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Flavor Constituents of Beef as Influenced by Forage- and Grain-Feeding

D. K. LARICK, H. B. HEDRICK, M. E. BAILEY, J. E. WILLIAMS, D. L. HANCOCK, G. B. GARNER, and R. E. MORROW

ABSTRACT

Hereford yearling steers (N = 144) were alloted to one of three pasture systems: tall fescue, smooth bromegrass-red clover or orchardgrass-red clover. After the grazing period, steers were finished in drylot and then serially slaughtered at 0, 56, 84 and 112 days. Carcass quality grades and yield grade numbers increased when steers were fed grain up to 112 days. Tenderness of loin steaks increased up to 84 days, after which no improvements were observed. Sensory panel scores for grassy flavor of steaks and ground beef decreased up to 112 days. Fifty-three compounds were identified in the volatiles of melted subcutaneous fat by direct sampling-gas chromatography/mass spectrometry. The major differences between volatiles from fat of forage-fed compared to grain-fed steers were the higher concentrations of 2,3-octanedione and various diterpenoids present in the samples of the forage-fed animals.

INTRODUCTION

NUMEROUS INVESTIGATORS have examined quality characteristics of beef from carcasses produced on high versus low energy diets (Aberle et al., 1981; Bowling et al., 1978; Brown et al., 1979; Cross and Dinius, 1978; Dolezal et al., 1982; Harrison et al., 1978; Hedrick et al., 1983; Reagan et al., 1977; Schroeder et al., 1980; Tatum et al., 1980). High energy diets consist mainly of grain, while low energy diets consist mainly of forages. Data from these studies indicate that as the energy content of the finishing diet fed to cattle is increased, there is an improvement in carcass quality grade and ultimately tenderness of the meat.

Recent sensory data reveal that beef from cattle finished on low energy forage diets has less desirable flavor than that from cattle finished on high energy grain diets (Brown et al., 1979; Bowling et al., 1978; Davis et al., 1981; Dolezal et al., 1982; Harrison et al., 1978; Hedrick et al., 1983; Melton et al., 1982a, 1982b; Reagan et al., 1977; Schroeder et al., 1980; Skelly et al., 1978; Smith et al., 1977; Tatum et al., 1980; Westerling and Hedrick, 1979). The less desirable flavor of forage-fed beef has been attributed to an intense milky-oily, sour and fishy flavor (Brown et al., 1979; Melton et al., 1982b) or a grassy flavor (Berry et al., 1980; Brown et al., 1979; Melton et al., 1982a; Schroeder et al., 1980).

The greatest sensory difference in beef from forage-fed and grain-fed steers appears to be in the flavor of the fat (Meyer et al., 1960; Harrison et al., 1978). Fat may affect flavor in two ways (Hornstein, 1971): (1) fatty acids, upon oxidation, can produce carbonyl compounds that are potent flavor compounds and (2) fat may act as a storage depot for odoriferous compounds that are released when heated. There has been little research published regarding the qualitative and quantitative differences in flavor components of beef produced on forage versus grain diets and their relationship to flavor.

The objective of the present investigation was to identify and quantitate volatile flavor constituents present in fat obtained from beef animals previously fed forage and grain diets and to determine the relationship between these constituents and sensory panel grassy flavor scores obtained for cooked meat. In addition, carcass characteristics and tenderness of the longissimus muscle of these animals were determined.

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MATERIALS & METHODS

SEVENTY-TWO YEARLING HEREFORDS steers were randomly allotted to one of three pasture systems (24 animals per system); tall fescue (*Festuca arundinacea*) fertilized with 100 pounds of nitrogen per acre, smooth bromegrass (*Bromus inermis* L.)-red clover (*Trifolium pratense* L.), or orchardgrass (*Dactylis glomerata* L.)-red clover pastures. The animals grazed these pastures from April through October. In November, the steers were placed in the feedlot and fed a corn silage-whole shelled corn diet (Table 1). This basis design was repeated for two years, yielding a total of 144 animals. In each year, six steers from each system were serially slaughtered after 0, 56, 84, and 112 days of grain feeding.

Approximately one-half kg of subcutaneous fat was removed from the chuck and rib of the left side of each carcass after slaughter and prior to washing the carcass. One-half of the fat sample was placed in glass jars with teflon-lined caps, purged with nitrogen, frozen immediately with dry ice, and then stored at −18°C for approximately 30 days and analyzed for volatile constituents. The remaining fat sample from each carcass was vacuum packaged in a cryovac bag and stored at −18°C for approximately 40 days and analyzed by sensory analysis. After a 24-hr chill period, USDA quality and yield grades were determined (Official United States Standards for Grades of Carcass Beef, 1975) for each carcass. Carcasses were stored at 2–3°C for 10 days prior to processing.

A 2.5 cm thick steak from the top round (semimembranosus muscle), two 2.5 cm steaks and one 3.8 cm steak from the anterior portion of the shortloin (longissimus muscle) were removed from the right side of each carcass for sensory evaluation and objective tenderness measurements. These samples were wrapped in laminated freezer paper, frozen and stored at −18°C.

Preparation and cookery of sensory samples

The loin steaks for sensory analysis were removed from frozen storage and thawed overnight in a 2–3°C cooler and the subcutaneous fat in excess of 0.5 cm was removed. The steaks were oven-broiled in a natural gas forced air convection oven at 177°C to an internal temperature of 70°C.

Samples (1 cm × 2 cm) were cut from the longissimus muscle of each freshly cooked loin steak and consisted of a portion of the lean and subcutaneous fat. The samples were immediately placed into preheated, amber-colored glasses imprinted with randomized codes which were kept in sand baths to assure hot samples; panelists were instructed to taste the entire sample.

A sample of ground beef was prepared from each animal using lean from the semimembranosus muscle and subcutaneous fat previously removed from the chuck and rib at the time of slaughter. The lean was thawed for 24 hr at 2–3°C prior to grinding, and the fat was kept frozen until immediately prior to grinding. The fat and lean portions were ground, mixed and then re-ground through a 3-mm plate using a table model meat grinder. The ground beef samples were formulated

Table 1—Diet for steers fed in drylot

Ingredient	Percent composition by weight[a]	
Whole shelled corn	70.90	
Corn silage	22.50	
Supplement	6.60	
Corn		1.00
Soybean		3.75
Urea		0.03
Limestone		0.79
Dicalcium phosphate		0.12
Potassium chloride		0.24
Trace mineralized salt		0.40
Vitamin A & D		+[b]
	100.00	

[a] As-fed basis.

[b] Add 0.89 kg Vitamin A & D supplement per 227 kg.

to contain 25% fat using the Latin Square method. Two 150-g samples of ground beef were prepared from each animal. The samples were vacuum packaged in Cryovac bags and stored at 2–3°C until the following day when they were analyzed by sensory analysis. The samples required for a particular session (150g) were placed in 600 ml, heavy-duty, Kimax beakers, covered with watch glasses and cooked in a natural gas convection oven with intermittent stirring to an internal temperature of 70°C.

Ground beef samples were presented in a similar manner as described for steaks except that each panelist received one tablespoon of cooked, well-mixed ground beef (approximately 28g) and they were instructed to stir the sample prior to tasting.

Sensory panel evaluation

To accommodate the large number of treatments in the experiment, a balanced incomplete block design was used for both the steak and ground beef analyses. Since no design for 12 treatments exists, a 13th treatment consisting of a shortloin and top round (semimembranosus muscle) from a typical high Choice grade steer carcass was added for sensory analysis. This sample served as a control throughout the entire period of evaluation. The particular design used was a Type IV balanced incomplete block design, Plan 11.22, with $t = 13$ treatments, $k = 4$ samples per judge, $b = 13$ panelists, and $\lambda = -1$ (Cochran and Cox, 1957). Six replications were required to evaluate all 72 samples each year. To accommodate the design, seven discriminating judges were used from the morning session, with one being randomly eliminated prior to the start of the analysis and all seven judges' scores were used from the afternoon session.

A seven-member trained sensory panel was used. The final panelists were chosen on the basis of their ability to differentiate between samples of cooked meat from forage- and grain-fed beef using a series of triangle tests. The term "grassy" was chosen by the panelists as one which was characteristic of the forage-fed beef and one they could use to quantitate flavor differences. Panelists were trained using samples of both corn- and forage-fed beef to familiarize them with varying intensities of "grassy" flavor. A 10-cm line anchored at each end with the terms "no grassy flavor" and "strong grassy flavor" was used. Quantitation of results was accomplished by measuring the distance away from the left side (no grassy flavor) at which the panelist placed a mark.

The sensory testing was conducted in an air-conditioned panel room with positive air pressure. The panelists were seated in individual booths with pass-through doors for serving samples and each booth was illuminated with red light to eliminated the influence of color differences between samples.

Instron Warner-Bratzler shear analysis

Loin steaks, 3.8 cm thick, were cooked for objective tenderness measurements as previously described for the sensory steaks. After cooking, the loin steaks were cooled to room temperature prior to removal of core samples. Three 2.5 cm core samples (dorsal, medial, and lateral) were removed from the longissimus muscle of each steak using a coring device attached to a drill press. Shear values were determined using an Instron, Universal Testing Machine (Model 1132) with a 100 kg load cell which had been interfaced with a computer to perform and record all measurements. The 2.5 cm cores were sheared on an Instron Warner-Bratzler shear attachment (compression mode). The cores were placed on the base of a blade guide and a triangle-shaped blade was forced down through the sample at 25.4 cm per minute. A full-scale load setting of 0 to 10 kg was used with a range setting of 1.0.

Sample preparation for gas chromatography

The subcutaneous fat was used for the analysis of volatile compounds. Volatile compound analyses were performed on 72 samples representing one-half the total number of animals in the study. Approximately 200g fat was minced into small pieces, placed into a 1000 ml heavy-duty Kimax Brand beaker and covered with a watch glass. This fat was then heated at 175°C for 20 min and the liquid transferred to a 100 mm × 13 mm screw-cap test tube with a teflon liner. The tube was then flushed with N_2 gas, capped tightly, and stored frozen (−18°C) until analyzed. Prior to analysis for volatiles, the fat was removed from the freezer after storage for approximately 30 days and melted by immersion in a 60°C water bath for 5 min.

Quantitative and qualitative analyses by GLC and GLC/MS

Volatiles were removed from 250 mg samples of fat by heating in a Pyrex tube in the apparatus described by Suzuki and Bailey (1985) under the identical conditions they reported for ovine fat. A 50m fused silica capillary column (0.32 mm id) coated with SE-54 was used to separate volatiles transferred to the column through a modified (Suzuki and Bailey, 1985) packed column injection port of a Perkin-Elmer Sigma 2 gas chromatograph equipped with a FID detector heated to 265°C. Chromatographic conditions for the separation and quantitation of volatiles were the same as those described by Suzuki and Bailey (1985).

The sampling procedure used for identifying volatiles by GLC/MS was identical to that used for separation and quantitation of volatiles except that the gas chromatograph was a Carlo Erba Model 41-60 and the sample split ratio was 1:10 through the conventional splitter. The SE-54 capillary column was connected to the mass spectrometer through a jet (Ryhage) separator. The mass spectrometer used was a double-focusing Kratos Model MS-25 equipped with a Kratos DS-55 data system with a NIH/EPA data base for library searching. The ionization voltage was set at 70 eV, and ion source temperature was 250°C, and the resolution was 600 (10% valley).

Statistical analysis

The data (excluding sensory analysis) for both years were combined and analyzed using analysis of variance for a fixed model, three-way factorial design (Cochran and Cox, 1957) with treatments being year (1 and 2), slaughter group (0, 56, 84 and 112 days), and pasture treatment (bromegrass-red clover, orchardgrass-red clover and fescue). The General Linear Model procedure for least square means was used to test differences in treatment means. Data from the sensory analysis of steak and ground beef samples were analyzed as a type IV balanced incomplete block design as described by Cochran and Cox (1957).

The forward selection procedure of stepwise discriminant analysis (SAS, 1982) was applied to the GC profiles to identify a subset of variables (volatile compounds) which best revealed differences due to length of time on corn after pasture. Discriminant analysis (SAS, 1982) was then used to obtain a more detailed evaluation of the subsets ability to classify cattle into the various treatments.

RESULTS & DISCUSSION

Carcass characteristics

Slaughter weights and carcass yield and quality grade characteristics are presented in Table 2. There were differences ($P<0.01$) in hot carcass weights among the slaughter groups. Hot carcass weight and 12th rib backfat thickness progressively increased as the period of time in the feedlot increased.

Ribeye area increased ($P<0.01$) during feeding for 56 days in the feedlot after which only slight increases were observed. USDA yield grades did not change during the first 56 days in the feedlot but there was an increase ($P<0.05$) in yield grade from 56 to 84 and 84 to 112 days of grain feeding.

