening at 20°C were variable. Sucrose and total sugar contents tended to be slightly higher for the ripened fruit. Sorbitol was significantly lower and glucose and fructose were not significantly different. Significant differences between as harvested and ripened fruit were found for the SS content (Fig. 3) for maturities 1, 2, and 4, while no significant differences were found for maturities 3, 5 and 6. In general, TA levels (Fig. 4) were significantly higher in the as harvested fruit, and the SS/TA ratio was significantly higher for artificially ripened peaches. Maturity 7 (tree-ripe fruit) had a SS/TA ratio of 22.5. It has been suggested that the ratio SS/TA indicates ripeness of fresh fruit and that fruit

Table 2—Effect of maturity and ripening on the sugar contents of peaches[a] (cv. Loring)

Maturity grade	Sucrose (%)		Glucose (%)		Fructose (%)		Sorbitol (%)		Total Sugars (%)	
	As harvested[e]	Ripened[f]	As harvested	Ripened	As harvested	Ripened	As harvested	Ripened	As harvested	Ripened
1	3.50[a]	4.33[a]	0.88[a]	0.93[ab]	1.02[a]	1.11[a]	0.41[bc]	0.22[a]	5.81[a]	6.59[a]
2	3.85[a]	4.26[a]	0.99[ab]	0.99[ab]	1.09[a]	1.16[a]	0.46[ab]	0.23[a]	6.39[a]	6.64[a]
3	5.15[b]	4.70[b]	1.04[b]	0.96[ab]	1.17[a]	1.14[a]	0.54[a]	0.25[a]	7.90[b]	7.05[b]
4	5.18[b]	6.12[c]	1.06[b]	1.12[b]	1.18[a]	1.32[b]	0.50[ab]	0.35[b]	7.92[b]	8.92[d]
5	5.69[bc]	6.81[c]	0.96[ab]	1.02[b]	1.10[a]	1.13[a]	0.32[c]	0.39[b]	8.06[b]	9.35[e]
6	6.23[c]	5.91[c]	1.03[ab]	0.85[a]	1.17[a]	1.03[a]	0.32[c]	0.30[ab]	8.76[b]	8.09[c]
7	6.30[c]	--	1.01[ab]	--	1.15[a]	--	0.30[c]	--	8.76[b]	--

[a-d] Mean of 15 fruit; mean values within a column followed by different letter superscripts are significantly different ($P < 0.05$).
[e] Characteristics determined on fruit as harvested.
[f] Characteristics determined on fruit after ripening at 20°C and 85–90% RH for 1 to 5 days to a firmness of less than 10N.

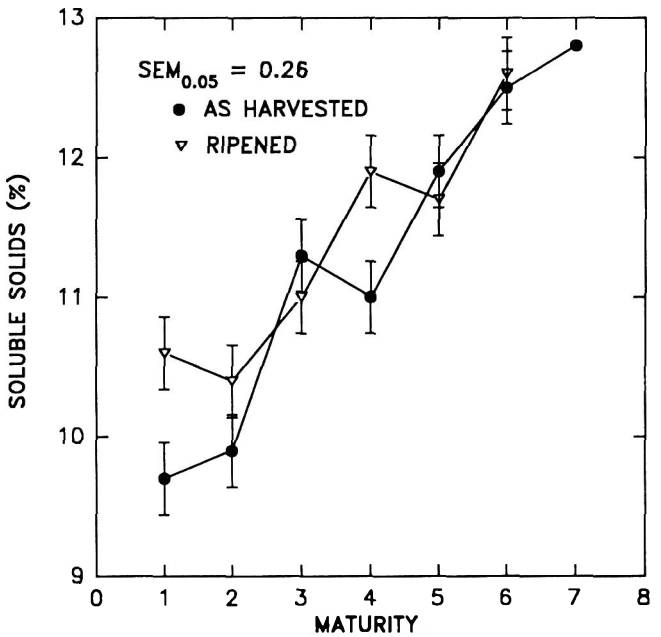

Fig. 3—Effect of maturity on soluble solids (SS) of Loring peaches as harvested and after ripening at 20°C.

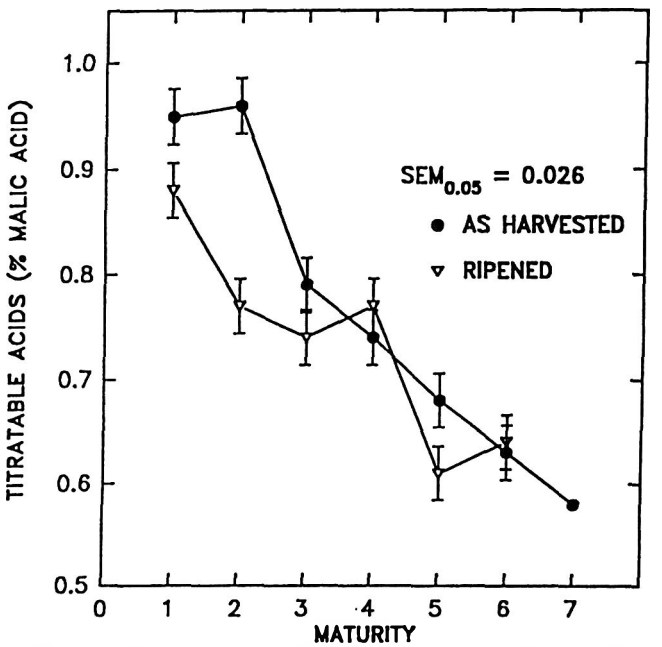

Fig. 4—Effect of maturity on titratable acidity of Loring peaches as harvested and after ripening at 20°C.

with a ratio greater than 15:1 will ripen to high-quality fruit (Despande and Salunkhe, 1964). Our data show this ratio was reached at about maturity 4, in agreement with data we reported for other cultivars (Meredith et al., 1989; Robertson et al., 1990, 1991). Results are similar to the characteristics of other cultivars grown at the University of Georgia Horticulture farm in previous years (Meredith et al., 1989; Robertson et al., 1990, 1991). The higher than normal rainfall and greater cloud cover in 1991 probably resulted in the Loring peaches being smaller and having lower total sugar contents than expected (Robertson et al., 1990). Grade 6 fruit was ripened for one day, and the physical and chemical characteristics of grades 6 and 7 peaches indicate that both as harvested could be considered tree-ripe, i.e. ready to eat quality.

Sensory scores for the seven maturity grades of Loring peaches are shown in Table 3. Maturity grades 1 and 2 were significantly less fruity than the more mature peaches. Maturity grades 5, 6 and 7 received similar scores for fruitiness. Intensity of the peach complex note also increased with maturity grade level up to maturity grade 5 and then tended to decrease. The sweet and sour notes showed inverse relationships. As sweetness increased with maturity, sourness decreased. Juiciness increased with maturity level to a maximum at maturity grade 5 and then decreased. Firmness values were relatively low for all maturity grades because all were artificially ripened at 20°C except maturity 7.

Data indicate that fruit of grades 1 and 2 did not attain sensory characteristics similar to tree ripe fruit (grades 6 and 7) during artificial ripening. The fruit of those grades were

Table 3—Sensory scores[a] for Loring peaches after ripening

Attributes	Maturity Grade at Harvest 1	2	3	4	5	6	7
Fruity[a]	6.05[c]	6.72[bc]	7.41[ab]	7.36[ab]	7.83[ab]	8.02[a]	7.87[a]
Peachy[a]	6.43[c]	7.09[bc]	8.00[ab]	7.53[abc]	8.73[a]	8.61[a]	7.88[ab]
Sweet[a]	4.12[d]	4.42[d]	6.21[bc]	5.29[cd]	7.71[a]	7.18[ab]	7.13[ab]
Sour[a]	7.33[a]	6.76[ab]	5.28[bc]	5.72[b]	3.95[cd]	4.00[cd]	3.39[d]
Juicy[a]	7.95[c]	8.72[bc]	9.34[ab]	9.19[ab]	9.99[a]	9.22[ab]	9.35[ab]
Firmness[a]	4.37[a]	3.86[abc]	2.96[c]	3.12[bc]	2.83[c]	4.17[ab]	3.38[abc]

[a-d] Values in rows followed by the same superscript letters are not significantly different ($P < 0.05$).
[a] Fruity, peachy, sweet, sour, juicy, and firmness were evaluated using line scales with values 0 (lowest intensity) to 15 (highest intensity). Values are means of responses of 15 panelists evaluating each sample in duplicate.

significantly ($P < 0.05$) less sweet and more sour than grades 5–7. Data also indicate that maturity grade 1 and 2 fruit were immature and had not reached physiological maturity (Watada et al., 1984). These results substantiate previous work (Fisher et al., 1943; Robertson et al., 1990, 1991) which showed that immature fruit would not ripen satisfactorily.

Correlation coefficients of selected physical, chemical and sensory characteristics of seven maturity grades of Loring peaches were developed (Table 4). Correlations between maturity and most selected quality parameters were significant at the 1% level except for peachy and sweet flavor attributes. Parameters not correlated at 5% level (not shown in table) were: Minolta *L* and *b* values, glucose, fructose and sorbitol

Table 4—Correlation coefficients of selected physical, chemical and sensory characteristics of 'Loring' peaches at seven stages of maturity[a]

	Weight	Firmness	DLE	"a" value	SS	TA	SS/TA	Sucrose	Total sugar	Fruity[b]	Peachy[b]	Sweet[b]	Sour[b]
Maturity	0.97	−0.95	−0.99	0.98	0.97	−0.97	0.99	0.97	0.93	0.92	0.77	0.87	−0.96
Weight		−0.95	−0.97	0.96	0.93	−0.92	0.94	0.93	0.88	0.92	0.83	0.90	−0.95
Firmness			0.92	−0.99	−0.94	0.90	−0.96	−0.89	−0.82	−0.83	−0.73	−0.87	0.93
DLE				−0.95	−0.96	0.95	−0.96	−0.97	−0.95	−0.96	−0.82	−0.87	0.95
"a" value					0.96	−0.95	0.98	0.94	0.88	0.88	0.77	0.88	−0.95
Soluble solids						−0.98	0.99	0.99	0.96	0.94	0.83	0.93	−0.98
Titratable acidity							−0.98	−0.99	−0.96	−0.93	−0.79	−0.89	0.96
SS/TA								0.97	0.93	0.89	0.75	0.88	−0.96
Sucrose									0.99	0.97	0.86	0.92	−0.97
Total sugar										0.97	0.86	0.88	−0.94
Fruity[b]											0.93	0.93	−0.95
Peachy[b]												0.94	−0.87
Sweet[b]													−0.97
Sour[b]													

[a] $P < 0.01$ for $r > 0.92$; $P < 0.05$ for $r > 0.84$.

[b] Peaches for sensory evaluation ripened at 20°C and 85–90% RH.

contents, and the juicy attribute. DLE and SS/TA had the highest correlations followed by Minolta *a* value. Weight, SS and sucrose contents significantly correlated at the 1% level.

The sour flavor attribute correlated significantly at the 1% level with all the selected quality attributes except peachy. Although an untrained panel was used, Robertson et al. (1991) reported that the sweet flavor attribute and overall peach flavor of Majestic peaches significantly correlated ($P < 0.01$) with maturity grade.

Results of this study illustrate the difficulty of determining at what stage of maturity peaches should be harvested so they would ripen into high-quality fruit and yet be firm enough to ship by existing marketing practices. Based on the sugar and sensory data the optimum maturity for harvesting Loring peaches for optimum quality is maturity grade 5. At that grade total sugars (Table 2) and the sweet sensory attribute (Table 3) have attained maximum values. Marketing practices may preclude harvesting at maturity grade 5 but our results show that the quality of peaches available to the consumer would be improved if marketing methods were changed so fruit could be harvested at a later stage of physiological maturity. These results and other studies in our laboratory (Meredith et al., 1989; Robertson et al., 1990, 1991) also show that under no circumstances should peaches be harvested before maturity grade 3 (equivalent to Clemson University color chip 3). Data substantiates that DLE has potential for being the best of the quality parameters for determining the maturity of peaches at all stages of marketing.

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Water Vapor Adsorption Isotherms of Guava, Mango and Pineapple

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ABSTRACT

Isotherms were determined at 25° and 50°C, using an electronic water activity meter. For the 3 fruits temperature had practically no effect on the isotherms. Sorption capacity of freeze-dried pineapple was higher than that for vacuum dried. The higher sorption capacity of mango and pineapple in comparison to guava corresponded to the respective sugar contents of the fruits.

Key Words: guava, mango, pineapple, water activity, sorption isotherm

INTRODUCTION

WATER SORPTION isotherms are important in considering drying, packaging and storing processes for foods (Bruin and Luyben, 1980). Most food products are complex in nature and theoretical prediction of the isotherms is not possible so experimental measurements are necessary. Reliable sorption data are also needed for prediction of food quality of stored foods.

Guava, mango and pineapple are important food crops in tropical and subtropical regions. Very little published information is available about the sorption isotherms of these products. Siddapa and Nanjundaswany (1960) determined sorption isotherms of mango and guava powders at 25° and 37°C by two different techniques. The experimental values reported by those authors varied considerably from one method to another and the experimental procedure did not allow one to conclude if data corresponded to the adsorption or desorption isotherms. Wolf et al. (1973) determined the adsorption isotherms of freeze-dried pineapple in the range 5–60°C, but did not investigate the effect of drying method on the sorption isotherm.

There are several experimental means to determine sorption isotherms. They can be grouped into hygrometric and gravimetric methods (Gal, 1981). The hygrometric method, based on the equilibration of the air/water activity to constant moisture content, has been applied with apparent good results (Pixton and Warburton, 1973; Kitic et al., 1986; Gruebel and Drewes, 1987; Falabella et al., 1989). This method has proven particularly convenient for determination of the isotherms at relatively high temperatures and water activities where foods may mold before equilibrium is attained (Falabella et al., 1989).

Our objective was to determine the sorption isotherms of guava, mango and pineapple for a range of water activities, using a hygrometric method. Also determined was the effect of temperature on the equilibrium data at 25° and 50°C.

MATERIALS & METHODS

Sample preparation

Mango (Tommy Atkins Cultivar), guava (white cultivar) and pineapple (smooth Cayenne Cultivar) were employed. The fruits were peeled and the pulp cut in slices 10 × 10 × 10 mm. These were blanched for 1 min in saturated steam, cooled in cold water and dipped in 0.5% sodium bisulfite for 1 min to avoid mold growth in samples exposed to high humidities. After that, the fruits were dried in a vacuum oven at 60°C in presence of P_2O_5 to constant weight. Samples of pineapple were also freeze dried in a Stokes freeze drier, Model EA4167, condenser temperature −40°C, pressure 50 μm and shelf temperature 20°C.

In order to determine equilibrium values corresponding to the adsorption branch of the isotherm, vacuum-dried samples of mango, guava and pineapple (freeze-dried pineapple, also) were partially rehydrated to different moisture levels. The rehydration times varied with moisture levels of the samples, but in all cases were no more than 2 wk. Samples were rehydrated by placing them into Petri dishes inside desiccators containing saturated salt solutions. The salts used were: NaOH, LiCl, NaCl, $MgCl_2$, KCl, $BaCl_2$, and $CuSO_4$. The desiccators were maintained at 4°C for different time intervals until the samples reached the desired moisture contents (no mold growth was observed). The rehydrated samples were sealed in plastic bags and maintained for 2 days in a refrigerator at 4°C before water activity measurements.

The weight of dry matter was determined by drying at 50°C in an air circulation oven for 24 hr followed by vacuum oven drying at 70°C in presence of P_2O_5 for 24 hr (Roman et al., 1982). Total sugar contents of mango, guava and pineapple were determined using AOAC (1984) procedure.

Water activity measurement

A Novasina Thermoconstanter Humidat TH2 meter manufactured by Novasina AG (CH-8500 Zurich, Switzerland) was used for water activity measurements of the treated samples. It consisted of a measurement chamber with a temperature regulator and a humidity sensor. The sensor consisted of a conductivity cell with a hygroscopic electrolyte. The measuring range of the apparatus was 0.1–1.0 water activity for a temperature range from 0 to 50°C. The sensor was calibrated using 3 saturated salt solutions as standards: $BaCl_2$, Mg $(NO_3)_2$ and LiCl. The calibration values at 25° and 50°C were taken from Greenspan (1977). The equilibrium time, which depends on water activity level, was no more than 90 min in all cases.

RESULTS & DISCUSSION

AS WAS POINTED OUT by Kitic et al., (1986) the time required to attain equilibrium within the sensor chamber is a function of various parameters. These include the physical nature of the sample, the water activity level, and the specific surface area and transport conditions in the free space above the sample. The rate of adsorption to equilibrium was rapid and was practically reached after 45 min depending on temperature and moisture content of the sample. To be on the safe side a reading time of 90 min was adopted (no drift of the experimental data was observed for the 45 min extra). Three replications were made for each moisture content.

The adsorption isotherms of guava (Fig. 1) mango (Fig. 2) and pineapple (Fig. 3) are shown for the two temperatures investigated (the complete lines were extrapolated to fit the data). The shape of the isotherms was very similar. At low water activities the products adsorbed relatively small amounts of water, while as water activity was increased the sorption of water increased considerably. A similar behavior was observed by other investigators for different fruits (Iglesias and Chirife, 1978); De Gois and Cal-Vital, 1985; Maroulis et al., 1988) and can be explained by the following. The solute solids, mainly

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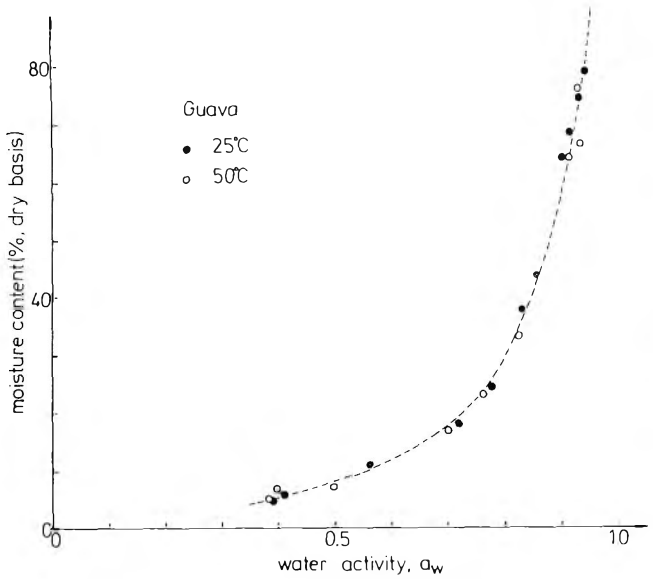

Fig. 1—Adsorption isotherms of guava (vacuum-dried).

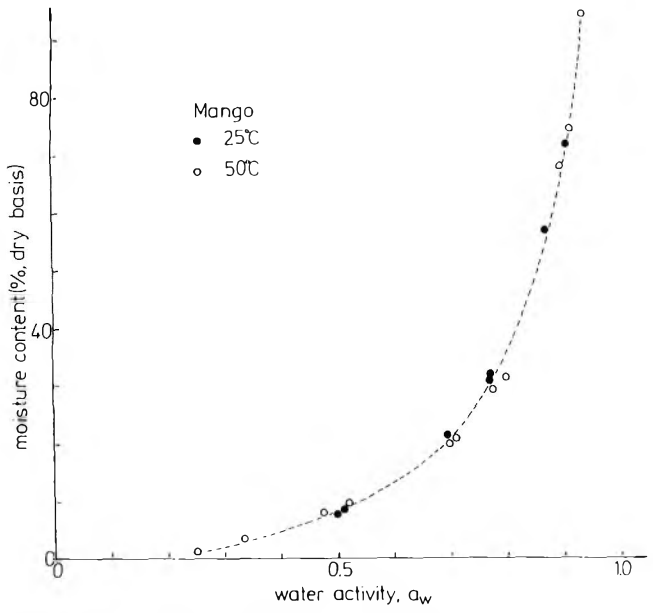

Fig. 2—Adsorption isotherms of mango (vacuum-dried).

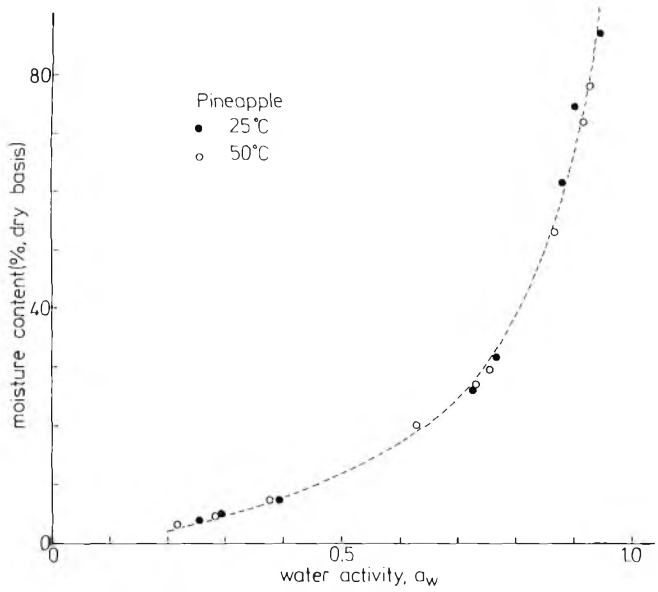

Fig. 3—Adsorptin isotherms of pineapple (vacuum-dried).

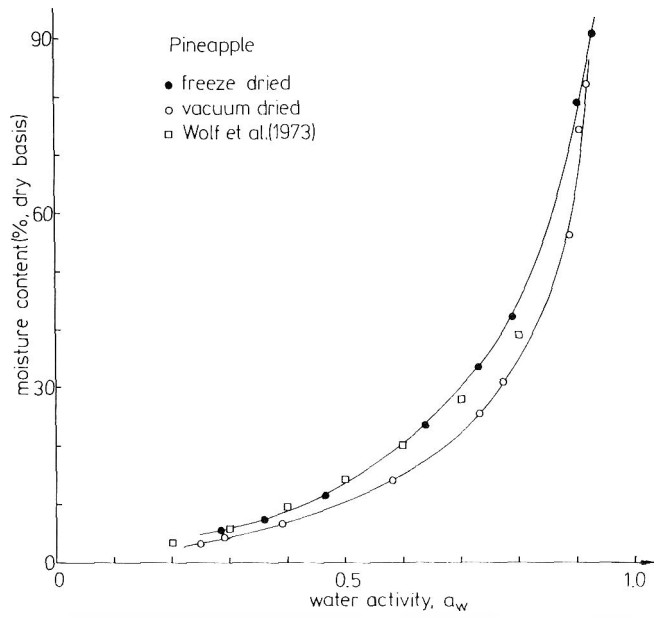

Fig. 4—Adsorption isotherms of vacuum-dried and freeze-dried pineapple at 25°C (comparison with the equilibrium data from literature).

sugars, adsorb little water at low water activities and sorption is mainly due to the biopolymers which usually accompany the sugars. The increase of sorption as water activity increases is mainly due to the dissolution of sugars.

It can also be seen that the temperature dependence of the isotherms was low and thus scarcely discernible. A neglegible temperature dependence of the isotherms has been reported by Bolin (1980) for prunes. De Gois and Cal-Vital (1985) have also found that the isotherms of papaya at 13° and 23°C were practically coincident. Similar results can be observed when the isotherms of pineapple (freeze dried) reported by Wolf et al., (1973) at 5° and 25°C are compared. However, for the other two isotherms reported by those authors for the same product at 45° and 60°C, they found the classical negative temperature dependency (an increase of temperature reduced sorption capacity). Unfortunately, the complex nature of the phenomenon under consideration makes it difficult to explain the differences between the results reported by Wolf et al., (1973) and those obtained here. On the other hand, negative temperature dependence followed by a positive temperature dependence as water activity increases (crossing of the isotherms) has also been reported for different foodstuffs (Berlin et al., 1969; Saravacos et al., 1980; Maroulis et al., 1988; Mujica et al., 1989). A possible explanation of this is as follows. As the energy involved in sorption is the algebraic sum of the endothermic heat of dissolution of solute and the exothermic heat of sorption of nonsolute solids, the net effect of temperature on the isotherms would depend on the magnitude of both. The low or negligible temperature dependence of the isotherms found here probably indicates that the heats of dissolution and adsorption were of similar magnitudes.

Guava, with a 34% sugar content (dry basis), adsorbed the least amount of moisture, which corresponded to the lower amount of sugar in the product (Young, 1975; Maroulis et al., 1988). For mango and pineapple the corresponding sugar contents were 71% and 63% (dry basis), respectively. For these two fruits the difference in sugar content seems not to be important. The corresponding isotherms were practically coincident at relatively high water activities, where the dissolution

effect of solutes was the principal factor responsible for the shape of the curve.

The adsorption isotherms at 25°C of vacuum dried and freeze-dried pineapple were compared (Fig. 4). Also included are the equilibrium data of pineapple (freeze dried) at 25°C reported by Wolf et al., (1973). It can be seen that freeze-dried pineapple adsorbed more water vapor than vacuum dried. The higher adsorption capacity of the freeze dried sample might be attributed to the more highly porous structure of that product in-comparison with vacuum dried. Mazza (1982) has reported that the isotherms of freeze-dried potatoes were higher than those for the vacuum dried product. The fact that at a constant moisture content the water activity of vacuum-dried pineapple was higher than that of the freeze-dried product would indicate that freeze drying promotes greater chemical and microbial stability than vacuum-drying, (i.e, the influence of water activity on deteriorative reactions).

CONCLUSIONS

A HYGROMETER METHOD was useful for determining adsorption isotherms of mango, guava and pineapple at 25° and 50°C. It was possible to combine rapid water activity measurement and sample rehydration at low temperature, two factors required if a deterioration of the sample as a consequence of mold growth is to be avoided. In such cases; this procedure may be more appropriate than the classical static method equilibration, particularly for the determination of hygroscopic equilibrium at high water activities and temperatures. Drying procedures affected the sorption characteristics of pineapple. For constant moisture content the water activity of freeze-dried pineapple was lower than that of vacuum-dried pineapple, making the freeze-dried product less susceptible to microbiological spoilage.

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Absorption of Aroma Volatiles of Orange Juice into Laminated Carton Packages Did Not Affect Sensory Quality

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ABSTRACT

Orange juice was stored in glass bottles and polyethylene/barrier material laminated cartons at 4°C for 24 wk. Studies were carried out on the absorption of 19 orange juice aroma compounds (e.g ethyl butyrate, d-limonene) into low density polyethylene (LDPE). Sensory evaluations were performed on the same orange juice in different packages over the storage period. A reduction of d-limonene of up to 50% by absorption into the LDPE inside coating was observed. However, an experienced sensory panel did not distinguish between orange juice stored in glass bottles and polyethylene laminated cartons.

Key Words: orange juice, aroma, absorption, packaging

INTRODUCTION

CITRUS JUICES are commonly packaged in cardboard packages and plastic containers with low density polyethylene (LDPE) as product contact layer. Interaction between these materials and food has been an increasing area of research. This has been prompted by the strong absorption of nonpolar aroma compounds of citrus juices into the inside layers of packaging materials or permeation through them. Many researchers have reported high losses of d-limonene and other aroma compounds from model solutions, vapors and citrus juices in contact with LDPE (Halek and Meyers, 1989; Kwapong and Hotchkiss, 1987; Shimoda et al., 1988; Marsili et al., 1989; Sadler and Braddock 1991). The consequence of this absorption on sensory quality of the packed products has been the subject of controversy although the expression "flavor scalping" for this absorption seems to be generally used. Kwapong and Hotchkiss (1987), and Hotchkiss (1987) showed that sensory changes due to absorption could be significantly detected in model systems. Mannheim et al. (1987) stated that d-limonene absorption shorted the shelf life of orange juice. Moshonas and Shaw (1989) claimed that absorption contributed to flavor changes, which were sensorially detectable in orange juice.

Other researchers showed that the absorption of up to 40% d-limonene had no effect on the sensory quality of orange juices during 90 days storage at 20°C (Dürr et al., 1981). They suggested that d-limonene made a low contribution to the aroma of orange juice.

Most such reported studies were either performed with model systems, not representing actual orange juice, or with orange juice which had not been commercially produced and packaged. In the majority of those investigations absorption measurements were performed without sensory evaluation.

The objective of our investigation was to evaluate absorption of orange juice aroma compounds in different package structures and compare with the sensory quality of the same orange juice. A further objective was to investigate whether different levels of absorption had a significant influence on the sensory quality of orange juice stored under conditions of commercial refrigeration (4°C).

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MATERIALS & METHODS

Orange juice samples and heat-treatment

At a Florida commercial citrus juice manufacturing plant, Valencia single-strength orange juice with the following characteristics was obtained: corrected Brix value 12.0°; Brix/Acid ratio, 15.8; suspended pulp, 4% (V/V). The orange juice was pasteurized at 96°C for 12 s, placed in steel drums (55 gallon) for freezing until used. After approximately 4 mo in storage at −18°C, the orange juice was thawed at room temperature ≈ 25°C during 48 hr and then rapidly heated to 73°C and held for 16 sec. The juice was rapidly cooled to 15°C and transported aseptically to filling machines. The heat-treatment and packaging was performed at Tetra Pak EquipUS test packing plant facilities in Minneapolis-St Paul, MN.

Packaging materials and packaging

The orange juice was filled into the following laminated carton types: (1) ≈ 1.9L gable-top packages with 4 g/m^2 adhesive polymer and 9 g/m^2 LDPE in product contact (5% o_2 in headspace); (2) ≈ 1.9L gable-top packages with 0.5 g/m^2 adhesive polymer and 26 g/m^2 LDPE in product contact (5% o_2 in headspace); and (3) 250 mL Tetra Brik Aseptic packages with a layer of 8 g/m^2 adhesive polymer and 34 g/m^2 LDPE in product contact (no headspace). For these packages area/volume ratios were calculated: 0.49 dm^2/100 mL juice for packages no. 1 and no. 2; 1.23 dm^2/100mL for package no. 3. The structures of the packaging materials are shown in Table 1. All structural materials in the packages contained a polymer barrier material or aluminium foil, both of which were nominally effective as oxygen barriers. The laminated carton packages with different thicknesses of LDPE as product contact layers were supplied from Tetra Pak, Lund, Sweden. Sterile glass bottles were used as the control packages. They were filled manually under sterile conditions, avoiding foaming and minimizing the amount of headspace. Packages number 1, 2 and 3 were filled aseptically. The cardboard packages and the glass bottles were placed in storage at 4°C immediately after filling.

Handling and storage

Samples were transported in refrigerated containers to the Univ. of Florida, Citrus Research & Education Center, Lake Alfred, FL for controlled temperature storage and sensory evaluation: and Tetra Pak GmbH, Stuttgart, Germany for controlled temperature storage and chemical analyses. To minimize product changes, the temperature of the orange juice was maintained just below 0°C during transportation. Upon arrival the temperature of the orange juice was −2°C. Samples were stored for 24 weeks at 4°C during the test period.

Table 1—Packaging material structures

Package no.	Packaging material structure[a]								
1	LDPE[b]	Board	LDPE	AP	EVOH	AP	LDPE		
						4	9		
2	LDPE	Board	LDPE	Aluminium		AP	PET	AP	LDPE
								0.5	26
3	LDPE	Board	LDPE	Aluminium		AP	LDPE		
						8	34		

[a] From outside to inside. The coating weight in g/m^2 is only included for the layers of importance in respect to aroma absorption.

[b] Abbrev.: LDPE, low density polyethylene; AP, Adhesive Polymer; EVOH, Ethylvinylalcohol; PET, Polyethylene terephthalate.

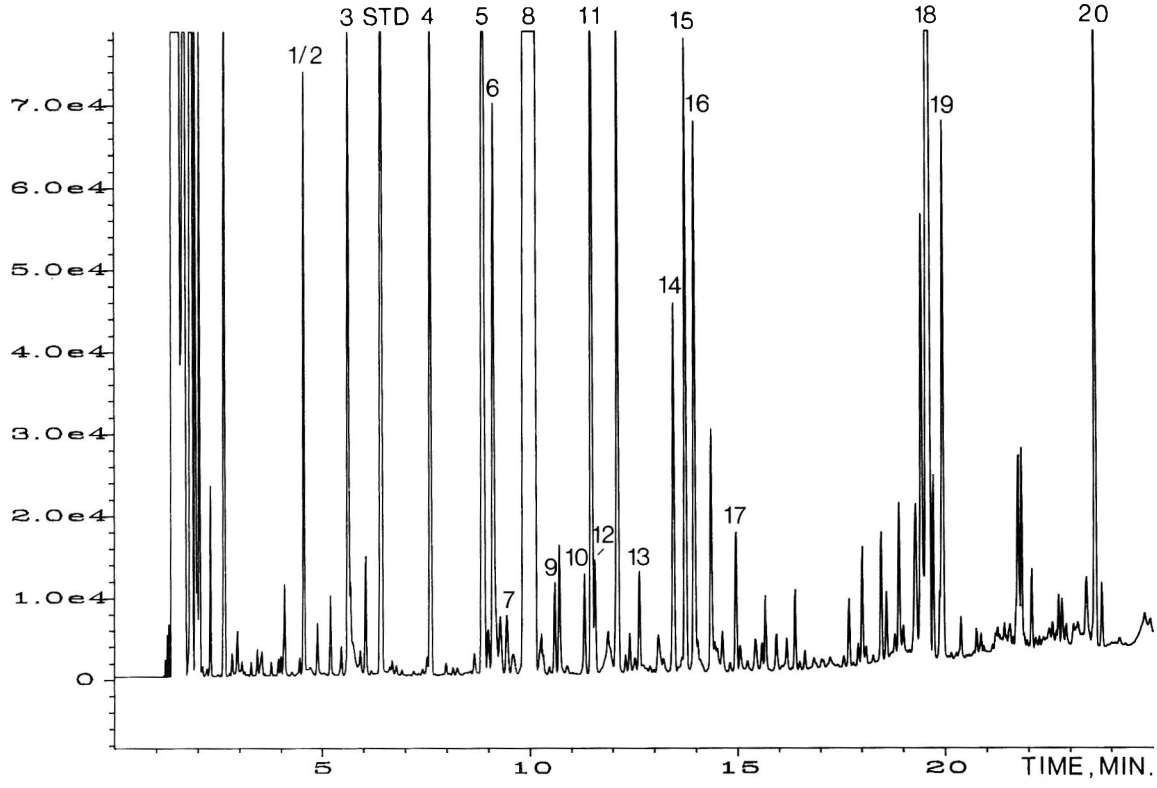

Fig. 1–GC-profile of volatile orange juice aroma compounds on a DB-5 column. Peak numbers correspond to Table 2. GC conditions described under analytical techniques.

Table 2–Volatile compounds identified in orange juice and used for flavor absorption studies

Peak # (Fig 1)	Compound name	Peak #	Compound name
	Hydrocarbons		**Aldehydes**
4	alpha-Pinene	2[b]	Hexanal
5	Myrcene	3[d]	trans-2-Hexenal
7	delta-3-Carene	6	Octanal
8[a]	Limonene	12	Nonanal
10	Terpinolene	16	Decanal
18	Valencene		
19	Cardinene		
	Ketones		**Esters**
17	Carvone	1[b]	Ethyl butyrate
20	Nootkatone		
	Alcohols		
9	Octanol		
11	Linalool		
13	beta-Terpineol		
14	Terpinene-4-ol		
15[c]	alpha-Terpineol		

[a] Not quantified by GC; not included in hydrocarbon retention.
[b] Separated on a DB-Wax column, all others on a DB-5 column.
[c] Not included in alcohol retention, due to increase during storage.
[d] Not included in aldehyde retention.

Chemical analysis

Standard flavor compounds were purchased from commercial sources. Round-shaped migration cells (2 × 0.5 dm^2) for the packaging material extracts were obtained from the Natec Institute, Hamburg, Germany. A modified Likens-Nickerson Distillation equipment was obtained from Analytichem International, Frankfurt, Germany.

Sample preparations

A simultaneous steam-distillation-extraction (Likens and Nickerson 1964) was used for the separation of volatile orange juice aroma compounds for gas chromatographic (GC) analysis. Juice (100 mL) with 1 mL of internal standard added (200 ppm 4-heptanol in methanol) was distilled and extracted with 25 mL of n-pentane. This solution of volatile compounds in n-pentane was concentrated from 25 mL to 1 mL with a Kuderna-Danish concentrator (International Organization of the Flavour Industry 1982).

Packaging material extracts

Aroma compounds, which had been absorbed into the inside layers of the packaging materials were extracted with 25 mL of n-pentane for 3 days at 6–8°C. Migration cells (Natec Institute) with a contact area of 2 × 0.5 dm^2 were used for the extraction. An internal standard (200 ppm 4-heptanol) in methanol was added before concentration of the solution to 1 mL with Kuderna-Danish equipment. The concentrated solution (1μL) was injected for GC analysis.

Analytical techniques

D-limonene was measured by a bromate titration (Scott and Veldhuis 1966). The extracted aroma compounds of orange juice were separated by GC (Hewlett-Packard 5890 series II, on-column injector and FID-detector) on two different fused silica, capillary columns: (1) DB-5 column (J&W Scientific, Folsom, CA); 30m × 0.32 mm I.D. × 1 μm film thickness, and (2) DB-Wax column (J&W Scientific); 30m × 0.32 mm I.D. × 1 μm film thickness with helium as carrier gas. The temperature program was set from 55–140°C at 6°C/min (45–140°C for DB-Wax) and from 140–220°C at 10°C/min with holding times of 1 min at 55°C (5 min at 45°C for DB-Wax) and 5 min at 220°C (10 min for DB-Wax). The pressure program was set from 15–25 psi at 0.5 psi/min with holding times of 1 min at 15 psi and 10 min (18 min for DB-Wax) at 25 psi. The injector was set at 58°C with the oven track modus on. The detector temperature was 250°C.

Compound identification was performed on a coupled system of GC infrared detector with mass-selective detector (Hewlett-Packard, Boeblingen, Germany) under similar chromatographic conditions as described above and via comparison with standard aroma compounds. Figure 1 shows a chromatogram of volatile orange juice aroma compounds using a DB-5 column. Table 2 shows the aroma compounds used for evaluation. Ratios of experimental peak areas to internal standard (4-heptanol for the DB-5 column; 4-heptanone for the DB-Wax column) were evaluated for every compound to compare aroma retention. Results for compounds of the same chemical classes were summarized (Table 2).

The peak area ratios for the aroma compounds extracted from 1 dm^2 each of the different packaging materials were multiplied with an

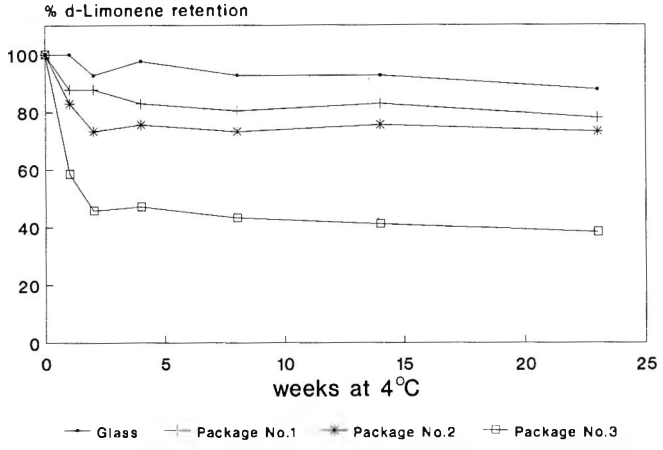

Fig. 2–D-Limonene retention in orange juice during storage at 4°C in different packages. The value found in glass bottles after 1 wk of storage (0.020% (v/v) d-limonene) was taken as initial concentration of d-limonene assuming that no chemical degradation had taken place within 1 wk at 4°C.

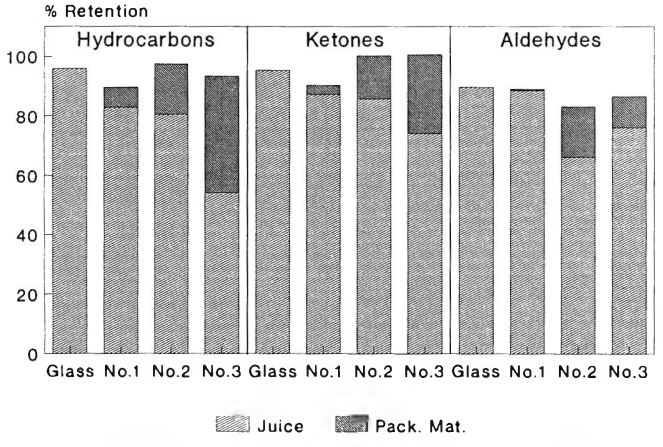

Fig. 3–Retention of hydrocarbons (d-limonene excluded), ketones, and aldehydes in orange juice and recovered hydrocarbons (d-limonene excluded), ketones, and aldehydes from packaging material extracts after 8 wk storage in different packages at 4°C.