All carcasses were A maturity. Marbling scores and USDA quality grades increased ($P<0.01$) as animals were grain-fed from 0 to 56, 56 to 84 and 84 to 112 days. These findings are

Table 2—Least squares means[a] of beef carcass yield and quality characteristics

Characteristic	Slaughter group (SG)[b]				Pasture treatment(PT)[c]			Year (YR)[d]	
	0 days	56 days	84 days	112 days	Fescue	Brome	Orchard-grass	1	2
Hot carcass wt., kg	181.4[g] (4.39)	224.5[h] (4.39)	261.5[i] (4.46)	289.1[j] (4.39)	217.5[h] (3.80)	251.4[h] (3.80)	248.3[h] (3.80)	257.3[g] (3.13)	221.0[h] (3.10)
Ribeye area, cm^2	53.39[g] (1.42)	66.3[h] (1.42)	68.6[h] (1.48)	70.19[h] (1.42)	62.2[g] (1.29)	66.3[h] (1.22)	65.7[h] (1.22)	63.8[g] (1.03)	65.6[g] (1.03)
12th Rib backfat, cm	0.23[g] (0.02)	0.46[h] (0.02)	0.86[i] (0.02)	1.09[j] (0.02)	0.53[g] (0.02)	0.74[h] (0.02)	0.74[h] (0.02)	0.76[g] (0.02)	0.56[h] (0.02)
USDA yield grade	1.69[g] (0.07)	1.77[g] (0.07)	2.44[h] (0.07)	2.92[i] (0.07)	2.02[g] (0.06)	2.29[h] (0.06)	2.30[h] (0.06)	2.57[g] (0.05)	1.84[h] (0.05)
Marbling score[e]	4.0[g] (0.27)	7.3[h] (0.27)	8.7[i] (0.28)	11.1[j] (0.27)	7.0[g] (0.24)	8.4[h] (0.24)	8.0[h] (0.24)	8.3[g] (0.19)	7.3[h] (0.19)
USDA quality grade[f]	10.4[g] (0.19)	12.6[h] (0.19)	13.7[i] (0.19)	15.6[j] (0.19)	12.4[g] (0.17)	13.5[h] (0.16)	13.2[h] (0.16)	13.4[g] (0.14)	12.7[h] (0.13)
Instron, kg	14.19[j] (0.301)	11.5[h] (0.301)	8.76[g] (0.306)	8.52[g] (0.301)	10.88[g] (0.265)	10.47[g] (0.261)	10.95[g] (0.216)	9.62[h] (0.215)	11.91[g] (0.213)

[a] Standard error of the least squares means presented in parentheses.
[b] Least squares mean of 36 observations.
[c] Least squares mean of 48 observations.
[d] Least squares mean of 72 observations.
[e] 12 = Small; 10 = Slight[0]; 9 = Slight; 8 = Slight[-].
[f] 16 = Choice[-]; 15 = Good[+]; 14 = Good; 13 = Good[-]; 12 = Standard[+].
[g-j] Means bearing different superscripts within SG, PT and YR on the same line differ significantly ($P<0.05$).

consistent with numerous published reports which indicate that as the period of time cattle receive a high energy finishing diet is increased, there is an improvement in marbling score and ultimately quality grade along with an increase in hot carcass weight and USDA yield grade number (Aberle et al., 1981; Bowling et al., 1978; Brown et al., 1979; Dolezal et al., 1982; Hedrick et al., 1983; Reagan et al., 1977; Schroeder et al., 1980; Tatum et al., 1980).

Steers that grazed fescue had lower hot carcass weights at slaughter (0 days) than those that grazed bromegrass-red clover or orchardgrass-red clover. Similarly, the ribeyes were smaller, backfat thickness was less and carcass quality grades were lower for the animals on fescue pasture. Hot carcass weights and most carcass characteristics were similar for animals that grazed bromegrass-red clover and orchardgrass-red clover pastures.

After 56 days of grain feeding, steers that previously grazed bromegrass-red clover and orchardgrass-red clover continued to maintain an advantage in hot carcass weight, ribeye area and carcass quality grade compared to steers that previously grazed fescue pasture. Fat thickness was similar for animals on all the prior grazing treatments. However, after 84 days of grain feeding, the differences in most carcass characteristics of the fescue animals became progressively less compared to animals receiving the other two grazing treatments. This indicated the animals that previously grazed fescue pasture exhibited greater compensatory growth in the feedlot than did steers which grazed either bromegrass-red clover or orchardgrass-red clover pastures. Similar results indicating compensatory growth for animals in drylot after fescue pasture grazing were reported by Hedrick et al. (1983). After 112 days of grain feeding, hot carcass weights continued to be lower for animals which grazed fescue, however, all other carcass characteristics were similar regardless of previous pasture group. At the same relative digestibility, grasses contain less lignin and more hemicellulose than do legumes (Heath et al., 1973). This promotes lower intake and net energy values for grasses than for legumes and would explain the differences observed between pastures in this study.

Animals from the first year of the study had greater hot carcass weights, 12th rib backfat thickness, marbling scores and USDA quality and yield grades compared to animals the second year. This was attributed to environmental effects which resulted in lower quality pastures during the grazing portion of the study the second year.

Instron shear force

Instron shear force values of cooked loin steaks decreased during the period of grain feeding from 0 to 84 days. However, grain feeding beyond 84 days did not improve tenderness. Previous research has revealed that finishing cattle on corn in drylot for any period of time beyond 30 days improved tenderness and that the maximum tenderness is achieved between 70 and 100 days of grain feeding (Aberle et al., 1981; Dolezal et al., 1982; Harrison et al., 1978; Tatum et al., 1980).

The initial pasture treatment on which steers were grazed did not appear to influence tenderness. Samples taken from carcasses the first year of study appeared to be more tender (less shear force required) than those the second year. As discussed earlier, carcasses from steers during the first year of this study exhibited higher marbling scores and higher carcass quality grades; therefore, a lower shear force may be expected if carcass quality grades are indicative of tenderness.

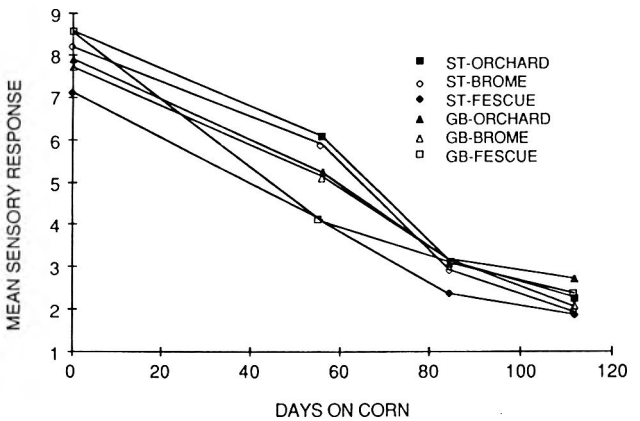

Fig. 1—Mean sensory panel scores for grassy flavor in ground beef (GB) and steak (ST) versus days on grain in feedlot for the three previous pasture treatments.

Subjective flavor analysis

The trained sensory panel's perception of grassy flavor for both cooked loin steaks and ground beef (25% fat) decreased ($P<0.01$) with period of grain feeding in drylot (Fig. 1). Loin steaks and ground beef of carcasses from steers slaughtered directly off pasture had the highest grassy flavor scores, followed by reductions in grassy flavor for samples from steers fed grain in drylot for 56, 84 and 112 days respectively.

Objective flavor analyses by GLC/MS

Melted subcutaneous fat samples from steers that grazed the three pastures (fescue, bromegrass-red clover and orchardgrass-red clover) and subsequently were grain-fed in drylot for 0, 56, 84 or 112 days were analyzed by the direct sampling-grass chromatography procedure for volatile compounds. Representative chromatograms of volatiles from fat of cattle fed orchardgrass-red clover (Slaughter Group 1) and cattle grain-

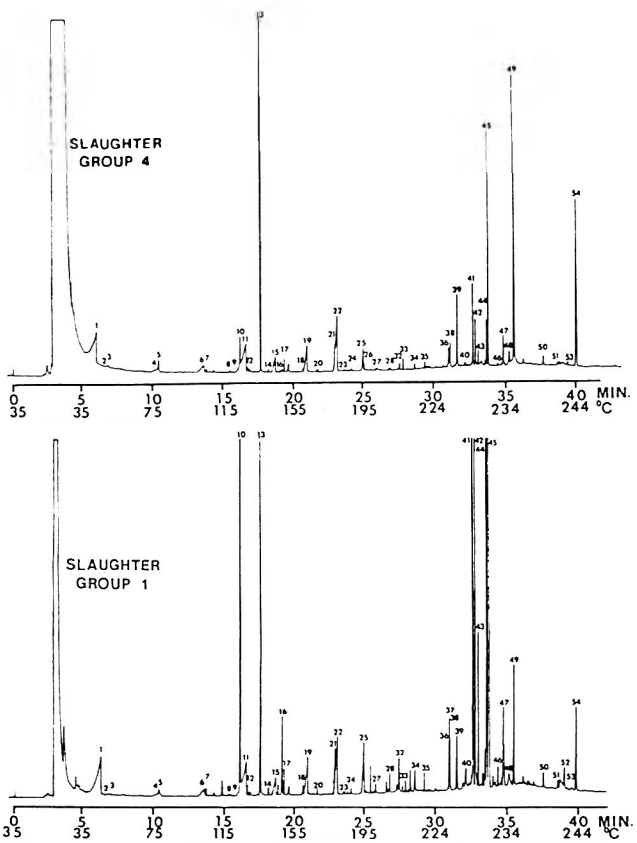

Fig. 2—Chromatograms of volatiles from fat of cattle fed orchardgrass-red clover (Slaughter Group 1) and cattle finished for 112 days in feedlot on grain after orchardgrass-red clover pasture (Slaughter Group 4).

fed for 112 days in feedlot after orchardgrass-red clover pasture are presented in Fig. 2. Chromatograms of fat from cattle fed fescue and bromegrass-red clover were qualitatively and quantitatively similar to these. Peak numbers refer to the compounds listed in Table 3 which contains the identity of the volatile constituents analyzed by GLC/MS and their identification method. Identification of unknown compounds was achieved by comparing MS and retention time (RT) data with that of authentic compounds. Where the authentic compounds were not available, unknowns were tentatively identified from MS data of published reference spectra and/or NIH/EPA reference spectra (NIH/EPA Chemical Information System, 1978). Many of these compounds are the same as those identified by Suzuki and Bailey (1985) from fat of forage-fed lamb. Fifty-three volatile compounds were identified which were grouped into 6 general classes including 7 hydrocarbons, 9 aldehydes, 11 acids, 5 ketones, 6 lactones and 15 terpenoid type compounds.

Thirty-one compounds out of the 53 previously identified were quantitatively analyzed by the direct sampling method for each of the 72 fat samples described earlier. Table 4 contains the Least Square Means of quantities of volatile compounds present in the beef fat samples as influenced by slaughter group, pasture treatment and year.

In general, the high molecular weight hydrocarbons present particularly hexadecane, heptadecane and octadecane decreased with time of grain feeding in the drylot with the most dramatic decreases occurring between 0 and 56 days of grain feeding. Similar results were previously reported for octadecane by Hedrick et al. (1980). Selke et al. (1975) explained the presence of n-hydrocarbons in beef fat volatiles as a result of the reaction of alkyl-free radicals, which are the product of thermally-decomposed hydroperoxides with hydrogen-free radicals.

Table 3—Volatile compounds in beef subcutaneous fat by direct sampling GLC/MS analysis

Peak #	Compound	Kovats Index[a]	ID[b]
	HYDROCARBONS		
3	heptane	700	MS
5	octane	800	MS
23	tridecane	1,300	MS
30	pentadecane	1,500	MS
35	hexadecane	1,600	MS
37	heptadecane	1,700	MS
42	octadecane	1,800	MS
	ALDEHYDES		
2	pentanal	685	MS
4	hexanal	798	MS
7	heptanal	905	MS
17	nonanal	1,110	MS
18	2-nonenal	1,160	MS
20	decanal	1,202	MS
21	2-decenal	1,260	MS
24	2,4-decadienal	1,325	MS
26	2-undecenal	1,365	MS
	ACIDS		
1	acetic acid	660	MS
6	pentanoic acid	900	MS
11	hexanoic acid	1,000	MS
15	heptanoic acid	1,070	MS
19	octanoic acid	1,170	MS
22	nonanoic acid	1,267	MS
25	decanoic acid	1,360	MS
34	undecanoic acid	1,560	MS
40	dodecanoic acid	1,755	MS
48	14-ME-pentadecanoic acid	1,940	$MS^{N/E}$
51	9-octadeceonic acid	2,140	$MS^{N/E}$
	KETONES		
10	2,3-octanedione	984	MS
16	3-hydroxyoctan-2-one	1,105	$MS^{P,1}$
32	2-tridecanone	1,513	MS
38	2-pentadecanone	1,702	(MS),(RT)
47	2-heptadecanone	1,910	(MS),(RT)
	LACTONES		
33	δ-decalactone	1,530	MS
36	γ-dodecalactone	1,695	MS
39	δ-dodecalactone	1,720	MS
49	δ-tetradecalactone	1,970	MS
50	δ-pentaldecalactone	2,050	(MS),(RT)
54	δ-hexadecalactone	2,190	$MS^{P,1}$
	TERPENOIDS		
8	β-pinene	961	MS
9	camphene	966	$MS^{N/E}$
12	α-pinene	1,005	MS
14	limonene	1,052	$MS^{N/E}$
27	1,3-dimethyl-8-(1,methyethyl) tricyclo[4.4.0.0.^{2,7}]dec-3-ene	1,413	$MS^{N/E}$
28	1,4-dimethyl-7-(1-methyethylidine) 1,2,3,4,5,6,7,8-octahydro-azulene	1,474	$MS^{N/E}$
29	4,11,11-trimethyl-8-methylene-bycyclo [7.2.0] undec-4-ene	1,493	$MS^{N/E}$
31	2,2,6,9-tetramethyl-(E.E.E)-1,4,8-cycloundecatriene	1,505	$MS^{N/E}$
41	phyt-1-ene	1,795	$MS^{P,1}$
43	phytane	1,810	$MS^{N/E}$
44	neophytadiene	1,840	$MS^{P,1}$
45	phyt-2-ene	1,853	$MS^{P,1}$
46	phytadiene (MW278)	1,747	(MS)
52	dihydrophytol	2,119	MS
53	phytol	2,130	MS

[a] Kovats indices were determined by using a series of hydrocarbons on the fused silica column (SE-54) described under the experimental section.

[b] MS (complete spectrum) and RT data were consistent with that of authentic compounds unless specified as follows: MS^{P} = MS data were consistant with that of published spectra, namely, (1) Nixon *et al.* (1979) and Urbach and Stark (1975). $MS^{N/E}$ = tentatively identified from NIH/EPA data. (MS) = tentatively identified from interpretation of MS data. (RT) = RT is consistent with the retention predicted from homologus compounds.

Saturated and unsaturated aldehydes were among the major volatiles present in heated beef fat. The concentration of aldehydes which were quantitated, with the exception of heptanal and 2-decenal (which decreased with time on grain), did not appear to be influenced by feeding system. The formation of both the alkanals and alk-2-enals can be attributed to the thermal (190°C) decomposition of hydroperoxides and peroxy

Table 4—Least squares means for quantities (ppm) of volatile compounds in fat analyzed by direct sampling-gas chromatography

Peak No.	Volatile compounds	Slaughter group (days)[a]				Pasture treatment[b]			Year[c]	
		0 days	56 days	84 days	112 days	Fescue	Brome	Orchard-grass	1	2
5	octane	1.44	0.70	1.39	0.60	1.20	0.65	1.25	1.26	0.81
6	pentanoic acid	0.75^{de}	0.90^{d}	0.82^{d}	0.54^{e}	0.83	0.70	0.73	0.73	0.78
7	heptanal	0.82^{d}	0.35^{e}	0.18^{e}	0.41^{e}	0.62^{d}	0.40^{e}	0.30^{e}	0.43	0.45
10	2,3-octanedione	7.19^{d}	1.51^{e}	1.01^{e}	0.79^{e}	2.65	3.01	2.20	2.21	3.04
11	hexanoic acid	2.01	2.06	2.63	1.77	2.49^{d}	1.78^{e}	2.09^{e}	2.00	2.24
15	heptanoic acid	1.13^{de}	1.20^{d}	1.12^{de}	0.89^{e}	1.19	0.97	1.09	1.04	1.13
16	3-hydroxyoctan-2-one	1.02^{d}	0.24^{c}	0.23^{e}	0.18^{e}	0.37	0.57	0.31	0.51	0.33
17	nonanal	0.61	0.77	0.60	0.60	0.61	0.57	0.74	0.63	0.66
19	octanoic acid	1.99^{d}	2.12^{c}	1.88^{d}	1.26^{e}	1.93	1.66	1.84	1.70	1.92
21	2-decenal	1.34^{d}	0.75^{e}	0.95^{e}	0.82^{e}	1.10	0.95	0.85	0.93	1.00
22	nonanoic acid	2.36^{d}	2.37^{d}	2.12^{d}	1.67^{e}	2.22	1.97	2.27	1.86^{e}	2.45^{d}
25	decanoic acid	2.02^{e}	2.66^{d}	2.57^{de}	1.00^{f}	2.22	1.88	2.09	1.82^{e}	2.31^{d}
26	2-undecenal	0.53	0.78	0.51	0.40	0.65	0.60	0.42	0.47	0.64
28	azulene 1,2,3,4,5, 6,7,8 octahydro-1, 4,-dimethyl-7-(-1-methylethylidine	1.24	0.44^{e}	0.40^{e}	0.28^{e}	0.63	0.62	0.52	0.62	0.56
32	2-tridecanone	1.19^{d}	0.49^{e}	0.37^{ef}	0.27^{f}	0.74^{d}	0.53^{e}	0.47^{e}	0.43^{e}	0.73^{d}
35	hexadecane	2.11^{d}	0.84^{e}	0.90^{e}	0.60^{e}	1.33	1.00	1.01	1.33^{d}	0.89;^{e}
37	heptadecane	1.81^{d}	0.74^{e}	0.67^{ef}	0.33^{f}	1.07	0.75	0.84	0.81	0.96
38	2-pentadecanone	0.85^{e}	1.38^{d}	1.53^{d}	0.72^{e}	1.24	1.03	1.09	1.12	1.12
39	δ-dodecalactone	2.05^{d}	1.72^{de}	1.57^{e}	1.09^{f}	1.71	1.51	1.61	1.39	1.83^{d}
40	dodecanoic acid	1.52^{e}	1.51^{ef}	2.47^{d}	0.65^{f}	1.64	1.67	1.30	1.21^{e}	1.87^{d}
41	phyt-1-ene	18.51^{d}	3.44^{e}	2.08^{e}	1.26^{e}	12.68^{d}	2.04^{e}	4.25^{e}	6.74	5.90
42	octadecane	4.30^{d}	1.27^{e}	1.12^{e}	0.62^{e}	2.31^{d}	1.72^{de}	1.46^{e}	1.41^{e}	2.24^{d}
43	phytane	1.71^{d}	0.89^{e}	1.05^{e}	0.48^{f}	1.26^{d}	0.90^{e}	0.94^{e}	0.70^{e}	1.36^{d}
44	neophytadiene	15.40^{d}	2.25^{e}	1.24^{e}	0.76^{e}	10.51^{d}	1.80^{e}	2.43^{e}	5.61	4.22
45	phyt-2-ene	38.39^{d}	7.36^{e}	4.40^{ef}	2.40^{f}	16.40^{d}	11.39^{e}	10.04^{e}	11.94	14.34
46	isomer of neophytadiene	1.12^{d}	0.73^{e}	0.83^{de}	0.36^{f}	0.96^{d}	0.74^{de}	0.58^{e}	0.59^{e}	0.93^{d}
47	2-heptadecanone	1.70^{e}	2.45^{d}	2.55^{d}	1.21^{e}	2.15	2.01	1.78	1.65^{e}	2.31^{d}
49	δ-tetradecalactone	2.20^{f}	4.10^{e}	5.84^{d}	3.71^{e}	4.32	3.73	3.84	3.07^{e}	4.86^{d}
52	dihydrophytol	1.47^{d}	1.06^{de}	0.73^{ef}	0.44^{f}	0.91^{de}	1.26^{d}	0.62^{e}	1.20^{d}	0.65^{e}
53	phytol	1.31^{d}	0.35^{e}	0.55^{e}	0.47^{e}	0.72	0.65	0.65	0.55	0.80
54	δ-hexadecalactone	2.16^{f}	2.96^{ef}	4.09^{d}	3.87^{de}	3.54	3.26	2.99	3.07	3.47

[a] Least squares mean of 18 observations.
[b] Least squares mean of 24 observations.
[c] Least squares mean of 36 observations.
[def] Means bearing different superscipts within Slaughter group, Pasture treatment and Year on the same line differ significantly ($P<0.05$).

radicals which are proposed to be the initial products of thermally-oxidized fats (Sink, 1973). Hexanal and 2,4-decadienal are two compounds which are produced during the thermal oxidation of $C_{18:2}$ (Frankel et al., 1961) and have previously been reported in higher concentrations in grass-fed beef compared to grain-fed beef (Bailey et al., 1980; Hedrick et al., 1980). Neither compound was found to exist in appreciable levels in any of the fat samples analyzed in the present study.

There were observable differences in the amounts of volatile fatty acids among samples from animals grain-fed for varying lengths of time. In general, complex relationships existed with the length of time steers were grain-fed after pasture. In each case though, the quantity of volatile fatty acids was observed to decrease between 84 and 112 days. Selke et al. (1975) reported the distribution pattern of aliphatic acids from tristearin and concluded their distribution closely resembled that of the aldehydes and that the various acids are formed via oxidation of the corresponding carbon chain aldehydes.

Large decreases were observed in the amount of 2,3-octanedione and 3-hydroxyoctan-2-one during the period of grain feeding, especially between 0 and 56 days. 2,3-Octanedione was identified as a cooked beef volatile by Liebich et al. (1972). The sensory property of this compound is a caramel-like, sweet aroma (Winter et al., 1976) so its exact relationship to grassy flavor is still not understood.

A series of methyl ketones (C_{13}, C_{15} and C_{17}) was detected in the beef fat samples. The concentration of these methyl ketones decreased during the period of grain-feeding with C_{15} and C_{17} exhibiting a quadratic relationship. Watanaba and Sato (1970) were among the first to identify C_{13}, C_{15} and C_{17} methyl ketones in heated or cooked beef fats. Thomas et al. (1971) proposed the autoxidation of fatty acids, particularly C_{18} unsaturates (via hydroperoxides) as a mechanism for the formation of methyl ketones. Selke et al. (1975) reported the formation of a homologous series of methyl ketones (C_3–C_{17}) from tristearin and concluded they could be a result of β-oxidation (from the carboxyl end) of the carbon chain followed by decarboxylation. Methyl ketones are known to cause off-flavors referred to as "perfume" rancidity in some foods (Stokoe, 1928). A similar series of methyl ketones was identified in much higher concentrations in fat from lambs grazed on pasture by Suzuki and Bailey (1985) and as such may contribute to lamb flavor.

Several δ-lactones (C_{10}, C_{12}, C_{14}, C_{15} and C_{16}) and one γ-lactone (γ-dodecalactone) were identified in the beef fat samples. While the recovery of high molecular weight lactones with this system is very temperature dependent (Sużuki and Bailey, 1985), an increase in the concentration of δ-tetradecalactone and δ-hexadecalactone and a decrease in the concentration of δ-dodecalactone were observed during the period of grain-feeding after pasture. Lactones are odorous compounds and may contribute either desirable or undesirable notes to meat flavor, depending on their concentration (Wasserman, 1972). Watanaba and Sato (1971) postulated two mechanisms for the formation of lactones. The first mechanism involves their formation from the corresponding γ- or δ-hydroxy acids which are known to be present in the fat. The second mechanism involves the conversion of low molecular weight saturated fatty acids, aldehydes and alcohols which, in general, are formed by oxidative degradation of meat fats.