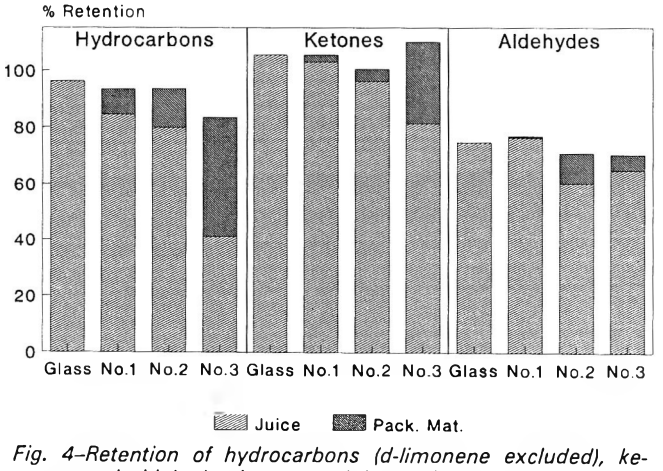

Fig. 4–Retention of hydrocarbons (d-limonene excluded), ketones, and aldehydes in orange juice and recovered hydrocarbons (d-limonene excluded), ketones, and aldehydes from packaging material extracts after 23 wk storage in different packages at 4°C.

appropriate factor to adapt the ratios to the areas, (which had actually been in contact with 100 mL of juice). After this normalization, ratios for the juices and the packaging material extracts could be added and compared to ratios for the glass bottles. This comparison gave recovery rates in the range of 87—105%, which confirmed the accuracy of the method. The percent retention of the aroma compounds was calculated by taking the peak area/peak area internal standard ratios of the compounds in the glass bottle after 1 wk at 4°C as initial values of 100%.

Sensory evaluation

The sensory evaluation was based on a hedonic scale method (Peryam and Pilgrim, 1957). The evaluation was performed by 12–16 experienced panelists in Florida. A 9-point hedonic scale was used: 1 = dislike extremely; 5 = neither like nor dislike; 9 = like extremely. Randomly coded samples were served at ≈ 13°C under red light conditions. The orange juices were evaluated after 1, 2, 3, 4, 6, 8, 10, 12, 14, 16, 19, 22, and 24 wk of storage. Data were analyzed statistically using computer-aided statistical software (Tektronix, Inc., 1976).

RESULTS & DISCUSSION

Aroma absorption studies

D-limonene retention in the stored juices showed increased absorption of d-limonene from material 1 to material 3 (Fig. 2). This was due to an increase in the amount of absorbing layers that were in contact with the juice. (Both LDPE and the adhesive layer were assumed to be absorbing layers). Because of the most unfavorable area/volume ratio, material 3 had by far the lowest retention of d-limonene. Steady-state absorption was reached after 2 wks storage. This was due to the barriers in the structures, which prevented further permeation. After reaching the steady state, only chemical degradation took place. This degradation was also clearly seen in the glass reference samples.

A similar absorption behavior during storage could be seen for other aroma compounds of the hydrocarbon type (Fig. 3,4) due to similar polarity and solubility of these compounds in LDPE. The thickness of the absorbing layers and the area in contact with the juice were again responsible for the difference between the materials. Between the 8th and the 23rd wk of storage, no significant change in absorption took place. Ketone absorption was less than that of hydrocarbons, but the pattern was similar, due to their relative nonpolarity.

The aldehyde absorption (Fig. 3 and 4) was the highest for material no. 2, which could not be correlated with thickness of the LDPE layer or with the area/volume ratio, because material no. 2 had an intermediate thickness of LDPE. In this case, the adhesive layer might have been an important factor by exhibiting significantly different absorption properties than LDPE or the other adhesive layers. A notable decrease of the total amount of aldehydes was found throughout storage in each of the packaging materials. Alcohols were only slightly absorbed. Octanol and linalool were the only alcohols which could be identified in the packaging-material extracts of materials no. 2 and 3 in amounts not higher than 3.5% of the total alcohol content. Orange juice retention of ethyl butyrate was high in all cases and was not significantly ($P > 0.05$) different for any of the packaging materials. The ester could not be identified in any of the packaging material extracts.

Sensory evaluation

The flavor scores for the orange juice in the different packages were compared (Table 3). There was no significant difference ($P > 0.05$) between any of the samples, at any of the testing occasions.

CONCLUSION

ABSORPTION of orange juice aroma compounds into laminated cardboard packages was strictly dependent on type of aroma compound and its solubility in the absorbing layer. Ab-

Table 3—Mean hedonic flavor scores[a] for orange juices during 24 wk storage at 4°C in different packages[b]

Wk of storage	Glass bottle	Package no. 1	Package no. 2	Package no. 3
1	not incl	6.4	6.4	6.9
2	6.8	6.8	7.0	6.8
3	6.5	6.9	6.6	7.0
4	6.7	6.8	7.0	6.9
6	6.6	7.1	6.8	6.5
8	6.2	6.1	6.3	6.4
10	6.7	6.3	6.1	6.6
12	6.4	6.5	6.5	6.3
14	6.3	6.1	6.5	6.6
16	6.2	6.1	6.4	6.2
19	6.5	6.2	6.5	6.4
22	5.8	6.1	6.2	6.0
24	5.6	5.7	5.8	5.7

[a] Where 9 = like extremely; 5 = neither like nor dislike; 1 = dislike extremely.
[b] At any given week, scores of different packaged samples were not significantly different (P > 0.05).

sorption generally decreased from hydrocarbons, ketones, and aldehydes to alcohols and esters. In contrast, ethyl butyrate, reported to be one of the most important compounds of orange juice aroma, was not absorbed into LDPE in measurable quantities. As was shown for the solubility rate, the absorption rate also depended on amount of absorbing polymer in contact with a unit volume of juice. This derived from the thickness of the LDPE and adjoining adhesive layers, and the area/volume ratios of the package. Qualitatively these results corresponded to findings reported earlier for model systems.

An experienced, independent panel did not distinguish between orange juice stored in glass bottles and that stored in laminated cardboard packages with LDPE in product contact. Thus, absorption of up to 50% of d-limonene and small amounts of some aldehydes and alcohols by the packages had no significant (P > 0.05) effect on sensory quality of juice stored at 4°C.

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Carbonation Interactions with Sweetness and Sourness

N.J.N. YAU and M.R. McDANIEL

ABSTRACT

The effects of CO_2 level on sweetness and of sweetener level on carbonation perception were measured in two sweetened systems. The effects of CO_2 level on sourness and of acid level on carbonation perception were measured in two acidulated systems. The effects were measured at concentrations in ranges of 2–16% (w/v) sucrose, 0.015–0.12% (w/v) aspartame, 0.02–0.29% (w/v) citric acid, and 0.015–0.06% (v/v) phosphoric acid. Little effect of carbonation on sweetness was found in either sweetened system. Sucrose at 16% (w/v) reduced carbonation perception. Carbonation enhanced sourness ratings at the lower acid levels and had no effect at higher acid levels for either acid. No effect of acid level on carbonation perception was found.

Key Words: carbonation, sensory, sweetness, sourness

INTRODUCTION

IN ADDITION to the classic sensations of taste and smell, the sensations from thermal, mechanical, and common chemical stimuli play are important in food and beverage perception. The interactions between these factors in the mouth are of interest in psychophysical research as well as in sensory evaluation. Our research studied the possible interactions between trigeminal and olfactory sensations (Cain and Murphy, 1980; Cain, 1981), as well as trigeminal and gustatory sensations (Berridge and Fentress, 1985; Lyman and Green, 1990).

The chemical irritation from capsaicin or piperine is believed to be mediated by trigeminal nerves. Lawless and Stevens (1984) studied the effect of oral chemical irritation on the four basic tastes. They reported that the taste intensities of citric acid and quinine when tested at two levels were reduced by chemical irritation from capsicum, oleoresin, and piperine. The sweetness of sucrose was reduced only at the higher level (11.5%, w/v). Saltiness of NaCl was not affected at the two levels tested. In contrast, Cowart (1987) found that natural capsaicin did not mask the four basic tastes. Stevens and Lawless (1986) found that citric acid, sucrose, NaCl, water, and quinine could reduce the perceived intensities of capsaicin to different degrees. The decline in irritation was the largest with citric acid and sucrose, intermediate with NaCl and water, and least with quinine.

Researchers have studied the interactions between the common chemical sensation from CO_2 and the classic olfactory or gustatory sensations. Cain and Murphy (1980) found mutual inhibition between odor and nasal irritation from both CO_2 and amyl butyrate. They reported that CO_2 (at 30 parts/100) reduced odor intensity by about 12%. McLellan et al. (1984) found that sourness was enhanced as carbonation levels increased in carbonated apple juice. Cometto-Muñiz et al. (1987) studied the interactions between oral pungency from CO_2 and sweetness (sucrose), sourness (tartaric acid), saltiness (NaCl), and bitterness (quinine sulfate). The concentration of CO_2 was controlled by diluting a supersaturated solution of CO_2 (4.7 vol CO_2) with distilled and deionized water to different percentages but CO_2 level was not measured. They reported no interaction between sucrose sweetness and CO_2 oral pungency, whereas tartaric acid sourness and CO_2 oral pungency enhanced each other. NaCl enhanced oral pungency throughout the CO_2 range employed, but CO_2 enhanced saltiness only at the lowest NaCl concentration (0.4% w/v). Quinine sulfate reduced oral pungency at low CO_2 concentrations and had no effect at high CO_2 concentrations. CO_2 enhanced bitterness at low quinine sulfate concentrations and suppressed bitterness at high quinine sulfate concentrations.

Sucrose and aspartame are known to differ in temporal (Larson-Powers and Pangborn, 1978) and other qualities (Tunaley, 1988), therefore their interactions with carbonation may also differ. Acidulants such as citric acid and phosphoric acid are also known to differ in temporal (Straub, 1991) and other qualities (Rubico and McDaniel, 1992), therefore, differences in their interactions with carbonation may be expected.

Our objectives were to measure how CO_2 level affected ratings of sweetness of sucrose and aspartame and to measure how sucrose and aspartame levels affected ratings of carbonation intensity. Other objectives were to measure how CO_2 level affected ratings of sourness of citric acid and phosphoric acid and to measure how citric acid and phosphoric acid levels affected ratings of carbonation intensity. All tests utilized simple systems of carbonated water with sweeteners or acidulants only.

MATERIALS & METHODS

Sample production

Concentrations of 2, 4, 8, and 16% (w/v) sucrose (commercial source), and 0.015, 0.03, 0.06 and 0.12% (w/v) aspartame (A00120, Nutrasweet Co., Skokie, IL), were used in experiment 1. Concentrations of 0.02, 0.048, 0.12, and 0.29% (w/v) anhydrous citric acid (FCC, Miles Inc., Elkhart, IN); and 0.015, 0.024, 0.038 and 0.06% (v/v) 75% phosphoric acid (Food grade, Monsanto Co., St Louis, MO) were used in experiment 2. For the noncarbonated samples, bottled water (Aqua-Cool, Portland, OR) was blended with the sweeteners or acidulants and stored in volumetric flasks. For the carbonated samples, 7.5L of each sweetened (or acidulated) solution, prepared as above, were carbonated in a Zahm and Nagel 18.9-L stainless steel carbonator (Zahm and Nagel Co., Buffalo, NY). The samples were bottled in 355 mL clear bottles and stored at 1° C for 1–3 weeks. Two carbonation levels, 2.0 and 3.0 volumes CO_2, were employed for each treatment.

Analytical measurement

Carbonation levels were measured at both 3°C and 12°C with a Zahm and Nagel piercing device (Zahm and Nagel Co., Buffalo, NY) and expressed as "volumes CO_2 per volume water" (Zahm and Nagel Co. Inc., 1964). For experiment 2, the pH values of the carbonated (not deaerated) and noncarbonated samples were measured with a Corning 125 pH meter (Scientific Instruments, Medfield, MA).

Sensory measurement

Nine students of the Department of Food Science & Technology at Oregon State Univ. volunteered to evaluate the samples. Magnitude estimation was employed for rating the intensities of attributes. The panelists practiced magnitude estimation to evaluate standards to become familiar with rating sweetness, sourness, and carbonation. The samples were served in 60 mL plastic opaque cups at 9–12°C. The volume of sample was 40–50 mL. The carbonated samples were poured

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Table 1—Split-plot design used in both experiments

Sweetness or Sourness		
Reference	Sets	Samples
C_0S_2	1	C_0S_1, C_0S_2, C_0S_3, C_0S_4
C_0S_2	2	C_1S_1, C_1S_2, C_1S_3, C_1S_4
C_0S_2	3	C_2S_1, C_2S_2, C_2S_3, C_2S_4
Carbonation		
Reference	Sets	Samples
C_1S_2	1	C_1S_1, C_1S_2, C_1S_3, C_1S_4
C_1S_2	2	C_2S_1, C_2S_2, C_2S_3, C_2S_4

C_0-0.0 vol CO_2, C_1-2.0 vol CO_2, C_2-3.0 vol CO_2.
S_1, S_2, S_3, S_4 represent the four levels of each sweetener or acidulant.

Table 2—Parameters from the regression analysis of sweetness intensity versus stimuli concentration

Sweetener	Carbonation level*	Slope (n)	Intercept (y)	r	p-value
Sucrose	none	1.34[a]	−0.90[a]	1.00	0.005
	2.0 vol	1.48[a]	−1.14[a]	0.99	0.014
	3.0 vol	1.18[a]	−0.87[a]	0.99	0.008
Aspartame	none	1.04[a]	1.50[a]	1.00	0.001
	2.0 vol	1.06[a]	1.48[ab]	0.99	0.009
	3.0 vol	0.90[a]	1.25[b]	1.00	0.005

* Carbonation level is expressed as volume CO_2 per volume water.
[a,b] Values with common superscripts within each sweetener are not different ($p > 0.05$) based on paired t-test.

at the time of serving. Each bottle was capped with a rubber stopper after opening. A bottle opened for 15 min or with less than 5 cm sample remaining was discarded. The panelists were instructed to hold the sample in the mouth for 3–5 sec before expectorating it. The attributes were rated after expectoration.

In order to limit the variation of carbonation level during serving, a split-plot design was employed for sensory measurement. For each sweetener (or acidulant), carbonation level was the whole plot and sweetener (or acidulant) level was the sub-plot under carbonation level (Table 1). Each sweetener (or acidulant) was tested independently. There were three carbonation levels (0, 2, and 3 vol CO_2) and four sweetener (or acidulant) levels for each sweetener (or acidulant).

Carbonation interaction with sweetness. For evaluation of sweetness of the sucrose sweetened samples, a noncarbonated 4% (w/v) sucrose solution was used as a reference and was assigned a sweetness intensity value of 100. Sucrose solutions at four concentrations, at a common carbonation level, were presented in one set along with the noncarbonated reference. Two sets of samples, a random combination of 2 of 3 carbonation levels (Table 1), were presented to each panelist per session. The presentation orders of the samples within sets as well as the carbonation levels (sets) were randomized. Each set was replicated three times. For aspartame, the same design was employed and a noncarbonated 0.03% (w/v) aspartame solution was the reference.

Carbonation intensity, defined as the overall perception in the whole mouth including both the bubble feeling and pain perception, was rated only for the carbonated samples. For evaluation of carbonation of sucrose samples, a 4% (w/v) sucrose solution at 2.0 vol CO_2 was used as a reference and was assigned a carbonation intensity value of 100. Sucrose samples at four concentrations, at a common carbonation level, were presented in one set along with the reference. Two sets of samples (two carbonation levels) were served to each panelist per session. The presentation orders of the samples within sets as well as carbonation levels (sets) were randomized. Each set was replicated three times. for aspartame, the same design was employed and a 0.03% (w/v) aspartame solution at 2.0 vol. CO_2 was the reference.

Carbonation interaction with sourness. For evaluation of sourness of citric acid, a noncarbonated 0.048% (w/v) citric acid solution was used as reference and was assigned a sourness value of 100. Citric acid solutions at 4 concentrations, at a common carbonation level, were presented in one set along with the noncarbonated reference. Two sets of samples, random combinations of two of three carbonation levels (Table 1), were presented to each panelist per session. The presentation orders of the samples within sets as well as the carbonation level (sets) were randomized. Each set was replicated twice. For phosphoric acid, the same design was employed and a noncarbonated 0.024% (v/v) phosphoric acid solution was the reference.

Carbonation intensity was again rated only for the carbonated samples. A 0.048% (w/v) citric acid solution at 2.0 vol CO_2 was chosen as a reference and was assigned a carbonation intensity value of 100. Citric acid samples at four concentrations, at a common carbonation level, were presented in one set along with the reference. Two sets of samples (two carbonation levels) were served to each panelist per session. The presentation orders of the samples within sets and the carbonation levels (sets) were both randomized. Each set was replicated twice. For the phosphoric acid, the same design was employed and a 0.024% (v/v) phosphoric acid solution at 2.0 vol CO_2 was the reference.

Data analysis

The geometric mean of magnitude estimates across replications, sweetener (or acidulant) levels, and carbonation levels for each panelist for each sweetener (acidulant) was calculated (Stone and Oliver, 1969). The reference was included in the calculation of each geometric mean (McDaniel and Sawyer, 1981). The magnitude estimates of each panelist were subsequently rescaled by dividing by the individual geometric mean.

For the evaluation of sweetness (or sourness), the rescaled data were then averaged across replications for each panelist, each sweetener (or acidulant) level, and each carbonation level in each experiment. The geometric mean of the sweetness ratings for the whole panel was taken for each sweetener (or acidulant) level. The power functions of sweetness (or sourness) were established by determining the regression line on a log-log basis for each carbonation level. Multiple regression analysis (MRA) (Neter et al., 1983) was employed to test the differences in slopes and elevations between the power functions. The paired t-test was used for the paired comparison.

The rescaled data was also tested by ANOVA for both the evaluation of sweetness (or sourness) and carbonation. The F-values were calculated by employing a compound F-test (Anderson and Bancroft, 1952) for a mixed model, split-plot design. The Least Square Difference test was employed for the multiple comparisons; the paired t-test was used for the paired comparisons.

RESULTS & DISCUSSION

Carbonation interaction with sweetness

The power functions of sweetness for both sweeteners were determined in a noncarbonated system and in a carbonated system at two carbonation levels. The parameters of the linear regression models are shown in Table 2. The slopes and intercepts of the models were compared by using MRA. There were no significant differences between the slopes of the sweetness power functions of the carbonated and noncarbonated systems for either sweetener. For the elevation of the functions, there was a difference ($p \leq 0.05$) among the functions for the aspartame-sweetened system, but not for the sucrose-sweetened system. For the aspartame samples, the elevation of the function of the noncarbonated samples was higher ($p \leq 0.05$) than that of the samples at 3.0 vol CO_2 (Table 2). This indicated that carbonation did not affect sweetness intensity in the sucrose-sweetened system, but it reduced the sweetness across all sweetener levels in the aspartame-sweetened system at 3.0 vol CO_2. However, this effect was slight since no differences ($p > 0.05$) among the samples at each sweetener level were found by using the paired t-test. This held true for both sweeteners.

From the ANOVA results, there was no effect ($p > 0.05$) of carbonation on sweetness for either sweetener (Table 3). This finding was similar to that of Cometto-Muñiz et al. (1987) who reported CO_2 oral pungency had no effect on sucrose sweetness. There was an effect ($p \leq 0.001$) of sweetener level on sweetness for both sweeteners (Table 3) with sweetness increasing with increasing sweetener concentrations as expected. There was also an interaction ($p \leq 0.001$) of panelist and sweetener level on sweetness for both sweeteners (Table 3). Panelists rated the sweetness in the same direction but at different rates of increase, which caused the interaction effect.

For rating carbonation intensity, there was an effect ($p \leq 0.001$) of carbonation level for both sweetener systems (Table 3), as expected. Carbonation perception was perceived to be 2.5 times higher at 3.0 vol CO_2 than at 2.0 vol CO_2 for both

Table 3—ANOVA table for sweetness and carbonation intensity of sucrose and aspartame samples

Source	DF	F value	
		Sucrose	Aspartame
Sweetness			
Panelist (P)	8		
Carbonation level (C)	2	2.08	1.20
PxC	16	1.61	1.19
Sweetener level (S)	3	19.50***	14.55***
PxS	24	7.77***	3.16***
CxS	6	1.77	1.73
Replication (R)	2	1.57	1.73
PxR	16	1.03	1.34
Carbonation			
Panelist (P)	8		
Carbonation level (C)	1	99.79***	31.15***
PxC	8	1.88	4.25**
Sweetener level (S)	3	4.64**	1.44
PxS	24	0.73	0.64
CxS	3	0.94	1.99
Replication (R)	2	3.59	1.14
PxR	16	0.49	0.63

*** $p \leq 0.001$ ** $p \leq 0.01$

Table 4—Carbonation ratings for the four sucrose levels at each carbonation level*

Sucrose levels (w/v, %)	Carbonation levels	
	2.0 vol	3.0 vol
2.0	0.81^{a}	1.80^{ab}
4.0	0.72^{a}	1.99^{a}
8.0	0.69^{ab}	1.95^{ab}
16.0	0.47^{b}	1.70^{b}

* normalized response intensity.

[a,b] Means with common superscripts within each column are not different ($p > 0.05$), tested by using LSD test.

Table 5—Parameters from the regression analysis of sourness intensity versus stimuli concentration

Acids	Carbonation level*	Slope (n)	Intercept (y)	r^2	p-value
Citric	none	0.88^{a}	0.87^{a}	1.00	0.001
	2.0 vol	0.54^{b}	0.68^{b}	1.00	0.001
	3.0 vol	0.39^{b}	0.57^{ab}	0.93	0.067
Phosphoric	none	1.05^{a}	1.52^{a}	0.99	0.011
	2.0 vol	0.56^{b}	0.93^{b}	0.96	0.037
	3.0 vol	0.38^{b}	0.70^{b}	0.87	0.129

* Carbonation level is expressed as volume CO_2 per volume water.

[a,b] Values with common superscripts within each acidulant are not different ($p > 0.05$) based on paired t-test.

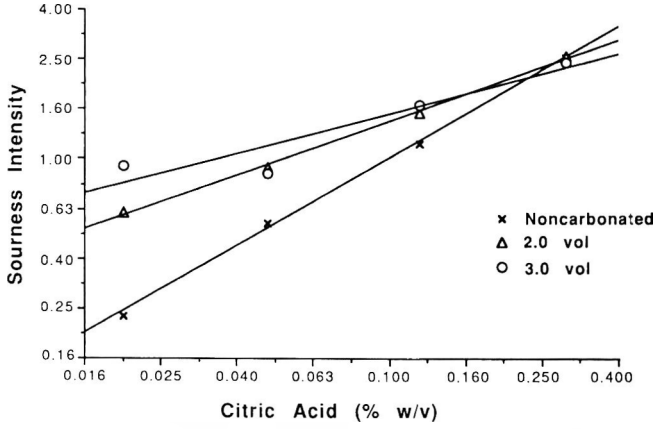

Fig. 1—Power functions of sourness for the citric acid-acidulated samples at the three carbonation levels on log-log scale (Y axis: rescaled sourness intensity).

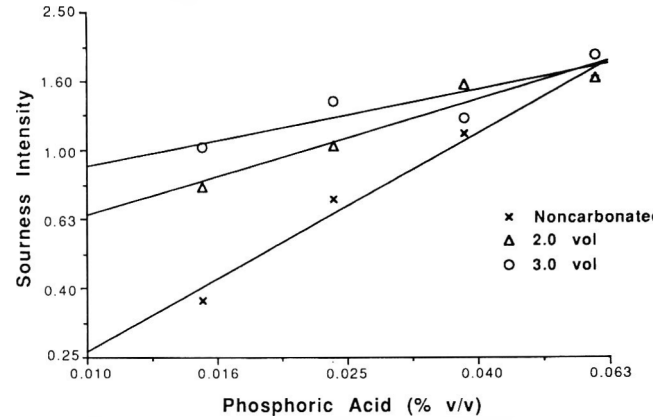

Fig. 2—Power functions of sourness for the phosphoric acid-acidulated samples at the three carbonation levels on log-log scale (Y axis: rescaled sourness intensity).

sweetened systems. For aspartame-sweetened samples, there was an interaction ($p \leq 0.01$) of panelist and carbonation level. All panelists rated carbonation intensity as increasing with increased carbonation level but at different rates. Again, the rates should be due to the different sensitivities to carbonation. This is supported by a previous study (Yau and McDaniel, 1990) in which differences in individual sensitivity to carbonation perception were reported. For sucrose-sweetened samples, there was an effect ($p \leq 0.01$) of sweetener level on carbonation. Samples sweetened with 16% (w/v) sucrose were rated significantly lower in carbonation intensity than the others. This was in agreement with the report of Sizer and Harris (1985) who found that their highest level of sucrose (9.9%, w/v) masked capsicin pungency. The ratings of carbonation intensity of the samples at the 4 sucrose concentrations at each carbonation level are presented in Table 4. At the 2.0 vol level, the 2% and 4% (w/v) sucrose samples were rated significantly higher than the 16% (w/v) sucrose sample. The higher concentration of sucrose reduced carbonation intensity. However, this phenomenon was not as clear for the 3.0 vol level samples. Only the 4% (w/v) sucrose sample was rated significantly higher in carbonation intensity than the 16% (w/v) sucrose sample. The effect of sucrose level on carbonation intensity may be carbonation level dependent.

This masking by a high sucrose concentration on carbonation intensity was in contrast with the finding of Cometto-Muñiz et al. (1987) in which no effect of sweetener level on CO_2 oral pungency was found. However, a range of 3–12% (w/v) of sucrose was employed in their study, whereas 16% (w/v) sucrose was the highest level employed in the current study. This suppression of carbonation may be due to the viscosity contributed by sucrose, or panelists may have been distracted by the high sweetness in the sample.

Carbonation interaction with sourness

The pH values of the citric-acidulated samples ranged from 2.5 to 3.3 for the high to low acid levels, respectively, and those of the phosphoric-acidulated samples ranged from 2.2 to 2.7 for the high to low acid levels, respectively. Carbonation did not change ($p > 0.05$) pH in the samples.

The power functions of sourness for both acids were determined in a noncarbonated system and in a system carbonated to two levels. The parameters of the linear regression models are shown in Table 5. Even though the models were not significant at the 3.0 vol level for both acids, they approached significance and were therefore compared to the other models. The 3.0 vol level data for both acids did not fit a second-order polynomial model either. There was a difference ($p \leq 0.05$) in slopes between the power functions in the carbonated and non-

Table 6—ANOVA table for sourness and carbonation intensity of citric and phosphoric acids

Source	DF	F value Citric	F value Phosphoric
Sourness			
Panelist (P)	8		
Carbonation level (C)	2	2.32	2.77
PxC	16	2.03	2.25*
Acid level (A)	3	31.80***	10.24***
PxA	24	2.17**	1.47
CxA	6	1.43	2.30*
Replication (R)	1	1.66	2.57
PxR	8	0.44	0.47
Carbonation			
Panelist (P)	8		
Carbonation level (C)	1	21.97***	32.63***
PxC	8	4.56***	3.01*
Acid level (A)	3	1.05	1.95
PxA	24	1.05	1.29
CxA	3	0.69	4.13**
Replication (R)	1	1.79	0.87
PxR	8	1.10	0.53

*** $p \leq 0.001$ ** $p \leq 0.01$ * $p \leq 0.05$

Table 7—Means of sourness intensity of the three carbonation levels and the four levels of citric and phosphoric acids*

Acid level	Carbonation level 0.0 vol	2.0 vol	3.0 vol
Citric acid(%, w/v)			
0.020	0.31[a]	0.69[b]	1.04[c]
0.048	0.61[a]	1.01[b]	0.96[b]
0.120	1.22[a]	1.59[ab]	1.69[b]
0.290	2.57[a]	2.74[a]	2.60[a]
Phosphoric acid(%, v/v)			
0.015	0.41[a]	0.94[b]	1.13[b]
0.024	0.81[a]	1.06[ab]	1.60[b]
0.038	1.21[a]	1.55[a]	1.28[a]
0.060	1.68[a]	1.72[a]	2.07[a]

* normalized response intensity.

[a,b,c] Means with common superscripts within each row are not different ($p > 0.05$), tested by using paired t-test.

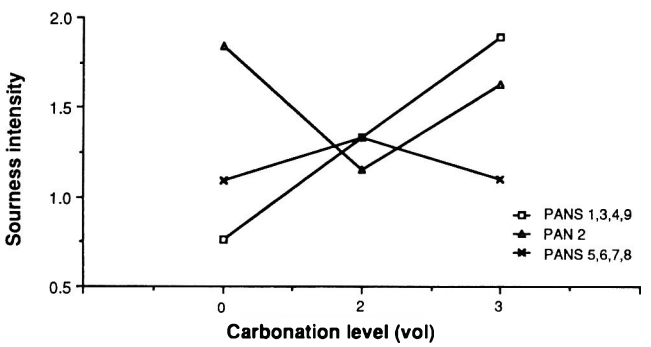

Fig. 3—Three patterns of panelist (PAN) and carbonation level interaction for sourness in the phosphoric acid-acidulated samples (Y axis: rescaled sourness intensity; PANS 1,3,4,9 and PANS 5,6,7,8 represents the average of panelists 1, 3, 4, and 9, and panelists 5, 6, 7, and 8, respectively).

carbonated systems for both acids (Table 5). The exponents in the noncarbonated systems were higher than those in the carbonated systems for both carbonation levels. Carbonation lowered the exponents of the power function of sourness (flattened the power function). The elevation of the linear functions for the carbonated samples, however, was above those for the noncarbonated samples, with all lines merging at the highest acid levels. This held true for both acids (Fig. 1 and 2).

Although the effect of carbonation level on sourness was not significant in the ANOVA test (Table 6), sourness was perceived to be higher in carbonated samples than in noncarbonated samples at the lower acid levels as tested by the paired t-test (Table 7). For citric acid, at 0.02% (w/v), the sourness of the samples at 3.0 vol CO_2 was 1.5 times higher than that of the samples at 2.0 vol CO_2 which was 2.2 times higher than that of noncarbonated samples. Therefore, the sample at 3.0 vol. CO_2 was 3.3 times higher in sourness than the noncarbonated sample. For the 0.048% (w/v) acid level, there was no difference ($p > 0.05$) between the samples at 2.0 and 3.0 vol CO_2, however, both were 1.6 times higher than that of the noncarbonated samples. For the 0.12% (w/v) citric acid level, the samples at 3.0 vol CO_2 were perceived to be more sour (1.4 times) than the noncarbonated samples. No difference was found among the 3 carbonation levels for the highest acid level tested.

For phosphoric acid at 0.015% (v/v), there was no difference ($p > 0.05$) between the two carbonated samples; both of them were rated 2.5 times higher in sourness than the noncarbonated sample. At the 0.024% (v/v) level, the sourness of the samples at 3.0 vol CO_2 was twice as high as that of the noncarbonated sample. No difference in sourness was found among the three carbonation levels for the two higher acid levels (0.038% and 0.06%). Carbonation enhanced sourness intensity at the lower acid levels and had no effect at the highest acid level for both acids. This finding agreed with the reports of McLellan et al. (1984) and Cometto-Muñiz et al. (1987). McLellan et al. (1984) reported that sourness of apple juice was enhanced by increasing carbonation levels. Cometto-Muñiz et al. (1987) found CO_2 oral pungency enhanced tartaric acid sourness within a range of 0.3–1.2% (w/v) tartaric acid.

Acid levels had an effect ($p \leq 0.001$) on sourness for both acids (Table 6). In general, sourness increased with acid concentration for both acids (Table 7). There was an interaction ($p \leq 0.05$) of panelist and carbonation level for phosphoric-acidulated samples (Table 6). However, this significant interaction was taken into account by using a compound F-test in testing the main effects in the ANOVA. The pattern of this interaction is shown in Fig. 3. Four panelists (5, 6, 7, and 8) rated sourness highest at 2.0 vol CO_2 and found reduced sourness at 3.0 vol CO_2, while another four panelists (1, 3, 4, and 9) found sourness increasing with each increased carbonation level. Panelist 2 rated sourness highest for the noncarbonated samples and lowest at the 2.0 vol level. For the panelists who rated the 3.0 vol CO_2 sample lower in sourness than the 2.0 vol CO_2 sample, the increased carbonation may have masked some of the sourness. This mixed pattern may also be due to the high astringency from phosphoric acid, which was reported to be more astringent than sour (Rubico and McDaniel, 1992). For citric-acidulated samples, there was a significant interaction of panelist by acid level. Again, the interaction resulted from the different increasing rates, although all the panelists rated samples in the same direction (increasing with increased acid concentration). There was an interaction ($p \leq 0.05$) of carbonation level by acid level for phosphoric-acidulated samples (Table 6). This interaction can be observed in Fig. 2. The F-value of this interaction for citric-acidulated samples did not reach significance (Table 6).

Carbonation level had an effect ($p \leq 0.001$) on carbonation intensity (Table 6), as intensity increased with carbonation level. Carbonation intensity was not affected by acid level for either acid (Table 6). This was not in agreement with the finding reported by Cometto-Muñiz et al. (1987). They reported that increasing level of tartaric acid enhanced CO_2 oral pungency. This discrepancy may be due to different properties of the different acids tested. There was a significant interaction of panelist and carbonation level for both acids (Table 6). Panelist's ratings of sourness increased as carbonation level increased but with different rates of increase. This was true for both acids. There was also on interaction ($p \leq 0.01$) of carbonation level and acid level for the phosphoric-acidulated samples (Table 6). Carbonation intensity was not affected by acid level at 2.0 vol CO_2 (Fig. 4). At the carbonation level of

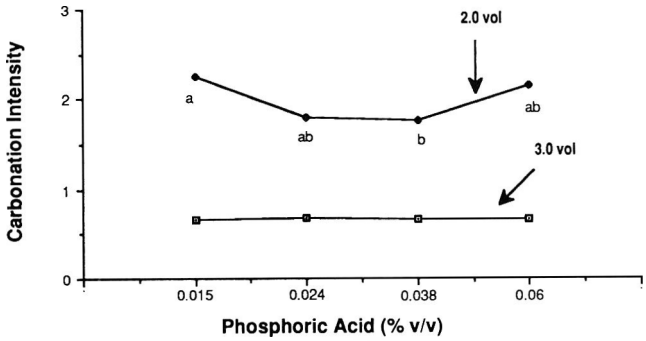

Fig. 4—Interaction of carbonation level and acid level for carbonation intensity in the phosphoric acid-acidulated samples (Y axis: rescaled carbonation perception intensity, ab- the points with common letters are not significantly different, p > 0.05).

3.0 vol, the carbonation intensity for acid level 1 was higher ($p \leq 0.05$) than that for acid level 3 as detected by a paired t-test. Therefore, there was an effect of acid level on carbonation intensity at 3.0 vol CO_2.

Interactions of tastes and carbonation

Overall, the effect of carbonation on sourness ratings was stronger and more clear compared to its effect on sweetness ratings. Cometto-Muñiz et al. (1987) suggested that sweetness may be more distant from CO_2 oral pungency than is sourness, (in terms of quality), since the trigeminal nerve has been reported to be activated by high concentration of tartaric acid but not by any level of sucrose. According to this concept of perceptual proximity, the more easily two stimuli can be distinguished from each other, the less likely there will be interaction between them. Our results support this theory.

Carbonation is complex (Yau and McDaniel, 1991), introducing mouthfeel components such as tingle, bite, burn and numbing. Sometimes pain is reported at high carbonation levels. Sourness may be contributed by carbonic acid which is produced when water is carbonated. The calculated amount of carbonic acid formed by carbonating water ranged from 0.036–0.57% (w/v) for the CO_2 concentration range of 1.2–3.7 vol. The sourness threshold of carbonic acid and the intensity of sourness contributed by varying amounts of carbonic acid are unknown. Further investigation is required to quantify this effect. Acidulants introduce astringency in addition to sourness into a system. How all these sensory components might interact in a beverage system has not been determined. The higher sourness in our carbonated vs noncarbonated samples may have resulted from an interaction between the mouthfeel from carbonation and sourness from an acidulant; or pain and sourness; or sourness from both the acidulant and carbonic acid.

Carbonation perception is believed to be mediated by the trigeminal nerve while sourness is mediated by the chorda tympani. The interaction between carbonation perception and sourness could occur at two steps (Lawless et al., 1985). First, the interaction may occur at a peripheral level. The signal (sensory input) mediated by trigeminal nerves interacts with that mediated by the chorda tympani as they are fired. The signals (inputs) may be suppressed or enhanced by each other to some degree, or the trigeminal stimuli may cause direct action on taste receptors. Secondly, the interaction may occur at a central level. As both signals (inputs) reach the brain, the information integrates. This second hypothesis is supported partially by the finding of Berridge and Fentress (1985). They found that there was an interaction between somatosensation (trigeminal) and gustation in the processing of palatability using rats as the model. The transaction of the trigeminal nerves reduced the ingestive action elicited by preferred tastes. The reduction, refering to preference and behavior, indicates that central (brain) integration is involved.

Another trigeminal stimulus, chemical heat (irritation), has also been studied. The interactions between chemical heat from capsaicin and basic tastes have been investigated intensively (Lawless and Stevens, 1984; Lawless et al., 1985; Stevens and Lawless, 1986). All studies found that there was an inhibitory effect of irritation on taste intensities of sucrose and citric acid. Stevens and Lawless (1986) also reported that the irritation intensity was reduced during trials with citric acid and sucrose. No enhancement in any taste intensity from chemical heat (capsaicin and piperine) was reported. This suggested that there may be an important difference in how chemical heat and carbonation stimulation are processed, even though both are mediated by trigeminal nerves.

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Compositional Changes in Brewed Coffee as a Function of Brewing Time

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ABSTRACT

Variations in the composition of brewed coffee as a function of brewing time were monitored using gas chromatography-mass spectrometry. Batches of coffee were produced using flow rates of 50–500 mL/min, which resulted in brewtimes of 4.42–31.07 min. While most components increased in concentration with longer brewing times, several showed significant losses beyond 8 min, indicating an optimum brewtime of 7–9 min.

Key Words: coffee, brew time, extraction, composition

INTRODUCTION

DUE TO its unique aroma and flavor, considerable research has been performed analyzing flavor-related compounds in roasted coffee grounds. Research on volatile flavor compounds have used several different techniques. Aishima and Ozawa (1989) used reversed phase HPLC to concentrate flavor compounds in aqueous systems. Tassan and Russell (1974) used headspace analysis to determine volatiles from coffee brews, while Vitzthum and Werkhoff (1975) analyzed cycloalkylpyrazines in coffee aroma using gas chromatography-mass spectrometry (GC-MS) and nuclear magnetic resonance (NMR) techniques. Shimoda and Shibamoto (1990) also used GC-MS with headspace analysis and identified 62 volatile compounds. Using such instrumental methods, over 600 chemical compounds have been identified from roasted coffee grounds.

Flament (1991) reported 18 general classes of flavor related materials from ground coffee. These classes and the number of components in each were: 50 hydrocarbons, 20 alcohols, 28 aldehydes, 70 ketones, 20 acids, 29 esters, 8 lactones, 42 phenols, 99 furans, 26 thiophenes, 67 pyrroles, 27 oxazoles, 28 thiazoles, 13 pyridines, 79 pyrazines, 24 amines, 16 sulfides, and 9 miscellaneous compounds. While each component or chemical class contributes to the overall nature of coffee aroma, it is difficult to assess the relative significance of any individual component. Of the hydrocarbons and alcohols found in coffee, only the terpenes are considered as contributing to coffee aroma (Gaudagni, 1966). Aldehydes are reported to impart a strong, fatty odor whereas the ketones, furans and pyrroles have a sweet, buttery to carmel note. The esters and lactones exhibit a fruity flavor and the phenols impart a smoky to burnt aroma (Furia, 1975). The presence of acids and sulfur-containing compounds are considered as imparting undesirable flavor notes ranging from sweat-like, to bitter and stale (Flament, 1991; Winter et al., 1972).