The major qualitative and quantitative differences associated with forage feeding were the presence of terpenoid type compounds particularly the class of compounds referred to as diterpenoids in the fat of forage-fed beef. Diterpenoids are C_{20}

Table 5—Simple correlation coefficients between quantity of volatile compounds present in bovine lipid and time on feed (slaughter group) or sensory panel scores for level of grassy flavor

Compound name	Peak number	Slaughter group	Ground beef	Steak
octane	5	−0.10	0.06	0.06
pentanoic acid	6	−0.20	0.20	0.03
heptanal	7	−0.32*	0.32**	0.21
2,3-octanedione	10	−0.53**	0.50**	0.45**
hexanoic acid	11	−0.11	0.02	−0.13
heptanoic acid	15	−0.23	0.24*	−0.01
3-hydroxyoctan-2-one	16	−0.34**	0.34**	0.29**
nonanal	17	−0.06	−0.02	0.02
octanoic acid	19	−0.36**	0.28*	0.11
2-decenal	21	−0.27*	0.23*	0.19
nonanoic acid	22	−0.24*	0.22	0.08
decanoic acid	25	−0.31*	0.17	0.19
2-undecenal	26	−0.11	−0.06	−0.08
1,2,3,4,5,6,7,8-octahydro-1,4-dimethyl-7-(-1-methyl-ethylidine) azulene	28	−0.60**	0.55**	0.57**
2-tridecanone	32	−0.59**	0.56**	0.47**
hexadecane	35	−0.49**	0.44**	0.47**
heptadecane	37	−0.61**	0.56**	0.52**
2-pentadecanone	38	−0.04	−0.05	−0.16
δ-dodecalactone	39	−0.44**	0.36**	0.30**
dodecanoic acid	40	−0.13	0.05	0.02
phyt-1-ene	41	−0.51**	0.49**	0.39**
octadecane	42	−0.60**	0.57**	0.46**
phytane	43	−0.50**	0.41**	0.33**
neophytadiene	44	−0.48**	0.47**	0.34**
phyt-2-ene	45	−0.71**	0.68**	0.62**
diene isomer (MW 278)	46	−0.40*	0.34**	0.19
2-heptadecanone	47	−0.13	0.04	−0.08
δ-tetradecalactone	49	+0.28*	−0.31**	−0.37**
dihydrophytol	52	−0.34**	0.46**	0.30*
phytol	53	−0.38**	0.40**	0.39**
δ-hexadecalactone	54	+0.35**	−0.25*	−0.34**

*(P<0.05)
**(P<0.01)

hydrocarbons consisting of four isoprene (C_5) units. The most dramatic decline in the concentration of each of the diterpenoids present occurred between 0 and 56 days of grain feeding.

According to Body (1977) diterpenoids are derived from phytol (3,7,11,14-tetramethyl-2-hexadecen-1-ol) of chlorophyll by the alction of rumen microorganisms. Dehydration of phytol forms neophytadiene (7,11,15-trimethyl-3-methylene-1-hexadecene). Phyt-1-ene (3,7,11,15-tetramethyl-1-hexadecene) is formed by dehydration of dihydrophytol (3,7,11,15-tetramethyl hexadecan-1-ol) which is derived from phytol via hydrogenation in the rumen. Further hydrogenation of phyt-1-ene produces phytane (2,6,10,14-tetramethyl hexadecane). The double bond at C_1 of phyt-1-ene migrates to C_2 to form phyt-2-ene (3,7,11,15-tetramethyl-2-hexadecene).

Urbach and Stark (1975) isolated phyt-1-ene, neophytadiene and phytane from butterfat and also isolated neophytadiene from ryegrass pastures on which the cows were grazed. These investigators concluded that the immediate precursor of phyt-1-ene and neophytadiene in the butterfat was neophytadiene in the pasture grass since the level of neophytadiene and phyt-1-ene were decreased when cows were transferred from grass pasture to drylot. Ferretti and Flanagan (1977) identified phyt-2-ene and neophytadiene as components of rabbit adipose tissue and stated that the most likely source of these diterpenoids was the rabbit feed (Purina Rabbit Chow) which contained large quantities of these compounds. The diterpenoids identified here as components of beef fat thus appear to be from a combination of microbial fermentation of phytol in the rumen and the preferential deposition into the adipose tissue of diterpenoids present in the forages themselves.

Correlation between subjective and objective flavor analyses

In order to relate the sensory data to the length of time on grain feeding and the chromatography data, simple correlation coefficients between the quantity of volatile compounds present in bovine lipid and time on feed (Slaughter Group) or sensory panel scores for level of grassy flavor were calculated (Table 5). Twenty compounds including heptanal; 2,3-octanedione; 3-hydroxyoctan-2-one; octanoic acid; 2-decenal; nonanoic acid; decanoic acid; 1,2,3,4,5,6,7,8-octahydro-1,4-dimethyl-7-(-1-methylethylidine)-azulene; 2-tridecanone; hexadecane; heptadecane; δ-dodecalactone; phyt-1-ene; octadecane; phytane; neophytadiene; phyt-2-ene; phytadiene; dihydrophytol and phytol were negatively ($P<0.05$) correlated with the length of time steers were grain-fed in drylot after grazing on pasture, i.e. their concentration decreased with grain feeding. δ-Tetradecalactone and δ-hexadecalactone were postively ($P<0.05$) correlated with time on feed. Fourteen of the compounds previously discussed including heptanal; 2,3-octanedione; 3-hydroxyoctan-2-one; 1,2,3,4,5,6,7,8-octahydro-1,4-dimethyl-7(-1-methylethylidine)-azulene; 2-tridecanone; hexadecane; heptadecane; δ-dodecalactone; phyt-1-ene; octadecane; phytane; neophytadiene; phyt-2-ene; dihydrophytol and phytol were positively ($P<0.05$) correlated with "grassy flavor" of steak samples. These fourteen compounds plus several of the acids including heptanoic acid, octanoic acid, nonanoic acid and decanoic acid were positively ($P<0.05$) correlated with "grassy flavor" of ground beef. The difference in cooking method, oven broiling for steaks versus cooking in a beaker where the meat juices accumulated for ground beef, would appear to be responsible for the increased sensory perception of the acids in the case of ground beef. Two compounds, δ-tetradecalactone and δ-hexadecalactone, were negatively ($P<0.05$) correlated with grassy flavor and as such, may be suitable as indicators of grain feeding.

Multivariant statistical analyses

The volatile compounds included in the model selected by the forward procedure of stepwise discriminant analysis (SAS, 1982) to predict differences due to length of time on grain after pasture, are listed in Table 6. The forward stepwise discriminant analysis procedure begins with no variables in the model. At each step, the variable is entered which contributes most to the discriminatory power of the model as measured by Wilks' lambda, the likelihood ratio criterion. The forward selection process is stopped when none of the unselected variables meets the entry criterion. The model (Table 6) selected for separating the cattle into the four slaughter groups contained 8 compounds and had an average squared canonical correlation (ASCC) of 0.5023. ASCC is close to 1.0 if all groups (times on grain after pasture) are well separated and if all or most directions in the discriminant space show good separation, for at least two groups (SAS, 1982). Results of discriminant analysis (SAS, 1982) indicated that there were observable and reproducible differences among the volatiles in this model since 91.67% of the samples were classified correctly. Mis-classified samples included one 56 day as an 84 day, one 84 day as a 56 day and three 84 day samples as 112 day. This model consists mainly of compounds such as phyt-2-ene, 2,3-octanedione and octadecane whose concentration decreased dramatically between 0 and 56 days on grain but changed only slightly after 56 days. This could explain in part the model's inability to correctly classify all the samples, especially those in the 56 and 84 day groups. The model (Table 6) for separating samples from pasture-fed cattle from cattle which were grain-fed for any period of time contained 9 compounds and had an ASCC of 0.7401. This model achieved 100% correct classification when tested using discriminant analysis as did the model (Table 6) containing 13 compounds (ASCC = 0.8477) which was used to classify samples according to days on grain. Phyt-2-ene was selected as the most discriminating compound in each model and as such, appeared to be an excellent indicator of forage feeding. Using the direct sampling procedure described earlier,

Table 6—Volatiles[a] in beef fat selected by stepwise discriminant analysis for inclusion into models to predict slaughter group, grain vs pasture feeding and days on grain in feedlot

Slaughter group (0 vs 56 vs 84 vs 112 days)		Grain vs pasture (0 vs 56, 84 and 112 days)		Days on grain (56 vs 84 vs 112 days)	
phyt-2-ene	(0.72)	phyt-2-ene	(0.71)	phyt-2-ene	(0.41)
decanoic acid	(0.33)	2,3-octanedione	(0.17)	decanoic acid	(0.32)
2,3-octanedione	(0.18)	octadecane	(0.14)	2-pentadecanone	(0.26)
2-undecenal	(0.15)	phytadiene isomer	(0.06)	2-tridecanone	(0.20)
octadecane	(0.15)	δ-tetradecalactone	(0.06)	2-decenal	(0.19)
dodecanoic acid	(0.14)	1,2,3,4,5,6,7,8-		nonanoic acid	(0.19)
2-pentadecanone	(0.13)	octahydro-(1,4-dimethyl)-		heptanal	(0.16)
phytadiene isomer	(0.13)	7-(1-methylethylidine)azulene	(0.06)	neophytadiene	(0.16)
		hexanoic acid	(0.05)	heptadecane	(0.14)
		octanoic acid	(0.04)	hexadecane	(0.13)
		phytol	(0.04)	octane	(0.13)
				phytol	(0.11)
				δ-dodecalactone	(0.10)

[a] Partial R^2 values for each volatile are listed in parentheses.

it is thus possible to classify cattle into groups of like feeding history in regards to forage versus grain feeding in drylot.

REFERENCES

Aberle, E.D., Reeves, E.S., Judge, M.D., Hunsley, R.E., and Perry, T.W. 1981. Palatability and muscle characteristics of cattle with controlled weight gain. Time on high energy diet. J. Anim. Sci. 52: 757.

Bailey, M.E., Dupuy, H.P., and Legendre, M.G. 1980. Undesirable meat flavor and its control. In "The Analysis and Control of Less Desirable Flavors in Foods and Beverages," G. Charalambous (Ed.), Academic Press, New York.

Berry, B.W., Maga, J.A., Calkins, C.R., Wells, L.H., Carpenter, Z.L., and Cross, H.R. 1980. Flavor profile analysis of cooked beef loin steaks. J. Food Sci. 45: 1113.

Body, D.R. 1977. Characterization of bovine rumen liquor isoprenoid hydrocarbons with reference to dietary phytol. Lipids 12: 204.

Bowling, R.A., Riggs, J.K., Smith, G.C., Carpenter, Z.L., Reddish, R.L., and Butler, O.D. 1978. Production, carcass and palatability characteristics of steers produced by different management systems. J. Anim. Sci. 46: 333.

Brown, H.G., Melton, S.L., Riemann, M.J., and Backus, W.R. 1979. Effects of energy intake and feed source on chemical changes and flavor of ground beef during frozen storage. J. Anim. Sci. 48: 338.

Cochran, W.G. and Cox, G.M. 1957. "Experimental Design," 2nd ed. John Wiley and Sons, New York.

Cross, H.R. and Dinius, D.A. 1978. Carcass and palatability characteristics of beef steers finished on forage diets. J. Anim. Sci. 47: 1265.

Davis, G.W., Cole, A.B., Backus, W.R., and Melton, S.L. 1981. Effect of electrical stimulation on carcass quality and meat palatability of beef from forage- and grain-finished steers. J. Anim. Sci. 53: 651.

Dolezal, H.G., Smith, G.C., Savell, J.M., and Carpenter, Z.L. 1982. Effect of time-on-feed on palatability of rib steaks from steers and heifers. J. Food Sci. 47: 368.

Ferretti, A. and Flanagan, V.P. 1977. Tissue variation in hydrocarbon composition in the rabbit. Lipids 12: 198.

Frankel, E.N., Evans, C.D., McConnel, D.G., Selke, E., and Dutton, H.J. 1961. Autoxidation of methylinolenate. Isolation and characterization of hydroperoxides. J. Org. Chem. 26: 4663.

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

Heath, M.E., Metcalfe, D.S., and Barnes, R.F. 1973. "Forages: The Science of Grassland Agriculture," 3rd ed. The Iowa State University Press, Ames, IA.

Hedrick, H.B., Bailey, M.E., Krouse, N.G., Dupuy, H.P., and Legendre, M.G. 1980. Relationship between volatile compounds in fat from forage and grain-fed beef and sensory characteristics of steaks and roasts. Proc. 26th European Meet. Meat Res. Workers.

Hedrick, H.B., Paterson, J.A., Matches, A.G., Thomas, J.D., Morrow, R.E., Stringer, W.C., and Lipsey, R.J. 1983. Carcass and palatability characteristics of beef produced on pasture, corn silage and corn gram. J. Anim. Sci. 57: 791.

Horstein, I. 1971. Chemistry of meat flavor. In "The Science of Meat and Meat Products," J.F. Price and B.S. Schweigert (Ed.) W.H. Freeman and Co., San Francisco, CA.

Liebich, H.M., Douglas, W.R., Zlatkis, A., Muggler-Charan, F., and Donzel, A. 1972. Volatile components in roast beef. J. Agric. Food Chem. 20: 96.

Melton, S.L., Amiri, M., Davis, G.W., and Backus, W.R. 1982a. Flavor and chemical characteristics of ground beef from grass-, forage-grain and grain-finished steers. J. Anim. Sci. 55: 77.

Melton, S.L., Black, J.B., Davis, G.W., and Backus, W.R. 1982b. Flavor and selected chemical characteristics of ground beef from steers backgrounded on pasture and fed corn up to 140 days. J. Food Sci. 47: 699.

Meyer, B., Thomas, J., Buckley, R., and Cole, J.W. 1960. The quality of grain-finished and grass-finished beef as affected by ripening. Food Technol. 14: 4.