The majority of reported work has involved the characterization of ground coffee or head-space analysis of coffee grounds or brew. Merrit and Proctor (1958) evaluated extraction rates for caffeine, tannin and some phenolic compounds in coffee brew. Niven and Shaw (1967) reported optimum brew conditions based on brew strength as a function of time, temperature and grind.

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Our objective was to apply a method involving extraction, concentration and GC-MS to the characterization of brewed coffee and compositional changes as a function of brewing time. The procedures employed were relatively uncomplicated and allowed monitoring of 44 species.

MATERIALS & METHODS

Materials

Prepackaged ground decaffeinated coffee was obtained from a local coffee service (American Brewed Coffee, Akron, OH). Several packages of coffee were mixed to provide a uniform batch of ground coffee sufficient to complete a given experiment. The filter papers used in the study were UF-50 filters (Mr. Coffee®, Inc., Bedford Heights, OH), and deionized water was used throughout. Phosphoric acid and sodium hydroxide solutions (4.0N) were used for pH adjustment. GC/MS grade methylene chloride (Fisher Scientific) was used to perform the extractions. Acenaphthene (Eastman Organic Chemicals) was used as internal standard and was dissolved in methylene chloride.

Apparatus

Water was heated using a constant temperature water bath (Model MW-1110A-1, Blue M, Blue Island, IL), which also served as a reservoir. Heated water was transferred using a Cole-Parmer Digi-Staltic pump (Model 7526-00) with silicon tubing, which was wrapped with insulating tape to minimize heat losses. The spray head assembly from a Mr. Coffee® EXP-1 coffee maker (Mr. Coffee, Inc. Bedford Heights, OH) was used as a basket support. The basket was equipped with a thermometer to allow monitoring of temperature during the brewing process and was insulated to minimize heat losses.

Methods

Two types of experiments were performed. The first involved brewing batches of coffee with different flow rates, and therefore different lengths of brewing time. The second involved collecting 200 mL aliquots of the coffee brew while the brewing process was occurring. For each study, 50.0g of coffee were placed into the brewing basket, using a generic filter paper. A fixed volume of heated water (1500 mL, 95°C) was pumped through the brewing basket, using the Digi-Staltic pump to control the flow rate. The brewed coffee was collected in a 2 μL Erlenmeyer flask and chilled in an ice bath. Flow rates of 50 to 500 mL/min were investigated.

For each extraction, 200.0 mL brewed coffee were pipetted into a separatory funnel, and the pH adjusted to 12.0 with sodium hydroxide. Following pH adjustment, 5.00 mL of internal standard (0.24 mg/mL acenaphthene in methylene chloride) were added and the solution extracted using 4 × 50 mL of methylene chloride. The combined extracts were dried using calcium chloride and then stored at 4°C. The basic coffee solution was then acidified with phosphoric acid to pH 2.0. An additional 5.00 mL internal standard solution were added, and the coffee extracted using 4 × 50 mL methylene chloride. The extracts were combined, dried and stored as described above. Each extract was then concentrated at 50°C using a Kuderna-Danish macroconcentrator to <2 mL. The volume was adjusted to 3 mL using methylene chloride and samples were split and transferred to GC autosampler vials for analysis.

The concentrated extracts were analyzed using a Hewlett-Packard (HP) 5890 GC configured for capillary column and interfaced to an HP 5970 Series B mass selective detector. A Supelcowax 10 capillary column (30m × 0.25 mm, 0.25 m film, Supelco) was used to separate the components. A Grob injection (1L, purge on after 1 min) was used, and the chromatograph was operated using a programmed tem-

Table 1—Halflife ($t_{1/2}$), time for 90% extraction ($t_{90\%}$) and for those compounds classified as volatile extractors, the time when maximum extraction occurred. (A) Denotes components found in acid extract, remainder are in base extract. 95% confidence limits are given for halflife values.

Component	$t_{1/2}$	$t_{90\%}$	Max. time
Fast extractors			
Caffeine	1.68 ± 0.16	5.54	
1-(1H-Pyrrol-2-yl)ethanone	1.82 ± 0.13	6.01	
3,4-Dimethoxyphenol (A)	1.84 ± 0.20	6.07	
Pentanoic acid (A)	1.90 ± 0.18	6.27	
1-(2-Furanyl)ethanone	1.96 ± 0.26	6.47	
2-Furancarboxaldehyde	2.04 ± 0.29	6.73	
Bicyclo[3.3.1]nonan-9-ol	2.07 ± 0.28	6.83	
2-Ethyl-5-methylpyrazine	2.09 ± 0.21	6.90	
5-Methyl-2-furancarboxaldehyde	2.11 ± 0.23	6.96	
2,5-Dimethylpyrazine	2.12 ± 0.25	7.00	
Benzeneethanol	2.27 ± 0.44	7.49	
Benzeneamine	2.29 ± 0.33	7.56	
2-Ethyl-6-methylpyrazine	2.30 ± 0.31	7.59	
2-Methylpyrimidine	2.50 ± 0.35	8.25	
Pyrazine	2.58 ± 0.42	8.51	
Benzaldehyde	2.59 ± 0.44	8.55	
5-Methyl-1H-pyrrole-2-carboxaldehyde	2.62 ± 0.19	8.65	
1-(2-Furanylmethyl)-1H-pyrrole	2.70 ± 0.11	8.91	
Decanoic acid (A)	2.98 ± 0.56	9.83	
Isopropenylpyrazine	3.06 ± 0.47	10.10	
3-Hydroxy-2-methyl-4H-pyran-4-one (A)	3.08 ± 0.48	10.16	
Phenol (A)	3.58 ± 0.51	11.81	
1H-Pyrrole-2-carboxaldehyde (A)	3.79 ± 0.38	12.51	
2-Formyl-1-methylpyrrole	3.84 ± 0.31	12.67	
Slow extractors			
3-Methyl-2-butenoic acid (A)	4.61 ± 0.62	15.32	
2-Methyl-2-cyclopentene-1-one	4.90 ± 0.31	16.17	
1-(4-Hydroxy-3-methoxyphenol)ethanone	4.96 ± 0.21	16.37	
1-Propyl-2-pyrrolidinone (A)	5.19 ± 0.44	17.13	
Methyl cinnamate	5.52 ± 0.82	18.22	
2-Methoxybenzeneamine	5.69 ± 0.59	18.78	
Pyrimidine	5.99 ± 0.48	19.77	
3,5-Dimethoxyphenol (A)	7.24 ± 0.49	23.89	
1H-Indole	8.85 ± 0.55	29.20	
Pyridine	9.56 ± 0.51	31.55	
Species which exhibit maxima			
4-Hydroxy-3-methoxybenzaldehyde (A)	1.54 ± 0.10	5.08	9.52
2,3-Dimethylpyrazine	1.95 ± 0.19	6.44	11.05
2-Iminocyclopentanecarbonitrile	2.35 ± 0.32	7.75	11.05
2-Furanmethanol	2.57 ± 0.08	8.48	7.87
5-(Hydroxymethyl)-2-furancarboxaldehyde	2.75 ± 0.36	9.07	16.85
2-Ethyl-3,5-dimethylpyrazine	2.80 ± 0.30	9.24	11.05
2-Hydroxy-3-methyl-2-cyclopenten-1-one	3.15 ± 0.45	10.39	11.05
Benzoxazole	3.50 ± 0.39	11.55	16.85
2-Ethenyl-6-methylpyrazine	4.18 ± 0.67	13.79	16.85
2-Methoxy-4-(2-propenyl)-phenol (A)	6.02 ± 1.12	19.87	9.52
Overall average	3.26 ± 0.18	10.76	

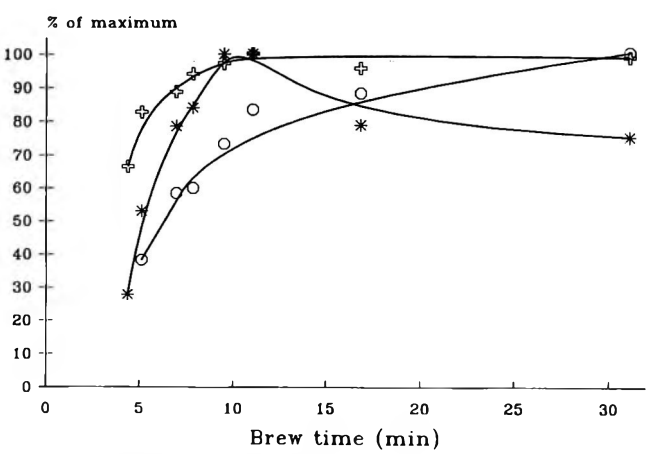

Fig. 1—Brewing curves of representative components showing slow extractors (O), fast extractors (+) and volatile extractors (*).

perature ramp with the following conditions: initial temperature, 35°C; initial hold time, 2 min; ramp, 8°C/min; final temperature, 280°C; final hold time, 7.5 min. An HP 7673A autoinjector was used to

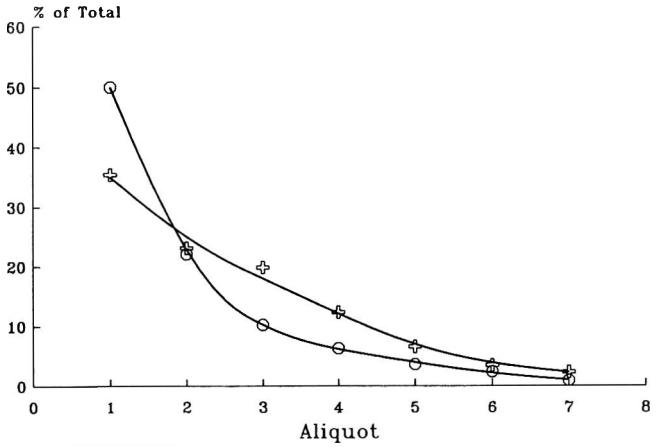

Fig. 2—Average concentrations at flow rates of 50 ml/min (+) and 250 ml/min (O).

minimize variations in sample volumes, and an HP 59940A MS Chemstation (HP-UX series) running version B.02.00 software was used for instrument control, data acquisition and data analysis. Mass spectral identifications of the 44 components were made using the HP implementation of the Wiley Database. All evaluations were made based on relative concentrations once normalized for the internal standard.

RESULTS & DISCUSSION

Evaluation of extraction efficiency

A study was conducted to evaluate the efficiency of the extractions as most of the components identified in the base/neutral extract were also present in the acid extract. It was determined that by using 4 × 50 mL of methylene chloride for each extraction average recovery was 98% with the worst case 94.5% for caffeine in the base/neutral extract. The components evaluated and the extract from which they were evaluated are listed in Table 1.

Effect of brewtime on total extraction

Three general classes of components were identified based on brewing curves. These components were classified as slow extractors, fast extractors and those components where a maximum was reached followed by subsequent losses most likely due to volatilization. Average extraction profiles for each class are shown in Fig. 1. The slow extractor components exhibited relatively low concentrations under rapid brewing conditions with 30–40% of the maximum amount extracted at 5 min. The fast extractors exhibited relatively high initial concentrations with 70–80% of the maximum extracted at 5 min. The 10 components which exhibited a maximum followed by losses had maximums at brewing times of 7.87–16.85 min. An exponential function was fitted to the brewing curves for each component, allowing calculation of the half-life for each component. This half-life represents the amount of brewing time required to achieve 50% of the maximum amount of a given component. Table 1 summarizes the components identified, their halflives ($t_{1/2}$) and the length of time required to extract 90% of the maximum amount of material ($t_{90\%}$). The average half-life was calculated by fitting the exponential function to a brewing curve prepared by averaging the relative percentage of each component at each brewing time for the fast and slow extractors.

Characterization of extraction profiles

Analysis of samples acquired at 200 mL intervals during brewing indicated that the highest concentrations of most components were found in the first 200 mL of the brew. A com-

Table 2—Percent of each compound for 250 mL/min brew vs. 50 mL/min brew.

Component	Percent
1H-Indole	16.7
Pyridine	27.3
Pyrimidine	54.8
3,5-Dimethoxyphenol	56.3
1-(4-Hydroxy-3-methoxyphenyl)ethanone	58.7
Methyl cinnamate	59.9
2-Methoxybenzeneamine	61.7
2-Methyl-2-cyclopentene-1-one	67.5
3-Methyl-2-butenoic acid	71.1
3-Hydroxy-2-methyl-4H-pyran-4-one	74.1
2-Formyl-1-methylpyrrole	75.3
Pentanoic acid	80.1
Phenol	83.4
1H-Pyrrole-2-carboxaldehyde	84.1
1-(2-Furanylmethyl)-1H-pyrrole	86.7
Benzoxazole	87.2
Isopropenylpyrazine	88.5
Benzaldehyde	89.1
1-Propyl-2-pyrrolidinone	89.7
5-Methyl-1H-pyrrole-2-carboxaldehyde	90.7
Caffeine	91.9
2-Furanmethanol	92.0
2,5-Dimethylpyrazine	92.0
Decanoic acid	92.6
3,4-Dimethoxyphenol	92.6
2-Methylpyrimidine	93.1
2-Ethyl-3,5-dimethylpyrazine	93.4
5-(Hydroxymethyl)-2-furancarboxaldehyde	93.6
Pyrazine	93.8
1-(2-Furanyl)-ethanone	94.2
Benzeneethanol	94.2
2-Furancarboxaldehyde	95.4
2-Ethyl-6-methylpyrazine	95.7
2-Imino-cyclopentanecarbonitrile	98.0
Benzeneamine	98.4
5-Methyl-2-furancarboxaldehyde	98.4
2-Ethyl-5-methylpyrazine	98.6
1-(1H-Pyrrol-2-yl)ethanone	99.2
2,3-Dimethyl pyrazine	100.2
2-Ethenyl-6-methylpyrazine	101.9
Bicyclo[3.3.1]nonan-9-ol	102.1
2-Hydroxy-3-methyl-2-cyclopenten-1-one	112.0
2-Methoxy-4-(2-propenyl)-phenol	118.0
4-Hydroxy-3-methoxybenzaldehyde	136.7

parison was made (Fig. 2) between the average percent extracted at flow rates of 50 and 250 mL/min. In the study performed at 250 mL/min, 50% of the total amount of materials were removed in the first 200 mL. Differences in rate of extraction as a function of flow rate were consistent with the hypothesis that at higher flow rates, the coffee grounds tend to mix with the water, resulting in rapid wetting and faster extraction. At low flow rates, we observed that very little mixing of the coffee grounds occurred. A comparison of the total amount of material removed at the 2 flow rates was made by calculating the ratio of material removed at 250 mL/min and 50 mL/min (Table 2). Of the 44 components evaluated, 22 were unaffected by the flow rate used for brewing, with at least 90% of material removed at 250 mL/min. Three components were found to have significantly higher amounts extracted at 250 mL/min than at 50 mL/min. These components (2-hydroxy-3-methyl-2-cyclopenten-1-one, 2-methoxy-4-(2-propenyl)phenol and 4-hydroxy-3-methoxybenzaldehyde) also exhibited losses with extended brewing time. This would indicate that significant volatilization may be occurring at the lower flow rate. The remaining components exhibited a decrease in the amount of material removed at 250 mL/min compared to 50 mL/min. This decrease ranged from 83.3% for 1H-indole to 10.3% for 1-propyl-2-pyrrolidinone.

CONCLUSIONS

BASED on the average halflife of 3.26 min and the corresponding $t_{90\%}$ of 10.76 min, the maximum length of time for brewing coffee should be 10–11 min. This would maximize the amount of fast extractor components in the brew. However, with brewing times longer than 8 min significant volatilization of some components begins to occur. With brew times less than 7 min, less than 80% of the brew's maximum concentration was achieved. Currently, most commercially available coffee makers that employ the drip method of brewing require from 8 - 12 min to produce 1500 mL of brewed coffee.

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Nutritional Evaluation of Breadfruit-containing Composite Flour Products

C. NOCHERA and M. CALDWELL

ABSTRACT

Composite flours containing wheat, breadfruit flour and soy protein, whey or peanut meal were formulated and incorporated into western style bread and biscuits. Bread containing 10% breadfruit flour and 5% whey, and biscuits containing 10% breadfruit flour and 5% soy protein were judged most acceptable in flavor, color, and texture. Breadfruit flour contained 4.4% protein and was lower in sulfur containing amino acids and higher in lysine than wheat flour. Breadfruit containing bread and biscuits had low amino acid scores. The protein efficiency ratio (PER) of the biscuit product, however, was similar to that of the casein control while the PER of bread was significantly lower. Results suggest that acceptable products can be made with composite flours containing 10% breadfruit flour.

Key Words: breadfruit, flour, wheat, protein, nutrients

INTRODUCTION

BREAD and other wheat-containing products are widely consumed throughout the world. In tropical countries, wheat production is limited and wheat flour must be imported to meet local demand. Composite flours have been developed in which flour from locally grown foodstuffs replace a portion of wheat flour for use in breads and other baked products. These flours are promoted to decrease the demand for imported wheat and to stimulate production and use of locally produced nonwheat agricultural products (Fellers, 1987). Rice, maize, cassava, yams, potatoes and breadfruit are among the crops used to create composite flours (Kim and Ruiter, 1968; Olatunji and Akinrele 1978). Kim and Ruiter (1968) reported that up to 10% nonwheat flour can be used in breads without undesirable changes in bread loaf volume and texture. Use of more than 10% nonwheat flour is possible depending on the source of the added flour and the type of product (Fellers, 1987).

Breadfruit is grown throughout Southeast Asia, the South Pacific and the West Indies. It is a rich source of carbohydrate, but use is limited because of poor storage properties of the fresh fruit. Conversion of the fresh product to dry flour increases storage life and also increases the versatility of the product. Work on breadfruit-containing composite flours is limited. However, Olatunji and Akinrele (1978) produced acceptable bread loaves when 10% of wheat flour was replaced by breadfruit flour. Addition of 20% breadfruit flour required use of additives to produce an acceptable western style bread loaf.

The nutrient content of breadfruit varies with the portion of the fruit used and the stage of maturity. Graham and deBravo (1981) reported that the protein content of the pulp of mature breadfruit ranged from 3.8–4.1%. The protein content of wheat flour is 10–12%. Therefore, when using breadfruit-containing composite flours, it may be necessary to add protein to produce products which are equivalent to those containing wheat alone. This could be done by including a protein source such as soy protein, peanut meal or whey in the composite flour.

Our objective was to (1) develop composite flours containing wheat flour, breadfruit flour and soy protein, peanut meal or whey; (2) formulate acceptable western style baked products using standard home recipes, and (3) assess the nutritional value of the products developed. The ultimate goal was to stimulate interest in the use of breadfruit as a partial substitution for wheat flour in areas of the world where breadfruit can be easily grown.

MATERIALS & METHODS

Harvest and preparation of breadfruit

Mature breadfruit (*Artocarpus communis*) were harvested by hand in Mayaguez, Puerto Rico, USA. Washed breadfruits were peeled and the pulp was sectioned and dried at 80°C for 24 hrs. Dried pulp was ground in a Waring mill to produce flour which passed through an 80 mesh (180 μm) sieve.

Proximate and amino acid analysis

Proximate analysis (protein, fat, crude fiber, ash and moisture) on breadfruit, soybean protein, peanut meal and whey were carried out

Table 1 – Ingredients for bread and biscuits containing wheat or a breadfruit-containing composite flour

Bread		Biscuit	
Component	%	Component	%
Water	33.0	Water	32.8
Vegetable Oil	1.3	Margarine	16.7
Sugar	1.4	Baking Powder	1.9
Salt	0.3	Salt	0.6
Yeast	1.0	Flour[a]	48.1
Flour[a]	63.0		

[a] For reference standards only white flour was used.

Table 2 – Composition of diets for PER determinations

	Bread		Biscuits	
Component	Test	Control	Test	Control
	g/100g			
Casein[a]	–	11.5	–	11.5
Biscuit	–	–	89.2	–
Bread	78.1	–	–	–
Corn Oil	6.9	7.8	–	17.6
Mineral mix[b]	4.8	4.9	0.1	4.9
Vitamin mix[c]	1.0	1.0	1.0	1.0
Alphacell[d]	0.5	1.0	0.3	1.0
Water	3.5	3.8	3.6	3.8
Carbohydrate[e]	4.9	69.8	5.6	57.8
Composition	% diet			
Protein	10.0	10.0	10.00	10.00
Fat	8.0	8.0	17.75	17.75
Mineral	5.0	5.0	5.00	5.00
Vitamin mix	1.0	1.0	1.00	1.00
Crude fiber	1.0	1.0	1.00	1.00
Moisture	5.0	5.0	5.00	5.00
Carbohydrate	70.0	70.0	60.25	60.25

[a] High protein casein ICN Pharmaceuticals (Nutritional Biochemical)
[b] Jones Foster mineral mix ICN Pharmaceuticals (Nutritional Biochemical)
[c] Vitamin mix, ICN Pharmaceuticals (Nutritional Biochemicals)
[d] Alphacell Non-Nutritional Bulk, ICN Pharmaceuticals, (Nutritional Biochemical)
[e] Carbohydrate used in test diet was sucrose; carbohydrate in control diet was 1/3 sucrose, 1/3 dextrose and 1/3 corn starch.

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Table 3—Proximate analysis of breadfruit flour, soy protein, peanut meal and whey

Flour	Moisture	Protein	Fat	Fiber	Ash
	% wet weight				
Breadfruit	7.2 ± 0.1[a]	4.4 ± 0.1	1.1 ± 0.2	3.8 ± 0	2.6 ± 0.1
Soy[b]	8.7 ± 0.0[c]	66.4 ± 0.8	0.4 ± 0.1	3.4 ± 0	6.0 ± 0.0
Peanut	7.0 ± 0.0[c]	50.1 ± 1.0	6.3 ± 0.2	3.1 ± 0	4.6 ± 0.0
Whey	14.1 ± 0.0[c]	12.6 ± 0.1	0.3 ± 0.1	3.1 ± 0	8.8 ± 0.0

[a] Mean ± standard deviation of five replicates
[b] Edible soybean protein (ICN Pharmaceutical), peanut meal and whey (United States Biochemical Corporation)
[c] Mean ± standard deviation of three replicates

Table 4—Sensory evaluation scores for bread and biscuits made with breadfruit-containing composite flours

Composition	Mean sensory scores[a]					
Breadfruit flour (%)	Bread			Biscuit		
	Color	Texture	Flavor	Color	Texture	Flavor
5	4.1[b]	3.6[c]	4.0[c]	4.0[c]	3.7[b]	4.4[b]
10	4.5[b]	5.0[b]	4.8[b]	4.7[b]	3.5[b]	4.4[b]
15	4.0[b]	3.6[c]	4.1[c]	3.7[c]	3.9[b]	3.9[b]
20	3.5[c]	4.0[e]	2.7[d]	3.8[c]	3.8[b]	4.2[b]

[a] For statistical analysis, sensory scores for each level of breadfruit were pooled across all levels and types of protein flour. A score of 5 indicates that the product was rated as equivalent to a standard product containing wheat flour only.
[b-e] Means followed by different letters within the same column are significantly different ($P \leq 0.05$).

according to procedures outlined by AOAC (1975). Amino acid analysis was performed on acid hydrolyzed breadfruit flour and composite flour products using a modified Technicon Autoanalyzer according to the method of Moore and Stein (1963). Tryptophan was determined by a commercial laboratory (Hazleton Laboratories, Inc., Madison, WN) using the method of Wooley and Sebrell (1945).

Preparation of food product

Composite flours were formulated by replacing 5,10,15 or 20% of enriched white flour by breadfruit flour and 5 or 10% of the white flour by soy protein, peanut meal or whey. Composite flours were then used to prepare biscuits and breads using standard recipes (Table 1).

Sensory evaluation and statistical analysis

Baked products were tested for acceptability of color, texture and flavor using a hedonic test (Larmond, 1977). Products were evaluated by an eight member taste panel who rated the products in comparison to a control which contained no breadfruit flour. A scale of 1-10 was used with 1 being inferior, 5 being equal and 10 being superior to the control. Data obtained from the taste panel were analyzed using ANOVA and Duncan's Multiple Range Test. To determine the extent to which wheat flour could be replaced by breadfruit flour, sensory scores for all bread and biscuits made with each level of breadfruit flour were pooled for statistical analysis. Sensory scores for each combination of breadfruit flour and soy protein, peanut meal or whey were examined. The biscuit and bread rated as most acceptable were selected for further analysis.

Nutritional evaluation of baked products

Amino acid score was calculated by dividing the mg of each essential amino acid in 1g test protein by the mg of that amino acid in 1g of the standard protein. The amino acid scoring pattern for children of preschool age was used as the standard protein (FAO/WHO/UNU Expert Consultation, 1985; FAO/WHO Expert Consultation, 1990). The essential amino acid present in the lowest amount compared to the standard was the limiting amino acid and determined the amino acid score. Protein efficiency ratio (PER) was also used to evaluate protein quality using standard methods (AOAC, 1975). Products were freeze-dried for 24 hr., ground to a fine powder and incorporated into the diet at a level calculated to provide 10% protein.

The bread diet and its casein control were formulated to contain 8% fat according to standard procedure. However, because the biscuits had high fat content, the biscuit-containing diet and its casein control had a fat content of 17.75%. To formulate the experimental diets, the amount of bread or biscuit necessary to obtain the desired amount of protein was determined. From proximate analysis of the freeze dried bread and biscuit, the fat, fiber, ash, moisture and carbohydrate provided by the bread or biscuit were calculated. Corn oil, fiber (alphacell, ICN), Ash (Jones Foster Mineral Mix, ICN) and carbohydrate (sucrose: dextrose: corn starch; 1:1:1) were added as needed to achieve the desired diet composition (Table 2).

Four groups of experimental rats were used. Ten male Sprague-Dawley weaning rats (Charles River Lab. Wilmington, MA) weighing 40–50g were assigned to each group to test diets of: bread, casein bread control, biscuit, and casein biscuit control. Initial mean weights did not differ by more than 3g between groups. Animals were housed individually and food and water were available ad libitum. Weight gain and food intake were recorded throughout the 28 day test period.

Table 5—Sensory evaluation scores for bread and biscuits made with breadfruit-containing composite flour

Breadfruit (%)	Protein (%)	Mean sensory scores[a]					
		Bread			Biscuits		
		Color	Texture	Flavor	Color	Flavor	Texture
	Whey						
5	10	3.7 ± 1.0	3.3 ± 1.1	4.5 ± 0.3	3.7 ± 0.7	4.3 ± 0.8	4.7 ± 0.0
10	5	5.2 ± 1.0	5.6 ± 0.9	5.5 ± 1.6	4.6 ± 0.4	3.3 ± 0.1	4.7 ± 0.7
15	5	4.6 ± 1.7	4.1 ± 1.6	4.3 ± 1.7	4.1 ± 1.0	4.5 ± 0.3	4.5 ± 0.9
15	10	5.2 ± 1.7	4.5 ± 1.1	3.0 ± 1.1	3.3 ± 0.6	3.6 ± 0.6	4.3 ± 0.9
20	5	4.1 ± 1.4	4.7 ± 1.4	3.6 ± 1.3	5.6 ± 0.4	3.8 ± 0.2	4.5 ± 0.2
20	10	4.6 ± 1.1	4.5 ± 1.7	3.8 ± 1.6	3.0 ± 0.1	2.8 ± 0.8	3.1 ± 0.1
	Soy protein						
5	10	4.6 ± 1.4	3.8 ± 1.1	4.5 ± 1.4	4.5 ± 0.8	4.0 ± 0.9	4.0 ± 0.1
10	5	4.7 ± 1.5	4.7 ± 1.0	4.1 ± 0.8	5.0 ± 0.1	4.2 ± 0.4	4.6 ± 0.6
15	5	3.6 ± 1.2	3.5 ± 1.6	4.5 ± 1.4	4.3 ± 0.7	3.7 ± 0.0	3.6 ± 0.2
15	10	4.7 ± 1.8	3.3 ± 1.6	3.0 ± 1.7	4.2 ± 0.2	3.2 ± 0.6	4.5 ± 0.9
20	5	4.1 ± 1.5	5.1 ± 1.6	3.0 ± 1.1	4.0 ± 0.7	4.1 ± 1.0	4.6 ± 0.5
20	10	4.3 ± 1.5	3.5 ± 0.9	2.7 ± 1.0	5.5 ± 0.6	4.7 ± 0.9	4.1 ± 0.6
	Peanut meal						
5	10	3.5 ± 0.9	3.3 ± 1.1	4.5 ± 1.3	2.7 ± 0.9	2.8 ± 0.2	3.6 ± 0.6
10	5	4.5 ± 1.1	4.5 ± 0.9	4.2 ± 1.9	4.0 ± 0.8	3.1 ± 0.6	3.5 ± 0.9
15	5	3.6 ± 1.9	3.5 ± 1.4	3.2 ± 1.0	3.2 ± 0.0	3.2 ± 0.9	3.5 ± 0.2
15	10	3.8 ± 1.7	3.3 ± 1.6	3.0 ± 1.7	3.0 ± 0.9	4.5 ± 0.2	2.7 ± 0.2
20	5	3.0 ± 0.9	4.0 ± 1.5	3.0 ± 1.2	3.6 ± 0.7	4.5 ± 0.5	4.5 ± 0.6
20	10	3.2 ± 1.8	4.1 ± 2.2	3.1 ± 2.2	3.2 ± 1.0	4.1 ± 0.1	5.0 ± 0.9

[a] A score of 5 indicates that the product rating was equivalent to a standard product containing wheat flour only.

Table 6—Essential amino acid content of breadfruit and white flour and bread and biscuits made from breadfruit-containing composite flours

Amino acid	FAO/WHO/UNU pattern[a]	Breadfruit flour	Wheat flour	Bread[b]	Biscuit[b]
		mg/g protein			
Isoleucine	28	43	45	40	39
Leucine	66	72	91	87	79
Lysine	58	81	19[c]	29	43
Methionine and Cystine (SAA)	25	6	49	17	9
Phenylalanine and Tyrosine	63	68	93	86	78
Threonine	34	40	27	31	24
Tryptophan	11	8	8	9	13
Valine	35	54	50	45	38

[a] FAO/WHO/UNU Expert Consultation (1985)
[b] Bread (10% breadfruit flour, 5% whey); biscuit (10% breadfruit flour, 5% soy protein)
[c] Values underlined indicate the limiting amino acid; amino acid score = breadfruit flour, 24; wheat flour, 49; bread, 50; biscuit, 36.

Differences between groups were evaluated for statistical significance using a Student's t-test.

RESULTS & DISCUSSION

PROXIMATE composition of breadfruit, edible soy protein, peanut meal and whey are shown in Table 3. Values for protein, fat, fiber and ash for breadfruit were similar to those reported by Graham and deBravo (1981) for pulp of the mature fruit. Breadfruit flour contained 4.4% protein, about one third of that reported for wheat flour (Johnson and Mattern, 1977). Therefore, in areas where protein sources are limited and wheat products normally provide significant amounts of dietary protein, it would be necessary to supplement breadfruit with a protein source such as soy protein, whey, or peanut meal in order to provide a level of protein equivalent to that of wheat. Addition of such proteins could also be beneficial in improving protein quality through improved amino acid balance.

When composite flours were used to prepare bread and biscuits at each level of breadfruit flour, those prepared with composite flours were judged less acceptable than the standard product containing only wheat flour (Table 4). Addition of 20% breadfruit flour to bread resulted in a product judged least acceptable in color and flavor. The level of breadfruit had less effect on sensory scores for biscuits than for bread. At a 10% level of breadfruit in biscuits, only the value for color was statistically different.

Individual scores for each product are presented in Table 5. Composite products prepared from flour containing peanut meal were consistently rated lower than comparable products containing soy protein or whey. Bread made with 10% breadfruit and 5% whey had the highest scores and was the only product rated as more acceptable than the standard in color, texture and flavor. This product was selected for further study. For biscuits, the two products with the highest scores contained 10% breadfruit, 5% soy and 20% breadfruit, 10% soy. Overall, 10% breadfruit products were most acceptable to the taste panel and the biscuit containing 10% breadfruit, 5% soy was selected for evaluation of protein quality.

Table 6 compares the essential amino acid content of breadfruit and wheat flour and of products containing composite flours to the amino acid scoring pattern (FAO/WHO Consultation, 1990). Breadfruit flour was most limiting in the sulfur-containing amino acids (SAA), while wheat flour was limiting in lysine. Combining breadfruit and wheat should improve protein quality with breadfruit supplying lysine and wheat supplying the SAA. The lysine contents of the composite flours were higher than that of wheat flour but did not meet the level in the scoring pattern. SAA, on the other hand, were lower in composite flours than in wheat flour and again did not meet the scoring pattern. In biscuits, the SAA were the most limiting amino acids while lysine was most limiting in bread. On the basis of essential amino acid content, both products appeared to be of low protein quality.

Two PER trials were performed (Table 7). To minimize interassay variation, PER values for casein controls were adjusted to 2.5 (Hopkins, 1978) and values for the composite flour products were adjusted proportionately. Combining the data for both trials, the adjusted PER for products made with breadfruit containing composite flours was 2.27 ± 0.22 for biscuits and 0.86 ± 0.08 for bread. Biscuits had a protein quality similar to that of the casein control. In contrast, the PER of bread made with composite flour was significantly lower ($p \leq .05$) than the control value. Although lower than that of biscuits, the PER obtained with bread made from the wheat, breadfruit and whey combination was similar to that reported for bread made with only white wheat flour (0.93) (FAO, 1973). An explanation for the discrepancy between the PER values obtained for biscuit and bread is not apparent as both products had low amino acid scores.

Soy or whey were added to the wheat-breadfruit mixture to improve the protein quality of the composite flours. Whether this goal was achieved is difficult to assess because of the lack of agreement in results between the two methods used to evaluate protein quality. The addition of soy resulted in a PER similar to that obtained with the casein control, but the amino acid score of the mixture was low.

The PER assay is the official method for evaluating protein quality in the United States and Canada, however the limitations of the method have been noted (Sawar and McDonough, 1990). Replacement of the PER with a protein digestibility-corrected amino acid score as the official method for routine evaluation of protein quality has been recommended (FAO/WHO Expert Consultation, 1990). Although the digestibility of our breadfruit containing wheat products was not determined, soy protein, milk protein and white wheat flour appear to be highly digestible (FAO/WHO Expert Consultation, 1990). The digestibility of breadfruit flour is not known, however, biscuit and bread did not differ in the percent of breadfruit added. Thus, digestibility of the breadfruit flour would not explain differences in estimates of protein quality obtained by the two methods.

CONCLUSIONS

BREADFRUIT flour could reduce by 10% the amount of wheat used in home prepared Western style bread and biscuits. Thus, in areas where breadfruit is grown, use of locally prepared breadfruit flour has the potential to decrease dependence on imported wheat and to stimulate use of a local crop. Since the protein content of breadfruit is lower than that of wheat, soy protein or whey may be needed to increase protein content and

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Table 7—Protein efficiency ratio (PER) for breadfruit containing bread and biscuits[a]

	Trial 1		Trial 2	
	PER	A-PER[b]	PER	A-PER
Biscuit	2.06 ± 0.32[c]	2.33 ± 0.21	2.47 ± 0.37[c]	2.22 ± 0.24
Casein Control	2.21 ± 0.31[c]	2.50 ± 0.22	2.78 ± 0.16[d]	2.50 ± 0.28
Bread	0.84 ± 0.25[d]	0.94 ± 0.07	0.93 ± 0.35[e]	0.78 ± 0.09
Casein Control	2.24 ± 0.20[c]	2.50 ± 0.22	2.97 ± 0.14[f]	2.50 ± 0.30

[a] Bread (10% breadfruit flour, 5% whey); biscuit (10% breadfruit flour, 5% soy)
[b] Adjusted PER: PER adjusted so that casein control equal 2.5.
[c-f] Values with different superscript are significantly different (≤ 0.05)

Amino Acid Metabolism by Yeasts and Lactic Acid Bacteria during Bread Dough Fermentation

CONCEPCIÓN COLLAR, ARTURO F. MASCARÓS, and CARMEN BENEDITO DE BARBER

ABSTRACT

Metabolism of 22 free amino acids (AA) during fermentation (early and later steps) of wheat dough samples started with pure and associated cultures of yeast and lactic acid bacteria, and commercial compressed yeast (CY) were investigated. Unfermented and fermented straight doughs were studied by Reversed-Phase HPLC of their dansyl derivatives. Used as starters were strains of *Saccharomyces cervisiae, Lactobacillus brevis, Lactobacillus plantarum* and *Enterococcus faecium*. Statistical data analysis indicated clustering of samples with yeast and samples without yeast (including uninoculated doughs) respectively, based on rate of metabolism of acidic, basic, aliphatic and aromatic AA. Differences in AA metabolism during the later fermentation step (DF2) categorized bacterial starters into groups according to balance between degree of assimilation (DF2 < 0) and exoproteolytic release (DF2 > 0) of amino acids.

Key Words: wheat, bread, fermentation, amino acid, metabolism

INTRODUCTION

DURING bread dough fermentation, involved microorganisms–yeast and lactic acid bacteria (LAB)–develop metabolic activities that govern dough functional properties and bread quality (Foramitti and Mar, 1982; Spicher and Stephan, 1982). The dynamics of basic metabolites of dough starters are influenced by different factors, which specific and overall action are little known. The species, strain and percent of inoculum as well as composition of growth media, presence and/or absence of some constituents and metabolites, specific enzyme activities, and variability of processing conditions, promote, inhibit or modulate metabolism of dough microorganisms (Spicher, 1983; Salovaara and Valjakka, 1987).

Among fermentation metabolites, amino acids are important in the metabolism of dough microoganisms, nutritional requirements and proteolytic activities (Baca and Golebiewski, 1977; Spicher and Schröder, 1979). They also serve as an important source of bread flavor precursors (Spicher and Nierle, 1988). During dough fermentation, some species of *Saccharomyces* (Marder et al., 1977), lactobacilli–*Lactobacillus brevis var. lindneri, L. fructivorans* and *L. farciminis*–(Spicher and Schröder, 1979) and streptococci–*Streptococcus termophilus*–(Desmazeaud and Hernier, 1973) evidence an important demand for amino acids.

On the other hand, the flour (Spicher and Nierle, 1988), the yeast (Dalaly and Al-Banna, 1983), and mainly LAB (Thomas and Mills, 1981; El-Soda et al., 1982; De Giori et al., 1985) contain proteases and peptidases. Their specific hydrolytic action on dough proteins during fermentation release amino acids and peptides. The microbial mass of microorganisms can also supply low molecular weight nitrogen metabolites by cellular lysis (Thorn, 1971; Rothenbuehler et al., 1982; Collar et al., 1991a). The release of amino acids and peptides by enzymatic reactions (MacWilliams and Clapperton, 1969; Spicher and Nierle, 1984a) and by metabolism of sugars (Okuhara and Harada, 1971) and other components (Nisbet and Payne, 1979), stimulates or inhibits the synthesis of proteases of some species of streptococci (Westhoff and Cowman, 1970). It also promotes the proteolytic activities of certain lactobacilli (El-Soda et al., 1978). The assimilation of amino acids promotes growth of microorganisms (Konova et al., 1986), increases fermentative activity and alcohol tolerance of yeasts (Rivareva et al., 1983) as well as lactic acid production of lactobacilli and streptocci (Desmazeaud and Hernier, 1972). These factors affect the sensory (Rothe and Ruttloff, 1983) and nutritional (Kasilova et al., 1973) quality of bread.

The dynamics have been reported in individual low molecular weight nitrogen metabolites during wheat dough fermentation, and the contribution of the inoculum of microorganisms to the nitrogen pattern (Collar et al., 1991a) of doughs started with pure cultures of yeast (Collar et al., 1989) and LAB (Collar et al., 1990; 1991b). Despite the interest in metabolism of amino acids during fermentation of selected microorganisms in connection with controlled flavor and performance of bread, few papers concerning wheat doughs have been reported. Since dough fermentation is conducted by associated cultures of microorganisms, our objective was to study the synergistic, antagonistic and/or indifferent effects of mixed populations of yeast and LAB on the metabolism of amino acids, in order to better understand starters for future applications in breadmaking technology.