Nixon, L.N., Wong, E., Johnson, C.B., and Birch, E.J. 1979. Nonacidic constituents of volatiles from cooked mutton. J. Agric. Food Chem. 32: 355.

Official United States Standards for Grade of Beef Carcasses. 1975. Title 7, Chapt. I, Part 53, Sec. 53.100-53.105 of the Code of Federal Regulations, reprinted with amendments effective April 14, 1975.

NIH EPA Chemical Information System. 1978. U.S. Government Printing Office, Washington, DC.

Reagan, J.O., Carpenter, Z.A., Bauer, F.T., and Lowrey, R.S. 1977. Packaging and palatability characteristics of grass- and grass-grain fed beef. J. Anim. Sci. 45: 716.

SAS. 1982. "User's Guide Statistics," SAS Institute, Inc., Cary, NC.

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

Selke, E., Rohwedder, W.K., and Dutton, H.J. 1975. Volatile components from tristearin heated in air. J. Am. Oil Chem. Soc. 52: 232.

Sink, J.D. 1973. Lipid soluble components of meat flavor odors and their biochemical origin. J. Am. Oil Chem. Soc. 50: 470.

Skelly, G.C., Edwards, R.L., Wardlaw, F.D., and Torrence, A.K. 1978. Selected high forage rations and their relationship to beef quality, fatty acid and amino acids. J. Anim. Sci. 47: 1102.

Smith, G.M., Crouse, J.D., Mandigo, R.W., and Neer, K.L. 1977. Influence of feeding regime and biological type on growth, composition, and palatability of steers. J. Anim. Sci. 45: 236.

Stokoe, W.N. 1928. The rancidity of coconut oil produced by mold action. Biochem. J. 20: 80.

Suzuki, J. and Bailey, M.E. 1985. Direct sampling capillary GLC analysis of flavor volatiles from ovine fat. J. Agric. Food Chem. 33: 343.

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

Thomas, C.P., Dimick, D.S., and McNeil, J.H. 1971. Sources of flavor in poultry skin. Food Technol. 25: 407.

Urbach, B. and Stark, W. 1975. The C-20 hydrocarbons of butterfat. J. Agric. Food Chem. 23: 20.

Wasserman, A.E. 1972. Thermally produced flavor components in the aroma of meat and poultry. J. Agric. Food Chem. 20: 737.

Watanabe, K. and Sato, Y. 1971. Gas chromatographic and mass spectral analyses of heated flavor compounds of beef fats. Agric. Biol. Chem. 35: 756.

Westerling, D.B. and Hedrick, H.B. 1979. Fatty acid composition of bovine lipids as influenced by diet, sex and anatomical location and relationship to sensory characteristics. J. Anim. Sci. 48: 1343.

Winter, M., Gautsch, F., Flament, I., and Stoll, M. 1976. U.S. Pat. 3989, 713. (cl. 260-362.2; c 07D 207/32).

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Binding, Sensory and Storage Properties of Algin/Calcium Structured Beef Steaks

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ABSTRACT

Structured beef steaks formed with the algin/calcium binder and with or without glucono-delta-lactone were compared with 100% beef controls and with structured steaks formed with salt and phosphate. Algin/calcium-treated products exhibited better binding and color in the raw state, but had lower palatability scores in the cooked state than salt/phosphate controls. Shelf-life of both algin/calcium products under aerobic conditions was similar to the all-beef control. In vacuum packages, however, the algin/calcium products showed more rapid gas production and microbial growth than all-beef and salt/phosphate controls. The algin/calcium treatment will allow increased marketing alternatives for meat products.

INTRODUCTION

VALUE ENHANCEMENT of raw materials, portion control, and consumer convenience are advantages of structured meat products (Simunovic et al., 1985). Currently, structured meat products are made by extracting muscle proteins, using salt, phosphates and mechanical action, which form a heat-set protein gel upon subsequent cooking (Schmidt and Trout, 1982). These products do not bind in the raw state and are marketed either frozen or precooked to retain structural integrity. There are problems, however, associated with frozen storage (discoloration, rancidity development, poor consumer appeal) and precooking (warmed-over flavor) (Pearson et al., 1977; Field, 1982). Therefore, development of technology to allow marketing of structured meat products in the fresh, refrigerated state would alleviate these problems and be beneficial for marketing underutilized red meat sources. Means and Schmidt (1986a) reported that an algin/calcium gelation mechanism can be successfully used to produce structured beef products which possess acceptable binding characteristics in both the raw and cooked state.

The non-meat ingredients (sodium alginate and calcium carbonate) of these beef products may possess unique microbiological properties not encountered with traditional structured meat products or raw meat. Algin/calcium coatings of meat products have a limited inhibitory effect on microbial growth (Earle, 1968; Lazarus et al., 1976; Marriott et al., 1977; Williams et al., 1978). Microbial spoilage of intact muscle products is generally limited to growth of bacteria on the meat surface (Ingram and Dainty, 1971; Gill, 1983). The microbiology of alginate coated products may also be different than structured meat products made using the algin/calcium gel as an integral binder. Sensory evaluation data have indicated that alginate coatings may positively influence color, odor and flavor, provided the calcium salt used does not cause bitter flavors (Allen et al., 1963; Williams et al., 1978; Wanstedt et al., 1981; Ahmed et al., 1983).

Preliminary studies indicated that the slowly released acid glucono-delta-lactone (GDL) may deter the undesirable mouthfeel of algin/calcium beef reported by Means and Schmidt (1986a). Also, GDL may promote stronger binding of meat pieces by the calcium alginate gel (Means, 1985).

The objectives of this study were to evaluate: (1) the effectiveness of the algin/calcium gelation mechanism as a binder in raw and cooked structured beef steaks made with and without addition of GDL; (2) the palatability of such steaks; and (3) their shelf-life when refrigerated under aerobic or anaerobic conditions.

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MATERIALS & METHODS

Experimental design and variables

A randomized block design was used to study four treatments (Table 1). The algin/calcium combination was evaluated with (AC+) and without (AC) addition of 0.2% GDL. The 0.78% sodium alginate (Na-alginate; Manugel DMB, Kelco, Division of Merck and Company, Inc.) and 0.14% calcium carbonate ($CaCO_3$; Gamma Sperse 80, Georgia Marble Co.) levels were based on the ideal algin:calcium ion ratio (2.5:0.18), on $CaCO_3$ solubility (Anonymous, 1984), and on previous studies (Means and Schmidt, 1986a). The reference control treatments included coarse ground beef patties with no additives (NA) and a 1.4% NaCl - 0.32% sodium tripolyphosphate (FMC Corp.) structured beef steak (RS) formulated to an ionic strength of 0.33. A split plot design was used to analyze two packaging techniques (vacuum, nonvacuum) to determine color changes and microbial growth during storage. The study was repeated in its entirety to give two complete replicates.

Product preparation

Fresh (2-day postmortem), boneless chuck pieces (*M. pectoralis* deep; from USDA Choice and Good, yield grade 2 and 3 cattle, Monfort of Colorado, Inc.) were ground once through a 1.9 cm × 4.8 cm kidney plate. The proximate composition of the fresh meat was 69.3% water, 8.3% fat and 19.2% protein. The ingredients were sifted into the meat (13.6 kg batches) with a kitchen flour sifter, and mixed in a 27 kg capacity, paddle-type mixer (Keebler Engineering Co.) for 2.5 min at 100 rpm. After mixing, the products were extruded as logs through a rib-eye shaped stuffing horn (87.1 cm^2 area) using a water powered piston-type stuffer (E-Z Pak, E. F. Zuber Mfg. Inc.). All beef logs (50 cm long) were wrapped in plastic-coated freezer paper (Crown Freshgard, Crown Zellerbach Corp.) and stored for 48 hr at 4 ± 1°C.

The logs were sliced into steaks (1.3 cm thick, 100 ± 10 grams) with a deli-style slicer (Hobart Mfg. Co.), and packaged in a retail-type tray-pack which consisted of a styrofoam tray (Mobil Chemical, Packaging Department) overwrapped with permeable polyvinyl chlo-

Table 1—Variables and experimental design to study the influence of the algin/calcium meat binder on microbial growth and quality

Treatment[a]	Ingredients (%) ALG	$CaCO_3$	GDL	NaCl	STP	Beef
NA	--	--	--	--	--	100.00
RS	--	--	--	1.40	0.32	98.28
AC	0.78	0.14	--	--	--	99.08
AC+	0.78	0.14	0.20	--	--	98.88

[a] NA = no additives, RS = NaCl and sodium tripolyphosphate (STP), AC = Na-alginate (ALG) and $CaCO_3$, AC+ = Na-alginate, $CaCO_3$ and glucono-delta-lactone (GDL).

ride (PVC), all-purpose, food film (PW-18, Anchor Industries), and in vacuum packages. Vacuum packages consisted of co-extruded nonshrink bags (Freshtuff, American Can Co.) with reported permeabilities of 9.3–12.4 ml $O_2/m^2/24$ hr measured at 22.8°C and 50% relative humidity, and 4.7–6.2g of $H_2O/m^2/24$ hr measured at 37.8°C and 90% relative humidity. Bags were evacuated (-8.4×10^4 N/m^2) and heat sealed using a chamber-type vacuum packaging machine (Multivac, type AG 800). Steaks for use in microbial evaluations were halved prior to packaging (i.e., 50g weight). PVC overwrap and vacuum packages were subsequently stored on shelves in a walk-in cooler (6 ± 1°C, relative humidity = 83%) and subjected to intermittent lighting (12 hr on, 12 hr off) from two Westinghouse Econowatt (F40 cool white, 34 watt) fluorescent lamps.

Objective evaluation

Cook yield. Five steaks from each treatment were weighed before and after broiling to determine cook yield, which was expressed as (weight of cooked steak/weight of raw steak) × 100.

Microbial evaluation. Individually packaged samples (50g steaks) were aseptically transferred into sterile blender jars and blended with the appropriate amount of 0.1% sterile peptone (Difco) buffer to give a 1:5 dilution. Serial dilutions were then made in 0.1% peptone diluent. The initial dilution was also used for pH determination. Duplicate pour plates from appropriate dilutions were made for two samples per treatment. Samples were plated on standard methods agar (SMA; Difco) and APT (BBL) agar on the day of product manufacture (Day 0) to obtain initial counts. Microbial evaluation of the raw meat was also done on Day 0. The PVC overwrapped products, which were packaged on Day 2, were subsequently plated on SMA on Days 3, 5, 7, 9, 11, and 16. Vacuum packaged products were plated on SMA and APT agar on Days 3, 8, 13, 18, 23, and 28. Plates were incubated aerobically at 6–7°C for 10 days prior to counting.

Hunter color analysis. Hunter L, a and b values were determined for the raw steaks (one per treatment) at time intervals during storage. A modified aperture opening of 2.5 cm × 5.0 cm was used for all measurements (three per steak) and large fat pockets were avoided. Measurements were made through one layer of PVC wrap after the Hunterlab colorimeter (Hunterlab Digital Color Difference Meter, model D25D2) was calibrated with one layer of PVC wrap and the white calibration standard (C2-3862). The same steak from each tray-peak treatment was used for each time interval. Color scores for vacuum packaged steaks were made through one vacuum bag layer before and after each sample had been allowed to bloom for 45 minutes at 23°C. The same side of each steak was used for nonbloomed and bloomed measurements, but different steaks had to be used for each time period.

Subjective evaluation

Raw product. An experienced five member panel evaluated the initial products (Day 0) for percentage of surface discoloration, color intensity and raw bind. The evaluators were trained and participated in a previous study with similar products (Means and Schmidt, 1986a). The six point descriptive scales of evaluation are given in Table 3 and Table 4 and were used in the previous study (Means and Schmidt, 1986a).

The data on microbiological growth were supplemented by data on changes in color, turbidity of exudate, and appearance of visible colonies and slime. The products were also evaluated for aroma, especially off-odors, in opened packages and after blending with peptone buffer. Separate packages were used for sensory and microbiological evaluation of spoilage. This evaluation was only intended to present an indication of how microbial growth was related to product rejection on appearance and off-odors. For this reason the evaluation was performed by two experienced individuals who also conducted the microbiological analyses. The evaluation was based on five-point descriptive scales presented in the legends of Fig. 3 and 7.

Cooked product. Cooked products were also evaluated by the five member experienced panel. Steaks were cooked on Faberware open hearth electric broilers (average temperature at steak surface = 160–170°C) for 6.5 min and 5.0 min per side to a medium degree of doneness, as judged by standard color photographs (AMSA, 1978). Mouthfeel, aroma, flavor and product bind in the cooked state were evaluated by the methods and criteria of Means and Schmidt (1986a). The six-point descriptive scales of evaluation were described by Means and Schmidt (1986a) and are given in Table 3.