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MATERIALS & METHODS

Starters composition and dough preparation

Cultures of pure strains of yeast (*Saccharomyces cerevisiae*, P-38) heterofermentative (*Lactobacillus brevis*, A-1) and homofermentative (*L. plantarum*, B-1, *Enterococcus faecium*, C-1) LAB, were prepared according to Barber et al., (1989). Bread doughs were elaborated by a straight dough process (Fig. 1). The dough formula consisted of 100 g wheat flour (energy of deformation W = $143 \cdot 10^3$ erg, curve configuration ratio P/L = 0.45; 11.8% protein and 14.90% moisture), 57.2 mL water, 1.8 g salt and pure and mixed cultures of yeast and LAB. Starter composition and dough formula are summarized in Table 1. Ingredients were mixed for 12 min in a 10 kg arm mixer at 60 turns/min, to obtain unfermented doughs (UD). Fermented doughs (FD) were obtained after bulk fermentation and proofing. In addition, uninoculated doughs and those containing bacterial starters were allowed to stand for 24 hr at 28°C to obtain long-time fermented doughs (LFD).

Extraction and purification of free amino acids

Freeze-dried UD, FD, and LFD (10g) were each extracted twice with 20 mL 40% ethanol (v:v). After centrifugation, deproteinization (Collar et al., 1991a), and chromatografic purification (Benedito de Barber et al., 1989; Collar et al., 1991b), dried extracts were derivatized and used for amino acid determination (Fig. 2)

Determination of free amino acids

Amino acid determination was carried out by reversed-phase high performance liquid chromatography (RP-HPLC) of their dansyl derivatives (Prieto et al., 1990). Qualitative identification was performed by comparing retention times of a standard mixture of 20 dansylated protein amino acids, γ-aminobutyric acid and ornithine (Benedito de Barber et al., 1989). Quantification was accomplished by using the internal standard addition method (norvaline)(Benedito de Barber et al., 1989; Collar et al., 1991a,b).

A Hewlett-Packard (Palo Alto, CA) HPLC system, composed of a 1050 pumping system, a rheodyne injector, a 1040 A diode array detector set at 254 nm, a 9000 Pascal Chem Station and an oven set

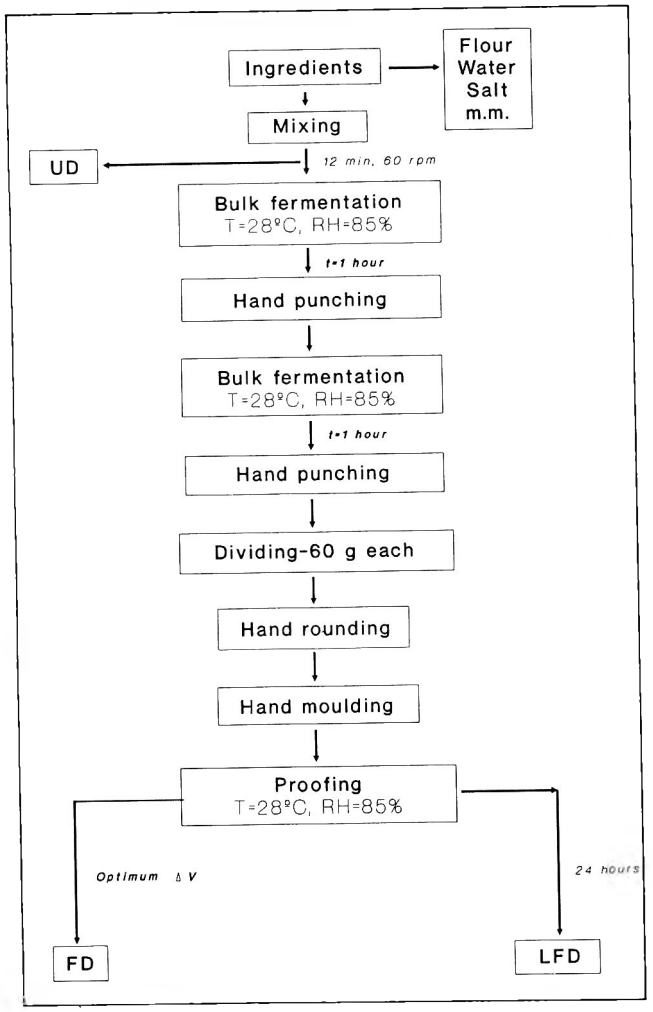

Fig. 1– Straight dough process used to obtain unfermented (UD), fermented (FD) and long-time fermented (LFD) wheat bread doughs.

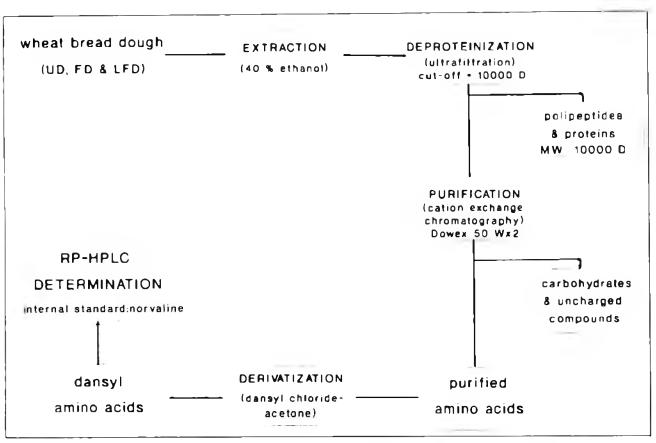

Fig. 2–Scheme of extraction, purification and determination of amino acids in wheat bread doughs.

at 30°C, was used. An ODS HS/3 column (3 μm particle size, 8.3 cm length, 4.6 mm i.d.) from Perkin-Elmer (Norwalk, CT) and a precolumn (5 μm, 2 cm, 4.6 mm) from Hewlett-Packard, were used as stationary phase. Separation of dansyl amino acids was conducted under gradient conditions. Mobile phase consisted of buffer 12 mM K_2HPO_4, pH 7 (A) and acetonitrile (B). Gradient program was from 10% to 43.6% B in 42 min, up to 70% B in 3 min, and isocratic elution during 10 min for washing purposes. Flow rate was 1.50 mL/min.

Table 1 – Starter composition and dough formula

Ingredients							
				Starter[a]			
Dough[b]	Flour (g)	Water (mL)	Salt (g)	*S. cerevisiae* (g)	*L. Brevis* (mL)	*L. plantarum* (mL)	*E. faecium* (mL)
D-1	100	57.2	1.8	4	1	–	–
D-2	100	57.2	1.8	4	–	1	–
D-3	100	57.2	1.8	4	–	–	1
D-4	100	57.2	1.8	4	0.5	0.5	–
D-5	100	57.2	1.8	4	0.5	–	0.5
D-6	100	57.2	1.8	4	–	0.5	0.5
D-7	100	57.2	1.8	4	0.33	0.33	0.33
D-8	100	57.2	1.8	4	–	–	–
D-9	100	57.2	1.8	–	1	–	–
D-10	100	57.2	1.8	–	–	1	–
D-11	100	57.2	1.8	–	–	–	1
D-12	100	57.2	1.8	–	–	–	–
D-13	100	57.2	1.8	–	0.5	0.5	–
D-14	100	57.2	1.8	–	0.5	–	0.5
D-15	100	57.2	1.8	–	–	0.5	0.5
D-16	100	57.2	1.8	–	0.33	0.33	0.33

[a] Yeast contained $1–2·10^{10}$ viable cells/g; lactic acid bacteria, $1–2·10^{10}$ viable cells/mL culture.

[b] A reference dough (D-0) started by compressed yeast (CY) was used (2g CY/100g, flour, moisture base)

Statistical treatment

Two parallel experiments and three replicates per experiment were made. Data of individual amino acid levels in UF, FD and LFD were statistically analyzed in a Microvax computer (Digital, Northboro, MA) using programs of the BioMedical statistical Package (BMDP). Univariate analysis (two-way analysis of variance) was performed to calculate significant interactions (starter × fermentation period), by applying 7D program (Tukey test). Multivariate data handling procedure (factor analysis) was conducted in order to classify starters on the basis of the differences during fermentation in individual amino acid levels, by applying 4M program.

RESULTS

GOOD RESOLUTION and reproducibility was achieved in the separation of 22 individual free amino acids in all samples. Quantification of total and individual amino acids in samples were summarized (Tables 2 and 3). The coefficient of variation for relative retention time was <1%, and for response factors, <6% for most amino acids. Variability in amino acid quantification was similar in all doughs to that for response factors in the standard mixture. Among doughs, total amino acid content (mg/100g dough, d.b.) ranged from 34.36 mg (D-11) to 89.54 mg (D-1), within UD; from 25.09 (D-6) to 62.21 mg (D13), within FD; and from 34.07 mg (D-9) to 92.24 mg (D-11), within LFD. Similar level of free amino acid (95.05 mg) was observed in 24 hr resting uninoculated doughs (D-12). Grouping amino acids by chemical structure, dicarboxylic acids and their amides, and aliphatic amino acids predominated in all doughs, accounting respectively for 40—60% of the total amino acid content in yeasted doughs (Table 2), and for 50—70% in unyeasted doughs (Table 3). The contribution of the remaining groups of amino acids was always <15%, especially for sulfur amino acids– methionine and cystine–(<1%). With respect to individual levels, prominent amino acids included aspartic, glutamic and γ-aminobutyric acids, alanine and arginine, whereas cystine was present in trace amounts, and methionine showed only noticeable levels (>1.3mg) after long fermentation periods (D-15, D-16), (Tables 2 and 3).

Amino acid metabolism of yeast and LAB

Biochemical changes during dough fermentation in free amino acid content clearly differentiated doughs with and without yeast as microbial starters. After proofing, general declines in total amino acid content around 40 mg/100 g dough, d.b. were

Table 2—Individual free amino acid content of yeasted bread doughs started with pure and associated cultures of S. cerevisiae, L. brevis, L. plantarum *and* E. faecium

Amino acid	Mean content (mg/100 g dough, d.b.)																	
	D-0		D-1		D-2		D-3		D-4		D-5		D-6		D-7		D-8	
	UD[b]	FD	UD	FD	UD	FD	UD	FD	UD	FD	UD	FD	UD	FD	UD	FD	UD	FD
Asp	6.41	0.88*[a]	9.21	1.94*	5.92	1.41*	6.51	0.64*	6.78	0.98*	6.09	2.02*	7.83	0.82*	6.75	1.73*	6.98	1.25*
Glu	6.95	3.40*	13.60	5.34*	8.30	4.56*	11.36	2.49*	6.36	2.47*	8.70	6.86*	10.70	3.17*	10.29	6.12*	10.14	6.85*
Asn	6.29	0.69*	8.27	0.99*	5.18	0.65*	4.59	0.97*	5.01	0.62*	4.83	0.83*	2.22	0.58*	4.83	1.05*	4.27	0.58*
Gln	6.85	1.56*	10.41	1.17*	7.02	2.28*	7.86	1.42*	9.44	3.45*	8.77	2.01*	7.48	0.80*	9.47	2.41*	7.53	2.22*
Ser	2.08	1.50*	4.02	0.75*	1.87	1.07*	1.91	0.41*	1.43	0.72*	1.97	1.51*	1.76	0.55*	1.68	1.01*	1.68	0.66*
Thr	2.78	1.47*	2.69	1.56	2.05	1.58	1.90	1.53	1.37	1.34	3.39	2.05*	0.60	2.07*	1.31	1.18	1.29	1.21
Gly	1.67	2.03	3.26	1.74*	2.20	1.56	2.57	1.35*	1.83	2.37	2.63	1.92*	2.57	0.93*	1.91	1.77	1.92	1.71
Ala	7.99	2.64*	8.84	2.47*	6.60	2.82*	6.99	2.63*	7.12	1.52*	7.61	3.96*	6.80	2.82*	7.00	4.57*	7.70	4.40*
Arg	4.54	4.25	5.63	4.94	6.26	4.66	4.44	5.55	3.80	3.53	4.98	4.05	5.16	2.82*	4.24	4.86	4.69	4.14
GABA	4.21	4.10	4.09	3.34	4.80	2.93*	5.25	3.07*	4.63	4.32	6.88	4.83*	2.33	1.89	6.47	6.65	5.95	4.30*
Pro	2.07	1.82	5.49	1.89*	2.62	2.10	2.60	2.81	2.23	1.52	3.64	2.57*	2.92	1.95*	2.99	2.80	2.81	1.95
Val	2.14	0.82*	2.84	0.88*	2.38	0.96*	2.53	0.41*	2.00	0.56*	2.89	1.01*	2.46	0.57*	2.38	0.90*	2.00	3.28*
Met	0.40	–	0.45	–	0.41	0.18	0.30	–	0.43	–	–	–	0.31	0.03	0.38	–	0.25	0.20
Ile	1.25	0.14*	1.02	0.22*	0.90	0.23*	1.29	0.34*	1.13	0.21*	1.26	0.40*	1.06	0.16*	1.03	0.23*	1.04	0.21*
Leu	1.07	0.42*	1.17	0.19*	1.58	0.22*	1.64	0.25*	1.38	0.30*	1.57	0.48*	1.34	0.25*	1.41	0.43*	1.37	0.28*
Trp	4.59	1.24*	3.58	1.10*	3.24	0.90*	3.50	2.40*	4.59	1.75*	4.16	1.67*	3.45	1.12*	3.34	1.97*	3.48	1.00*
Phe	1.02	0.25*	0.96	0.12*	0.84	0.40*	1.08	0.12*	1.11	0.18*	1.14	0.39*	0.96	0.17*	0.94	0.25*	1.05	0.20*
Cys	–	–	–	–	–	0.12	–	–	–	0.23	–	0.44*	–	0.28*	0.10	–	–	–
Orn	0.76	0.63	0.71	1.01	1.20	0.96	0.86	0.81	4.37	0.61*	0.99	1.17	1.15	0.52	0.87	0.83	1.00	1.10
Lys	2.47	0.97*	1.55	1.21	3.52	0.41*	2.68	0.37*	2.42	0.64*	3.10	1.16*	3.18	0.63*	3.60	1.08*	3.34	0.54*
His	0.50	0.40	0.55	0.48	1.08	0.28*	0.86	0.12	0.54	0.26	0.47	0.42	1.51	0.24*	0.75	0.39	1.56	0.27*
Tyr	1.27	0.72	1.21	1.21	1.25	2.23*	1.32	2.57*	1.16	0.47	1.05	0.30	1.65	2.73*	1.33	2.12	1.82	1.36
Total	67.2	29.9*	89.5	32.0*	69.2	32.4*	72.0	29.4*	65.7	28.1*	76.1	40.0*	68.7	25.1*	73.0	42.4*	72.4	34.9*

[a] Symbols and formula used for starters as in Table 1.

[b] UD: unfermented dough; FD: fermented dough (after proofing).

* Indicates a statistically significant difference (95%) between mean value for FD and UD.

denoted within doughs containing yeast (Table 2). In contrast, within doughs inoculated with bacterial starters (Table 3), no significant changes in AA level were observed after proofing, except for the increase ($P<0.01$) in those started with binary mixtures of homo and heterofermentative LAB (D-13, D-14).

Doughs containing yeast as starter

After proofing, doughs containing *S. cerevisiae* (D8) and commercial compressed yeast (D-0), underwent variable decreases in levels of all groups of amino acids, mainly dicarboxylic acids and amides (−78% D-8, −75% D-0, Table 2). Big declines in individual amino acids (D-8, D-0) were observed for asparagine (−86, −89%), aspartic acid (−82, −86%), glutamine (−70, −77%), lysine (−61, −84%) and glutamic acid (−32, −51%). These amino acids are rapidly absorbed by *S. cerevisiae* strains (Jones and Pierce, 1964; Rose and Harrison, 1987). In addition, alanine and tryptophan are also metabolized in high extent. No significant changes were detected in glycine, arginine, proline and ornithine contents.

During proofing, doughs started with associated cultures of yeast P-38 and LAB A-1, B-1, C-1, (D-1 to D-7) followed, in general, the same behavior reported for doughs containing pure cultures of P-38 (D-8) and CY (D-0), respectively (Table 2). The only difference concerned the significant decrease in proline (D-1, D-5), and arginine (D-6). In contrast, insignificant changes were observed in both amino acid levels during fermentation of doughs started with individual microorganisms (D-8 to D11). Consequently, a synergistic effect on metabolism of proline and arginine was induced among strains of yeast and LAB.

Uninoculated doughs and those with bacterial starters

In doughs with no added starter (D-12), the resting period led to an increment of the total amount of amino acids by 80%. Up to 4 hrs, the amount of γ-aminobutyric (+67%), aliphatic- (+38%) and aromatic- (+82%) amino acids increased (Table 3). Further resting period up to 24 hrs induced increase of those same amino acids (+69, +117, +49%, respectively), In addition, a significant increase ($P<0.05$) was observed for basic- (267%) and sulfur-amino acids.

In doughs started by pure strains of LAB (D-9, D-10, D-11), main amino acids (dicarboxylic acids and amides, aliphatic- and basic, are responsible for 90—95% of the changes registered in total amino acid content. Higher absolute values corresponded in general to long-time fermented doughs (LFD), except for dicarboxylic acids and amides of doughs started by both lactobacilli (Table 3). For individual amino acids the fall in some suggested that they were assimilated in higher extent than they were released by proteolysis. The drop in level of glycine–20% (D-9), 35% (D-10), 10% (D-11)—showed a strong demand for this amino acid at the beginning of fermentation (0-4 hr) by the strains of LAB considered. Asparagine is consumed later (4—24 hr), mainly by *E. faecium* (−60%). Contents of glutamic acid, asparagine, tryptophan and proline, described as essential amino acids for some strains of *L. brevis* (Spicher and Schröder, 1979) were depleted by 15% (glutamic acid) and 100% (tryptophan) during the later fermentation steps (Table 3). This heterofermentative lactobacillus also metabolizes glutamine (−22%) and serine (−70%) from 0 to 4 hr, and aspartic acid (−45%) and alanine (−66%) throughout the fermentation. Doughs fermented by *L. plantarum*, B-1 (D-10) showed significant ($P<0.05$) metabolism of aspartic acid (−35%), glutamic acid (−25%), glutamine (−70%), serine (−65%), alanine (−40%) and threonine (−80%) with 0 to 4 hr fermentation. Proteolytic activity of individual LAB was evidenced by the increase in level of some protein amino acids. Valine, leucine and lysine contents gradually increased up to 24 hr fermentation. The level of arginine, essential amino acid for the majority of LAB (Spicher and Schröder, 1979) only increased significantly in doughs started with *L. plantarum*, B-1 (D-10) during the second period of fermentation. On the opposite side, in doughs with no added starter (D-12), arginine gradually increased throughout fermentation (Table 3).

However, among doughs started with associated mixtures of LAB (D-13 to D-16) dynamics during fermentation in aliphatic amino acids accounted for 30—40% of total changes. Doughs containing binary mixtures of *L. brevis*, A-1 (D-13, D-14) showed a constant increase in aliphatic amino acids during fermentation. Whereas, dynamics in doughs started by the

Table 3 – Individual free amino acid content of unyeasted bread doughs started with pure and associated cultures of L. brevis, L. plantarum *and* E. faecium

Amino acid	Mean content (mg/100 g dough, d.b.)																					
	D-9			D-10			D-11			D-12			D-13			D-14			D-15			D-16	
	UD[b]	FD	LFD	UD	FD	LFD	UD	FD	LFD	UD	FD	LFD	UD	FD	LFD	UD	FD	LFD	UD	FD	LFD	UD	FD	LFD
Asp	7.32	5.97*[a]	4.26*	10.55	7.01*	7.20	5.18	14.02*	18.88*	6.00	5.04	6.74*	4.49	6.19*	6.96	4.76	7.03*	5.55*	7.10	7.87	5.65*	3.94	5.16	5.05
Glu	5.43	5.30	4.51	5.18	3.91	6.06*	5.71	7.52*	12.35*	4.19	2.84*	5.74*	3.06	2.30	5.43*	3.05	3.29	5.63*	4.63	3.73	5.96*	2.63	2.50	5.32*
Asn	3.46	2.49*	1.67	3.61	2.34*	1.92	3.76	4.46	1.68*	5.97	5.78	5.22	4.38	5.27	3.42*	4.03	4.22	4.55	5.38	4.48	4.50	3.29	3.12	4.53*
Gln	1.38	1.01	1.17	1.23	0.34	0.77	1.21	1.19	1.25	4.57	3.67	1.84*	4.07	4.89	2.51*	4.38	2.48*	1.77	7.06	4.59*	1.56*	3.92	2.78	2.37
Ser	1.38	0.40*	0.31	1.13	0.37*	1.21[d]	1.00	1.30	2.23*	1.45	0.92*	1.31	1.14	1.21	0.47*	0.98	1.51*	0.30*	1.32	1.09	0.52*	0.88	0.84	0.43*
Thr	0.85	0.62	1.10	0.81	0.11	0.60	0.68	0.90	2.23*	1.12	2.04	1.71	3.34	2.57	0.98*	0.63	1.40	0.64	1.98	0.70*	0.92	0.41	1.29	0.94
Gly	2.07	1.62	1.39	1.13	0.72	1.59*	2.16	1.95	3.10*	1.30	1.69	2.15	1.10	1.61	2.03	1.34	4.32*	1.83*	1.21	1.56	1.71	1.21	0.89	1.40
Ala	3.83	2.57*	1.31*	2.77	1.58*	2.47	3.52	3.58	5.17*	2.92	3.67	8.98*	4.07	4.75	2.69*	3.40	4.90*	2.67*	3.45	3.55	2.35*	2.81	2.48	1.64
Arg	1.07	0.62	—	1.33	1.75	5.03*	0.82	1.12	2.25	1.77	3.80*	12.54*	3.71	4.18	5.55	1.25	2.64	3.69	1.99	3.17	2.06	0.77	2.36	2.39
GABA	1.13	1.41	0.79	1.07	1.71	1.80	1.52	1.40	1.95	2.58	4.30*	7.28*	2.93	6.69*	6.56	1.91	5.91*	5.24	3.15	4.96*	5.40	2.14	4.69*	5.48
Pro	1.56	1.97	1.48	0.99	1.29	1.57	1.39	1.54	3.13*	1.66	2.30	3.16	1.70	2.69*	3.73*	1.33	1.85	2.47	1.89	1.50	2.10	1.02	1.42	2.59*
Val	1.49	1.63	2.26	1.14	1.25	2.77*	1.40	1.57	5.13*	2.00	3.28*	5.19*	1.79	2.65*	3.73*	1.46	3.86*	4.38	2.02	1.96	4.57*	1.69	2.05	5.70*
Met	0.15	0.19	—	—	0.47*	0.14	—	0.35	1.05*	0.37	0.44	1.29*	0.11	0.63*	0.93	0.31	0.49	1.19*	0.12	0.41	1.35*	0.36	0.53	1.35*
Ile	1.00	0.89	1.54*	0.79	0.68	2.16*	0.78	0.86	2.75*	0.88	0.99	1.92*	1.03	1.45*	2.31*	0.91	1.43*	2.66*	1.06	0.95	2.14*	0.85	0.84	2.31*
Leu	2.09	3.32*	5.29*	1.35	2.69*	6.61*	1.67	1.86	10.34*	1.27	1.93*	6.78*	1.34	2.51*	8.10*	1.17	2.88*	9.63*	1.43	2.01*	8.25*	1.17	1.67	8.14*
Trp	0.97	1.15	—	0.63	0.89	0.61	1.15	1.12	1.46	2.99	4.21*	5.26*	3.96	4.79*	4.99	3.41	4.97*	4.87	3.74	3.26	3.87	2.91	3.07	3.95*
Phe	1.11	1.11	2.05*	0.71	0.62	2.74*	1.00	1.01	4.95*	0.84	1.51*	5.45*	0.93	1.60*	3.92*	0.87	1.76*	4.10*	1.04	1.23	3.51*	0.77	1.06	3.83*
Cys	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.05	—	—	—	—	—	—	0.17	—
Orn	1.04	1.29	2.14	0.17	0.08	0.61	0.32	0.65	5.21*	0.14	0.28	4.13*	0.13	0.39	5.01*	0.17	0.81	4.35*	0.19	0.59	3.35*	0.15	0.60	4.00*
Lys	1.47	1.73	1.99	1.30	1.52	3.46*	0.74	1.43	4.47	1.02	1.38	4.16*	0.95	2.10*	4.65*	0.95	1.71*	3.98*	1.09	1.37	3.17*	0.79	1.61*	3.85*
His	0.35	0.42	0.46	0.35	0.29	0.45	0.24	0.43	1.00	0.63	1.19	3.55*	0.21	1.38*	1.58	0.83	—	1.61*	0.95	1.57	1.29	0.80	1.98*	2.24
Tyr	0.76	0.61	0.34	0.58	0.33	1.51*	0.37	0.79	1.66	0.35	1.91*	0.65*	0.72	2.21*	1.66	0.68	1.24	1.05	0.96	1.46	1.92	0.29	1.79*	0.46*
Total	39.9	36.3	34.1	36.8	29.9	49.4*	34.4	49.0	92.2*	44.0	53.2	95.1*	45.1	62.2*	77.2	38.1	58.7*	72.0	51.7	51.0	66.2	32.8	31.4	68.0*

[a] Formula used for starters as in Table 1.
[b] UD: unfermented dough; FD: fermented dough (after proofing); LFD: long-time fermented dough (24 hr).
[c] Indicates a statistically significant difference (95%) between mean value for FD and UD.

two homofermentative strains (D-15) and by the ternary mixture of LAB (D-16), took place mainly during the second period (4—24hr, Table 3).

Predominant dicarboxylic acids and amides did not undergo noticeable changes during fermentation. Aspartic acid, described as an essential amino acid for enterococci (Bracquart and Lorient, 1979) was released first (0-4 hr), and metabolized later (4-24 hr) in doughs started by *E. faecium*, C-1 (-21% (D-14), -28% (D-15)). Glutamic acid, essential amino acid for lactobacilli (Spicher and Schröder, 1979), and enterocci (Reedy and Ranganathan, 1983) was accumulated with 4 to 24 hr in all doughs resulting in net increases from 30% (D-15) to 100% (D-16). Asparagine content did not undergo significant changes, but glutamine levels were depleted in all doughs from 38% (D-13) to 78% (D-15). Increasing trends in basic amino acids during fermentation were observed in all doughs. The extent of such changes depended on the inoculum (Table 3). Lysine and histidine, amino acids stimulating the growth of some strains of enterococci (Bracquart et al., 1978; Reedy and Ranganathan, 1983), and essential for some lactobacilli species (Spicher and Schröder, 1979), accumulated in all LFD. Arginine followed a similar trend, but it was assimilated by *E. faecium* (Reedy and Ranganathan, 1983). Consequently, fermented doughs without *E. faecium* as microbial starter yielded higher levels of this amino acid -5.5 mg (D-13). With respect to minor amino acids, aromatic-, cyclic-, sulfur- and γ-aminobutyric acid accumulated in LFD, whereas hydroxy-amino acids showed a decreasing trend, mainly up to the later period of fermentation (Table 3).

DISCUSSION

BIOCHEMICAL DYNAMICS in 22 individual amino acids during bread dough fermentation (early and later steps) characterize metabolic activities of yeast and LAB starting strains. Metabolism of predominating amino acids during proofing—dicarboxylic acids and their amides, aliphatic- (except alanine), lysine and histidine, phenylalanine and tryptophan and γ-aminobutyric acid–clearly differentiated doughs with (D-0 to D-8) and without yeast (D-9 to D-16, including uninoculate doughs) on the rate of metabolism of these same amino acids, (Tables 2 and 3). At the beginning of fermentation, yeast *S. cerevisiae* rapidly reached the log phase, and assimilated nitrogen due to cell growth and multiplication (Pekur and Bur'yan, 1981), in good accordance with the high consumption and rapid assimilation during proofing of some amino acids in yeasted doughs. Proline (predominant amino acid of gluten protein, (Pomeranz, 1971) is slowly assimilated by *S. cerevisiae* as pointed out before (Rose and Harrison 1987). The addition of LAB strains to *S. cerevisiae* as fermentation starters, do not modify noticeably the degree of metabolization of main amino acids, but a synergistic effect on arginine and proline assimilation is induced.

On the other hand, during proofing LAB still have a long lag phase (Morimoto, 1966) and develop metabolic activities at a slow rate. In this respect, homo- and heterofermentative species only evidence early requirements for glycine, which was significantly depleted after 4 hr fermentation. Differences in amino acid metabolism during the later fermentation step (with 4 to 24 hr) categorize amino acids into two groups on the basis of their dynamics during this period. The first group includes aspartic and glutamic acids, isoleucine, leucine, lysine, ornithine, proline, phenylalanine and methionine; whereas, the second group comprises alanine, γ-aminobutyric acid, arginine and histidine. Trends observed for both groups classify uninoculated and bacterial doughs in four groups according to the balance between degree of assimilation and exoproteolysis. Depletion in levels of the first group of amino acids predominates in fermented doughs started with pure lactobacilli (D-9, D-10) mainly with *L. brevis*, A-1, in good accordance with the nutritional exigences on essential amino acids described for

that microorganism (Spicher and Schröder, 1979). Doughs containing *E. faecium*, C-1 (D-11) and uninoculated samples (D-12) undergo the highest accumulation of amino acids included in the first and second groups, respectively.

Accumulation of amino acids during fermentation of rye sour doughs has been previously reported and attributed to proteolysis action of specific proteases from LAB species on high molecular weight nitrogen compounds (Spicher and Nierle, 1984b; 1988). In addition, the effect of hydrolytic action on dough proteins by flour enzymes and flour endogenous microflora (Grant and Wang, 1972; Spicher and Nierle, 1988) must be considered. This explanation is supported by the significant increase in total and some individual amino acids observed in doughs with no added starter (D-12) after resting. Doughs started with associated mixtures of LAB (D-13 to D-16) constitute a cluster characterized by a slight production of the first group of amino acids and a slight assimilation of the second group of amino acids.

Results indicate that LAB strains have a strong demand for some free amino acids during bread dough fermentation. The nature, extent and specificity of the absorption depend on both the microorganism and the time of fermentation (Collar et al, 1991b). The presence of two or more strains of LAB modulates the rate of assimilation and production of free amino acids. Accumulation of free amino acids in long-time fermented doughs (overall trend) due to exoproteolysis is closely dependent on the bacterial population. The slow removal of basic amino acids and the release of arginine by yeasts can be important for selection of microbial starters since basic amino acids are most reactive during baking (El-Dash and Johnson, 1970) and enhance bread flavor (Kiely et al., 1960). Since the importance of amino acid metabolism in dough functionality and bread flavor has been recognized (Maga, 1974; Spicher and Nierle, 1988), complementary studies on breadmaking performance of doughs starters (yeast and LAB and associated cultures) will contribute to selection of starters with suitable metabolic activities for bread production.

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Retrogradation Behavior of Wheat Starch Gels with Differing Molecular Profiles

W. ZHANG and D. S. JACKSON

ABSTRACT

Differing molecular profiles of starches were developed by acid treatment of native wheat starch. Molecular weight distribution, branching, and linear chain length distribution was characterized. Starches with fewer large size amylopectin fragments, increased smaller size amylopectin fragments, decreased branch points, decreased overall sizes, and narrower linear chain length distributions, had higher initial retrogradation rates. However, all aged starch gels had similar final crystalline enthalpy levels. Different retrogradation behaviors were due to smaller size amylopectin fragments, narrowed linear chain length distribution, and decreased branching. Retrogradation behavior was modeled using classical Avrami kinetics to allow comparison with other retrogradation studies.

Key Words: wheat, starch, gels, molecular structure, retrogradation

INTRODUCTION

DURING storage of gelatinized starch, extensive reassociation of starch polymers occurs, resulting in crystallite formation and gel firming. This phenomenon, retrogradation (Collison, 1968; Atwell et al., 1988), is of considerable importance to the food industry, since it may cause starch-based foods to have undesirable textural properties (Collison, 1968; Kulp and Ponte, 1981). It is essentially a crystallization process. Retrograded starch has been found to have crystalline X-ray diffraction patterns (Sterling, 1978; Gidley and Bulpin, 1987). Marsh and Blanshard (1988) reported that the maximum crystallinity for 50% wheat starch gel stored at 25°C was 15%.

Schoch and French (1947) pointed out that amylopectin had a major effect on bread-staling (retrogradation), and suggested that linear side chains of amylopectin associated gradually and caused firming. Amylose has less effect because it rapidly associates immediately after baked bread cools. Other researchers have confirmed that during the storage of starch-based foods, amylose crystallizes rapidly and amylopectin crystallizes slowly (Miles et al., 1985; Orford et al., 1987; Ring et al., 1987; Russell, 1987). The melting temperatures for retrograded gel crystallites are 55°C for amylopectin crystallites and 130°C for amylose crystallites (Eberstein et al., 1980).

Limited work has been reported relating starch molecular characteristics and retrogradation behavior. Whistler and Johnson (1948) found there was an optimum chain length (undetermined) for amylose retrogradation; Gidley et al. (1986) found the minimum chain length required for retrogradation was 8 or 9 glucose units. Both results, however, were obtained using starch solutions (0.5–3.0%), rather than gel systems. Sterling (1978) reported that β-amylase treated amylopectin (external branches reduced to 2–3 glucose units) retrograded less readily than native amylopectin.

The Avrami model of polymer crystallization has been widely used to kinetically model retrogradation (McIver et al., 1968; Colwell et al., 1969; Kim and D'Appolonia, 1977; Longton and LeGrys, 1981; Russell, 1987; Marsh and Blanshard, 1988). Levine and Slade (1988), however, have disputed the use of the Avrami model because of the non-equilibrium behavior of starch during recrystallization. A more appropriate theoretical model should rely on measurement of the glass transition state of the starch polymers at given water contents and the Williams-Landel-Ferry equation should be applicable to explain polymer crystallization (1988). Unfortunately, because of instrumental variability, it can be difficult to experimentally determine accurate glass transition temperatures of aqueous polymer systems when the glass transition is near the melting temperature of ice (0°C). Levine and Slade (1988), however, acknowledged the utility of the Avrami equation when used to represent empirical data from retrogradation experiments.

Based on crystalline phase changes vs. time, the Avrami equation is expressed as follows:

$$\Theta = 1 - e^{(-kt^n)} \quad (1)$$

where Θ is the percent crystallized starch at time **t**, **k** is a rate constant, and **n** is the Avrami exponent (Jankowski and Rha, 1986) used to assign corresponding mechanisms of crystallization. The Avrami exponent (**n**) provides qualitative information on the nature of nucleation and growth processes. An exponent of **1** is said to represent rod-like crystalline growth from instantaneous nuclei, and an exponent of **2** can either represent rod-like crystalline growth from sporadic nuclei or disc-like growth from instantaneous nuclei (Sharples, 1966). The rate constant (**k**) is a combined function of nucleation and growth rate, providing quantitative information on the course of crystallization (Sharples, 1966). In order to describe the temperature dependence of crystallization, Marsh and Blanshard (1988) suggested that the Arrhenius equation could be used to set up a relationship between the Avrami rate constant and temperature. Since starch molecular associations are the basic cause of retrogradation, starch molecular sizes, structure, and homogeneity, must influence retrogradation behavior. The objective of this research was to study how starch polymers with different molecular sizes and structures influence retrogradation (crystalline formation) behavior.

MATERIALS & METHODS

Starches

Commercially isolated wheat starch was used to produce starches with differing molecular weight (MW) profiles. Wheat starch (400g) was treated with sulfuric acid (26% w/w, 500 mL) at ambient temperature, with shaking once every 100 min (Kainuma and French, 1972). A portion of the starch slurry (80 mL) was removed at 5, 10, 15, 20, 25, 30, and 35 hr. To adjust the pH of acid treated starch to neutral, 4 L of distilled water were added to each subsample and the resulting suspension held at room temperature for 3 hr to allow starch to settle and protons to diffuse out of the granules (establish an equilibrium). The supernatant was decanted and the washing procedure repeated. The resulting starch slurry was further diluted with distilled water (100 mL slurry, 900 mL water) and centrifuged at 2,300 × *g* for 5 min. In all cases, pH of the final starch suspension was near neutral. The starch sediment was collected and dried in a forced-air oven at 42°C. Starch moistures were determined by AACC method 44-15A (AACC, 1983).

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Characterization of starch MW distribution

High performance size exclusion chromatography (HPSEC) was used to characterize starch MW distributions (Jackson, 1991; Jackson et al., 1989, 1992). Starch suspensions (0.4%, w/v, dry basis) were prepared in test tubes by bringing 0.04 g of starch and 1 mL of deionized distilled water to a volume of 10 mL with methyl sulfoxide (DMSO). Sample test tubes were placed in boiling water for 15 min, and shaken by hand every 3 min. Solutions were filtered through a 5 μm nylon filter and injected (25 μL) into the HPSEC system consisting of four KS-series (806, 804, 803, 802) Shodex Ionpak columns (Showa Denko K. K., Tokyo, Japan) connected in series (column temperature 50°C). Deionized distilled water was used as the mobile phase with the flow rate 1 mL/min. Separated compounds were detected by a refractive index detector (Waters model 410, Millipore Co., Milford, MA). The apparent MWs of starches were determined based on a quadratic regression ($r^2 = 0.999$) of pullulan MW standards.

Debranching starches

Isoamylase [EC 3.2.1.68], purchased from Sigma Chemical Co. (St. Louis, MO; lot# 69F0041), was used to debranch starches. Just prior to use, the enzyme was diluted with deionized distilled water to obtain a concentration of 624 units/mL. Chang Rupp and Schwartz's (1988) method was modified by decreasing the starch concentration from 0.5% to 0.4% in the starch/enzyme/buffer system to obtain better starch dispersion, and 19.5 units of isoamylase/mg starch was used to decrease the reaction time to 15 hr. The enzyme reaction system contained 1.50 mL 1.6% (dry basis) starch solution (in 90% DMSO), 2.22 mL 0.1M acetate buffer (pH 3.80), 0.75 mL isoamylase solution (624 units/mL), and 1.53 mL deionized distilled water.

The enzyme reaction end point was determined by incubating the reaction system in a 40°C water bath for 15, 16, and 17 hr, followed by HPSEC characterization of debranched starch MW profiles. After 15 hr enzymic treatment, the HPSEC MW profiles remained unchanged. Therefore, all starches were treated with isoamylase for 15 hr. The enzyme was inactivated by placing the solution in boiling water for 3 min. The enzyme debranched materials were divided into 2 portions: 1 portion (1.5 mL) for deionization and HPSEC analysis, and the rest (4.5 mL) for reducing power and branch point number analysis.

Deionization and characterization of debranched starches

Because ions in the isoamylase debranched starch samples can interfere (via ion exclusion) with HPSEC results they were removed prior to injection with an ion exchange resin (AG501-X8, Bio-Rad Lab., Richmond, CA). Resin (120 mg) was added to a screw cap culture tube containing 1.5 mL of enzyme treated sample (80 mg resin/mL sample). The tube was shaken in an incubator at room temperature for 30 min. During shaking, the inside wall of the tube was rinsed every 6 min with the sample by hand tilting the tube several times. The amount of resin used (80 mg resin/mL sample) was determined by monitoring the HPSEC profiles of starch/enzyme/buffer mixtures when treated with 30–70 mg resin/mL sample. At 70 mg resin/mL sample, the ion peak disappeared. For added assurance, 80 mg resin/mL sample was used for deionization of all debranched starches. To characterize the linear chain MW distribution, debranched deionized starch was filtered through a 5 μm nylon filter and injected (25 μL) into the HPSEC system.

Determination of branch point number index (BPNI)

Reducing power of starches before and after isoamylase treatment was measured according to the modified reagent procedure outlined by Miller (1959). Light absorbance was measured by a Perkin-Elmer spectrophotometer (Lambda 3B, Perkin-Elmer Co., Norwalk, CT) at 575 nm. Since each starch molecule has only one reducing end, when isoamylase breaks down starch at α-D(1→6) branch points, a new reducing end is formed. Therefore, the increase in reducing power (expressed as glucose after isoamylase treatment,) gave the number of branch points in starch. The branch point number index (BPNI) of starch was calculated using the following formulas:

$$BPNI = N_2 - N_1 \quad (2)$$

$$N = X/B \quad (3)$$

where N = reducing end number, moles/g dry starch; X = Reducing end number, moles/mL sample; B = Starch solution concentration, g/mL sample; N_1 = N before isoamylase treatment; N_2 = N after isoamylase treatment (debranched starches). Each sample was analyzed three times, and statistical analysis was done using the Duncan's multiple range test (SAS Institute, Inc., 1987). BPNI (although in moles/g) should be considered a relative index of branching.