Statistical analysis

The data were analyzed as complete block designs, with split plots when appropriate (Cochran and Cox, 1957; Snedecor and Cochran, 1976; Steel and Torrie, 1980), and the use of the statistical package for the social sciences (Nie et al., 1975; Hull and Nie, 1981). Mean separation tests, either least significant difference or Duncan's multiple range test, were used when a significant ($P<0.05$) F-test for main effects was found (Steel and Torrie, 1980). Mean separation tests were made using an $\alpha = 0.10$ in some cases where $0.05 < P < 0.10$ for F-test of main effects.

RESULTS & DISCUSSION

Microbial growth and spoilage

Aerobic. All products seemed to follow a normal microbial growth curve during aerobic storage (Fig. 1). The all-beef control and algin/calcium samples showed very similar rates of increase in psychrotrophic counts and exceeded 10^8 CFU/g in 7 days of storage. The rate of microbial growth, however, was slower ($P=0.053$) in the NaCl/phosphate (RS) steaks which exceeded 10^8 CFU/g in 9–11 days of storage. Thus, the algin/calcium treatment did not enhance microbial growth in beef, while the slower microbial growth in the RS products may have been due to the antimicrobial activity of NaCl and phosphate (Sofos, 1984; Madril and Sofos, 1984).

Initial raw product pH values (Table 2 and Fig. 2) were higher ($P<0.05$) with algin/calcium than controls. Gluconodelta-lactone reduced the pH of algin/calcium products to intermediate levels which were similar to RS products. The pH of the algin/calcium treatment (AC) decreased ($P<0.05$) between five and nine days of storage. The pH of all other treatments was fairly constant for up to 9 days of storage. Except for the RS treatment, pH values increased by about 0.5 units between Day 9 and Day 16, indicating possible degradation of proteins and production of amines (Ingram and Dainty, 1971; Gill, 1983). The data of Table 2 also indicate that there were no treatment differences ($P>0.05$) in cook yield.

Visual changes and off-odors indicating product spoilage under aerobic (PVC overwrap) conditions (Fig. 3) developed at a rate similar to microbial growth. At Day 9 all treatments,

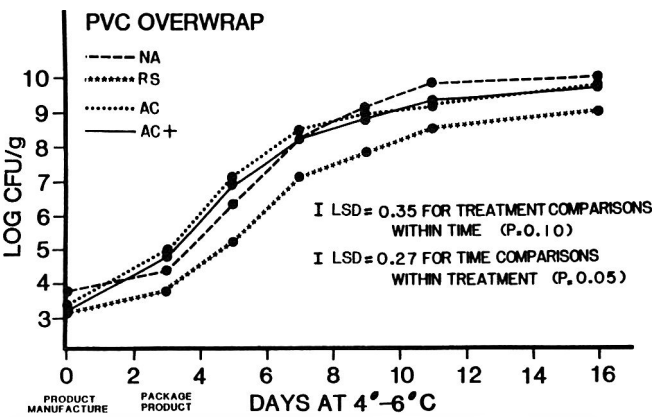

Fig. 1—Total psychrotrophic counts on standard methods agar of PVC packaged structured beef steaks formulated with the algin/calcium binder (treatment codes are presented in Table 1).

Table 2—Effect of the algin/calcium binder on the pH and cook yield of structured beef steaks

Treatment	Raw pH	Cooked pH	Cook yield (%)
NA	5.60[a]	5.82[a]	75.9[a]
RS	5.96[b]	6.10[b]	78.1[a]
AC	6.15[c]	6.32[c]	78.9[a]
AC+	6.00[b]	6.15[b]	78.9[a]

[a,b,c] Numbers in same column with different superscripts are different ($P<0.05$). Standard error for cook yield = 0.79, raw pH = 0.02, cooked pH = 0.02.

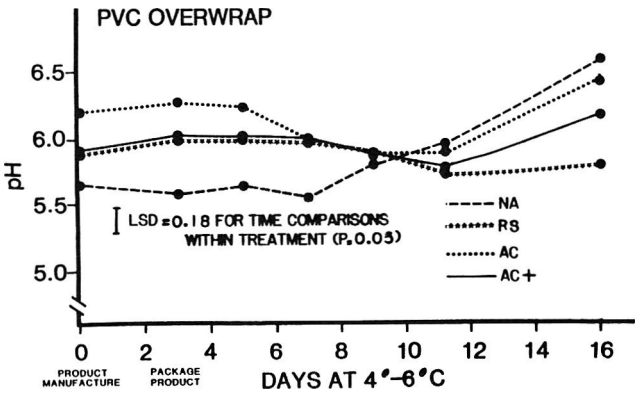

Fig. 2—pH of raw structured beef steaks formulated with the algin/calcium binder and packaged in PVC (treatment codes are presented in Table 1).

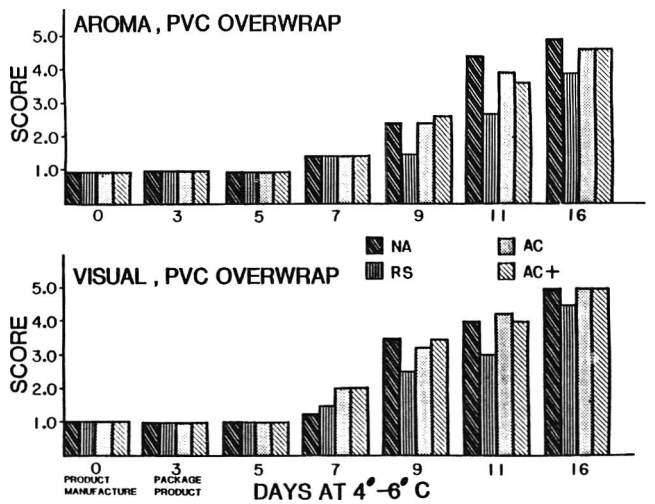

Fig. 3—Aroma and visual sensory spoilage scores of PVC packaged structured beef steaks formulated with the algin/calcium binder (1 = no detectable spoilage, 3 = marginally spoiled and 5 = grossly spoiled; treatment codes are presented in Table 1).

except RS, were at least marginally spoiled. The off-odors, however, did not exceed the marginally spoiled condition until Day 11 (Fig. 3). The RS treatment demonstrated less spoilage odor than the other three treatments through the entire storage period of 16 days. The AC+ treatment developed a unique odor described by evaluators as "wet lettuce." This aroma persisted through Day 16 at which time other, more acrid spoilage odors predominated. The "wet lettuce" odor was more predominant after samples were blended in a 1:5 dilution of 0.1% peptone, and may indicate variation in microbial profiles or metabolites produced. Samples of the RS treatment were first detected as marginally spoiled on Day 11. In general, samples of all treatments were spoiled when psychrotrophic counts exceeded 10^8 CFU/g.

The results suggested that, overall, algin/calcium-structured beef steaks had a shelf-life comparable to that of the 100% beef treatment (NA) under aerobic conditions. In addition, the shelf-life of these products (7–9 days) was comparable to that (4–14 days) reported for aerobically stored intact beef cuts (Greer and Jeremiah, 1981; Eqan, 1984). Since the study was not designed to delineate microbial types of growing in the various treatments, it is not known if the same organisms were responsible for spoilage of all treatments. Although, RS steaks had a longer shelf-life than other treatments studied, they are not likely to be marketed in the uncooked, unfrozen state.

Vacuum. In samples stored in vacuum packages total psychrotrophic counts and microbial counts on APT agar exceeded

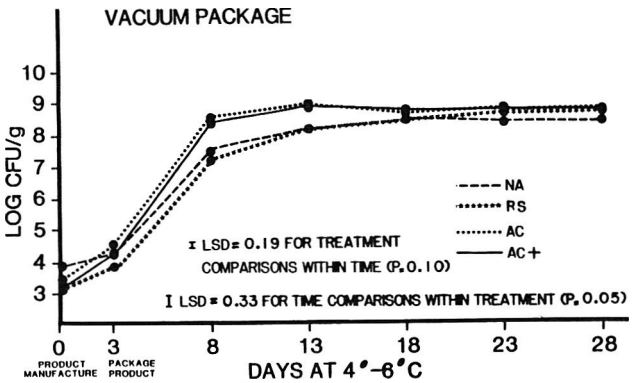

Fig. 4—Total psychrotrophic counts on standard methods agar of vacuum packaged structured beef steaks formulated with the algin/calcium binder (treatment codes are presented in Table 1).

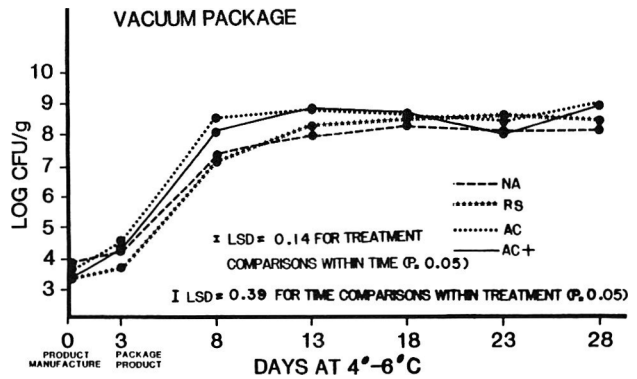

Fig. 5—Microbial counts on APT agar of vacuum packaged structured beef steaks formulated with the algin/calcium binder (treatment codes are presented in Table 1).

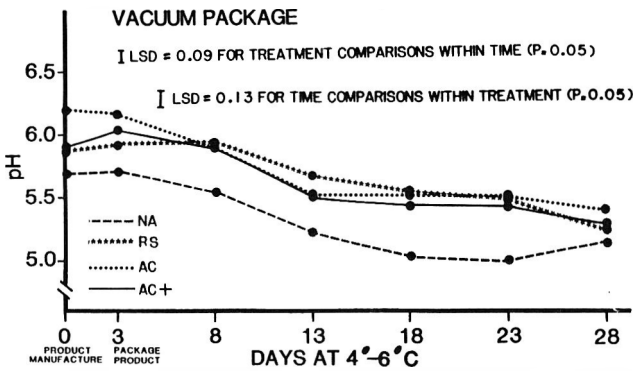

Fig. 6—pH of raw structured beef steaks formulated with the algin/calcium binder and packaged under vacuum (treatment codes are presented in Table 1).

10^8 CFU/g after storage of 8-13 days at 4–6°C depending on the treatment (Fig. 4 and 5). Microbial growth was more rapid in algin/calcium treatments, which exceeded 10^8 CFU/g at Day 8, compared to the all-beef control and the RS treatment, which exceeded 10^8 CFU/g on Day 13. Due to small sample and replicate variations in microbial counts, these differences in microbial growth were significant ($P<0.05$). With extended storage (> 13 days) all samples reached similar counts in the range of 10^8–10^9 CFU/g. Thus, it appears that the algin/calcium binding agent supported more rapid microbial growth in products stored in vacuum packages.

Product pH values decreased gradually during storage (Fig. 6). These pH decreases became significant ($P<0.05$) for each treatment after 8–13 days of storage. Eventually pH values

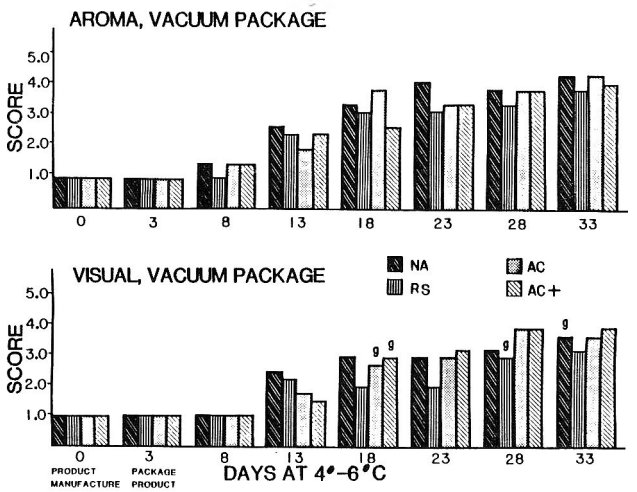

Fig. 7—Aroma and visual sensory spoilage scores of vacuum packaged structured beef steaks formulated with the algin/calcium binder (1 = no detectable spoilage, 3 = marginally spoiled and 5 = grossly spoiled; treatment codes are presented in Table 1).

reached levels in the range of 5.0–5.5. These pH declines under anaerobic conditions of storage indicate that microbial changes may have mostly consisted of proliferation of acid producing organisms. There was also a statistical difference ($P=0.001$) between total psychrotrophic counts on SMA and APT agar with the latter being higher in numbers. Although small, this statistical difference may have indicated that heterofermentative lactobacilli were the most predominant type of flora growing under vacuum.