Characterization of starch retrogradation behavior

Retrogradation behavior of native and acid-treated starch was investigated using differential scanning calorimetry (DSC). Duplicate samples of 6.80 ± 0.01 mg of starches (dry basis) were prepared in aluminum DSC pans (TA Instruments Inc., New Castle, DE) and deionized distilled water was added to obtain a starch/water weight ratio of 40:60 (starch dry basis). The pan was hermetically sealed after adding water and allowed to equilibrate at room temperature overnight (Deffenbaugh, 1990). The pans were heated in a Du Pont Thermal Analyzer 2000 (Du Pont Co., Wilmington, DE) at 10°C/min from 5°C to 120°C to gelatinize starch (Zeleznak and Hoseney, 1987). A sealed empty pan was used to balance the heat capacity of the sample pan. The resulting gel was stored at 23°C for 70 hr and then reheated in the DSC at 10°C/min to melt the recrystallized starch. The thermogram, and its parameters such as enthalpy (J/g dry starch) of crystalline melting, onset temperature (°C), and peak temperature (°C), were recorded.

Retrogradation behavior was also investigated by monitoring retrogradation energy changes of the starches vs time using DSC (Jankowski and Rha, 1986). Four starches (Native [0 hr], 5hr, 20hr, and 35hr acid treatments) were chosen to represent the range of different MW profiles. Triplicate samples were prepared for DSC analysis as above. Starch in water was heated in the DSC at 5°C/min from 23°C to 100°C to gelatinize starch. The resulting gels were stored at 23°C for 0, 1, 2, 3, 5, 8, and 11 days and then reheated in the DSC at 5°C/min to melt the crystallites. Statistical analysis for the DSC samples was performed using the Duncan's multiple range test (SAS Institute, Inc., 1987).

Avrami kinetic models

The Avrami equation of polymer crystallization was used to study retrogradation kinetics and to compare our results to those obtained by other workers. Equation (1) can be rewritten as follows (Jankowski and Rha, 1986):

$$\Theta = \frac{\Delta H_t}{\Delta H_\infty} = 1 - e^{-kt^n} \quad (4)$$

where Θ = the percent crystallized starch at time t (%); ΔH_t = the enthalpy change at time t, J/g dry starch; ΔH_∞ = the limiting enthalpy change (11th days in this study), J/g dry starch; t = time, days; k = rate constant, day^{-n}; n = Avrami exponent.

Two types of Avrami models were studied:

Model 1, where n and k were allowed to vary (Free n and k):

$$\Delta H_t = \Delta H_\infty(1 - e^{-kt^n}) \quad (5)$$

Model 2, where n = 1, and k was allowed to vary (n = 1, free k):

$$\Delta H_t = \Delta H_\infty(1 - e^{-kt^1}) = \Delta H_\infty(1 - e^{-kt}) \quad (6)$$

The kinetic parameters (n and k) were obtained by regression analysis of recrystallization enthalpies from DSC. The linear regression form for Eq (5) is:

$$\log\{\ln[\Delta H_\infty/(\Delta H_\infty - \Delta H_t)]\} = \log k + n\log t \quad (7)$$

and the regression form for Equation (6) is:

$$\ln[\Delta H_\infty/(\Delta H_\infty - \Delta H_t)] = kt \quad (8)$$

RESULTS & DISCUSSION

MW distribution of starches

HPSEC MW profiles of native and acid treated wheat starches were compared (Fig. 1). Native wheat starch has two distinct peaks, the sharper one is amylopectin, which eluted first due to its larger size (MW 3.7E8), and the second peak is amylose (MW 2.0E5). The MWs of both components agreed with other

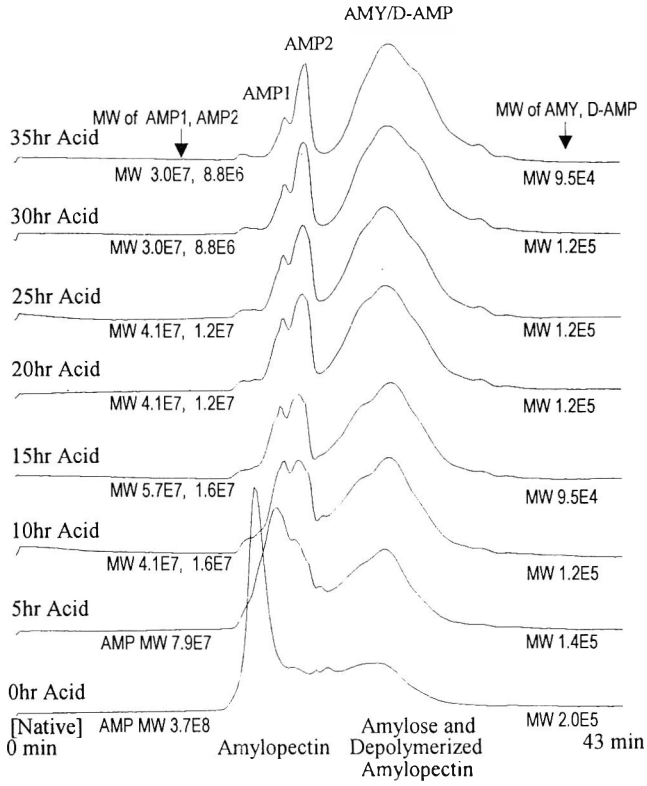

Fig. 1—HPSEC MW profiles of native and acid treated wheat starches. AMP = amylopectin; D-AMP = depolymerized AMP; AMY = amylose. Labeled MW refers to the MW at the corresponding maximum peak height.

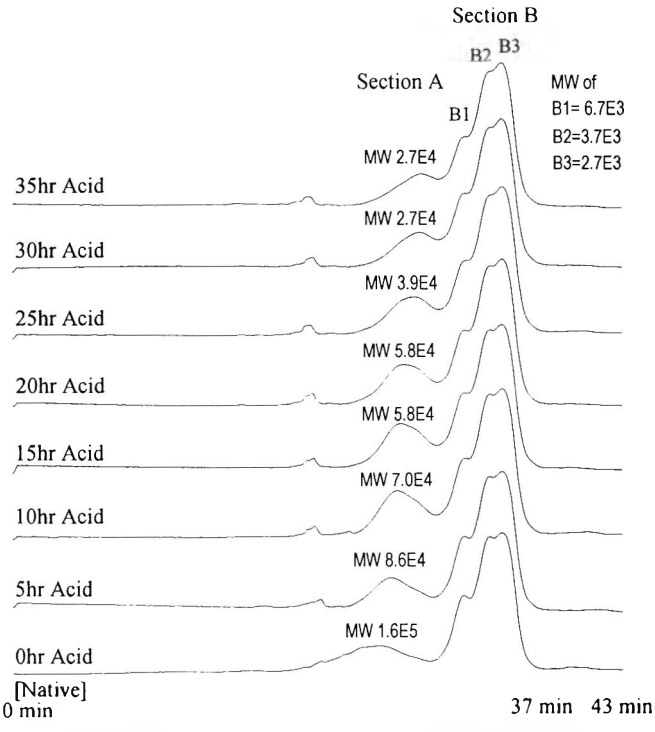

Fig. 2—HPSEC MW profiles of starches after isoamylase [EC 3.2.1.68] treatment. Labeled MW refers to the MW at the corresponding maximum peak height.

estimations of MW, 4E8 – 5E8 for amylopectin and 2E5 – 1.3E6 for amylose (Geddes et al., 1965; Banks et al., 1972).

The MW distribution of two main polymer populations were generally maintained during acid treatment (Fig. 1). One population was the large size amylopectin (AMP), which later became 2 subcomponents (AMP1 & AMP2) after 5 hr acid treatment. The other population was a mixture of amylose and small size (severely depolymerized) amylopectin fragments, with a size similar to native amylose (2.0E5 MW). The presence of depolymerized amylopectin eluting with amylose was confirmed by the HPSEC peaks representing isoamylase debranched amylopectin (discussed below). If the peak MW is considered the approximate average MW for that component, then the large amylopectin fragments have an average MW ranging from 8.8E6 (35 hr) to 3.7E8 (Native). The average MW of smaller amylopectin fragments range from 9.5E4 (35 hr) to 2.0E5 (Native). The size of large AMP is approximately 100 (Acid 35hr) to 500 (Acid 5hr) times larger than smaller AMP (Fig. 1). Acid treatment created a series of starches with differing molecular profiles. As acid treatment time increased, the resulting starches had fewer large size amylopectin fragments, increased small size amylopectins fragments (size similar to amylose), and all groups of polymers shifted to lower MWs.

MW distribution of debranched starches

The HPSEC MW profiles of isoamylase debranched starches (linear polymers) also were compared (Fig. 2). Isoamylase specifically breaks down starch at its branch points (α-D(1→6) glucosidic bonds) and releases linear polymers. Native amylose has an average degree of polymerization (DP) of 1,200 (Fig. 1) and very few branch points. Amylopectin has larger numbers of branch points; the linear chains between branch points in amylopectin polymers have two distinctive groups, with average DP's of 45 and 15 (Robin et al., 1974). Therefore, polymers in section A (Fig. 2) were from amylose, and polymers in section B were (predominantly) from amylopectin (representing two debranched amylopectin peaks with B1 = DP41 and B3 = DP17). The large peak area of section B (Fig. 2) confirms that depolymerized AMP in acid treated starches eluted with amylose, resulting in the increased AMY/D-AMP peak area in Figure 1. As acid treatment time increased, section A shifted to a lower MW and section B ended at the same MW for all starches. Therefore, acid treatment results in a narrower linear chain length distribution within starches. That is, as acid treatment time increased, starches had increased molecular homogeneity in terms of MW distribution of linear segments within the starch.

Branch point number index (BPNI)

BPNI values decreased as acid treatment time increased, ranging from 2.57×10^{-3} to 2.73×10^{-3} moles/gram dry starch. There was a statistically significant difference (α = 0.05) between [5hr acid], [15hr, 20 and 25 hr acid], and [35hr acid] starches. After debranching, all starches had similar reducing values (N_2). Prior to debranching, however, the starches showed an increase in reducing value (N_1) as acid treatment time increased. Therefore, these results suggest that as acid treatment time increased, there were fewer branched starch polymers. This result coincides with the well accepted starch granule structure model and the mechanism for acid hydrolysis of starch granules (Robin et al., 1974). Based on these theories, the majority of branch points are located in amorphous regions of the starch granule, and acid first attacks the amorphous region. Therefore, there is an increased chance for acid to attack branched areas in starch, and to reduce the number of branch points.

Retrogradation behavior and Avrami analysis

An endotherm transition was observed in the 45 to 75 °C range for all eight starch gels stored at 23 °C for 70 hrs. This peak, identified by previous workers, is the melting peak of

Table 1—DSC thermogram values of starch gels (40% dry basis) stored at 23°C for 70 hrs[a]

Starch	ΔH	T_0	T_p	β
Native	2.52[b,c]	48.7[b,c]	56.3[b,c]	24'00"[d]
5 hr Acid	2.79	50.4	57.2	25'45"
10 hr Acid	3.15	48.8	57.6	30'00"
15 hr Acid	2.91	50.2	57.8	36'00"
20 hr Acid	2.94	50.1	56.3	36'15"
25 hr Acid	3.23	50.5	57.3	36'15"
30 hr Acid	3.10	49.2	57.2	37'20"
35 hr Acid	3.20	48.6	57.3	37'40"

[a] ΔH = enthalpy of endotherm peak, J/g starch; T_0 = onset temperature of endotherm peak, °C; T_p = endotherm peak temperature, °C; β = the angle between baseline and tangent of endotherm peak front.
[b] Means of two determinations.
[c] Means in each column are not significantly different (α = 0.05).
[d] Values are from representative thermograms.

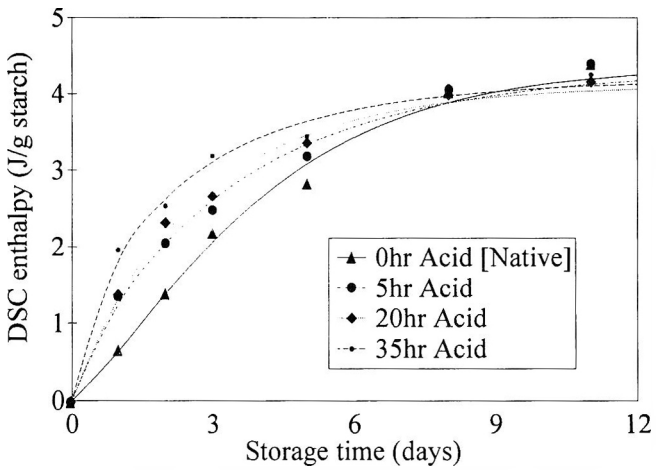

Fig. 3—The enthalpy changes of starch gels (40/60 starch/water) versus time during the storage at 23 °C compared with the regression lines from the Avrami model 1 (free n and k).

Table 2—Avrami kinetic parameters of recrystallization for different starches obtained from regression[a]

Model 1 (Free n and k)				
Starch	Native	5 hr Acid	20 hr Acid	35 hr Acid
n	1.260	0.896	0.954	0.775
k	0.162	0.344	0.395	0.575
r^2	0.989	0.976	0.985	0.956
Model 2 (n = 1, free k)				
Starch	Native	5 hr Acid	20 hr Acid	35 hr Acid
n	1	1	1	1
k	0.271	0.302	0.379	0.407
r^2	0.930	0.974	0.979	0.953

[a] n = Avrami exponent; k = rate constant, day^{-n}; r^2 = regression coefficient.

amylopectin crystallites (Eberskin et al., 1980; Zeleznak and Hoseney, 1986). Parameters corresponding to the endotherm peaks are shown in Table 1.

Using two replicates, there were no statistically significant differences (α = 0.05) in enthalpy (ΔH), onset (T_o) and peak (T_p) temperatures between the starches (Table 1), though the ΔH values appeared to increase slightly as acid treatment time increased. The onset temperatures (T_o) were similar for all starches (average 49.6 °C), but the angle between the baseline and the tangent of the endotherm peak front (β angle) increased as acid treatment time increased (Table 1). This indicates that peaks were sharper and higher. In a sharper DSC peak, the unit temperature interval around the peak temperature covers a larger number of crystallites with similar energy levels (Note: a sharper peak is basically an indication of a perfect/homogeneous crystal i.e., water ice crystal). Therefore, a larger number of crystallites with similar energy levels (more homogeneous crystallites) were obtained as acid treatment time increased. Kinetic studies (discussed later) confirmed that enthalpy increased as acid treatment time increased, which also indicated an increase in the total amount of crystallites.

Ageing of starches 70 hrs was only the midpoint of the recrystallization process. Figure 3 shows the enthalpy changes of four starch gels (0 hr [Native], 5hr, 20hr, and 35hr acid treatment starches) during 11 day storage, and the associated regression lines from Avrami kinetic model 1. The curve shape of enthalpy change vs. time for native starch agreed with previous reports (Longton and LeGrys, 1981; Russell, 1987; Roulet et al., 1988). The limiting enthalpy values also agreed, for example: Longton and LeGrys (1981) reported that a 47% gel stored at 21 °C for 11 days had a melting enthalpy of 4.2 J/g. Our results were 4.35 J/g, for a 40% gel at 23 °C for 11 days. The slight difference between these data is explainable: the higher the starch concentration, the slower the retrogradation (Orford et al., 1987), and the lower the temperature, the more crystallites formed (Colwell et al., 1969).

Enthalpy changes of starches during 11 day storage demonstrated (Fig. 3) that starches with longer acid treatment time had higher initial rates of recrystallization. After 11 days, however, all starches reached similar enthalpy levels. These differences in retrogradation behavior could be attributed to the molecular changes of starches. After acid treatment, some amylopectin fragments decreased to a size similar to amylose (Fig. 2), but the resulting smaller amylopectin fragments still retained the native amylopectin branching pattern. These smaller amylopectin fragments, due to their increased mobility (smaller size), could more rapidly form nucleation sites and promote crystal growth and therefore, show higher initial rates of recrystallization (Fig. 3). However, because these polymers retained the native amylopectin branching pattern, the final crystalline states were not significantly different among starches (almost the same enthalpy levels after 11 days). This agreed with previous reports that amylopectin was responsible for long-term retrogradation (Schoch and French, 1947; Miles et al., 1985; Ring et al., 1987; Russell, 1987), and that amylopectin side chain association affects retrogradation (Sterling, 1978).

The enthalpy values of four starch gels during 11 day storage were fit into two Avrami models: model **1** (with free **n** and **k** parameters) and model **2** (with **n** = 1, free **k** parameter). The parameters of the two Avrami models are shown in Table 2. Avrami model **1** had larger regression coefficients for all starches than model **2**. Therefore, model **1** was more descriptive of recrystallization than model **2**. The regression lines from model **1** are shown in Fig. 3; the curves fit the experimental data. By comparison of regression lines, at any given time during the first seven days of retrogradation, as acid treatment time increased, the starch had higher enthalpy levels. This confirmed the previous observation that for 70 hr storage, starch Δ H values appeared to increase as acid treatment time increased (Table 1).

In model **1** (Table 2), **n** is 1.260 for native starch. This value generally agrees with those reported by others: i.e., **n** = 0.99 for bread (Kim and D'Appolonia, 1977), and **n** = 1.02 for 50% wheat starch gels (McIver et al., 1968). Also, Table 2 shows that the longer the acid treatment time, the smaller the Avrami exponent **n**. Because **n** values ranged from 1.26 to 0.775, recrystallization can be said to generally follow the **n** = 1 type of rod-like growth (one-dimensional growth) from instantaneous nuclei (zero order nucleation; Sharples, 1966). The deviation of **n** from 1 is likely due to the starch gel system itself: a more complicated starch/water two-component system than common (plastic) polymers (because water acts as a polymer plasticizer). Native starch has a larger average molecular size (Fig. 2) and therefore, more restricted mobility within a concentrated gel. Sporadic nuclei (given **n** = 2 rod-like growth (Sharples, 1966), since disc-like growth is unlikely) might occur in some regions of the starch gel. This may contribute to the increase in **n** from 1 (instantaneous nucleation only) to 1.26 (combined sporadic and instantaneous

nucleation). For an acid treated starch, in addition to the decreased molecular size (increased mobility), the MW distribution of the linear portion is narrowed (Fig. 2) and branching is decreased (Table 2), thus the starches have increased homogeneity of molecular structure. Therefore, nucleation is "more" instantaneous, and results in a $\mathbf{n} < 1$. This is in agreement with the earlier explanation regarding the sharper/narrower DSC peaks, resulting from more homogeneous/total crystallites formed during retrogradation (70 hr storage).

From model **1** (free **n** and **k**), the rate constant **k** (= 0.162) for native starch is smaller than McIver et al's (1968) value for **k** (= 0.266). However, with a fixed **n** (= 1, model 2), **k** (= 0.271) for native starch (Table 2) agrees with most reports, i.e. **k** = 0.267 (Kim and D'Appolonia, 1977) and **k** = 0.278 (Longton and LeGrys, 1981). Several factors might have caused the differences in **k** i.e., sample source, gel concentration, method of gel preparation, storage temperature, DSC scanning rate, etc. However, both models show an increased rate constant **k** for the starches with increased acid treatment time. This indicates an increase in overall recrystallization (both nucleation and growth) rate. The increased rate constants for acid treated starch support the hypothesis that smaller size amylopectins contribute to increased initial crystallization rates in acid treated starches.

CONCLUSIONS

RETROGRADATION behaviors can be attributed to molecular changes of starches. The increased initial retrogradation rate was due to increased mobility of smaller size amylopectin fragments, and the narrowed linear chain length distribution/decreased branch points (increased molecular homogeneity). The final similar enthalpy level of retrogradation indicated the retention of native amylopectin side chain patterns in the smaller amylopectin fragments. The Avrami kinetic model of polymer crystallization, which allowed both exponent **n** and rate constant **k** to vary, better fit the experimental data than the model with **n** = 1 and a varying **k**. Based on the Avrami model, retrogradation appears to follow a mechanism of "rod-like growth from instantaneous nuclei." The Avrami exponents, however, were not integers. This suggests that starch gels are more complex than conventional polymer systems. It further confirms that despite some utility, the Avrami equation is not a good theoretical model to explain crystalline growth and nucleation during starch retrogradation.

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Raw-Starch Degrading Amylase(s) Affect Enzyme-resistant Starch

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ABSTRACT

Enzyme-resistant starch (RS) was degraded, in part, by amylase(s) that act on raw starch. The degradation (solubilization) was accompanied by changes in several characteristics (peak temperature and enthalpy) and, probably, by the presence of a lipid-amylose complex. The degradation resulted in a significant reduction in one of the two main contiguous peaks of RS around 155°C.

Key Words: starch, enzymes, amylase

INTRODUCTION

RETROGRADATION of amylose-containing starches may result in the formation of an enzyme-resistant starch (RS) fraction that comprises short-chain linear α-glucans (Russell et al., 1989). This form of starch, undigestible in vitro and in vivo, is found in food products processed at relatively high moisture contents, such as cooking, baking and autoclaving (Englyst et al 1983). Resistant starch (RS) is composed of noncovalently bonded crystallites of amylose. Crude RS preparations contain native starch lipids (Sievert and Pomeranz, 1990). Interchain amylose association in the RS fraction is indicated by an endothermic transition around 155°C in differential scanning calorimetry thermograms (Sievert and Pomeranz, 1989; Szczodrak and Pomeranz, 1991). There is considerable interest in the nutritional implications of RS in foods, since a relatively slow rate of starch hydrolysis in the gastrointestinal tract of man is associated with low glycaemic responses and may have some of the physiological effects of dietary fiber (Englyst and Macfarlane, 1986). RS is formed during cooling of gelatinized starch and its generation, presumably, involves reordering or retrogradation phenomena. Reistance to amylolysis is lost after dissolving RS in dimethylsulfoxide or 2N KOH, which also suggests that the resistance is due to physical changes that take place during heating and cooling rather than due to covalent chemical modification(s) (Russell et al., 1989). RS can be produced by autoclaving high-amylose starch (i.e. from maize) and cooling under controlled conditions. RS can be concentrated by removing nonretrograded highly amorphous parts with thermostable bacterial α-amylase (Sievert and Pomeranz 1989). Our objective was to determine whether, and to what extent, some amylase would affect RS.

MATERIALS & METHODS

HIGH-AMYLOSE STARCH, Amylomaize VII, was obtained from the American Maize Products Co., Hammond, IN, USA. Heat stable α-amylase (Takalite L-340) from *Bacillus licheniformis* was from Miles Laboratories, Inc., Elkhart, IN, USA. Raw Starch Degrading Amylase (RSDA) from *Aspergillus* sp. K-27 was obtained from S. Hizukuri, Kagoshima University, Japan, through the courtesy of J. G. Ponte, Jr., Kansas State University, Manhattan, KS.

RS was prepared and concentrated from Amylomaize VII by the procedure of Sievert and Pomeranz (1989). In that procedure, RS is concentrated by sequential treatment with Takalite at 100°C and with amyloglucosidase at 60°C.

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Enzyme treatment of RS

One g (d.m. basis) of RS was prepared from Amylomaize VII suspended in 100 mL water containing 100 mg RSDA or 0.5 mL Takalite. The suspensions were shaken while heated at 60°C for 12 hr. The cooled suspensions were filtered. The residues were washed and dried at 55°C. The amounts of residue were determined gravimetrically. Differential scanning calorimetry (DSC) measurements were made on the washed residues as described by Szczodrak and Pomeranz (1991).

RESULTS & DISCUSSION

DSC DATA for RS before and after treatment with enzymes are summarized in Table 1. The results show amylolysis resulting from the action of the 2 enzyme preparations on RS. Under these conditions, amylolysis by RSDA was much more extensive (≈40%) than by Takalite (≈15%). RSDA seemed also to affect the RS structure, which is responsible for its thermal characteristics. Treatment with RSDA lowered the enthalpy by about 25% compared with the control or Takalite treated sample.

RS as isolated and purified (as a result of Takalite treatment) by the method of Sievert and Pomeranz (1989) had a much higher enthalpy than the original mixture of amorphous and crystalline components. Treatment of RS-lipid complexes with Takalite practically eliminated the enthalpy of 2.6 J/g around 110°C that is associated with the complexes (Czuchajowska et al. 1991). In the case of RSDA, however, the enthalpy of the enzymatically treated RS was reduced and a small, but quite prominent, peak around 110°C was recorded (Fig. 1). Those changes were accompanied by two additional observations.

RS is known to be composed of diverse, variable forms resulting from manifold intermolecular binding forces among α-glucan chains (Czuchajowska et al 1991). Thus, RS comprises crystalline regions and less ordered domains, the proportions of which depend on the heat treatment history of the starch. RS might be composed of parallel-stranded double helices with different tertiary organizations. The indistinct X-ray diffraction β-type pattern shown by Russell et al. (1989) and Sievert et al. (1991) confirmed the presence of a mixture of

Table 1—Effects of enzyme treatment on degradation and thermal characteristics of RS

RS Sample	Solubilized RS (%)	Transition temperatures[a] (T, °C)[b] and Transition enthalpies (ΔH, J/g)[c]			
		T_i	T_p	T_c	ΔH
Control	–	103.9	154.1, 161.0	169.9	37.2
RS after Takalite treatment	15.5	120.5	156.2	171.6	38.4
RS after RSDA treatment	40.8	124.0	152.1	170.2	28.2

[a] T_i, T_p, T_c = initial, peak, and completion transition temperatures, respectively.
[b] SD < 2°C; n = 3.
[c] SD < 10% of the mean; n = 3.

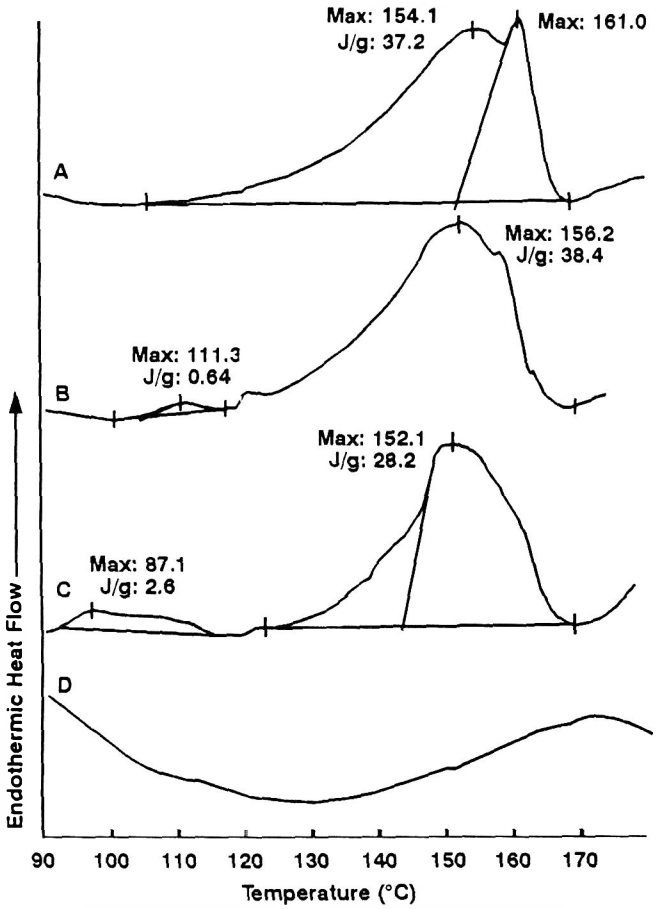

Fig. 1—Differential scanning calorimetry thermograms of RS: control sample (A), after treatment with Takalite (B), after treatment with RSDA (C), and RSDA blended with Al_2O_3 in the ratio 1:9 (D).

crystallites and amorphous regions. Such a mixture is indicated by the presence of two distinct peaks (T_p of 154.1°C and 161.0°C) in the control sample (Fig. 1). The Takalite and RSDA treatments, apparently, degraded-solubilized some moieties in the control sample. Two peaks, a large one at 156.2°C and a small one at about 159°C, were shown by the Takalite-treated sample. In the RSDA-treated sample, the transition underlying the RS peak around 155°C was reduced in amplitude and sharpness and only a shoulder to the large peak was recorded. Those changes were accompanied by lowered peak temperatures (T_p) from about 158°C (average of 154 and 161°C) in the control to 156°C in the Takalite-treated, and to 152°C in the RSDA-treated samples.

The nature of the changes and identity of the modified (eliminated) RS components (crystalline and/or amorphous) are the subject of further ongoing investigations. Some RS components that are not degraded by amylase(s) from *B. licheniformis* used in purfication are susceptible to degradation by the amylolytic enzyme(s) from *Aspergillus* sp. K-27. The latter preparation was shown to contain a thermostable glucoamylase that seemed to have a "starch-binding site" and an α-amylase which synergistically enhanced the degradation of starch with glucoamylase (Abe et al., 1988a).

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Gelling Characteristics of Pectin From Sunflower Head Residues

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ABSTRACT

Gelling properties of sunflower head pectin were studied using two instrumental methods: the standard sag method and the Instron textural profile method. For the Instron method, the jellies were formulated with 0–60% sugar content with various amounts of calcium chloride (30 to 90 mg/g pectin) at pHs 5.4 and 6.0. Sunflower pectin had a gel power of 110 compared to 100 for a citrus low-methoxyl pectin and 120 for an amidated low-methoxyl pectin. Sunflower pectin formed jellies under all experimental conditions. Sunflower pectin has a high potential for producing low-caloric foods, particularly when a near-neutral taste is required.

Key Words: sunflower, pectin, gels, texture, sugar

INTRODUCTION

THE ABILITY of low-methoxyl pectin (LMP) to form jellies is affected by both intrinsic and extrinsic factors, including uronic acid content, molecular size, degree of methoxylation, distribution of the carboxyl groups, acetylation, neutral sugar composition, amidation, pH, sugar content, calcium content, ionic strength, and temperature (BeMiller, 1986; May, 1990; Axelos and Thibault, 1991). The interactions among these factors in pectin gelation are very complex (Axelos and Thibault, 1991). The LMP, which was naturally present in the head residues of sunflower, had a high molecular mass than a commercial LMP (Miyamoto and Chang, 1992). The sunflower pectin also had a high viscosity at pH 3. Cultivar, maturity of sunflower heads, and extraction methods also affected pectin quality (Campbell et al., 1978; Lin et al., 1975, 1978).

Generally, high-methoxyl pectin (HMP) cannot form jellies without a high sugar content and/or proper acidity, and LMP can gel in the presence of calcium without sugar. However, addition of sugar to LMP can increase gell strength, reduce syneresis and increase setting temperature (Axelos and Thibault). Kim et al. (1978) and Sosulski et al. (1978) studied gelation of sunflower pectin, ranging from pH 2.5 to 4.7, with calcium content ranging from 15 to 35 mg/g pectin and sucrose content varying from 10 to 50%. Sunflower pectin (1% concentration) formed firm jellies with 100 gel power, which was measured by a sag method with a ridgelimeter, in formulations containing 30 to 40% sugar, 25 to 30 mg calcium (in the form of calcium chloride)/gram pectin in a pH range 2.5 to 4.5 (Sosulski et al., 1978). Sunflower pectin was less sensitive to the changes of pH or calcium concentration than citrus pectin (Sosulski et al., 1978).

Several food applications of the LMP, including the manufacturing of confectionery jelly products and milk gel, require a neutral taste (pH 4.6 to 5.5) (Hercules Inc., 1990; Hoefler, 1991; Sanofi Bio-Industries, 1990). Gelation of sunflower pectin at a pH higher than 4.7 and a greater range of calcium and sugar content than Sosulski et al. (1978) reported has not been reported. In addition, objective textural profile analysis (Friedman et al., 1963), including brittleness, adhesiveness, cohesiveness, hardness, and gumminess, of sunflower pectin have not been reported.

The objectives of this study were (1) to investigate the textural properties of sunflower pectin, using the standard sag method and the Instron textural profile method, particularly under near neutral pHs, and (2) to compare the gelling properties with two types of commercial citrus LMP.

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MATERIALS & METHODS

Materials

Sunflower pectin was isolated from the head residues of oilseed-type sunflower grown in Carrington, North Dakota (Miyamoto and Chang, 1992). A standardized citrus low-methoxyl pectin (Genu LM12CG) (CLMP) and a standardized citrus amidated low-methoxyl pectin (Genu 104AS) (CALMP) were used as references (Hercules Inc., Middletown, NY).

Preparation of jellies

Sunflower pectin and the 2 reference pectins were used for all gelation experiments. For measuring gel power using the sag method, the standard Food Chemical Codex (FCC) procedure for LMP was used (National Research Council, 1972).

For textural profile analysis, the FCC procedure was modified for jelly preparation. The pectin concentration used was 1% for the sunflower pectin and 1.6% for the standardized citrus pectin references. Using 1.6% for the standardized pectin brought the uronic acid content to the same level as the sunflower pectin since the reference pectin had been standardized with sucrose at the factory (Miyamoto and Chang, 1992). After mixing pectin with a half amount of sugar (if required), citric acid, and water, the suspension was adjusted to pH 5.4 and 6.0 with 1N NaOH and 2.5 N NaOH, respectively. The

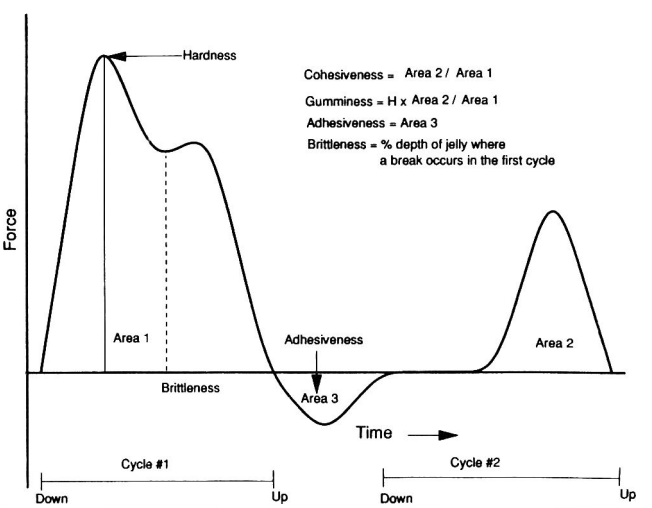

Fig. 1—An illustration of a typical textural profile curve determined using the Instron Universal Testing Instrument (Sanderson et al., 1988).

Table 1—Effect of sugar and calcium chloride content on the hardness (g force) of the jellies made from sunflower pectin and commercial pectins at pH 5.4 (A) and 6.0 (B)[a]

	Ca++ (mg/g)	Sugar (%) 0 SLMP[b]	0 CLMP[c]	0 CALMP[d]	20 SLMP	20 CLMP	20 CALMP	40 SLMP	40 CLMP	40 CALMP	60 SLMP	60 CLMP	60 CALMP
A	30	508.6 (5.0)	NA	NA	277.9 (50.0)	NA	NA	663.3 (9.5)	84.9 (7.0)	473.9 (26.5)	141.6 (26.9)	413.4 (4.7)	1849.7 (426.4)
	50	860.8 (11.6)	422.3 (30.1)	63.5 (6.4)	765.3 (5.3)	182.1 (9.1)	676.1 (2.9)	972.3 (44.1)	397.6 (27.4)	244.0 (0)	1354.0 (89.1)	1213.0 (14.1)	263.5 (20.3)
	70	818.3 (30.6)	1018.9 (23.0)	1351.5 (181.7)	1038.5 (2.1)	573.4 (86.8)	1767.6 (47.3)	857.8 (89.4)	752.5 (51.6)	1709.5 (27.6)	673.6 (26.0)	1198.5 (193.0)	855.0 (99.0)
	90	454.0 (5.7)	1296.4 (160.7)	1126.0 (284.0)	250.9 (9.8)	772.4 (51.4)	1314.5 (108.2)	354.2 (20.6)	367.9 (9.9)	636.3 (22.1)	693.4 (39.4)	710.7 (48.2)	936.3 (12.0)
B	30	52.5 (5.7)	NA	NA	57.1 (4.3)	NA	NA	142.6 (0.1)	NA	NA	780.2 (5.2)	NA	172.8 (15.6)
	50	456.8 (69.7)	NA	NA	404.6 (12.2)	NA	NA	504.6 (31.7)	NA	84.7 (4.0)	983.8 (108.5)	337.4 (12.5)	272.7 (1.4)
	70	817.0 (107.4)	212.0 (8.8)	NA	846.0 (87.7)	123.1 (0)	371.3 (13.1)	739.2 (55.4)	197.0 (0)	564.6 (57.8)	1439.6 (207.1)	538.0 (36.1)	810.1 (46.7)
	90	626.3 (15.2)	603.7 (5.2)	265.5 (56.9)	881.8 (8.1)	557.2 (31.3)	1035.5 (27.7)	523.9 (22.8)	970.5 (62.6)	1472.5 (95.5)	520.9 (1.1)	1019.3 (74.6)	1190.3 (124.0)

[a] Data represent the means of two determinations with standard deviations in parentheses. NA stands for not available. Under these conditions, jellies did not form.
[b] Sunflower LMP.
[c] Citrus LMP (LM12CG) from Hercules Inc., Middletown, NY.
[d] Citrus amidated LMP (104AS) from Hercules Inc., Middletown, NY.

Table 2—Effect of sugar and calcium chloride content on the cohesiveness (dimensionless) of the jellies made from sunflower pectin and commercial pectins at pH 5.4a A and 6.0 (B)[a]

	Ca++ (mg/g)	Sugar (%) 0 SLMP[b]	0 CLMP[c]	0 CALMP[d]	20 SLMP	20 CLMP	20 CALMP	40 SLMP	40 CLMP	40 CALMP	60 SLMP	60 CLMP	60 CALMP
A	30	0.10 (0.01)	NA	NA	0.26 (0.02)	NA	NA	0.22 (0.01)	0.45 (0.12)	0.63 (0.01)	0.21 (0.06)	0.50 (0.04)	0.49 (0.01)
	50	0.13 (0.02)	0.25 (0.01)	0.21 (0.05)	0.14 (0.02)	0.37 (0.02)	0.28 (0.13)	0.15 (0.02)	0.31 (0.02)	0.58 (0.06)	0.15 (0.02)	0.23 (0.01)	0.54 (0.01)
	70	0.08 (0.01)	0.10 (0.02)	0.06 (0.00)	0.08 (0.01)	0.19 (0.03)	0.15 (0.03)	0.10 (0.01)	0.12 (0.00)	0.17 (0.02)	0.35 (0.09)	0.16 (0.04)	0.54 (0.02)
	90	0.12 (0.01)	0.08 (0.00)	0.12 (0.01)	0.19 (0.06)	0.07 (0.01)	0.14 (0.02)	0.33 (0.11)	0.26 (0.01)	0.11 (0.01)	0.37 (0.01)	0.58 (0.06)	0.26 (0.01)
B	30	0.31 (0.07)	NA	NA	0.53 (0.01)	NA	NA	0.35 (0.01)	NA	NA	0.32 (0.08)	NA	0.61 (0.02)
	50	0.13 (0.01)	NA	NA	0.23 (0.00)	NA	NA	0.23 (0.04)	NA	0.52 (0.05)	0.25 (0.00)	0.54 (0.05)	0.63 (0.01)
	70	0.11 (0.03)	0.29 (0.07)	NA	0.11 (0.01)	0.65 (0.07)	0.42 (0.01)	0.13 (0.01)	0.54 (0.03)	0.49 (0.03)	0.15 (0.01)	0.41 (0.08)	0.75 (0.08)
	90	0.09 (0.00)	0.14 (0.01)	0.17 (0.02)	0.07 (0.01)	0.22 (0.02)	0.06 (0.02)	0.13 (0.01)	0.17 (0.01)	0.18 (0.02)	0.38 (0.05)	0.22 (0.00)	0.52 (0.06)

[a] Data represent the means of two determinations with standard deviations in parentheses. NA stands for not available.
[b] Sunflower LMP.
[c] Citrus LMP (LM12CG) from Hercules Inc., Middletown, NY.
[d] Citrus amidated LMP (104AS) from Hercules Inc., Middletown, NY.

remaining sugar was added, and the mixture was heated. Final sugar concentrations were 0, 20, 40, and 60%.

After bringing the mixture to a boil, an appropriate amount of 2.25% calcium chloride solution was added slowly to give a concentration of 30, 50, 70, and 90 mg calcium/g pectin. After mixing all ingredients, the concentration of pectin in the solution was slightly diluted. The solution was heated continuously until the required weight was obtained to achieve the desired pectin concentration (1% for sunflower pectin or 1.6% for the citrus reference pectin) and poured into plexiglass cylinders (1.5 cm height and 5.08 cm in diameter), which had been prepared by wrapping the top with a piece of transparent tape to extend the height by 1 cm. The bottom of the cylinder was sealed with a piece of plastic film, with a small amount of grease at the bottom of the cylinder and a rubber band to prevent leakage (Friedman et al., 1963). The gels were stored at 4°C for 18 to 24 hr before textural analysis.