Slower growth of microorganisms, even at relatively high pH (Fig. 4 and 5), for the RS treatment can be attributed to the antimicrobial activity of NaCl and possibly sodium tripolyphosphate (Sofos, 1984; Madril and Sofos, 1984). The slower microbial growth in the NA products may have been due to the lower initial pH of that treatment compared to the algin/calcium products. The non-meat ingredients in the AC and AC+ treatments, however, may have also stimulated growth of certain organisms.

Although microbial growth for all treatments exceeded 10^8 CFU/g in 8–13 days of storage under vacuum, visual and aromatic changes did not indicate spoilage until the length of storage had exceeded 18 days (Fig. 7). This is in agreement with the conclusion of Egan and Shay (1982) that sensory changes were not detected in vacuum packaged meats until several weeks after lactic acid producing bacteria counts reached 10^7 CFU/cm^2. Undesirable visual and aromatic changes developed slower in RS products.

The vacuum packages were also visually checked for development of gas (bubbles) during storage. The first day that gas was detected is indicated by the letter "g" on Fig. 7. Products with algin and calcium were the first treatments to exhibit formation of gas (day 18). Gas was first detected in the other treatments on Days 28 and 33. More rapid gas production in the algin/calcium treatments may be due to added ingredients (algin, $CaCO_3$) and/or a higher pH. Additional studies are needed, however, to determine the influence of algin/calcium on individual types of organisms and gas production.

Interrelationships between pH and film permeability are very important in spoilage of vacuum packaged fresh meat. Spoilage occurred faster when both pH and oxygen transmission rates were high (Taylor and Shaw, 1977; Egan, 1984). Although the vacuum bags used in this study had relatively low permeabilities, the pH of AC and AC+ treatments was approximately 0.5 units higher than the NA control (Fig. 6). It is unclear whether the increased pH, per se, was directly responsible for the more rapid microbial growth in algin/calcium treatments than in the NA control. The added ingredients themselves could also play a role in affecting microbial growth (Souw and Rehm, 1975; Cranston, 1983). Accelerated gas production and higher counts at 8 and 13 days, however, do indicate that microbial growth in these treatments may be different from NA and RS treatments.

Subjective evaluation

Raw product. Scores for discoloration and color intensity were lower ($P<0.05$) for RS products than for other treatments (Table 3). This supports previous reports on the deleterious effects of NaCl on fresh meat color (Ockerman and Organisciak, 1979; Booren et al., 1981; Means and Schmidt, 1986b). In the present study, addition of GDL resulted in more intense ($P<0.05$) color (darker), compared to algin/calcium or NA treatments (Table 3).

As expected, binding scores of the NA and RS treatments in the raw state were very low (Table 4). Both algin/calcium treatments, however, were scored much higher ($P<0.05$) for binding characteristics in the raw state. This supports data reported by Means and Schmidt (1986a) who found the algin/calcium mechanism to be an effective binder in the raw and cooked state. It appeared that GDL offered no benefit to binding in the raw state.

Cooked product. Panelists detected no major differences in the aroma of cooked products (Table 3), indicating that none of the added ingredients contributed to detectable off-odors. Flavor scores of alginate treatments, however, were lower ($P<0.05$) than other products. Results of a previous study (Means and Schmidt, 1986a) suggested that Na-alginate which had not yet reacted with calcium ions was responsible for detected off-flavors. Algin/calcium treatments also received lower ratings ($P<0.05$) for mouthfeel than controls (Table 3). This has also been attributed to Na-alginate which has not undergone gelation by reacting with calcium ions (Means and Schmidt, 1986a). Thus, inclusion of GDL in the formulation did not improve mouthfeel. Future research, using lower levels of Na-alginate and $CaCO_3$ in conjunction with better methods of ingredient distribution, may alleviate this problem. Although binding in the cooked state (Table 4) was variable among treatments, the differences were not significant ($P=0.194$).

Table 3—Effect of the algin/calcium binder on sensory evaluation scores of raw and cooked structured beef steaks

	Raw		Cooked		
Treatment	Discoloration[d]	Color intensity[d]	Aroma[d]	Flavor[d]	Mouthfeel[d]
NA	4.6[a]	5.0[a]	5.5[a]	5.6[a]	5.7[a]
RS	3.0[b]	3.1[b]	5.4[a]	5.1[b]	5.7[a]
AC	5.4[a]	4.9[a]	5.1[a]	4.7[c]	3.9[b]
AC+	5.4[a]	4.4[c]	5.3[a]	4.7[c]	4.0[b]

[a,b,c] Numbers in same column with different superscripts are different ($P<0.05$). Standard error for discoloration = 0.37, color intensity = 0.11, aroma = 0.19, flavor = 0.02 and mouthfeel = 0.18.

[d] Surface discoloration: 1 = > 80%, 6 = no discoloration.
Color intensity: 1 = dark purple, 6 = light cherry red.
Aroma: 1 = very strong off-odor, 6 = no off-odor.
Flavor: 1 = strong off-flavor, 6 = no off-flavor.
Mouthfeel: 1 = very strong mealy/slippery, 6 = like intact muscle.

Table 4—Binding scores of raw and cooked structured beef steaks formulated with algin/calcium binder

	Binding Scores[a]	
Treatment[b]	Raw[b]	Cooked
NA	1.4[x]	2.5[z]
RS	2.1[x]	5.5[z]
AC	4.6[y]	3.6[z]
AC+	4.9[y]	4.3[z]

[a] 1 = virtually no bind; 6 = very strong bind, intact muscle.
[b] Numbers in same column with different superscripts are different ($P<0.05$).

Table 5—Effect of the algin/calcium binder on Hunterlab L values of structured beef steaks[a]

Days at 4–6°C	Package type: PVC overwrap, Treatment NA	RS	AC	AC+	Vacuum-nonbloom, Treatment NA	RS	AC	AC+	Vacuum-bloomed, Treatment NA	RS	AC	AC+
3	78.9	78.2	78.8	78.7	78.6	78.2	78.5	78.3	78.9	78.5	78.9	78.9
5	78.7	78.0	78.4	78.4	78.6	77.9	78.1	78.3	79.0	78.4	78.4	78.7
9	78.8	78.2	78.6	78.5	78.5	78.1	78.3	78.4	78.8	78.3	78.4	78.6
16	78.6	78.3	78.4	78.6	78.6	78.1	78.5	78.4	79.0	78.4	78.7	78.8
31					78.4	78.0	78.5	78.6	78.9	78.4	78.8	78.8

[a] Significant main effects for PVC overwrap *vs* vacuum-nonbloomed *vs* vacuum-bloomed included package ($P<0.01$), se = 0.019; treatment ($P<0.05$), se = 0.049; time ($P<0.01$), se = 0.029. Significant main effects for vacuum nonbloom vs vacuum-bloomed were package ($P<0.01$), se = 0.010; time ($P<0.01$), se = 0.042; with treatment approaching significance ($P=0.06$), se = 0.064.

Table 6—Effect of the algin/calcium binder on Hunterlab a values of structured beef steaks[a]

Days at 4–6°C	Package type: PVC overwrap, Treatment NA	RS	AC	AC+	Vacuum-nonbloom, Treatment NA	RS	AC	AC+	Vacuum-bloomed, Treatment NA	RS	AC	AC+
3	1.9	1.8	1.8	1.5	1.6	1.3	1.5	1.5	2.3	1.7	2.1	2.1
5	1.6	1.4	1.4	1.3	1.6	1.3	1.4	1.5	2.6	1.8	2.0	2.1
9	1.3	1.1	1.1	1.2	1.6	1.3	1.5	1.6	2.4	1.7	2.0	2.2
16	1.2	0.9	1.0	1.1	1.7	1.5	1.7	1.7	2.4	1.7	2.2	2.3
31					1.8	1.4	1.6	1.6	2.1	1.6	2.1	2.0

[a] Significant main effects for PVC overwrap *vs* vacuum-nonbloomed *vs* vacuum-bloomed included package ($P<0.01$), se = 0.011; treatment ($P<0.01$), se = 0.019; time ($P<0.01$), se = 0.017. Significant main effects for vacuum-nonbloom *vs* vacuum-bloomed were package ($P<0.01$), se = 0.009; time ($P<0.01$), se = 0.016; time ($P<0.01$), se = 0.027.

Table 7—Effect of the algin/calcium binder on Hunterlab b values of structured beef steaks[a]

Days at 4–6°C	Package type: PVC overwrap, Treatment NA	RS	AC	AC+	Vacuum-nonbloom, Treatment NA	RS	AC	AC+	Vacuum-bloomed, Treatment NA	RS	AC	AC+
3	−1.0	−1.3	−1.1	−1.2	−1.4	−1.7	−1.6	−1.5	−1.4	−1.7	−1.6	−1.6
5	−0.9	−1.3	−1.1	−1.1	−1.1	−1.7	−1.5	−1.4	−1.2	−1.8	−1.6	−1.5
9	−1.0	−1.5	−1.2	−1.3	−1.2	−1.6	−1.5	−1.4	−1.2	−1.8	−1.5	−1.4
16	−1.1	−1.5	−1.2	−1.2	−1.1	−1.7	−1.5	−1.3	−1.2	−1.8	−1.5	−1.4
31					−1.2	−1.6	−1.4	−1.3	−1.4	−1.8	−1.4	−1.4

[a] Significant main effects for PVC overwrap *vs* vacuum-nonbloomed *vs* vacuum-bloomed included package ($P<0.01$), se = 0.010; treatment ($P<0.01$), se = 0.022. Significant main effects for vacuum-nonbloom *vs* vacuum-bloomed were package ($P<0.01$), se = 0.007; treatment ($P<0.01$), se = 0.020; time ($P<0.01$), se = 0.025.

Color analysis

Hunterlab color values are given in Tables 5, 6 and 7. The low redness (a) values are due to the white standard used for instrument calibration. Type of package had a significant ($P<0.05$) influence on L, a and b values, with the bloomed samples being lighter, redder and bluer than the non-bloomed products. A noticeable decrease in redness (a) values was found for PVC overwrapped samples during storage. Treatment effects were also significant ($P<0.05$) for a and b values and approached significance ($P=0.06$) for L values. The RS treatment was the most discolored treatment, with lower L, a and b values which supports earlier data (Means and Schmidt, 1986a). Addition of GDL apparently had no major influence on Hunterlab color values.

CONCLUSIONS

THE ALGIN/CALCIUM GEL mechanism can be used to produce structured beef steaks under simulated commercial conditions. These products possess adequate binding characteristics in both the raw, refrigerated state and the cooked state. More research is needed to explain the more rapid microbial growth and quality deterioration of algin/calcium products stored in vacuum packages. It does not appear, however, that the refrigerated shelf-life of algin/calcium structured beef steaks stored in a commercial PVC overwrap will hinder marketing of these products in the fresh state. This new technology will allow development of new meat products with increased consumer appeal and marketing alternatives.

REFERENCES

Ahmed, E.M., Cornell, J.A., Tomaszewski, F.B., and Deng, J.C. 1983. Effects of salt, tripolyphosphate and sodium alginate on the texture and flavor of fish patties prepared from minced sheepshead. J. Food Sci. 48: 1078.

Allen, L., Nelson, A.I., Steinberg, M.P., and McGill, J.N. 1963. Edible corn-carbohydrate food coatings. II. Evaluation on fresh meat products. Food Technol. 17: 1442.

American Meat Science Association. 1978. Guidelines for cookery and sensory evaluation of meat. American Meat Science Association, Chicago, IL.

Anonymous. 1984. "Alginates: Seaweed Extracts." CECA, Inc. St. Louis, MO.

Booren, A.M., Mandigo, R.W., Olson, D.G., and Jones, K.W. 1981. Effect of muscle type and mixing time on sectioned and formed beef steaks. J. Food Sci. 46: 1665.

Cochran, W.G. and Cox, G.M. 1957. Balanced and partially balanced incomplete block designs. Ch. 11. In "Experimental Designs," 2nd ed., p. 439. John Wiley and Sons, New York.

Cranston, P.M. 1983. Alginic acid derivatives as a solidifying agent for microbiological nutrient suspension. Food Technol. Aust. 35: 134. [In Food Sci. Technol. Abstr. (1983) 15(12): 12B259].

Earle, R.D. 1968. Algin coating for extending shelf life of seafood and meat. U.S. Patent 3,395,024.

Egan, A.F. 1984. Microbiology and storage life of chilled fresh meats. European Meeting of Meat Res. Workers Proc., Bristol, England, 9-14 Sept., Session 5.

Egan, A.F. and Shay, B.J. 1982. Significance of Lactobacilli and film permeability in the spoilage of vacuum-packaged beef. J. Food Sci. 47: 1117.