Textural analysis

For the sag method, the ridgelimeter (Bulmer Food Co., Hereford, UK) was used. The gel power was calculated according to the method described in the *Food Chemical Codex* (National Research Council, 1972). For textural profile analysis, the procedure of Friedman et al. (1963) was followed, using an Instron Universal Testing Instrument (Instron Inc., Canton, MA) (Sanderson et al., 1988). The Scotch tape was removed from the gel cylinder, and the top of the jelly was cut along the top edge with a sharp knife. The cylinder containing the

Table 3—Effect of sugar and calcium chloride content on the gumminess (dimensionless) of the jellies made from sunflower pectin and commercial pectins at pH 5.4a (A) and 6.0 (B)[a]

	Ca++	Sugar (%) 0			20			40			60		
	(mg/g)	SLMP[b]	CLMP[c]	CALMP[d]	SLMP	CLMP	CALMP	SLMP	CLMP	CALMP	SLMP	CLMP	CALMP
A	30	51.6 (1.6)	NA	NA	71.7 (8.4)	NA	NA	133.0 (6.5)	38.2 (13.3)	295.5 (13.4)	297.8 (86.7)	206.6 (15.2)	895.7 (192.8)
	50	106.3 (22.0)	103.4 (4.3)	12.9 (1.8)	109.2 (16.4)	67.5 (5.9)	189.7 (86.9)	144.7 (17.8)	121.0 (0.1)	140.3 (15.6)	195.7 (13.1)	272.9 (5.4)	140.9 (9.0)
	70	65.6 (6.0)	96.6 (19.4)	73.4 (0.01)	80.0 (10.2)	107.0 (28.2)	255.8 (30.6)	91.3 (12.2)	90.3 (6.2)	290.8 (28.8)	228.3 (52.9)	182.4 (12.4)	206.4 (35.9)
	90	51.4 (0.6)	103.7 (12.9)	128.5 (24.7)	47.9 (16.4)	50.4 (8.8)	183.3 (3.5)	115.1 (34.9)	93.9 (5.2)	66.9 (6.8)	260.3 (4.1)	413.6 (68.2)	248.1 (3.5)
B	30	15.6 (1.9)	NA	NA	30.5 (1.8)	NA	NA	51.1 (1.3)	NA	NA	250.6 (71.1)	NA	105.6 (12.0)
	50	61.6 (12.5)	NA	NA	93.5 (2.8)	NA	NA	116.4 (28.5)	NA	43.5 (2.1)	244.4 (26.9)	180.8 (23.3)	170.6 (0.9)
	70	85.2 (10.6)	61.2 (12.4)	NA	93.5 (1.9)	80.1 (8.7)	155.9 (0.2)	99.6 (15.7)	106.4 (5.6)	277.5 (44.3)	222.1 (34.7)	240.6 (29.4)	605.3 (33.7)
	90	59.3 (7.0)	81.5 (4.9)	44.7 (5.9)	59.0 (5.7)	122.4 (1.0)	57.3 (23.5)	70.1 (7.3)	160.4 (17.2)	248.8 (13.6)	194.1 (25.5)	224.2 (16.4)	617.0 (139.7)

[a] Data represent the means of two determinations with standard deviations in parentheses. NA stands for not available.
[b] Sunflower LMP.
[c] Citrus LMP (LM12CG) from Hercules Inc., Middletown, NY.
[d] Citrus amidated LMP (104AS) from Hercules Inc., Middletown, NY.

Table 4—Effect of sugar and calcium chloride content on the adhesiveness (dimensionless) of the jellies made from sunflower pectin and commercial pectins at pH 5.4 (A) and 6.0 (B)[a]

	Ca++	Sugar (%) 0			20			40			60		
	(mg/g)	SLMP[b]	CLMP[c]	CALMP[d]	SLMP	CLMP	CALMP	SLMP	CLMP	CALMP	SLMP	CLMP	CALMP
A	30	0.67 (0.04)	NA	NA	1.47 (0.04)	NA	NA	2.34 (0.18)	0.53 (0)	1.69 (0.21)	2.62 (0.17)	2.64 (0.11)	3.75 (0.71)
	50	0.48 (0.11)	0.70 (0.18)	0.21 (0.01)	1.30 (0.16)	1.21 (0.02)	1.28 (0.90)	1.24 (0.12)	0.31 (0.02)	1.38 (0.04)	3.60 (0.15)	3.78 (0.74)	1.38 (0.14)
	70	0.20 (0.05)	0.58 (0.08)	0.65 (0.00)	0.66 (0.06)	1.52 (0.11)	0.38 (0.10)	0.90 (0.08)	1.68 (0.03)	0.68 (0.18)	3.81 (1.58)	1.30 (0.14)	1.70 (0.25)
	90	0.24 (0.06)	0.19 (0.01)	0.25 (0.05)	0.53 (0.16)	0.37 (0.04)	0.77 (0.10)	1.52 (0.40)	1.08 (0.07)	6.49 (0.11)	3.49 (0.31)	6.92 (1.03)	1.65 (0.28)
B	30	0.28 (0.10)	NA	NA	0.41 (0.02)	NA	NA	1.04 (0.11)	NA	NA	3.67 (0.16)	NA	1.11 (0.01)
	50	0.84 (0.06)	NA	NA	1.62 (0.05)	NA	NA	2.32 (0.15)	NA	0.44 (0.05)	4.20 (0.71)	2.80 (0.25)	1.39 (0.15)
	70	0.89 (0.21)	0.67 (0.01)	NA	1.02 (0.11)	0.92 (0.05)	1.33 (0.16)	1.73 (0.10)	1.64 (0.16)	2.30 (0.23)	2.32 (0.05)	4.53 (0.01)	3.45 (0.21)
	90	0.41 (0.15)	0.96 (0.18)	0.55 (0.04)	0.37 (0.03)	1.56 (0.27)	0.69 (0.08)	0.66 (0.13)	0.95 (0.03)	0.70 (0.14)	4.09 (0.62)	1.89 (0.35)	3.35 (0.64)

[a] Data represent the means of two determinations with standard deviations in parentheses. NA stands for not avai able.
[b] Sunflower LMP.
[c] Citrus LMP (LM12CG) from Hercules Inc., Middletown, NY.
[d] Citrus amidated LMP (104AS) from Hercules Inc., Middletown, NY

jelly was separated from the cylinder. A cylindrical plunger with 5 cm diameter and a weight beam of 5 kg were used. The speed of the cross head and the recording chart was set at 20 cm/min. The plunger was set to move 1.15 cm into the jelly. Textural properties, including hardness, cohesiveness, brittleness, adhesiveness, and gumminess, were calculated from the curve according to the method of Friedman et al. (1963). An example of the calculation of the values of the textural properties from the curve is presented in Fig. 1. A total of 32 conditions (treatments) (sucrose x $CaCl_2$x pH) was measured in duplicate at room temperature.

Statistical analysis

The General Linear Model, using the SAS software (SAS, 1986), was performed to determine differences in jelly characteristics among treatments and the interactions among the factors such as sucrose, $CaCl^2$, and pH. For the standard sag gel power analysis, Duncan's multiple range test was used to determine the differences among the 3 pectin samples. For the textural profile analysis, the Duncan's multiple range test was not used to analyze the significance among treatments since the interactions among factors were strongly significant.

RESULTS & DISCUSSION

Gel power from sag analysis

The mean gel power values were sunflower pectin 111.1 CLMP 109.0 and CALMP 119.7. The CALMP was significantly ($p < 0.05$) higher than the sunflower pectin. However, sunflower pectin and CLMP did not differ significantly. The sag method has been used to grade pectin since 1959 (IFT, 1959). It is an inexpensive method. However, the sag method does not estimate internal gel strength and does not predict gel performance under actual use (Crandall and Wicker, 1986).

Table 5—Effect of sugar and calcium chloride content on the brittleness (%) of jellies made from sunflower pectin and commercial pectins at pH 5.4 (A) and 6.0 (B)[a]

	Ca^{++}	Sugar (%) 0			20			40			60		
	(mg/g)	SLMP[b]	CLMP[c]	CALMP[d]	SLMP	CLMP	CALMP	SLMP	CLMP	CALMP	SLMP	CLMP	CALMP
A	30	NB	NA	NA	56.7 (0)	NA	NA	60.0 (4.7)	NB	NB	65.0 (2.4)	53.3 (0)	NB
	50	50.0 (0)	NB	NB	56.7 (0)	50.0 (0)	NB	63.4 (4.7)	58.4 (2.3)	NB	63.3 (0)	71.7 (2.3)	NB
	70	55.0 (2.4)	60.0 (0)	56.7 (0)	55.0 (2.4)	60.6 (0)	NA	56.7 (0)	65.0 (2.4)	NB	53.4 (4.7)	61.2 (2.3)	65.0 (2.4)
	90	51.2 (2.3)	53.4 (4.7)	NB	41.2 (2.3)	55.0 (7.1)	66.7 (0)	46.7 (4.7)	40.0 (0)	56.7 (0)	48.4 (2.3)	NB	61.2 (2.3)
B	30	NB	NA	NA	31.0 (1.4)	NB	NA	43.3 (0)	NA	NA	56.7 (0)	NA	NB
	50	66.7 (4.7)	NA	NA	58.4 (2.3)	NA	NA	60.0 (4.7)	NA	NB	58.4 (2.3)	56.7 (0)	NB
	70	58.4 (2.3)	NB	NA	51.2 (2.3)	31.7 (2.3)	55.0 (2.4)	60.0 (4.7)	48.4 (2.3)	NB	58.4 (2.3)	46.7 (0)	NB
	90	52.3 (0)	61.2 (2.3)	51.2 (2.3)	50.0 (4.7)	61.2 (2.3)	65.0 (2.4)	48.4 (2.3)	58.4 (2.3)	NB	50.0 (0)	66.7 (0)	NB

[a] Data represent the means of two determinations with standard deviations in parentheses. NA stands for not available. NB stands for not brittle. Under these NB conditions, there were no peak splits during the first cyle of the compression.
[b] Sunflower LMP.
[c] Citrus LMP (LM12CG) from Hercules Inc., Middletown, NY.
[d] Citrus amidated LMP (104AS) from Hercules Inc., Middletown, NY.

Textural profile analysis

The textural profile estimations using instrumental analysis correlate well with human sensory response (Szczesniak et al., 1963). Textural profile analysis provides internal gel strength information that the sag method does not provide. The sunflower pectin formed jellies under all conditions used (Tables 1A and 1B). However, at pH 5.4 and 30 mg calcium, CLMP did not gel at low sugar concentration (0 and 20% sugar) (Table 1A). At pH 6.0, CLMP also did not gel under some (7) conditions, particularly at 30 and 50 mg calcium (Table 1B). Amidated LMP did not gel under similar conditions as the LMP. Thus, sunflower pectin was less sensitive to high pH conditions than CLMP and CALMP. Strong interactions (significant, $p < 0.05$) among the 3 factors (sucrose, calcium, and pH) were observed.

Commercial CLMP is derived from HMP through chemical or enzymatic de-esterification (May, 1990). The higher molecular size of the sunflower pectin than the CLMP may be partly responsible for its ability to gel at high pH and low sugar content. Other structural features of the pectin, such as intramolecular methoxylated group distribution, may affect sensitivity to pH and calcium ions (BeMiller, 1986; DeVries et al., 1986). All three types of pectin formed gels at 90 mg calcium content.

Hardness is an estimation of the force required to penetrate the jellies (Szczesniak et al., 1963). Generally, when sugar concentration or calcium content increased, the hardness of the jellies increased (Tables 1A and 1B). However, this was not true for all conditions. Amidated low-methoxyl pectin gels seemed to have greater hardness than the sunflower pectin when calcium content was at 90 mg. Sunflower pectin formed firm jellies under most conditions.

Cohesiveness is related to the work required to overcome the internal bonding of the materials (Friedman et al., 1963). Generally, at low sugar content (0 and 20%), the cohesiveness of pectin jellies decreased (Tables 2A and 2B) as the calcium content increased. However, at high sugar content (40 and 60%), there was not a definite trend of increase of cohesiveness as calcium content increased. Generally, cohesiveness increased with the increase of sugar content at a specific calcium content. The CLMP and CALMP had higher cohesiveness than the sunflower pectin at high sugar content (40 and 60%).

Gumminess can be described as a denseness that persists through mastication (Szczesniak et al., 1963). Gumminess of pectin generally increased as sugar content increased (Tables 3A and 3B). Depending upon specific conditions, gumminess changed with calcium concentrations but showed no definite trends of increase or decrease as calcium content was increased.

Adhesiveness is a measure of the degree of stickiness of the gel. It is related to the work that is required to pull the plunger from the sample (Friedman et al., 1963). Generally, pectin jellies had low stickiness (Tables 4A and 4B). The adhesiveness increased as sugar content increased. At pH 5.4, the adhesiveness of the sunflower pectin, CLMP, and CALMP tended to decrease as the calcium content increased under low sugar content (0 and 20%). The adhesiveness varied nonsystematically for all other conditions.

Brittleness is related to the percent strain required to fracture the jellies (Sanderson et al., 1988). Brittleness was measured as the percent of the plunger path (full path is 1.15 cm) into the jelly when the jelly broke. Therefore, the higher the percentage, the less brittle the jelly. In most of the conditions for all pectin sources, brittleness was in the range 50% to 70%. The effect of pH, sugar, and calcium content on brittleness showed no systematic trends (Tables 5A and 5B). However, CALMP gels were nonbrittle under most conditions with 40% and 60% sugar content.

Low-methoxyl pectin can be used for a number of food applications, including the production of low-sugar (sucrose) jellies, an emulsion stabilizer for frozen yogurt, fruit gum, confectionery jellies, milk gels, a bakery filling, a thickening agent for low-caloric creams, sauces and dressings (Pilgrim et al., 1991; Hoefler, 1991). Because of the smooth mouth feel of the pectin jellies and the ability of the LMP to gel in the presence of calcium chloride, the potential of LMP to replace fat in several reduced caloric food formulations is high. The results from this study clearly indicate that sunflower pectin possesses the functional properties, which can be used for low-caloric food formulation, particularly when a near-neutral pH is required.

CONCLUSIONS

SUNFLOWER pectin formed firm jellies in formulations with a wide range of calcium and with a wide range of sugar at pH

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Extraction and Physicochemical Characterization of Pectin from Sunflower Head Residues

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ABSTRACT

Pectin was isolated from sunflower head residues, using 0.75% sodium hexametaphosphate extraction followed by acid precipitation. The yield of pectin was7.3% of the head residues. The isolated pectin contained 89.2% anhydrogalacturonic acid, 2% acetyl ester, and 4.2% neutral sugars, which were mainly rhamnose and glucose. The degree of methylation was 38.5%. The pectin had a high viscosity (527 cp at 1% level) at pH 3 and a high water-holding capacity (57g water/g organic matter). The peak molecular mass of the sunflower pectin was >523,000 daltons.

Key Words: sunflower, pectin, extraction, sugars, methylation

INTRODUCTION

GENERALLY, citrus peels and apple pomace are good sources of high-methoxyl pectin (HMP). Commercial low-methoxyl pectin (LMP) products from citrus or apple are de-esterified from the extracted HMP (Axelos and Thibault, 1991). However, sunflower head residues, which are the heads that remain after seeds have been harvested, are naturally rich in LMP. The LMP in sunflower head residues can be extracted with solutions containing chelating agents, including sodium hexametaphosphate (SHMP), ammonium oxalate, or disodium ethylenediamine tetraacetic acid (EDTA) (Sosulski et al., 1978). The extraction process, the variety, and the stage of sunflower maturity (Campbell et al., 1978) can affect the quantity and quality of extracted pectin. Low-methoxyl sunflower pectin can be used to prepare jellies with low sugar content or without sugar in the presence of a small amount of calcium and could be used as an ingredient for formulating low-calorie foods.

Generally, pectins are substances composed primarily of galacturonic acid and some neutral sugars, such as rhamnose, arabinose, and galactose. Rhamnose coexists with galacturonic acid in the main chain of pectin, and arabinose and galactose are found in the side chains. Depending upon sources, galacturonic acid residues of the pectin are esterified to various degrees, and the size and composition of the neutral sugar side chains vary. Degree of esterification, molecular size, attached chains of neutral sugars, acetylation, amidation and cross-linking of pectin can affect the textural properties of pectin gels (BeMiller, 1986; May, 1990). Ash content also can affect the ability of pectin to gel.

Researchers differ on the reported molecular size of sunflower pectin. Neutral sugar composition, viscosity at various pH values, and water-holding capacity of sunflower pectin have not been reported. By understanding the physicochemical properties of pectin extracted from sunflower heads, food technologists would be able to better use sunflower pectin in food product development and processing.

The objectives of this study were (1) to isolate pectin from sunflower heads; (2) to study the physicochemical properties of the sunflower pectin; and, (3) to compare sunflower pectin with selected commercial high- and low-methoxyl citrus pectins.

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MATERIALS & METHODS

Materials

Oilseed-type sunflower head residues were obtained from the Carrington Research Center (Carrington, ND). The head residues were dried in a cabinet oven with air circulation at 55°C. The dried head residues were crushed, ground by a hammer mill to pass a 60-mesh size screen, and stored at −20°C until use. Citrus high-methoxyl pectin (Genu BB Rapid Set) (CHMP1) and citrus low-methoxyl pectin (Genu LM 12CG) (CLMP) were obtained from Hercules Incorporated (Middletown, NY). A second sample of citrus high-methoxyl pectin (CHMP2) was obtained from Sigma Chemical Co. (St. Louis, MO). All experiments in this study were conducted in duplicate.

Proximate Chemical Analysis

The moisture content was determined by an air-oven method (AACC method 44.15A, 1983). Ground sunflower samples and pectin were dried in an air-circulated oven at 135°C for 2 hr. The ash content was measured by incinerating the sample overnight in a muffle furnace at 550°C (AOAC method 14.006, 1984). Protein was measured by the Kjeldahl method (AOAC method 2.057, 1984). Lipid was extracted with chloroform:methanol (3:1, v/v).

Pectin isolation

Pectin was extracted from sunflower heads by a modification of the method of Sosulski et al. (1978). After acid precipitation, the pectin was washed three times with 1.5 volumes of 60% alcohol and dried in an vacuum oven. After drying, the pectin was washed with pH 1 HCl twice and rewashed with 60% alcohol to reduce ash content. Instead of using a laboratory hydraulic press, the precipitated pectin was pressed by hand as much as possible.

Analysis of sunflower pectin

Nonstarchy polysaccharides in ground head residues and SHMP-extracted residues were determined as the sum of neutral sugars and uronic acid according to the method of Englyst et al. (1988). The residues were subjected to sequential treatments with dimethyl sulfoxide, α-amylase and pullulanase to remove starch. The non-starchy polysaccharides were precipitated with ethanol and subjected to acid hydrolysis to form monosaccharides, followed by reduction and derivatization to alditol acetates, which were analyzed using gas chromatography (Hewlett 5890 Packard Series II, Avondale, PA). Galacturonic acid was determined by a colorimetric procedure, using 3,5-dimethylphenol as a color reagent (Scott, 1979).

The method of Englyst et al. (1988) also was used to determine neutral sugar composition of the standardized pectin (CHMP1 and CLMP). Sucrose in the standardized pectin was removed in the precipitation and washing steps as described by Englyst et al. (1988). Neutral sugars in unstandardized pectin, including CHMP2 and sunflower head pectin, was determined by a modification of the Englyst et al. (1988) method. The unstandardized pectin samples were hydrolyzed directly with 12 M sulfuric acid at 35°C for 1 hr; then 22 mL of water were added. The mixture was placed in a boiling water bath for 2 hr. After hydrolysis, the monosaccharides were reduced, acetylated and determined using gas chromatography as described by Englyst et al. (1988).

The methyl ester content was determined, using an alcohol oxidase method (Klavons and Bennett, 1986). The degree of methylation of

Table 1—Sugar composition of nonstarchy polysaccharides in oil-seed type sunflower head residues[a]

Sugar	Percentage
Rhamnose	1.25 (0.11)
Arabinose	1.47 (0.03)
Xylose	3.50 (0.14)
Mannose	1.13 (0.08)
Galactose	1.23 (0.04)
Glucose	11.02 (0.17)
Total neutral sugar	20.50 (0.16)
Uronic acid	19.70 (0.02)
Total neutral and acidic sugar	40.20

[a] Data represent the means of four determinations with standard deviations in the parentheses and are expressed on a dry-weight basis.

Table 2—Sugar composition of nonstarchy polysaccharides of the insoluble fraction after sodium hexametaphosphate extraction[a]

Sugar	Percentage
Rhamnose	1.88 (0.05)
Arabinose	2.73 (0.12)
Xylose	8.87 (0.26)
Mannose	2.23 (0.07)
Galactose	1.87 (0.06)
Glucose	26.07 (0.36)
Total neutral sugar	43.61 (0.62)
Uronic acid	19.58 (1.85)
Total neutral and acidic sugar	63.19

[a] Data represent the means of four determinations with standard deviations in the parentheses and are expressed on a dry-weight basis.

the pectin was calculated by multiplying the molar ratio of methyl groups to anhydrogalacturonic acid by 100. Acetyl ester content was determined by an enzymatic method (Boehringer Mannheim Biochemicals, 1984). The determination was based on the formation of NADH, which was measured by the increase in absorbance at 365 nm. Pectin samples from sunflower heads were hydrolyzed by 1N KOH for 30 min at room temperature. The acetyl content was estimated, using a standard curve prepared with known amounts of acetic acid.

Ferulic acid was analyzed spectrophotometrically (IBM Instruments Inc., Model 9410) at 320 nm (Fry, 1979). The polymer esterified ferulic acid in pectin samples was hydrolyzed in 0.1N NaOH. The ferulate concentration was estimated, usinga ferulic acid standard curve.

The molecular mass was determined using gel filtration (Pharmacia, 1979). Sepharose CL-6B was packed in a 1.6 × 96 cm column (bed volume, 193 mL) and equilibrated with 0.01M sodium hexametaphosphate (SHMP) buffer pH 6.0, containing NaCl (0.1M) and sodium azide (1 mM). The exclusion and inclusion volumes (V_o and V_i) of the column were determined using blue dextran (average MW 2 million daltons) and sucrose, respectively. Molecular mass standard curve was determined, using dextran standards (average MW 523,000, 150,000, 39,200, and 9,000 daltons). Pectin samples were dissolved in the same buffer and eluted at a flow rate of 12.5 mL/hr. Total sugars were determined on the column eluent fraction using a colorimetric method (Dubois et al., 1956). Galacturonic acid in the fractions was measured by a colorimetric method (Scott, 1979).

Water-holding capacity (WHC) was determined using the osmotic suction pressure procedure (McBurney et al., 1985). The WHC of pectin samples was measured by weighing the hydrated samples after they were placed under the osmotic-suction pressure of 89 mosmol/L (atm) across a dialysis membrane. The WHC was expressed as water held (g) organic matter (g).

Absolute viscosity was determined using a Wells-Brookfield Cone/Plate Viscometer (Model RTV). A citrus pectin (CHMP2) was used as a reference. Pectin (1%, w/v) samples were prepared in a 0.5M sodium citrate buffer (pH 3, 4, 5, 6) and in a 0.5M sodium phosphate buffer (pH 7) and hydrated at 4°C overnight before measuring viscosity (cp) at 25°C. Viscosity of pectin heated at 100°C for 2 min also was determined after cooling to 25°C.

RESULTS & DISCUSSION

Proximate chemical analysis

The dried oil-type sunflower head residues contained 7.7% moisture and 18.5% ash, 6.5% crude protein, 3.1% crude lipids, and 71.9% carbohydrate (by difference) on a dry weight basis. The total amount of the nonstarchy polysaccharides in the head residues was ≈40% (Table 1). Sugars, starch, and lignin may have contributed the difference (≈32%), which was the value between 71.9% of the total carbohydrate and 40.2% of the total amount of nonstarchy polysaccharides. The pectin content, determined as anhydrogalacturonic acid, in the head residues was ≈20% (dry weight basis). Therefore, about half of the non-starchy polysaccharides were pectin. Lin et al. (1975) reported the pectin content of four cultivars of sunflower ranged from 15–24%. The predominant neutral sugar in the nonstarchy polysaccharides of sunflower head residue was glucose, which might be attributed to cellulose.

Bishop (1955) reported that acid hydrolysis of the neutral polysaccharides in sunflower head residues yielded an aldobiuronic acid (1.53%) and a mixture of neutral sugars: xylose (59.3%), glucose (23.5%), galactose (16.2%), arabinose (trace), and rhamnose (trace). According to Edrees et al. (1976), the major components of sunflower head residues were comprised of galacturonic acid (8%), rhamnose (21%), galactose (3.4%), glucose (1.7%), arabinose (0.63%), and xylose (0.7%).

Table 3—Sugar and ash compositions of sunflower pectin and commercial pectins[a]

Composition (%)	Sunflower	CHMP1[b]	CHMP2[c]	CLMP[d]
Rhamnose	1.65(0.02)	1.01(0.10)	1.49(0.13)	0.57(0.11)
Arabinose	0.70(0.07)	1.73(0.08)	1.23(0.17)	ND
Xylose	0.29(0.01)	0.09(0.03)	0.14(0.03)	ND
Mannose	0.09(0.01)	ND	0.18(0.04)	ND
Galactose	0.63(0.02)	2.90(0.07)	4.62(0.36)	1.45(0.02)
Glucose	1.10(0.23)	0.70(0.16)	0.76(0.16)	0.54(0.06)
Total neutral sugar	4.26(0.32)	6.46(0.23)	8.42(0.85)	2.58(0.01)
Uronic acid	89.2 (0.20)	47.0 (1.30)	77.0 (1.92)	53.4 (2.45)
Total sugar (neutral and acidic)	93.3	53.5	85.4	55.98
Ash	1.24(0.00)	2.57(0.00)	5.22(0.02)	1.91(0.01)

[a] Data represent the means of four determinations with standard deviations in the parentheses and are expressed on a dry-weight basis. ND stands for not detectable.
[b] A citrus high-methoxyl pectin (BB RAPID SET) from Hercules Inc., Middletown, NY.
[c] A citrus high-methoxyl pectin (P-9135) from Sigma Co., St. Louis, MO.
[d] A citrus low-methoxyl pectin (LM 12CG) from Hercules Inc., Middletown, NY.

Table 4—Relative percentages of sugars of sunflower pectin and commercial pectins[a]

Composition (%)	Sunflower	CHMP1[b]	CHMP2[c]	CLMP[d]
Rhamnose	1.77(0.02)	1.89(0.19)	1.74(0.15)	1.02(0.20)
Arabinose	0.75(0.08)	3.23(0.15)	1.44(0.20)	ND
Xylose	0.31(0.01)	0.17(0.06)	0.16(0.04)	ND
Mannose	0.10(0.01)	ND	0.21(0.05)	ND
Galactose	0.68(0.02)	5.42(0.13)	5.41(0.42)	2.59(0.04)
Glucose	1.18(0.25)	1.30(0.29)	0.89(0.19)	0.96(0.11)
Total neutral sugar	4.57(0.34)	12.1 (0.43)	9.86(1.00)	4.61(0.02)
Uronic acid	95.6 (0.21)	87.9 (2.43)	90.2 (2.25)	95.39(4.38)
Total sugar (neutral and acidic)	100.0	100.0	100.0	100.0

[a] Data represent the means of four determinations with standard deviations in the parentheses and are expressed on a dry-weight basis. ND stands for not detectable.
[b] A citrus high-methoxyl pectin (BB RAPID SET) from Hercules Inc., Middletown, NY.
[c] A citrus high-methoxyl pectin (P-9135) from Sigma Co., St. Louis, MO.
[d] A citrus low-methoxyl pectin (LM 12CG) from Hercules Inc., Middletown, NY.

Extraction yield of pectin

The average yield of the sunflower pectin was 7.3% of the dried residues. The pectin yield produced in our laboratory was lower than the 10% value Lin et al. (1978) reported, using a similar extraction procedure. The SHMP-insoluble fraction (Table 2) contained ≈63% of nonstarchy polysaccharides. The pectin content, determined as anhydrogalacturonic acid in the SHMP-insoluble residues, was still high, indicating some pectin might exist as insoluble protopectin.

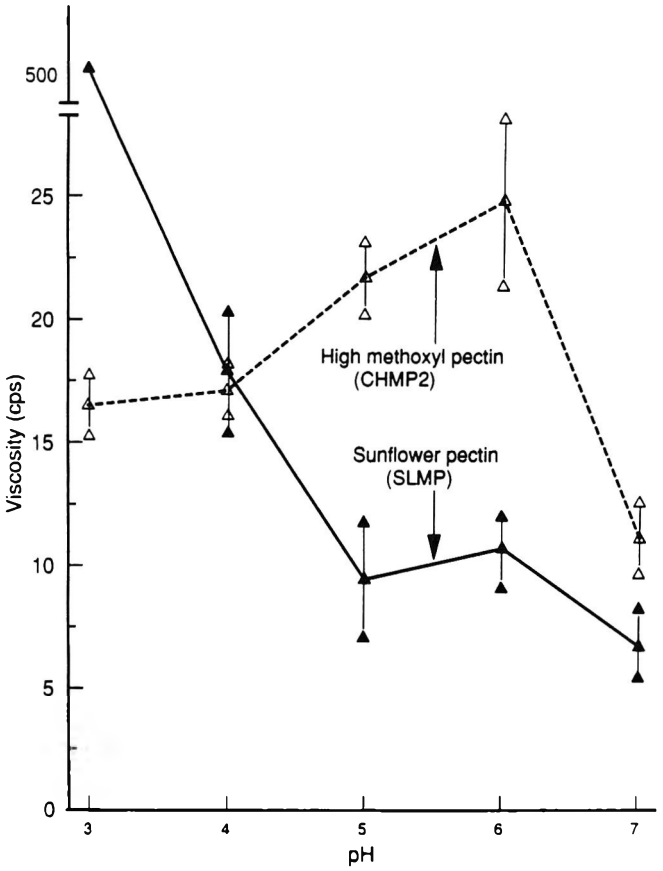

Fig. 1—Viscosity of 1% (w/w) sunflower pectin at 25°C and at various pH values without heat treatment.

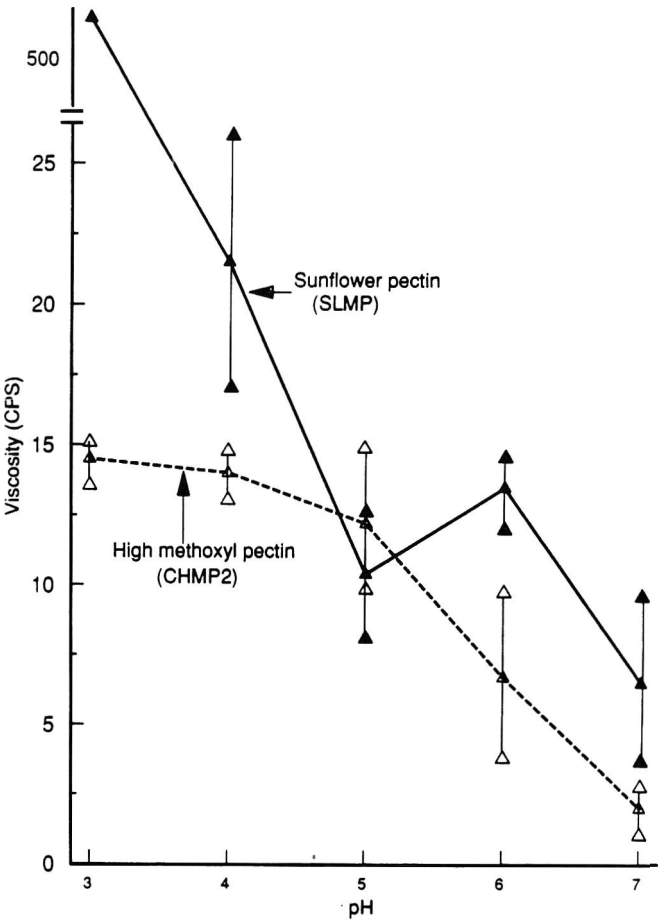

Fig. 2—Viscosity of 1% (w/w) sunflower pectin at 25°C and at various pH values with heat treatment.

Physicochemical characterization of pectin

Sugar and ash compositions of sunflower pectin and commercial pectins were compared (Table 3). Relative percentages of the sugar composition of sunflower pectin and commercial pectins are shown (Table 4). The sunflower pectin contained 89.2% anhydrogalacturonic acid, which was similar to the 87.1% value Sosulski et al. (1978) reported, but was much higher than the commercial citrus pectin products (Table 3). The low galacturonic acid content and the low total sugar in CHMP1 and CLMP was because pectins in those samples had been mixed with sucrose to a standard grade. When the individual sugars were calculated as percentages of the total sugar of the pectin (Table 4), the uronic acid content of sunflower pectin was higher than SHMP1 and SHMP2. Lin et al. (1978) showed that sunflower pectin isolated by SHMP extraction andacid precipitation contained 98.0% galacturonic acid. A commercial standardized citrus pectin and an unstandardized citrus pectin contained 49.9% and 83.9% uronic acid, respectively (Sosulski et al., 1978).

Neutral sugars (Table 4), including rhamnose, glucose, arabinose, galactose, xylose, and mannose, also were found in the sunflower pectin. Rhamnose normally is inserted in the main chain of the polygalacturonic acid. Arabinose and galactose could be present as side chains of the pectin polymers (Zitko and Bishop, 1965; Rees and Wight, 1971; DeVries et al., 1986). Other researchers had not reported a complete profile of the neutral sugars in sunflower pectin. Commercial high-methoxyl pectin had a higher relative percentage of total neutral sugars (Table 4).

Galactose was the major neutral sugar in commercial citrus low- and high-methoxyl pectins. Arabinose in HMP also was significantly greater than that in sunflower pectin (Table 4). In addition to uronic acid, the presence of neutral sugars in pectin may affect its jellying capacity (Speiser and Eddy, 1946; Hills et al., 1949; Kertesz, 1951). Generally, pectin with a high neutral sugar content has a low gelling power (Schneider and Bock, 1937). The ash content of the sunflower pectin in our laboratory was 1.24% (Table 3). Ash contents of the commercial high- and low-methoxyl pectin varied from 1.9 to 5.2%. Ash content also can affect the gelling of pectin. A low ash content is more favorable for gel formation (Lin et al., 1976; 1978; Sosulski et al., 1978).

Methyl ester content in terms of percent degree of methylation of the sunflower pectin was 38.5%, which is in the range (28.8% to 40.4%) of low-methoxyl content reported by Lin et al. (1976; 1978) and Sosulski et al. (1978). The acetyl content (2.01%) in the unmodified sunflower head pectin was similar to that (1.90%) Kim et al. (1978) reported. The acetyl content may adversely affect functional properties, such as pectin solubility, pregelation, brittleness, and granuality. Kim et al. (1978) studied the effect of amidation and de-acetylation on the gelling capacity of the sunflower head and stalk pectin. They found several treatments with ammonium hydroxide could improve the gelling capacity of sunflower pectin.

Ferulic acid is present in spinach and sugar beet pectin, but has not been reported in sunflower pectin (Fry, 1983; Phatak et al., 1988). Ferulic acid was not detected in the sunflower pectins studied here.

The water-holding capacity of sunflower pectin was 57.9 g water/g organic matter, which does not differ statistically from the water-holding capacity of the commercial CHMP (61.8% g water/g organic matter). A high water-holding capacity is important to get good quality gels. When pectin can hold high amounts of water, it can reduce the free water within the gel matrix. Water holding capacity is one of the physical properties of soluble dietary fiber to affect nutrient digestion and absorption. A high water-holding capacity may increase the viscosity

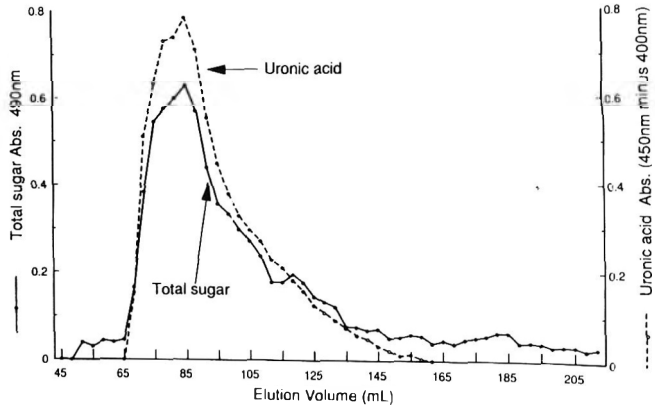

Fig. 3—The elution pattern of sunflower pectin from a Sepharose CL-6B column. The void volume and inclusion volume were 74 mL and 182 mL, respectively. The peak elution volumes for molecular mass markers 523,000, 150,000, 39,000 and 9,000 daltons were 98.9 mL, 115.6 mL, 139.8 mL, and 168.4 mL, respectively.

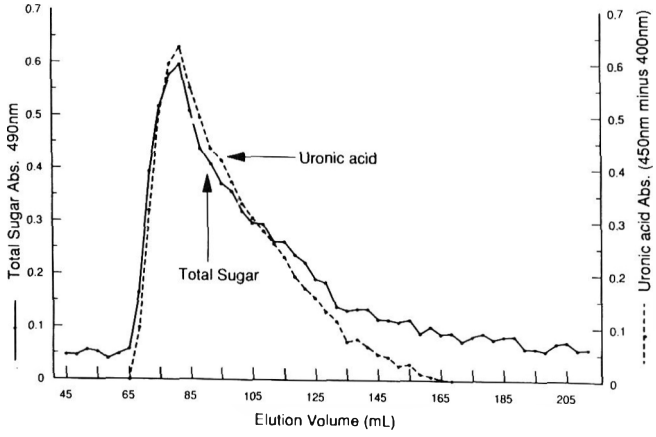

Fig. 4—The elution pattern of a commercial citrus high-methoxyl pectin from a Sepharose CL-6B column. The void and inclusion volumes and molecular mass marker elution volumes are the same as in Fig. 3.

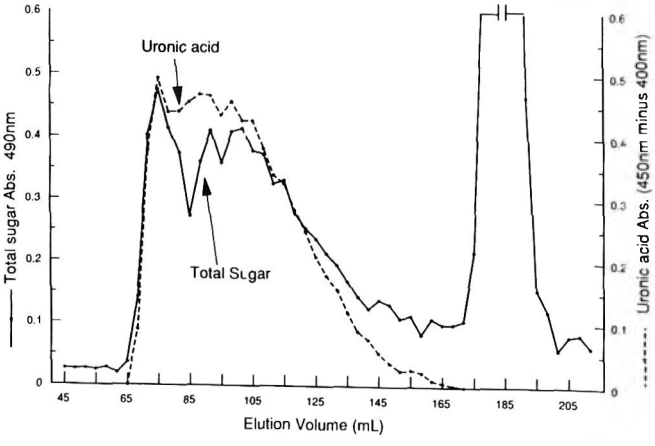

Fig. 5—The elution pattern of a commercial citrus low-methoxyl pectin from a Sepharose CL-6B column. The void and inclusion volumes and molecular mass marker elution volumes are the same as in Figure 3.

of the intestinal contents and slow nutrient absorption (Schneeman, 1986).

The viscosities of 1% (w/v) unheated (Fig. 3) and heated pectins (Fig. 4) at 25°C at various pH values are shown. The pH significantly affected the viscosity of sunflower pectin. At pH 3, the viscosities of the heated and unheated sunflower pectins were much higher than a CHMP (Fig. 1 and 2). At pH 4 to 7, the viscosities of unheated sunflower pectin ranged from 7 to 17 cp, which were lower than those observed in citrus pectin. Heating (100°C for 2 min) slightly increased viscosity of the sunflower pectin. However, heating (100°C for 2 min) slightly decreased viscosity of the citrus pectin. Pectin with a high viscosity at an acidic range may be used to thicken fruit juice and beverages.

Typical elution patterns of the sunflower pectin (Fig. 3), a commercial CHMP (Fig. 4), and a commercial CLMP (Fig. 5) from a Sepharose CL-6B column are shown. The molecular mass (relative to standard dextrans) of the sunflower pectin was estimated to range from ≈150,000 to >523,000 daltons, with a major peak molecular mass of >523,000 daltons (Fig. 3). The molecular mass of the CHMP ranged from 80,000 daltons to >523,000 daltons (Fig. 4), while CLMP ranged from 63,000 daltons to 523,000 daltons (Fig. 5).

The range of molecular mass distribution of sunflower pectin (Fig. 3) was narrower and relatively greater than those of the CLMP (Fig. 5). The elution pattern of the CHMP1 was similar to that of the sunflower pectin though the CHMP1 pattern had a slightly larger tailing (Fig. 4). A large peak of free sugar was found at elution volume 175 mL to 195 mL in the elution curve of the CLMP because this pectin had been standardized with sucrose. The molecular mass of sunflower head pectin has been reported to be 30,000-300,000 daltons (Gross et al., 1982), 147,000 daltons (Kim et al., 1978), 105,000 daltons (Alarcao e Silva, 1990) and 27,300 daltons (Turmucin et al., 1983). The molecular mass of pectin could be affected by extraction method, quality of the heads at harvest, cultivar, and method of determination. Generally, a high molecular mass is more favorable for gel forming. Low-methoxyl pectin produced by alkaline treatment or ammonia de-esterification may break down the pectin to smaller molecular species (Whistler and BeMiller, 1958; Padival et al., 1979; Kim et al., 1978).

CONCLUSIONS

Sunflower pectin contained a high amount of galacturonic acids and had 38.5% methoxyl ester content. The pectin also contained neutral sugars and acetyl ester groups. The pectin had a higher viscosity at pH 3.0, higher water-holding capacity and higher molecular sizes than CLMP. Sunflower head pectin has a high potential for commercial food applications.

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*GELATION OF SUNFLOWER PECTIN. . .*From page 1438

5.4 and 6.0. In general, an increase in sugar or calcium concentration increased firmness of jellies. Strong interactions among the factors of pH, sucrose, and calcium existed for all three sources of pectin. Generally, the textural profile of sunflower pectin was comparable to the commercial citrus low-methoxyl pectin. The sunflower pectin could form jellies with no or low sugar content with 30 to 90 mg calcium content. The ability of the sunflower pectin to form jellies at near-neutral pHs at low sugar concentrations would be useful for producing low-caloric foods with a more neutral taste than the acidity and high sugar content required for the HMP to gel.

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Determination of Low Level Trans Unsaturation in Fats by Fourier Transform Infrared Spectroscopy

FRANZ ULBERTH and HANS-JÖRG HAIDER

ABSTRACT

Fourier Transform infrared spectroscopy (FTIR) was an excellent tool for rapid determination of trans unsaturation in edible fats. Methyl esters were determined as a neat solution, thereby avoiding cumbersome weighing operations and handling of CS_2. The method was calibrated by gravimetrically prepared mixtures of methyl elaidat in methyl esters of a trans fatty acid (TFA) free soy oil. In contrast to TFA concentrations > 10%, which could be determined easily by the classic baseline technique, low TFA values were only quantifiable (with high accuracy) after a computer-assisted spectral subtraction procedure.

Key Words: soy, fats, FTIR spectroscopy, trans unsaturation, fatty acids

INTRODUCTION

PARTIAL hydrogenation of vegetable oils results inevitably in the formation of octadecenoic acid isomers with different positional and geometrical configuration of the double bond. Trans unsaturated fatty acids (TFA) in dietary fats have been implicated by some researchers in the etiology of various metabolic disorders (Hanis et al., 1989, Mensink, 1990). Therefore, the accurate estimation of TFA in edible fat products is of major concern. Since isolated trans double bonds exhibit an IR absorption band at 967 cm^{-1}, the determination of TFA by means of IR spectroscopy is widely used in fat and oil industries. In order to ensure an acceptable precision of this method, it has been standardized by IUPAC (1987) and AOCS (1981). In those protocols the application of dispersive IR instruments is specified. The Fourier Transform IR (FTIR) technique has been introduced successfully for the determination of isolated TFA isomers in dietary fats (Lanser and Emken, 1988; Sleeter and Matlock, 1989). This type of instrumentation offers several advantages over conventional IR spectrometers (Günzler and Böck, 1983): (a) improved S/N ratio due to the averaging of multiple scans, (b) high accuracy of the wavelength calibration due to a reference He-Ne laser, (c) improved light through-put and speed of analysis due to the Michelson interferometer principle and (e) spectra manipulation by means of computers due to the digital format of the spectroscopic data.

Despite the numerous advantages of FTIR, the principal problem of an accurate quantitation of low TFA concentrations (< 15%) is not completely resolved. Inherent to the direct measurement of TFA in native oil or fat samples is the interference of acylglycerols, because of their broad absorption band in the region of the trans C-H out-of-plane deformation (Kaufmann et al., 1959). This disadvantage is normally circumvented by transesterification of the starting material to form methyl esters, but they produce TFA values which are 1.5–3% low (Firestone and LaBouliere, 1965). Arithmetic compensation or a differential IR measurement procedure have been proposed to overcome this limitation of the IR technique (Huang and Firestone, 1971a). Reports dealing with the determination of TFA by FTIR did not address those general problems, although Sleeter and Matlock (1989) indicated that a quadratic equation should be used to correct for the observed curvature in the calibration graph (% trans vs band area). Direct read-out of the area under an absorption peak is a prerequisite for this kind of calibration. This feature is not always available with commercial software packages used for quantitative FTIR analysis. Therefore, we investigated a spectral subtraction technique which enables the assessment of low concentrations of isolated trans double bonds in hydrogenated fats. Results of this procedure were in good agreement with the known TFA concentration of gravimetrically prepared standard mixtures. Furthermore, we explored the application of partial least squares (PLS) analysis for the quantification of low TFA levels in edible fats, and compared those results with the results of the spectral subtraction technique.

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MATERIALS & METHODS

Materials

Cold-pressed soy oil was donated by Farina Mühlengesellschaft (Graz, Austria); all other edible fats were purchased at retail outlets. Methyl elaidat (purity > 99%) was from E. Merck (Darmstadt, Germany).

Methods

Fat samples were melted in an oven at 55 °C, the oily layer decanted and filtered over anhydrous Na_2SO_4. Methyl esters were produced via alkaline transesterification of the melted fat with methanolic 2N KOH (Christopherson and Glass, 1969). After 5 min transesterification 0.05 mL glacial acetic acid was added and the organic phase was washed twice with an aqueous NaCl solution (50 g/L). The ester solution was dried over anhydrous Na_2SO_4, filtered and the solvent removed in a vacuum evaporator (Büchi; Flawil, Switzerland).

A Bomem Michelson MB-100 interferometer with DTGS detector (Bomem Inc., Québec, Canada) connected to an IBM/AT compatible PC equipped with a 80287-coprocessor was used for recording the IR spectra and data manipulations. The region from 5 000 cm^{-1} to 400 cm^{-1} was scanned with a resolution of 2.00 cm^{-1} (15 scans/sample). Esters were analysed as neat solutions as described by Sleeter and Matlock (1989) in a fixed pathlength cell (nominal thickness = 0.100

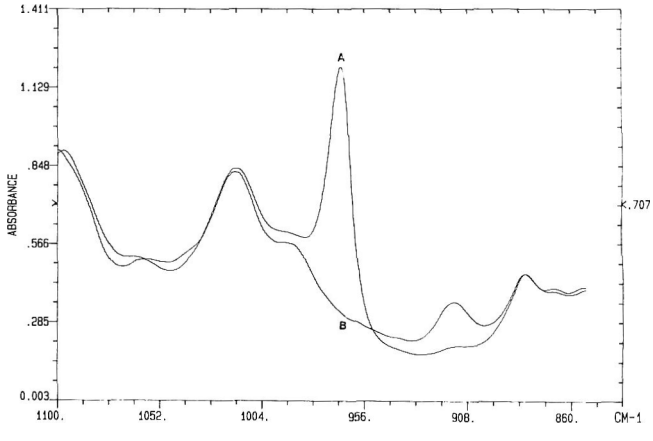

Fig. 1. – FTIR spectrum of a sample high (A) and low (B) in TFA.

Table 1—Effect of different calculation methods on % of TFA (as methyl esters)

	AOCS[a]		IUPAC[b]		LE[c]	PLS-1	Corrected[d]	
Weighed	Area	Height	Area	Height	Area	Area	Area	Height
0.00	−2.34	−2.49	−23.14	−4.20	2.53	0.08	--	--
1.92	−0.26	−0.32	−19.71	−1.91	4.36	1.87	1.92	1.96
4.14	2.22	2.13	−15.10	0.73	6.34	4.18	4.22	4.21
6.77	5.04	4.97	−10.02	3.75	8.62	6.81	6.78	6.77
10.79	9.48	9.56	−2.73	8.57	12.40	10.96	10.76	10.89
15.34	14.42	14.34	6.77	13.71	16.21	15.28	15.30	15.25
18.31	17.64	17.63	12.62	17.23	19.08	18.28	18.25	18.25
22.00	21.85	21.78	20.18	21.65	22.24	22.03	22.08	22.00
27.53	27.72	27.70	30.78	27.94	26.97	27.49	27.38	27.35
38.71	40.33	40.41	53.37	41.48	37.23	39.10	38.83	38.86
SE[e]	1.51	1.57	14.75	2.65	1.64	0.14	0.08	0.10

[a] Baseline according to AOCS (from 998 to 944 cm^{-1})
[b] Baseline according to IUPAC (from 1000 to 925 cm^{-1})
[c] Baseline according to Lanser and Emken (1988) (baseline from 1045 to 945 cm^{-1} and area integration from 982 to 945 cm^{-1})
[d] Baseline according to AOCS applied to corrected FTIR spectra (sample spectrum minus spectrum of TFA free soy oil)
[e] Standard error of estimate

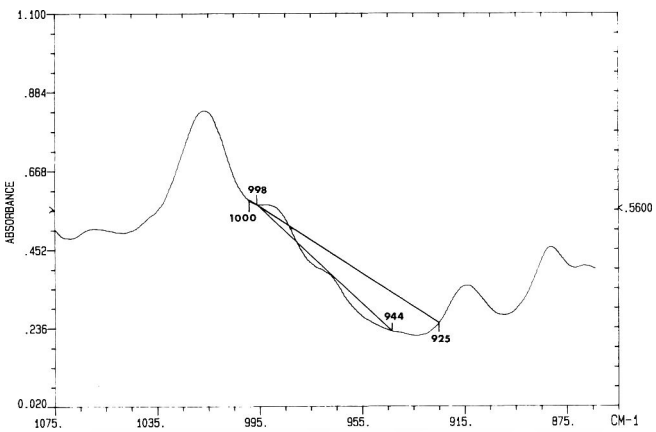

Fig. 2.—Baselines drawn according to IUPAC (1000 to 925 cm^{-1}) and AOCS (998 to 944 cm^{-1}), respectively.

mm) with NaCl windows (Perkin-Elmer Corp., Norwalk, CT). After each measurement the cell was thoroughly rinsed with n-hexane and dried with N_2. Standard mixtures were prepared by blending accurately known amounts of cold-pressed soy oil methyl esters and methyl elaidate. The TFA concentration of the standards ranged from 1.92 to 38.7% (w/w).

The Bomem Easy Ver. 1.41c and the SpectraCalc PLSplus Ver. 2.0 (Galactic Industries Corp., Salem, NH) software packages were used for quantification purposes. The absorption band between 998 and 944 cm^{-1} was the basis for computation of peak area or peak height according to AOCS Method Cd 14–61 (1983), and 1000 and 925 cm^{-1} according to IUPAC Method 2.207 (1987), respectively. With both evaluation methods the K-matrix option (Bomem Easy) was the basis for the calculation. For the PLS-1 procedure (SpectraCalc) the AOCS wave number boundaries were used.

RESULTS & DISCUSSION

THE EXCELLENT performance of the FTIR technique in terms of the precision of the TFA data and the long term stability of the calibration function has been described by Sleeter and Matlock (1989). A major advantage of this method is that fatty acid methyl esters (FAME) can be analysed as neat solutions, thereby avoiding handling of CS_2 (health hazard). The IR spectrum of the neat methyl esters of a margarine oil and a cold-pressed soy oil were compared (Fig. 1). Due to the exceptional high light through-put of the Michelson interferometer it was not necessary to dilute the samples in order to keep the transmission between 20–50% (0.70–0.15 absorbance units). Sleeter and Matlock (1989) also pointed out that vapor or air bubble formation in the sample cell, as a consequence of warming-up the relative low boiling CS_2 solution by IR radiation, often leads to useless results.

Determinations of TFA are based on the "background corrected absorptivity" method. This means that a baseline is drawn between 2 predetermined wave numbers and the absorption of the peak maximum is corrected by the absorbance value of the corresponding baseline point (Firestone and LaBouliere, 1965). In the standard methods described by IUPAC

Table 2—Percent TFA (as methyl esters) of various fats obtained with different evaluation methods

		AOCS[a]		IUPAC		PLS-1	Corrected	
Product		Area	Height	Area	Height	Area	Area	Height
Margarines								
Brand	A	29.16	29.87	34.68	30.27	29.41	28.62	29.30
	B	26.21	26.54	26.93	26.55	26.71	25.92	26.32
	C	20.76	21.80	19.54	21.71	21.76	20.98	21.98
	D	16.87	17.79	12.18	17.40	17.59	17.43	18.33
	E	22.69	23.63	22.42	23.56	22.45	22.69	23.60
	F	19.12	20.13	16.58	19.94	20.28	19.52	20.48
	G	27.80	28.32	30.25	28.49	28.09	27.39	27.94
Dietetic margarines								
Brand	H	−1.70	−1.58	−20.54	−3.03	1.65	0.71	0.93
	I	−1.91	−1.66	−21.96	−3.19	1.01	0.52	0.85
	J	−2.70	−2.30	−25.95	−4.07	−0.70	−0.12	0.27
Shortenings								
Brand	K	18.79	19.17	15.14	18.82	19.36	19.18	19.62
	L	6.17	6.48	−8.65	5.32	7.85	7.83	8.17
	M	−2.24	−1.17	−21.56	−2.69	−0.40	0.15	1.17
	N[b]	−5.32	−3.13	−32.02	−5.29	−5.04	−2.73(1.87)[c]	−0.66(1.88)
	O[b]	−8.34	−5.86	−38.23	−8.27	−8.27	−5.44(−0.82)	−3.13(−0.58)

[a] Abbreviations see Table 1
[b] Based on coconut fat
[c] Values in parenthesis are based on correction of the sample spectra by a TFA free coconut fat

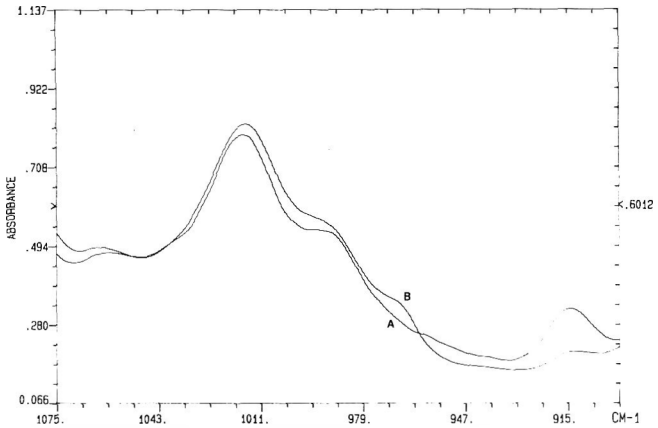

Fig. 3.—FTIR spectrum of TFA free soy oil (A) and (B) dietetic margarine H (Table 2).

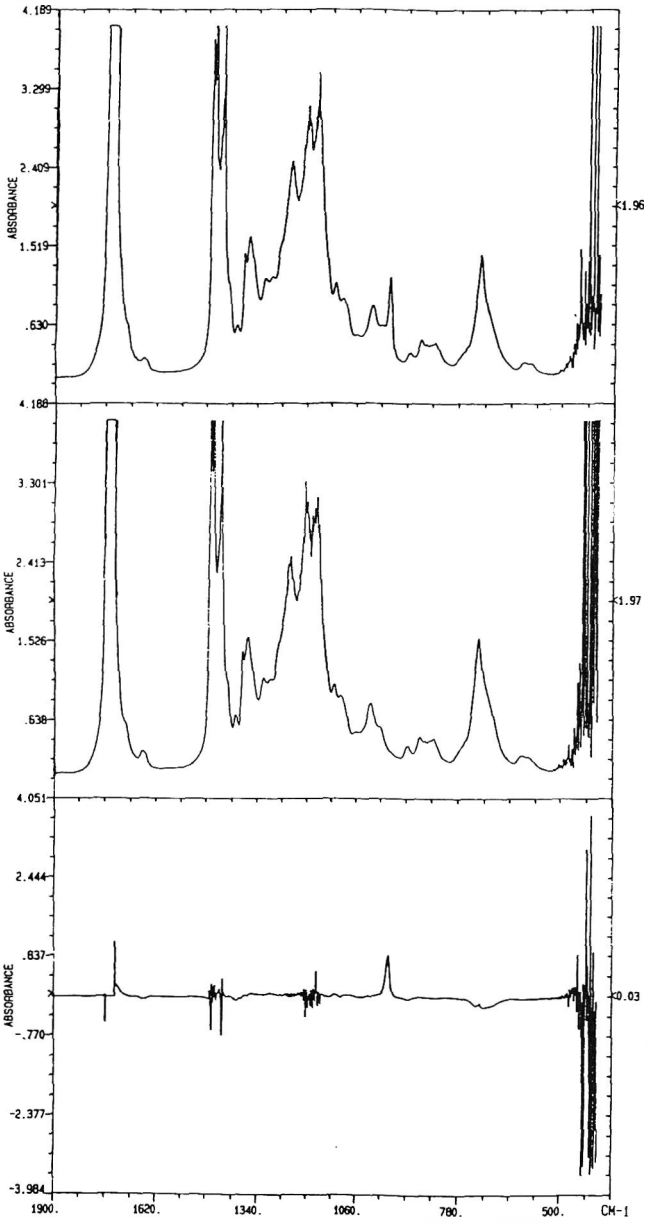

Fig. 4.—Difference spectrum (A - B) of a TFA containing margarine sample (A) and the TFA free soy oil (B).

and AOCS different wave numbers are specified for drawing the baseline. We tested the influence of different baseline

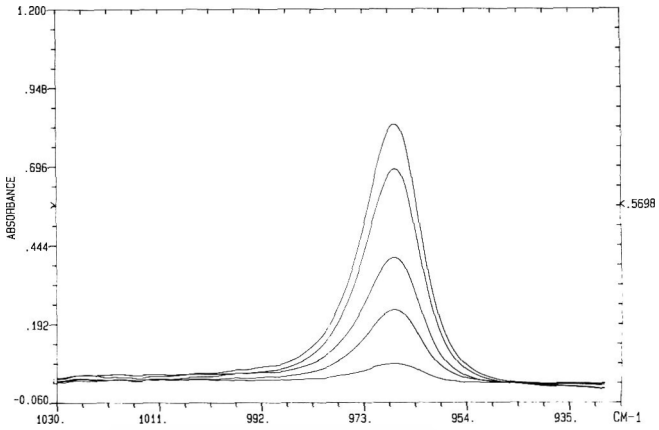

Fig. 5.—Corrected FTIR spectra of standard mixtures containing between 22.00% (most intense peak) and 1.92% (least intense peak) TFA.

boundaries and the computation method (peak height or peak area) on the outcome of TFA measurements of accurately known standards. As can be seen from the standard error of the estimates (SE)(Table 1) quantitative evaluations based on either the peak height or peak area were equivalent with the AOCS baseline boundaries, but not with the IUPAC baseline. In this case, TFA quantification based on peak area measurement showed a very large SE, i.e. 14.75, indicating inadequate accuracy. Changing the computation method from peak area to a peak height measurement improved accuracy (SE = 2.65). The main reason for the deviating results is clearly depicted in Fig. 2. At low TFA concentrations there is nothing left to measure, if a baseline according to IUPAC is used, regardless of the peak evaluation technique (height or area). The AOCS baseline seems to be more appropriate for samples with low TFA values. Nevertheless, huge deviations from the known values were observed at concentrations below 15% TFA.

Lanser and Emken (1988) evaluated different baseline termini and their implications on the accuracy of the FTIR technique. They recommended to adjust the baseline points and the integration limits according to the actual TFA content to obtain valid results. A new calibration was set up with the following baseline points and integration limits: baseline from 945 to 1045 cm^{-1} and area integration between 945 and 982 cm^{-1}. The complete series of calibration mixtures was recalculated with this set of baseline points (Table 1). At the lower range of TFA values the sign of the deviations, i.e. difference between the expected and the actual values, changed, compared with the IUPAC and the AOCS baseline conditions, but accuracy did not improve. The obtained values were ca. 2% (absolute) high, whereas the AOCS values were ca. 2% low for TFA concentrations < 10% (Table 1). Lanser and Emken (1988) reported excellent agreement between the known and the obtained TFA values, even at low concentrations. Their method for calculating % TFA included the term "cell constant" in the denominator of the given equation. However, the method used to calculate that value, particularly the baseline and integration points employed, was not clearly outlined.

PLS is a powerful technique for quantitative analysis of a complex system, even if component bands are highly overlapping. We applied the PLS-1 procedure of the PLSplus software package to the quantitative estimation of TFA by FTIR. Five vectors were included in the computation model and the results of the calibration trial are given in Table 1. An R^2 value of 0.99985 indicated an excellent fit of the data to a straight line. Moreover, SE, a predictor of the average error in the analysis, was as low as 0.142.

A variety of margarines and shortenings were analyzed for TFA by the proposed FTIR method and different quantification methods were applied to the spectroscopic data (Table 2). In

general, the actual TFA content of samples containing > 10% were in good agreement. However, the results obtained with the IUPAC method based on peak area measurement deviated extremely from those of the other methods. Therefore, we concluded that the IUPAC baseline *per se* was not suitable for TFA determinations by FTIR. The AOCS method produced negative TFA values for dietetic margarines and shortenings based on coconut fat. With PLS-1 only 1 of 3 dietetic products were rated with negative values. A visual inspection of the spectra clarified that some of the items with negative TFA results in fact exhibited a small TFA absorption band, which was clearly descernible from the spectra of the trans free soy oil (Fig. 3). Relatively large negative TFA values were observed with the coconut based shortenings, regardless of calculation method.

The deviation of TFA values by IR spectroscopy from the known values is well documented (DeMan and DeMan, 1983). Huang and Firestone (1971) devised a simple differential IR method to correct for the low TFA values when analysing FAME. Because FTIR spectrometers are usually single beam instruments, a direct differential measurement is not possible, but post-run data manipulation by a PC is extremly easy due to the digital format of the FTIR spectra. The outcome of a spectral subtraction experiment (TFA containing margarine sample - TFA free soy oil) is shown in Fig. 4. Even the lowest TFA concentration in the calibration mixtures (1.92%) produced a completely resolved, Gaussian shaped absorption band upon subtraction of the TFA free soy oil (Fig. 5). The AOCS procedure was recalibrated with the background corrected series of calibration standards. Excellent agreement was found between expected and obtained values (Table 1). Based on these findings, the TFA free soy oil spectrum was subtracted from the spectra of all samples tested and the quantitation program was re-run (Table 2). Negative TFA values were only found in the case of the coconut based shortenings. Since the chain length of the FAME mixture may influence the final results of the TFA determination by IR (Huang and Firestone, 1971b), a TFA free coconut fat was alternatively used for background correction of the coconut based shortenings. With that application, a correct classification of the coconut samples was achieved.

The precision of the spectral subtraction was tested by running a margarine fat low in TFA (7.83%) and another one high in TFA (26.57%) in quintduplicate. Each spectrum was corrected by the TFA free soy oil and the TFA content determined by the AOCS peak area method. A RSD of 1.34% for the low and of 0.92% fcr the high TFA fat was found, indicating that no substantial error was introduced by the subtraction process. Despite the notable advantages of the FTIR technique (Sleeter and Matlock, 1989, Lanser and Emken, 1988), the difficulties associated with :he analysis of fats low in TFA were not completely solved by this technique *per se*. The introduction of a differential measurement is a very simple means to improve accuracy of the FTIR technique at low TFA concentrations. It is easy to perform because of various data manipulation options afforded by the computer assisted spectra evaluation system.

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Miss M. Henninger provided technical assistance.

Interaction of Flavor Compounds with Microparticulated Proteins

J.-P. SCHIRLE-KELLER, H. H. CHANG, and G. A. REINECCIUS

ABSTRACT

The vapor pressures of acetaldehyde, diacetyl, ethyl benzene, ethyl caproate, ethyl heptanoate, ethyl sulfide, hexanal, trans-2-hexenal, D-limonene, octanone, 1-pentanol, 2-pentanone, and styrene (50 ppm each) over a vegetable oil emulsion, water or selected fat replacer (aqueous solutions, 37°C) was monitored. The five fat replacers were a whey-based and an egg based microparticulated protein, a pregelatinized tapioca maltodextrin, microcrystalline cellulose (MCC) and a MCC/carboxymethyl cellulose blend. The flavor compounds interacted with oil in a predictable manner following Raoult's Law. The fat replacers generally had little influence on vapor pressure of the flavor compounds behaving as in a water system except for the Simplesse products which had substantial interaction with aldehydes.

Key Words: Fat replacers, flavor, interaction, vapor pressure

INTRODUCTION

FAT SUBSTITUTES comprise an active and intense area of food research directed towards their use in current products and developing new products for the market place. Replacement of fats in foods necessitates overcoming new and unique flavor challenges. Fats serve several functions in food products (e.g. mouthfeel and richness, interact with flavor components for sensory balance and precursors to flavor formation as in deep-fat fried foods or cheese products). Approved fat substitutes are based on carbohydrates and proteins rather than being lipid-based. Lipophilic particles can be formed from carbohydrate or protein to achieve a fat-like texture. Neither of these classes of substitutes would be degraded or metabolized to the same flavor compounds as lipids during processing nor would they interact with any added flavor to give the same sensory profile. Such flavor/fat substitute interaction was the subject of our study.

Substantial research has been devoted to the study of flavor/protein and flavor/carbohydrate interaction. Of these, more research has been devoted to studying flavor interaction with proteins (O'Neil and Kinsella, 1987a; O'Neil and Kinsella, 1987b; Crowther et al., 1980; O'Keefe et al. 1991a, b) than with carbohydrates (Land and Reynolds, 1981; Solms et al., 1973). Some studies on proteins included flavor-protein interaction with soy (O'Neil and Kinsella, 1987a; O'Neil and Kinsella, 1987b; Crowther et al., 1973; O'Keefe et al. 1991a, b; Aspelund and Wilson 1983), whey (Mills and Solms, 1984; Jasinski and Kilara, 1985), egg albumin (EA) (Land and Reynolds, 1981), bovine serum albumin (BSA) (Dumont, 1987) and β-lactoglobulin (BLG) (Dufour and Haertle, 1990; O'Neil and Kinsella, 1987a; Farrell et al., 1987; Diaz de Villegas et al., 1982; Brown, 1984). Some studies have focused on the binding of selected flavor compounds such as aldehydes, ketones, esters, aromatic compounds, or terpenes. BLG was reported to bind β-ionone (ratio 1:1) but not α-ionone, d or l-limonene or geraniol (Dufour and Haertle, 1990). Other aromatic compounds were noted to react with BLG at a 1:1 ratio (Farrell et al., 1987). They explained that the binding site on this protein would have the size of an aromatic ring. O'Neil and Kinsella (1987a) showed that the binding strength of the alkanones (affinity for BLG) increased with the length of the carbon chain but the number of binding sites was independent of the carbon chain length. With aldehydes (Mills and Solms, 1984), an increase in length of the carbon chain increased the quantity that was bound. It is generally accepted that flavor/protein binding increases with the hydrophobicity of the flavor compound (Dumont, 1987; Noar, 1986; Brown, 1984).

If a fat substitute is to successfully replace fat in a food product (normally containing fat), the overall aroma profiles of the two products should be similar. Therefore, the flavor formulation must be modified to reflect the differences that occur in the flavor/fat vs flavor/fat-substitute interactions. Flavors interact with fat in a very predictable manner. This interaction can be approximated using Raoult's law. The interaction we achieve with the various fat substitutes is much less predictable and thus was the subject of our study. Our objective was to study the binding of 13 flavor compounds in five different model systems containing either a fat replacer based on protein (egg or whey protein, Singer and Dunn, 1990) or on carbohydrate and to observe relative vapor pressures in the headspace of the model systems.

MATERIALS & METHODS

Materials

Five fat replacers were used: (1) whey-based microparticulated protein, Simplesse 100 (S-100); (2) egg-based microparticulated protein, Simplesse 300 (S-300) (The NutraSweet Co., Mt Prospect, IL); (3) N-Oil II (National Starch Corporation, Bridgewater, NJ); (4) microcrystalline cellulose/carboxymethyl cellulose Avicel • type RC 591 (MCC/CMC); and (5) microcrystalline cellulose Avicel • type FD-100 (MCC) (FMC Corp., Newark, DE). S-100 has 23.37% protein, 1.72% fat, 16.84% carbohydrate and 1.97% ash. S-300 has less solids than the S-100 (11.5% protein, 0.05% fat, and 10.0% carbohydrate). N-Oil II is a pregelatinized tapioca maltodextrin (Dextrose equivalent < 20). An oil system (Crisco vegetable oil-blend of canola, sunflower and soybean oils) was used as a reference for a fat-containing product, and a water system (emulsifier and water) was used to normalize data. The S-100 and S-300 (fully hydrated products with a paste-like consistency) were received freshly made from the supplier and were stored at 4°C until use (<14 days from receipt). The other matrices are powders and required no refrigeration.

Model flavor system

A solution containing acetaldehyde, diacetyl, ethyl benzene, ethyl caproate, ethyl heptanoate, ethyl sulfide, hexanal, trans-2-hexenal, D-limonene, octanone, 1-pentanol, 2-pentanone, and styrene was prepared as a stock solution at 5,000 ppm (each component except for diacetyl at 10,000 ppm) in propylene glycol. This solvent was chosen because of its low vapor pressure; i.e., it did not interfere with the analysis of the very volatile compounds such as acetaldehyde or diacetyl.

Methods

Model systems were prepared as follows: 177g of distilled water was thoroughly blended with 20g (10%) of a fat replacer or Crisco oil and 1g (0.5%) of Tween 80 (emulsifier). Thereafter, 2g (1%) of

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the stock flavor solution was added and mixed by hand. The model system (15-mL aliquots) was then placed in 20mL-headspace vials that were sealed and stored in a cooler at 4°C. For the MCC/CMC system, only 4g (2%) of the fat replacer could be used due to its very high viscosity in solution. A water system was also prepared which contained water instead of the fat substitute.

Headspace analysis. A Hewlett-Packard (HP) Headspace Autosampler (Model HSS 19375A, Avondale, PA) was used to monitor static headspace concentration of the model system volatiles. The sampling conditions of the HSS 19375A were as follows: 30 sec pressurization, 30 sec filling and 30 sec injection. The headspace loop (3 mL) temperature was 45°C. No cryofocussing was needed. All samples were tempered at 37°C for 40 min prior to analysis.

Gas chromatography. A Hewlett-Packard (Model 5880A, Avondale, PA) gas chromatograph equipped with a flame ionization detector or mass selective detector (MSD) was used for analysis. Separation was achieved on 30 m × 0.32 mm i.d. × 1 μm film thickness fused silica column (J&W Scientific; Rancho Cordoba, CA) coated with 5% phenylmethylsilicone (DB-5). All samples were analyzed under the same GC conditions. The oven temperature was held at 40°C for 1 min and then increased at 5°C/min to 130°C. The injection port was 250°C and the detector was 275°C. Helium was used as carrier gas at column flow ca. 2 mL/min and 15 psi head pressure. The split ratio was 30 to 1. The data were recorded on a HP Level Four integrator. Compounds were initially identified by mass spectrometry and thereafter, retention indices. The data are averages of duplicates.

Experimental design

In the time effect (TE) study, samples were stored at ca. 4°C until they were analyzed. Samples were analyzed in duplicate about every 48 hr for a total of 10 days storage.

In the effect of equilibration temperature (EQ), study samples (prepared the previous day) were tempered at 25, 35, 55, or 70°C for 45 min immediately prior to headspace analysis.

Data presentation

The model system without fat or fat replacer (water system) was used as the reference system for calculation of relative vapor pressure (rvp). The headspace concentrations of each test compound above each fat or fat replacer system were divided by the values for the water system of the corresponding day (or temperature) to determine the flavor compound relative vapor pressure (rvp) in the different matrixes versus water system where no interaction was expected, as shown:

$$\text{rvp} = \frac{\text{matrix X day A value 1} + \text{matrix X day A value 2}}{\text{water day A value 1} + \text{water day A value 2}}$$

RESULTS & DISCUSSION

Interaction as a function of time

Oil matrices. The rvp of flavor compounds in the oil system was predictable and in agreement (Fig. 1) with that expected based on solubility of the flavor compound in oil. In addition, there was no obvious effect of storage time on flavor rvp. Flavor compounds which had limited solubility in oil (i.e., mostly water soluble), exhibited an rvp of ≈1 since there was little solubility of that compound in oil and thus no lowering of vapor pressure. Compounds which had significant oil solubility had reduced rvp in the oil systems. Thus as expected, ethyl benzene, limonene and styrene (Fig. 1) resulted in the lowest rvp values. These components are very nonpolar and their partition ratio (water:oil) is very small. Ethyl caproate as well as octanone showed similar results as hydrocarbons due to their nonpolar nature. Compounds with less oil solubility, (e.g., diacetyl, pentanone, pentanol, hexanal, trans-2-hexenal and ethyl sulfide) exhibited a greater rvp approaching 1 for acetaldehyde which is very water soluble.

Protein-based fat replacers. The results for both the whey-based (S-100) and egg-based (S-300) systems were less predictable. In the S-300 system, little flavor interaction was observed. The compounds tended to exhibit rvp values as if they were in water (except for trans-2-hexenal). All rvp values were near 1. There appeared to be little effect of time on interaction of the flavor compounds. The substantial decrease in rvp for the trans-2-hexenal was assumed due to Schiff's base formation although this has not been confirmed. The unsaturated aldehyde is apparently quite reactive with the S-300; much more so than acetaldehyde or the saturated hexanal. This was in agreement with results of Gremli (1974) who also reported that the unsaturated aldehydes were much more reactive than saturated aldehydes (soy protein in aquenous solution).

The S-100 results were more compound dependent than the S-300 results. In S-100 system, nonpolar flavor compounds (e.g., ethyl benzene, styrene, limonene, octanone, ethyl heptanoate, and ethyl caproate) exhibited rvp values of $0.6 \leq 0.8$. While these values were substantially greater than the oil-based system, the S-100 interacts with flavor compounds more like oil than any of the other fat replacers. Note that the S-100 contains a small percentage of fat (1.72%). Since this replacer was used at 10%, there would be about 0.17% fat in the S-100 model system studied. Thus, the increased interaction of S-100 with nonpolar flavor compounds may be largely due to this small amount of fat. Recent work in our laboratory has demonstrated that as little as 0.1% fat would decrease the pressure of nonpolar flavor compounds by 10-15% in fat replacer systems (Schirle-Keller et al., 1992).

The most water-soluble flavor compound (acetaldehyde) showed little interaction with the S-100. Also of interest, both hexanal and trans-2-hexanal interacted quite strongly with the S-100 but acetaldehyde did not. Diacetyl, a compound with both water and oil solubility, exhibited rvp values in both protein-based fat replacers very similar to those in the oil system.

Carbohydrate-based fat replacers. In the MCC matrix, compounds showed little interaction yielding rvp values of about 1 +/-0.2 (Fig 1). Any effect observed is probably a salting out by the carbohydrate. Some of the rvp values were > 1. Again there was no obvious increase or decrease in rvp of the model flavor compounds with time.

With the exception of ethyl sulfide, the rvp values for flavor compounds added to N-Oil II systems were very similar to those observed for the MCC system. We have no data to explain the behavior of ethyl sulfide in N-Oil II (or MCC/CMC). The rvp was obviously lower in both fat replacer systems. Additional work needs to be done to explain this observation. Of the carbohydrate-based fat replacers, the MCC/CMC material showed an interaction (i.e., lowering of rvp) with the most nonpolar flavor compounds and had virtually no interaction with the more polar flavor compounds. The level of interaction was not as great as that observed for the S-100 or oil but was intermediate between the S-100 system and water.

Temperature effects on interaction

This series of experiments was to determine whether interaction between flavor compounds and the protein-based fat replacers was weak and readily negated by increasing sample temperature or whether the bonding was too strong to be influenced by temperature. This was evaluated by increasing sample equilibration temperature (i.e., in analysis) anticipating an increase in rvp to that of the water system reference if the bonding was sufficiently weak. Results indicated no observable effect of temperatures up to 70°C on the rvp of any of the flavor compounds. While the absolute vapor pressure of each of the compounds increased (evident from higher headspace concentrations), increases relative to water were not significant. Thus interactions responsible for the decreased rvp values observed with the protein-based fat replacers were so strong as to not be influenced by temperatures within the reported conditions.

CONCLUSIONS

THE S-300, MCC and N-Oil II fat substitutes were similar, exhibiting minimal interaction with flavor compounds. Their

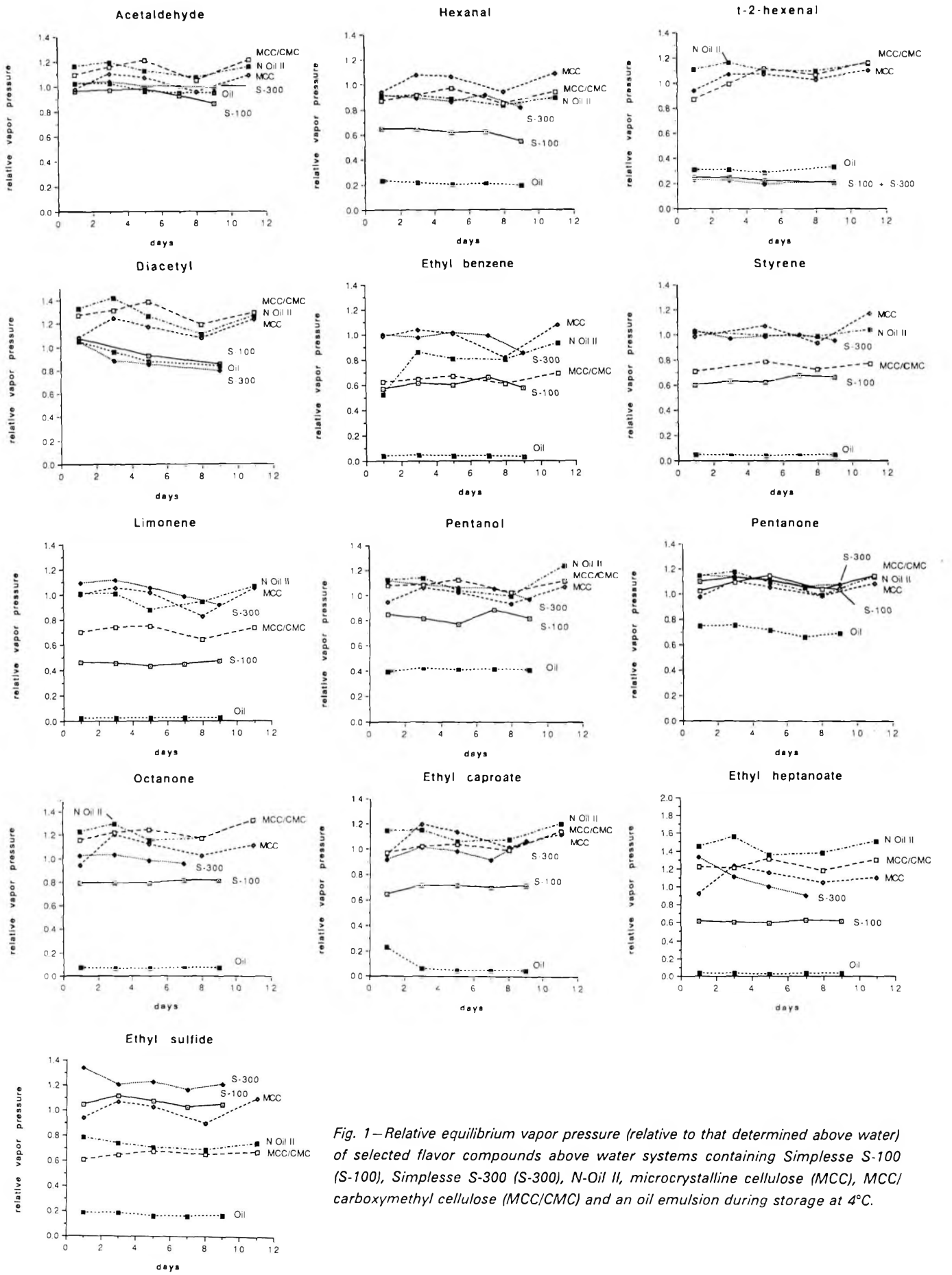

Fig. 1—Relative equilibrium vapor pressure (relative to that determined above water) of selected flavor compounds above water systems containing Simplesse S-100 (S-100), Simplesse S-300 (S-300), N-Oil II, microcrystalline cellulose (MCC), MCC/carboxymethyl cellulose (MCC/CMC) and an oil emulsion during storage at 4°C.

use in a food product may require major changes in flavor formulations (assuming a flavor designed to work in fat-containing system had been used) to be acceptable. Overall, the S-100 and MCC-CMC systems were relatively similar in interaction with flavor compounds with the S-100 acting more like fat than MCC/CMC in some instances (e.g. aldehyde interaction). While these two replacers had the greatest flavor interactions, neither S-100 nor MCC/CMC interacted with most flavor compounds as much as fat would. Fat soluble flavor compounds dissolve in the fat and this lowers its vapor pressure (thus a reduction in perceived intensity). Therefore, the use of any fat substitute in a food product would require changes in flavor formulation.

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*BREADFRUIT COMPOSITE FLOUR PRODUCTS. . .*From page 1422

to improve protein quality. Inclusion of soy protein in the composite flour resulted in PER similar to a casein control while the PER obtained when whey was included was significantly lower. Further study is needed to better predict the ability of a protein source to complement the breadfruit wheat flour mixture. Whether addition of a protein source would be advantageous depends upon the ability of that protein to improve protein quality, the importance of wheat products as a protein source in local diets and the availability and cost of the protein flour.

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Fatty Acid Composition of Suspension Cell Cultures of *Theobroma cacao* are Altered by Culture Conditions

M. C. WEN and J. E. KINSELLA

ABSTRACT

The triglyceride content of cacao suspension cell cultures increased from 0.6% to 2.3% by addition of coconut water, and by reducing rate of agitation. The palmitic acid content of total lipids decreased from 28% to 17% by culturing cacao cells in media containing 0.50% casein hydrolysate. Oleic acid was increased from 10% to 45% following addition of coconut water. Linoleic acid was reduced by the addition of coconut water, or by omitting sucrose.

Key Words: cacao, cells, fatty acids

INTRODUCTION

THE FAT CONTENT and fatty acid profile of lipids in plant tissue and cell cultures are usually different from seeds of the source plant. The quality and quantity of lipids in plant tissue cultures can be modified by cultural conditions and by components of the medium (Radwan and Mangold, 1976; Wen and Kinsella, 1987). Understanding these factors may help future exploitation of plant tissue and cell cultures as sources of edible fats, flavors, etc.

Because of the unique physical properties of cocoa butter, attempts to produce this product by tissue culture from cacao bean embryos is the objective of ongoing research. The lipid content of cacao tissue calli or suspension cells is lower than in cells of cotyledons of ripe cocoa beans (Tsai and Kinsella, 1982). Immature beans (110 days after pollination) contained 11% total lipids, which increased to 54% in ripe beans (Lehrian and Keeney, 1980, Wright et al., 1983). The total lipids of immature beans contained 80% triglycerides, 10% phospholipids, and small amounts of other lipid classes. During maturation, the percentage of triglycerides increased to 97% while other lipid classes decreased to less than 1% (Lehrian and Keeney, 1980; Tsai et al., 1982). In calli or suspension cells, however, glycolipids are the major lipid component. Lipids from cacao cell suspension cultures contain small amounts of triglycerides, which account for 13% of the total lipids (Tsai and Kinsella, 1982).

The major fatty acids in ripe cacao beans are stearic, oleic, and palmitic acids (Lehrian and Keeney, 1980; Tsai, et al., 1982). Unsaturated fatty acids, including linoleic and oleic acids, are the dominant fatty acids in suspension cultures with 50% linoleic acid and about 30% palmitic acid, a fatty acid pattern similar to immature beans (Tsai and Kinsella, 1982). Small changes in total lipid content and fatty acid composition can be brought about by varying culture media (Tsai et al., 1982; Wen et al., 1984). Exogenous fatty acids are incorporated into lipids of cultured cacao cells (Tsai and Kinsella, 1982) and exogenous stearic acid enhanced the triglyceride content about two-fold (Tsai et al., 1982). However, no significant change occurred in the fatty acid composition of the cellular triglycerides.

The objective of our research was to determine if culturing conditions could alter the lipid content and composition of cultured cells of *Theobroma cacao.*

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MATERIALS & METHODS

Preparation of cultural media

The basal medium was composed of 30 g/L sucrose, MS (Murashige and Skoog, 1962), salts, glycine and vitamins as described by Murashige and Skoog (1962). The medium was either liquid or gelled with 0.7% Gibco Phytagar. Casein hydrolysate, coconut water, bactopeptone (Difco Lab., Detroit, MI) and plant growth regulators were added as specified in the experiment. The standard agar medium contained 4.52 μM 2,4-dichlorophenoxyacetic acid (2,4-D) and 1.0 μM kinetin as growth regulators while the standard liquid medium continued 2.25 and 0.5 μM of 2,4-D and kinetin according to the suggestion of Tsai and Kinsella (1981). After adjusting the pH to 5.7 by adding 0.1 N KOH, the medium was autoclaved at 121°C and 1.1 kg/cm^2 pressure for 15 min.

Establishment of callus cultures

Cacao calli were initiated as described by Tsai and Kinsella (1981) from cotyledon explants obtained from pods 120 to 130 days after pollination. The calli were maintained as actively growing cells by transferring them onto fresh agar media every 4 wk and incubating at 29 ± 1°C under dark conditions. Suspension cultures were initiated by dispersing 400 mg fresh weight of calli in the liquid standard medium (Tsai and Kinsella, 1981). Cacao cell suspension cultures (45 mL) were grown in 125-mL Erlenmeyer flasks in darkness on Environ-shakers (140 rpm) at 29 ± 1°C. The suspension cultures, which were subcultured every 2 wk, exhibited an approximate doubling time of 3 days with fresh weight increasing 20-fold, from 300 mg to 6g in 2 wk (Tsai and Kinsella, 1981). Cultures were usually harvested 2 wk after subculturing or specific treatments.

Extraction of lipids

The total lipids of cells were extracted by the method of Bligh and Dyer (1959) following disruption using a Polytron and sonication using a Heat Systems Ultrasonics sonicator. Extraction was repeated twice. The combined chloroform layers was dried under nitrogen and the total lipids were dissolved in 1 mL of chloroform:methanol (v/v 1:1). Lipid classes were fractionated by thin-layer chromatography on Silica Gel G 60 plates, 0.25 mm thickness (Kinsella and McCarthy, 1968). The plates were sprayed with 0.2% 2,7-dichlorofluorescein in ethanol, viewed under UV light and the lipid classes identified. Triglycerides were analyzed by recovering the triglyceride band from TLC plates after separation of the lipid classes. Aliquots (3 mL) of chloroform were used to elute the triglycerides. The eluted triglycerides were dried, saponified and analyzed colorimetrically (Lowry and Tinsly, 1971). Fatty acids were analyzed as described by Kinsella et al. (1977).

Culture conditions

Several experiments were conducted to determine the effects of different factors on the growth, triglyceride content and fatty acid composition of lipids of cultured cells.

Shaker speed. The effects of shaking conditions (stationary, 90 rpm, and 140 rpm) on growth and lipid production were investigated.

Light and sucrose. The concentration of sucrose was varied at 0%, 1.5% or 3% in the standard MS liquid medium. Treatments included: 3.0% sucrose + darkness (control); 3.0% sucrose + light; 1.5% sucrose + light and 0% sucrose + light. The light intensity (2000 lux) was provided by white fluorescent tubes.

Table 1—Effects of differing culture conditions on the fatty acid composition (weight percent) of cultured cacao cells

Fatty acid	Culture variable[a]									
	Shaker speed (rpm)			Sucrose and light[b]				Coconut water (%)		
	0	90	140	0,L	1.5,L	3,L	3,D	O	5	10
16:0		28.0	23.2	43.0	34.5	37.4	32.8	33.5	29.0	26.0
18:0	4.9	7.0	3.9	6.0	5.0	5.8	5.7	8.6	9.0	9.5
18:1	38.5	41.2	37.1	35.0	12.8	11.5	12.1	20.6	41.6	44.3
18:2	25.3	16.4	27.6	12.8	40.1	39.3	41.9	29.3	14.1	13.0
18:3	6.5	7.3	7.0	6.2	5.4	4.9	7.4	7.0	6.0	7.2

[a] See methods for conditions. Fatty acid weight percent values are means of triplicate experiments.
[b] Numbers represent % sucrose in medium, L-light, D-dark.

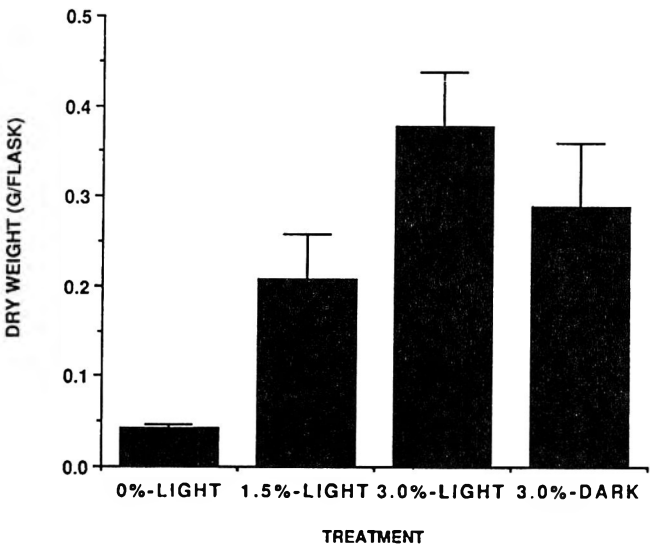

Fig. 1—Effect of combination of sucrose and light content on growth (g cell/dry wt/culture flask) of cacao suspension cultures. Cacao suspension cells (5 mL) were cultured in 45 mL basal Murashige and Skoog (1962) liquid medium with different sucrose content at 28°C under light or dark for 2 wk. Cells were harvested and dry weights determined.

Casein hydrolysate. 0%, 0.05%, 0.1%, 0.5%, 1.0% type II enzymatic casein hydrolysate (Sigma, St. Louis, MO) dissolved in standard liquid medium.

Coconut water. 0%, 5%, 10%, 15%, 20% coconut water (Gibco, Grand Island Biological Co., NY) mixed in standard liquid medium.

In all experiments, conducted in triplicate, 300 mg of 2-wk-old healthy cells ($\approx 3 \times 10^7$ cells) in early stationary phase were used as inoculum (Tsai and Kinsella, 1981). The cells were inoculated into the sterile basal liquid media containing additional components as specified in the experiment and cultured at 29 ± 1°C usually under dark conditions for 2 wk. Cells were then harvested, quantified (dry weight), and the lipid content and composition analyzed.

RESULTS & DISCUSSION

CELL GROWTH increased by increasing agitation, i.e., shaker speed. The average dry weight of cells/culture flask were 25, 90 and 223 mg following culturing at 0, 90 and 140 rpm, respectively. The triglyceride content of cells decreased with increasing shaker speed from 2.1 to 0.8% of dry weight of cells. The fatty acid profiles of cells grown at different shaker speed are shown in Table 1. Increasing the shaker speed from 0 to 90 rpm caused a decrease of linoleic acid. However, a further increase in shaker speed from 90 rpm to 140 rpm stimulated an increase in linoleic acid.

Increased shaker speed enhanced cell growth possibly by facilitating uptake of oxygen and nutrients. This facilitated and increased the desaturation of oleic acid to linoleic acid consistent with the finding of Rebeille et al. (1980) who observed that aeration of sycamore cell cultures induced rapid conversion of oleate to linoleate.

Increasing the concentration of sucrose enhanced the growth of cultured cacao cells (Fig. 1). At 3% sucrose level, cacao cells grew faster under illumination. The triglyceride content of the cultured cells was affected by sucrose in the medium, i.e., triglyceride was 0.14, 0.5, 0.37 and 0.28 g/100g dry cell mass when grown with 0, 1.5, and 3% sucrose with light and dark, respectively. The fatty acid profiles of the lipids were similar for the cells grown under light with 1.5 and 3% sucrose. In contrast, cell suspensions cultured under light but without sucrose contained more than twice the amount of oleic acid, but only one-third the content of linoleic acid compared to the other cells. These observations were consistent with results obtained with *Brassica napus*, and *Glycine soja* (Radwan and Mangold, 1976). The low linolenic acid content and pale color of the cultured cells of *Theobroma cacao, Brassica napus,* and *Glycine soja* (Radwan and Mangold, 1976; Tsai and Kinsella, 1981) reflected the heterotrophic nature of these cultures.

The growth of cacao cell suspensions was stimulated by the addition of casein hydrolysate to the standard MS culture medium, i.e., from 0.1 to 0.23, 0.26, and 0.29 dry weight (g/flask) with 0, 0.25, 0.5 and 1.0% casein hydrolyzate in the medium. The triglyceride content of cultured cells was also enhanced from 0.5 to 0.65% dry weight of the cells by the addition of 0.5% casein hydrolysate to the media. The addition of casein hydrolysate progressively decreased palmitic acid and increased oleic acid.

Coconut water increased the total lipids from 0.35 to 0.60% of dry weight and the proportion of triglyceride in the total lipid extracts of cultured cells. The optimum concentration of coconut water was 5.0% which increased the triglyceride in the total lipids to 24% compared with 9.2% in the control medium. The addition of 5% coconut water caused a two-fold increase in oleic acid with a concomitant two-fold decrease in linoleic acid. In contrast, a decrease in stearic and oleic acid and an increase in linolenic acid content in triglycerides was observed in soybean suspension cultures grown in modified MS medium containing 15% coconut water (Shin et al., 1971).

These results extend earlier reports (Tsai et al., 1982; Wen et al. 1987) and indicate the difficulty of increasing TG content in undifferentiated cells, even those derived from immature beans (Janick et al., 1982). Embryogenesis from these cells or tissue explants appear to provide a better approach for obtaining triglycerides (Pence et al., 1981).

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Agar Medium for Enumeration of Fecal Coliforms

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ABSTRACT

Spread-inoculated plates of fecal-coliform agar (FCA) were pre-incubated 2 hr at 35°C, and then transferred to 44.5°C for 22 hr. Fecal coliforms on FCA produce acids and CO_2 which react with bromocresol purple and calcium lactate to form yellow to yellow-green colonies surrounded by a pale yellow zone. Recovery of control or freeze-stressed *Escherichia coli* on FCA was about 1 log cycle lower than that with nonselective media. The accuracy for FCA to enumerate fecal coliforms from 138 typical colonies of three food samples was 98.6%; while the false positive was 1.4%. When the method was compared with the standard 3-tube MPN method for fecal coliforms, counts from two methods were not significantly different ($p > 0.05$).

Key Words: fecal coliforms, enumeration, agar medium

INTRODUCTION

FECAL COLIFORMS have long been used as an indicator of fecal contamination in food and water. Standard most probable number (MPN) method (FDA, 1984) is officially used to enumerate fecal coliforms. In the last decade, several approaches have been developed for detecting fecal coliform microorganisms, mainly including impedimetric procedures (Firstenberg-Eden et al., 1984) and radiometric methods (Reasoner and Geldreich, 1989). However, these require special instruments.

Desoxycholate agar and eosin-methylene blue agar are employed to count total coliforms and *Escherichia coli*, by counting colored colonies resulted from decomposed products or metabolites, but not from CO_2. Fecal coliform organisms can ferment lactose to produce CO_2 at 44.5°C (Prescott et al., 1990), and calcium lactate can react with CO_2 to form precipitates revealing background color in a plating medium (Cirigliano, 1982). However, nonfecal coliforms cannot produce acids and CO_2 at that temperature. Selective agents, like bile salts, can inactivate gram-positive bacteria and spore-formers (Cooke et al., 1985). Based on these observations, an agar medium for enumeration of fecal coliforms was developed.

Our objective was to assess the practicability of the medium developed. Reference strains were used to test the medium comparing with MPN procedure. Typical and atypical colonies cultivated from food samples on the medium were also examined.

MATERIALS & METHODS

Bacterial strains

Reference strains (36) applied to observe the growth response on the fecal coliform agar (FCA) were collected from several sources. *Acinetobacter calcoaceticus* ATCC 23055, *Aeromonas hydrophila* ATCC 7966, *Flavobacterium aquatile* ATCC 11947, *Moraxella phenylpyruvica* ATCC 2333, and *Salmonella typhimurium* ATCC 15277 were purchased from American Type Culture Collection (Baltimore, USA); *Bacillus cereus* CCRC 10446, *B. circulans* CCRC 11590, *B. subtilis* CCRC 11602, *Citrobacter freundii* CCRC 12291, *Enterobacter aerogenes* CCRC 10370, *Escherichia coli* CCRC 11634, *E. coli* CCRC 10675, *E. coli* CCRC 11446, *Hafnia alvei* CCRC 10906, *Klebsiella oxytoca* CCRC 10026, *K. pneumoniae* CCRC 10694, *K. pneumoniae* CCRC 10692, *Pseudomonas aeruginosa* CCRC 10261, *P. fluorescens* CCRC 10304, *P. putida* CCRC 10459, and *Staphylococcus aureus* CCRC 10780 were purchased from Culture Collection and Research Center (Food Industry Research & Development Institute, Hsinchu, Taiwan); *Aeromonas shigelloides* 1799475, *Alcaligenes faecalis*, *Micrococcus luteus*, *M. roseus*, *Proteus vulgaris*, *Salmonella derby*, *S. enteritidis*, *Serratia marcescens*, *Streptococcus faecalis* DS-5, *Vibrio cholerae* FRB 014, *V. cholerae* 0145A non-01, *V. cholerae* E9120 El Tor, *V. cholerae* 14033 Inaba, *V. parahaemolyticus* 17802, and *V. mimicus* 2001H were from Mr. S. S. Chang (Taiwan Fisheries Research Institute, Keelung, Taiwan). The cultures were cultivated on nutrient agar slants (Difco, containing 0.5% NaCl; except, 3% for *V. parahaemolyticus*) and stored at 4°C.

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Formulation of medium

In 1000 mL distilled water were dissolved: tryptone (Difco) 20g, bile salts No.3 (Difco) 1.5g, lactose (Difco) 10g, yeast extract (Difco) 5g, sodium chloride (Merck) 5g, β-glycerophosphate (disodium salt, Sigma) 1g, calcium lactate (pentahydrate, Hayashi) 14g, bromo-cresol purple (BCP) solution (1g BCP [Sigma] in 100 mL 20% ethanol) 3 mL, and agar (Difco) 12g. The tryptone, bile salts, lactose, yeast extract and sodium chloride were dissolved in 850 mL 50°C distilled water (solution A). Calcium lactate was dissolved in 150 mL 50°C distilled water to which the β-glycerophosphate had been added (solution B). Solution B, subsequently, was mixed gradually into solution A. The pH of the mixture was adjusted to 7.0 ± 0.2 using 1 N NaOH. BCP solution and agar were finally added to the mixture. The resultant mixture was boiled for 5 min, and then pour-plated and solidified before spread-plating.

Moisture and water activity

To examine changes in moisture and water activity of FCA plates during incubation, the plates were incubated at 44.5°C for 0, 8, 16 and 24 hr. Conventional vacuum-oven (≤100 mmHg, 100°C) procedure (AOAC, 1984) was applied for moisture determination. Novasina humidity meter (Humidat-IC II, Swiss) was used for water activity measurement.

Pre-incubation test

For observation of recovery of injured bacteria, *E. coli* CCRC 10675 was activated in nutrient broth (NB, Difco), and then suspended in fresh NB again. Colony-forming unit (CFU) of the suspension (3.3×10^8/mL) was enumerated using plate count agar (PCA, Difco) after 48-hr incubation at 35°C. Suspension (10 mL) in a tube was frozen at −20°C for 7 days. At the termination of storage, frozen suspensions were thawed at 25°C for 1 hr. Enumeration of *E. coli* was carried out by spreading on PCA and FCA and by MPN procedure. FCA-plates were pre-incubated at 35°C for 0, 2, and 4 hr prior to incubation at 44.5°C for 24–20 hr. Pre-incubation test was also conducted for the suspension without freezing (control).

Selective tests using reference strains

For observation of growth response of 36 reference strains on FCA, the strains were activated in NB containing 0.5% NaCl (3% for *V. parahaemolyticus*) at 35°C before streaking on FCA. Size and color of colonies on FCA were examined after 24-hr incubation at 44.5°C.

To compare results from our FCA medium and standard 3-tube MPN procedure, two strains of fecal coliforms, *E. coli* CCRC 11634 and *K. pneumoniae* CCRC 10694, were employed. Each strain was activated in NB and then diluted in phosphate buffer solution (PBS) to get suspensions containing 10-10^9 CFU/mL. The plates spread with

Table 1—Recovery of freeze-stressed (−20°C, 7 days) Escherichia coli *CCRC 10675 suspended in nutrient broth after pre-incubation at 35°C for 2 or 4 hr prior to incubation at 44.5°C*[a]

Treatment	PCA	MPN	FCA		
			Pre-incubation time (hr)		
			0	2	4
Control	8.52±0.09B	9.50±0.10A	7.63±0.21C	7.68±0.20C	7.58±0.22C
Frozen	7.07±0.12A	7.28±0.44A	5.87±0.18B	6.06±0.13B	6.12±0.14B

[a] FCA: fecal coliform agar; PCA: plate count agar; MPN: standard 3-tube MPN method. Original count in suspension was 3.3×10^8 CFU/mL; mean±SE, the counts/mL were transformed to $\log_{10}$ counts; figures in the same row with the same letter are not significantly different ($p>0.05$); six replicates were conducted. For control, suspension was not frozen.

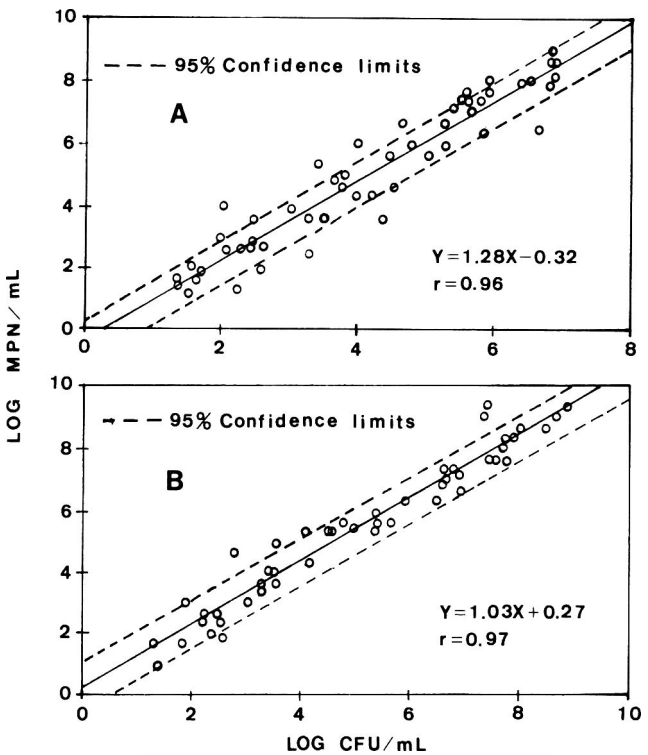

Fig. 1—Correlations of $\log_{10}$ colony-forming unit (CFU)/mL enumerated using FCA medium and $\log_{10}$ most probable number (MPN)/mL obtained using standard three-tube MPN method from Escherichia coli *CCRC 11634 (A) and* Klebsiella pneumoniae *CCRC 10694 (B) suspended in phosphate buffer solution.*

the bacteria were inverted and placed at 35°C for 2 hr and then transferred to 44.5°C for 22 hr. When incubation was terminated, yellow to yellow-green colonies surrounded by pale yellow zone (2–7 mm diam) on FCA were enumerated. For control, each strain was spread on NA and incubated 48 hr at 35°C.

For investigating the selectivity of fecal coliform bacteria and nonfecal coliform bacteria by FCA, *Enterobacter aerogenes* CCRC 10370, a nonfecal coliform, was mixed with *E. coli* CCRC 10675 as the inocula. Activated *E. coli* was suspended in PBS to obtain a series of suspensions containing 50–50,000 CFU/mL. Into each suspension, a constant amount (10,000 CFU/mL) of activated *E. aerogenes* was added. Enumerations by FCA and MPN methods were carried out. A control, without seeding *E. aerogenes*, was also conducted.

Selective tests using food samples

Food samples (32) including seafoods, poultry, pork, beef, milk, and processed food products were purchased from retail markets. Each food sample was homogenized (except milk) and diluted decimally using PBS. Fecal coliform microorganisms in each food were enumerated using FCA plates and MPN procedure. From pork, chicken, and oyster, 92, 50, and 38 typical and atypical colonies, respectively, on three FCA plates were selected and purified twice using NA. For each isolate, Gram stain and IMViC tests (Balows, 1991) were carried out, and they were finally identified using API 20E (API System S.A. France).

Statistical methods

The counts/g (or mL) were transformed to $\log_{10}$ counts to ensure normality of the data. Counts reported as <0.47 or <−0.5 were set to equal 0 during statistical analysis. An analysis of variance (spss/pc program, SPSS Inc., 444 North Michigan Avenue Chicago, IL 60611. USA) was used to compute tests of significance. Correlation of scatterplots between plate counts on FCA and most probable numbers was determined according to Snedecor and Cochran (1973).

RESULTS & DISCUSSION

Growth response of reference strain

In a preliminary study on formulation of FCA, we found that K_2HPO_4 or KH_2PO_4 reacted with calcium lactate to produce precipitates during preparation of the medium. Therefore, β-glycerophosphate was used instead of potassium phosphate for the growth requirement of the bacteria. Calcium lactate reacts with CO_2 forming calcium carbonate for easy observation of colonies (Cirigliano, 1982). Also BCP would become yellow when pH values range from 5.2–6.8. Thus, the colonies of fecal coliform bacteria on FCA appeared yellow to yellow-green surrounded by a pale yellow zone.

Since FCA plates were incubated at 44.5°C for 22 hr, moisture on FCA may be lost. Alexander and Marshall (1982) stated that agar medium in plates should not lose more than 15% of its weight during 48 hr incubation. In addition, the minimal water activity for the growth of *E. coli* and *K. pneumoniae* is 0.94–0.97 and 0.95–0.98, respectively (Speck, 1984). When our FCA plates were placed at 44.5°C for 24 hr, moisture content and water activity of the medium were 93.3% and 0.97, respectively (data not shown). At the end of incubation, the loss of moisture from the medium averaged 0.3%; while water activity in FCA changed little (0.002) during incubation.

Being stressed in the environment, fecal coliforms may be underestimated when they are cultivated in selective medium and incubated at 44.5°C (Corry et al., 1982). Resuscitation is therefore required before they are incubated at higher temperatures. The recovery of *E. coli* in frozen NB (−20°C for 7 days) was examined by pre-incubating freeze-stressed bacteria at 35°C for 2 and 4 hr prior to incubation at 44.5°C (Table 1). No significant differences ($p>0.05$) were found between the values of $\log_{10}$ CFU/mL before and after pre-incubation in either frozen or nonfrozen suspension. Counts using MPN procedures were more than 1 log-cycle higher than those using FCA-plating procedure. This may be because cell count was high ($\log_{10}$ CFU/mL using PCA was 8.52 or 7.07) in control or frozen suspensions, resulting in a high estimation using the MPN method (APHA, 1985). During growth, coliform bacteria will produce organic acids which are harmful to the cells (Hussong et al., 1981). FCA is a selective medium which will affect growth of unhealthy cells both in control and freeze-stressed suspension. Accordingly, there was ≈1 log cycle difference in counts enumerated by PCA and FCA in control and treated samples. A comparison of enumerations of *E. coli* CCRC 11634 and *K. pneumoniae* CCRC 10694 (10^3–10^4 CFU/mL) using our FCA medium and MPN method showed no significant differences ($p>0.05$, data not shown). Correlations between the $\log_{10}$ counts/mL of both strains (Fig. 1) obtained using FCA medium and MPN method had correlation coeffi-

Table 2—Comparison of recovery of Escherichia coli *CCRC 10675 mixed with* Enterobacter aerogenes *CCRC 10370 in phosphate buffer solution using FCA and MPN procedure*

Cells mixed (CFU/mL)		*E. coli* + *E. aerogenes*		*E. coli*
E. coli	*E. aerogenes*	FCA	MPN	FCA
50~100	10,000	2.05±0.04A[a]	2.59±0.06B	2.01±0.05A
100~500	10,000	2.75±0.03B	3.16±0.15C	2.68±0.03B
500~1,000	10,000	3.13±0.04C	3.53±0.07D	3.14±0.05C
1,000~5,000	10,000	3.72±0.02D	3.99±0.09E	3.70±0.02D
5,000~10,000	10,000	4.15±0.06EF	4.34±0.13F	4.11±0.05EF
10,000~50,000	10,000	4.76±0.03G	4.78±0.8G	4.68±0.10G

[a] Mean±SE, ; the counts/mL were transformed to $\log_{10}$ counts; figures in the same row or column with the same letter are not significantly different ($p>0.05$).

Table 3—Growth response of some bacterial strains inoculated on FCA

Bacterial strain	Growth response[a]
Acinetobacter calcoaceticus ATCC 23055	NG
Aeromonas shigelloides 1799475	purple
A. hydrophila ATCC 7966	NG
Alcaligenes faecalis	NG
Bacillus cereus CCRC 10446	NG
B. circulans CCRC 11590	NG
B. subtilis CCRC 11602	NG
Citrobacter freundii CCRC 12291	purple
Enterobacter aerogenes CCRC 10370	pale purple
Escherichia coli CCRC 11634	Y, SPYZ
E. coli CCRC 10675	Y, SPYZ
E. coli CCRC 11446	Y, SPYZ
Flavobacterium aquatile ATCC 11947	NG
Hafnia alvei CCRC 10906	purple
Klebsiella oxytoca CCRC 10026	NG
K. pneumoniae CCRC 10692	Y, SPYZ
K. pneumoniae CCRC 10694	Y, SPYZ
Micrococcus luteus	NG
M. roseus	NG
Moraxella phenylpyruvica ATCC 2333	NG
Proteus vulgaris	NG
Pseudomonas aeruginosa CCRC 10261	NG
P. fluorescens CCRC 10304	NG
P. putida CCRC 10459	NG
Salmonella derby	purple
S. enteritidis	purple
S. typhimurium ATCC 15277	purple
Serratia marcescens	NG
Staphylococcus aureus CCRC 10780	NG
Streptococcus faecalis DS-5	NG
Vibrio cholerae FRB014	NG
V. cholerae 0145A non-01	purple
V. cholerae E9120 El Tor	purple
V. cholerae 14033 Inaba	purple
V. parahaemolyticus 17802	NG
V. mimicus 2001H	NG

[a] Y, SPYZ: yellow, surrounded by a pale yellow zone
NG: no growth.

cients (r) of 0.96 and 0.97, for *E. coli* and *K. pneumoniae* respectively.

The recovery of *E. coli* CCRC 10675 in PBS mixed with a nonfecal coliform microorganism, *Enterobacter aerogenes* CCRC 10370, was examined by FCA as compared with the MPN method (Table 2). MPNs were significantly higher than plate counts when cell counts of nonfecal coliforms in suspension were higher than those of fecal coliforms. This has been found in other studies (Franzblau et al., 1984; Robinson, 1984; Pierson and Stern, 1986). When the count of *E. coli* in suspension equalled that of *E. aerogenes*, the differences of counts enumerated using the two methods were not significant ($p>0.05$). Therefore, the enumeration of *E. coli* using FCA was not affected by addition of *E. aerogenes*. However, it was influenced significantly using MPN procedure when nonfecal coliforms were added.

Growth response of 36 bacterial strains on FCA is shown in Table 3. Only *E. coli* CCRC 11634, *E. coli* CCRC 10675, *E. coli* CCRC 11446, *K. pneumoniae* CCRC 10692 and *K. pneumoniae* CCRC 11694 could grow and create typical colonies (yellow, surrounded by a pale yellow zone). *Bacillus*, *Streptococcus*, *Staphylococcus*, *Micrococcus*, and some gram-negative bacteria could not grow on FCA. *Aeromonas shigelloides*, *Citrobacter freundii*, *Enterobacter aerogenes*, *Hafnia alvei*, *Salmonella* and some *Vibrio* sp. could grow on FCA, but produced purple colonies. All of the tested nonfecal coliforms created purple colonies.

Table 4—Fecal coliforms detected from food samples using FCA plates and MPN procedure

Food sample and storage condition	Log CFU(or MPN)/g(or mL)	
	FCA	MPN
Beef, frozen	0	<0.47[a]
Chicken, ambient	3.70	4.38
Clam, refrigerated	0	<0.47
Crab meat, frozen	0	<0.47
Cuttle fish, frozen	0	<0.47
Fish ball, refrigerated	0	<0.47
Fish ball, refrigerated	0	<0.47
Ground Pork, refrigerated	6.21	6.38
Ground Pork, refrigerated	6.22	6.66
Ground Pork, refrigerated	5.75	5.97
Ground Pork, refrigerated	6.26	5.97
Ground Pork, refrigerated	1.30	1.59
Ground Pork, refrigerated	2.40	2.63
Ground Pork, refrigerated	3.06	3.32
Ham, ambient	0	<0.47
Milk, refrigerated	0	<−0.5
Milk, refrigerated	1.30	1.36
Milk, refrigerated	1.85	2.32
Milk, refrigerated	2.63	2.63
Oyster, refrigerated	4.52	5.18
Oyster, refrigerated	3.48	3.48
Oyster, refrigerated	3.45	3.97
Oyster, refrigerated	3.58	3.32
Pork, refrigerated	4.03	4.38
Pork, refrigerated	3.68	3.36
Pork, refrigerated	4.00	3.97
Raw fish slice, frozen	0	<0.47
Sausage, refrigerated	2.00	1.60
Sausage, refrigerated	3.60	4.36
Sword shrimp, frozen	0	<0.47
Tiger prawn, frozen	0	<0.47
Tilapia, refrigerated	2.00	1.60
Mean±SE	2.34±0.38A[b]	2.45±0.39A

[a] Counts showed as < −0.5 or <0.47 were set to equal 0 during statistical analysis
[b] Figures with same letter are not significantly different ($p>0.05$), using a paired-comparisons t-test.

Growth response of bacteria in food

For 32 food samples, counts of fecal coliforms enumerated from FCA and 3-tube MPN method were not significantly different ($p>0.05$, Table 4). Most of 11 food samples in which no fecal coliforms were detected were processed or frozen foods. Restaino et al. (1990) compared a direct-plating method using peptone-tergitol agar base (PTX) with a standard 3-tube MPN procedure for enumeration of *E. coli* on 50 naturally contaminated ground beef samples, and found no significant difference between the methods.

During the enumeration using FCA, solid food samples were homogenized. The homogenate was decimally diluted and 0.1 mL homogenate was used. Since the plate count methodology is not accurate with less than 25 colonies/ plate (FDA, 1984), the FCA method is theoretically not accurate to enumerate solid foods or liquid foods for counts below 2,500 or 250 CFU/ g of mL. The sensitivity may be improved by increasing the number of plates in a given dilution. For improvement of sensitivity, we tried a pour-plating (1 mL homogenate seeded) procedure, however, gas bubbles were produced in the medium which confused observation.

From three food samples (pork, chicken and oyster), 138 typical (including a yellow green) and 42 atypical (purple) colonies growing on FCA were purified and identified (Table 5). Among the typical, nine strains which could grow in EC broth but could not produce gas were identified as *E. coli*; three strains which could not grow in EC broth were identified

Table 5—Identification of typical and atypical colonies growing on three FCA plates from pork (92 colonies), chicken (50), and oyster (38)

Category	Counts of colonies	Cell morphology and Gram reaction[a]	Growth in EC broth[b]	IMViC test I M V C	Color of colonies on FCA[c]	Identification[d]
1	116	SR(−)	+	+ + − −	Y, SPYZ	*Escherichia coli*
2	6	SR(−)	+(NG)	+ + − −	Y, SPYZ	*E. coli*
3	3	SR(−)	+(NG)	− + − −	Y, SPYZ	*E. coli*
4	9	SR(−)	+	− + − −	Y, SPYZ	*E. coli*
5	1	SR(−)	+	− − + +	Y, SPYZ	*Klebsiella pneumoniae*
6	1	SR(−)	−	− − + +	YG, SPYZ	*K. pneumoniae*
7	2	SR(−)	−	+ + − +	Y, SPYZ	*Enterobacter agglomerans*
8	38	SR(−)	−	+ − − −	purple	*Plesiomonas shigelloides*
9	4	SR(−)	−	− + − +	purple	*Citrobacter freundii*

[a] SR: short rod; −: Gram negative
[b] +: growth; −: no growth; NG: no gas produced
[c] Y: yellow; SPYZ: surrounded by a pale yellow zone; YG: yellow green
[d] Identified using API 20E.

as *K. pneumoniae* and *Enterobacter agglomerans*, respectively. Since *E. coli* and *K. pneumoniae* are members of fecal coliforms, the accuracy for FCA to enumerate fecal coliforms from food samples was 98.6% (136/138); while the false positives were 1.4% (2/138). The accuracy for enumeration of fecal coliforms by m-FC is 89%, by m-TEC is 94% (Pagel et al., 1982), by 7h-FC is 97% (Reasoner et al., 1979), and by VRB agar is 98.4% (Klein and Fung, 1976). Thus the accuracy of our method is relatively high. Recovery accuracy of 100% was shown by m-FC2 method (Pagel et al., 1982), but the false positive was as high as 18%.

Since nine of the 138 strains of fecal coliforms isolated from foods did not produce gas, 6.5% false negative was observed from MPN method (Table 5). Rippey et al. (1987) pointed out that a selective medium can be used in a microbial test when its accuracy is >90% and the false negative is <10%. Accordingly, our FCA is suitable for enumeration of fecal coliform bacteria in foods and liquid samples.

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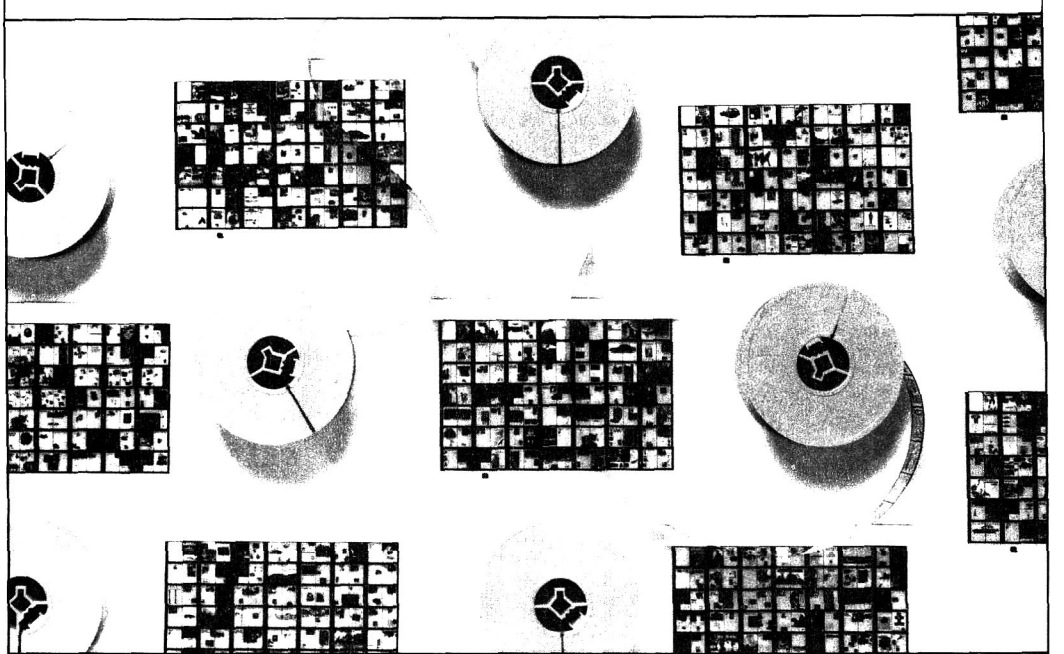

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