Field, R.A. 1982. New restructured meat products — foodservice and retail. In "Meat Science and Technology International Symposium Proceed-

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Potassium Sorbate Inhibition of Microorganisms Growing on Refrigerated Packaged Beef

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ABSTRACT

The influence of potassium sorbate concentration (27–1325 ppm of sorbic acid) on the growth of natural flora in raw beef samples wrapped in plastic films of different oxygen permeability was studied at 0° and 4°C. Changes in aerobic plate counts, *Pseudomonas* spp., *Brochothrix thermosphacta, Lactobacillus* spp., *Enterobacteriaceae* and yeasts were monitored. Sorbate treatment effect on percentage increment of lag phase and reduction of growth rate during exponential phase for the different microorganisms were analyzed and antimicrobial action was evaluated by the Inhibition Index. A significant increase in the time to reach aerobic counts of $10^{6.5}$ CFU/cm^2 was observed at low storage temperatures and pH values in vacuum packaged treated samples.

INTRODUCTION

EXTENSIVE RESEARCH on the use of sorbic acid and its salts as preservatives in foods was reviewed by Sofos and Busta (1983). Sorbic acid and potassium sorbate were originally used to inhibit the growth of molds and yeasts, but it has been found that they also act upon *Staphylococcus aureus*, *Clostridium botulinum*, salmonellae and pseudomonads. Antimicrobial effect in meat products has been studied for inhibiting bacterial growth in sausages (Tompkin et al., 1974), for prolonging shelf-life of poultry (Robach and Sofos, 1982), for preventing growth and production of toxin by *Cl. botulinum* in canned pork (Ivey and Robach, 1978) and for inhibiting growth of *S. aureus* in bacon (Pierson et al., 1979). Robach and Ivey (1978) found that total bacterial counts were reduced when chicken breasts were dipped in a 5% solution of potassium sorbate compared to control samples. The same authors also showed a reduction in the rate of growth of salmonellae inoculated on the surface of treated samples. Cunningham (1979) determined the shelf-life and quality of chicken parts treated with 5%, 10% and 15% solutions of potassium sorbate. The same author studied the effectiveness of sorbate in baked drumsticks stored at 4°C, 10°C and 22°C (Cunningham, 1981). Myers et al. (1983) studied the effect of sorbate to prevent the growth of *Yersinia* spp. in vacuum-packaged pork meat; the results were highly satisfactory. Elliot et al. (1985) studied the growth of lactic acid bacteria and pseudomonads at 10°C on fresh chicken thighs exposed to a combination of potassium sorbate/carbon dioxide modified atmosphere packaging system. There are few studies on the effect of potassium sorbate on beef. Greer (1982) investigated the influence of this preservative on the growth of psychrotrophic bacteria which deteriorate beef.

The purposes of the present study were: to evaluate the effectiveness of potassium sorbate solutions on refrigerated beef to prolong its shelf-life and to analyze the influence of the concentration of preservative, storage temperature, pH of the meat and gas permeability of the wrapping film on each of the microorganisms which are found in the natural flora of the product.

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MATERIALS & METHODS

Preparation and storage of samples

Storage experiments with packaged refrigerated beef were performed at two temperatures (0 ± 0.3°C and 4 ± 0.5°C), using two types of plastic films with different degrees of permeability to oxygen: low density polyethylene, 60 μm thick (Ipesa Bs. As., water vapor permeability (WVP) = 12g vapor/m^2 day at 30°C and RH = 78%, oxygen transmission rate (OTR) = 6500 cm^3/m^2 atm day at 23°C), and a composite EVA/SARAN/EVA coextruded film, 60 μm thick, for vacuum packaging (trade name Super Cryovac, Darex, Bs. As., WVP = 7.2 g/m^2 day at 30°C and RH = 78%, OTR = 37.5 cm^3/m^2 atm day at 25°C and RH = 75%).

Beef samples were obtained from semitendinosus muscles removed from steers classified as U_2 grade A according to the Argentine National Meat Board Classification (average carcass weight of 250 kg) with a postmortem time of 48 hr at 4°C. Effect of muscle pH was analyzed working with samples of pH between 5.7 and 6.0. The muscles were cut in slices, transversely to the fibers, 1.5 cm thick and 10 cm in diameter. These slices (weight = 117g; surface area = 200 cm^2) were sprayed with 12.5 mL sorbate solution (0.21% to 10% w/v); this was equivalent to 0.11 mL/g or 0.06 mL/cm^2. The nozzle of the spray bottle was held 25 cm from the meat samples which were turned to expose all surfaces during spraying. Samples were allowed to drip for 30 sec before wrapping in plastic bags; controls were sprayed with a sterile distilled water. Samples were packaged under vacuum using a Minidual equipment model MW 4980 (Schcolnik SAIC, Buenos Aires) with a single chamber at 4.5 mm Hg and heat sealing; the average pressure of the internal atmosphere in the packages was 40 mm Hg. Heat-shrinkage of the low permeability film was performed by dipping the packages in a water bath at 84°C for 1 sec. The temperature of the storage cabinets was recorded by means of thermocouples inserted within the beef slices, connected to a Data Logger model 2240-C (John Fluke M. Fg. Co, Inc, Mountlake Terrace, WA); a meat electrode model 405-M4 (Ingold Lot, Switzerland) was used for pH determination. Colorimetric measurements were performed using a Hunter Lab Tristimulus Colorimeter, Model D25-A3 (Fairfax, VA) calibrated with standard plates (White C 29322, pink C 29323).

Sorbate analysis

Sorbate assay in meat was performed by triplicate on samples 10 cm in diameter and 1.5 cm thick using ultraviolet spectrophotometry with ethyl ether extraction in acid medium according to AOAC (1980). A Double-Beam UV-visible digital spectrophotometer model UV-150 (Shimadzu Corp., Tokyo) was used, and the absorbance was determined at 250 nm. Measurements were performed throughout and at the end of the storage both on raw and cooked meat. In the last case beef samples were roasted (oven temperature: 200°C) wrapped in aluminum foil until the center reached 78°C measured with a Cu-Co thermocouple (Omega Engineering, Ing., Stamford, CT). Total concentration of sorbate was expressed as ppm sorbic acid based on the weight of the sample.

Microbiological analysis

After each storage interval, the packages were opened aseptically at random. The bags were removed by cutting with a sterile scalpel through the film along the perimeter of the meat and using sterile forceps to separate the bag from the meat. A ribbon (20 cm^2 in surface and approximately 0.3 cm thick) was removed along the lateral area of the muscle by means of a sterile forceps and scalpel. Each sample was then placed in 500 mL flask containing 180 mL of sterile 0.1% peptone broth and shaken for 15 min at 250 rpm and 30°C (Shaker Model G-52, New Brunswick Scientific Co, Inc., NJ); appropriate

dilutions were made with sterile 0.1% peptone broth and duplicate plates were used.

Total aerobic counts were made on Plate Count agar (Oxoid Hampshire, England) with incubation at 30°C for 2 days. For enumeration of *Pseudomonas* spp., Masurovsky agar (Masurovsky et al., 1963) was used. Lactobacilli were counted on MRS agar (Oxoid) following incubation at 30°C for 3 days. For the detection of *B. thermosphacta*, STAA agar (Gardner, 1966) was used, and the plates were incubated at 22°C for 2 days. Yeast counts were made on Wort agar (Oxoid) following incubation at 30°C for 2 days. *Enterobacteriaceae* counts were made on Violet Red Bile Dextrose agar (Oxoid) following incubation at 30°C for 2 days (Mossel et al., 1975).

Diagnostic schemes and procedures used for identification of isolates were those presented by Vanderzant and Nickelson (1969). Standard biochemical tests were supplemented with Enteric 20 strips Inolex (Biomedical Division Inolex Corp, Glenwood, IL) for *Enterobacteriaceae* identification.

All results were average values of duplicate samples from three animals and were expressed as log CFU/cm^2 (CFU = colony forming units). The effect of sorbate treatment on lag time (θ) and growth rate during exponential phase (μ) for the different microorganisms were analyzed determining the percentage increment of θ and reduction of μ defined as:

$$\Delta\,\theta\% = \frac{\theta_t - \theta_c}{\theta_c} \times 100 \qquad (1)$$

$$\Delta\,\mu\% = \frac{\mu_c - \mu_t}{\mu_c} \times 100 \qquad (2)$$

where subindex t referred to treated samples and c to control samples.

Sorbic acid effectiveness was represented by the Inhibition Index (I), which was evaluated as:

$$I = 1 - \frac{t_c}{t_t} \qquad (3)$$

where: t_c and t_t are the times necessary to increase in one log cycle the initial microbial population for control and sorbate treated samples, respectively. Considering the typical equation for the exponential microbial growth phase (Zamora and Zaritzky, 1985):

$$N = N_o e^{\mu\,(t\,-\,\theta)} \qquad (4)$$

with N = number of total CFU/cm^2 at time t; N_o = initial number of total CFU/cm^2; μ = growth rate (1/days); θ = lag time (days) and assuming, according to the definition of I, $\log \frac{N}{N_o} = 1$, the following expression was obtained:

$$t = \frac{2.3}{\mu} + \theta \qquad (5)$$

showing that I values included the effects of sorbate inhibitory action on μ and θ.

As additional information to compare the effectiveness of sorbate concentrations, the time at which aerobic counts reached 10^7 CFU/cm^2 ($t_{10}7$) in the case of beef packaged in high oxygen permeability films and the time to reach $10^{6.5}$ CFU/cm^2 ($t_{10}6.5$) for vacuum packaged beef were determined. The value of $t_{10}7$ is used since it correlates with the onset of spoilage in aerobic storage of beef; $t_{10}6.5$ was selected because in many cases of vacuum packaged treated samples, microbial counts do not reach levels of 10^7 CFU/cm^2 in the stationary phase.

Statistical analysis

The lag phase was determined as the minimum time necessary to show significant differences ($P < 0.05$) between microbial population and initial counts. Growth rates were obtained from the slopes of the microbial count regression lines (log N vs time) during the exponential phase. The method of Neter and Wasserman (1974) was used to compare regression lines, establishing the existence of significant differences ($P < 0.05$) among treatments.

RESULTS & DISCUSSION

THE INITIAL SORBATE concentrated layer at the surface of the sprayed samples diffuses into the meat bulk due to the concentration gradient (Torres et al., 1985). During prolonged storage time sorbate profile tends to reach a uniform distribution, and it is convenient to express this concentration based on sample weight. Solutions of potassium sorbate in the range 0.21–10% left residues between 27–1325 ppm of sorbic acid in meat (based on sample weight, Table 1). Observed differences between residual sorbate levels in refrigerated meat samples during storage time (Table 2) were not statistically significant ($P > 0.05$); these results agreed with data reported by Robach et al. (1980). The assays performed on roasted meat showed no indication that residual sorbate level decreased with cooking. This result can be attributed to the fact that decomposition temperature of sorbic acid, 228°C, (Weast, 1976) was not achieved during cooking.

Storage in high oxygen permeability films

Sorbate effect in polyethylene wrapped samples stored at 0°C (muscle pH = 5.70) was observed both in the lag phase and in the growth rate of the spoilage flora.

Concentrations of 100, 360 and 400 ppm sorbic acid in beef samples affected the growth of *Pseudomonas* spp. (Fig. 1A) producing lag phase percentage increments ($\Delta\,\theta\%$) of 16.7, 33.3, 40% and growth rate reductions ($\Delta\,\mu\%$) of 20.1, 38.1, 43.7%, respectively. These results showed that sorbic acid influenced both parameters. Microbial counts of *Pseudomonas* spp. were not significantly different ($P > 0.05$) in samples with 0–42 ppm sorbic acid.

Robach (1978) working with two strains of *P. fluorescens* in laboratory media at different pH values and concentration of potassium sorbate found that the preservative decreased the number of viable cells acting on both growth parameters; similar results were obtained in the present study. However, Greer (1982) showed that sorbate prolonged the lag phase without affecting the growth rate of *P. fluorescens*. The observed discrepancy with the results of Robach (1978) can be attributed to differences in culture media, incubation temperature and bacterial strain, making comparisons difficult.

In the case of *B. thermosphacta* (Fig. 1b), for the same sorbic acid concentration values (100, 360 and 400 ppm) the lag phase percentage increments ($\Delta\,\theta\%$) were 0, 110, 160% and the growth rate reduction ($\Delta\,\mu\%$) 35.6, 54.3, 61.6%, respectively. Differences in microbial counts corresponding to

Table 1—Average residual potassium sorbate in beef (as sorbic acid)

% Potassium sorbate in sprayed solution	Sorbic acid[a] (ppm)
10.00	1325[b]
5.00	668
4.00	530
3.00	400
2.70	360
1.00	132
0.76	100
0.43	60
0.21	27

[a] Mean of three samples

[b] Concentrations correspond to cylindrical samples (diameter = 10 cm, height = 1.5 cm) and were based on sample weight.

Table 2—Effect of storage time on residual sorbic acid in vacuum packaged beef samples

Sorbic acid in beef (ppm)	Storage time at 4°C (days)		
	0	20	38
100	96	108	96
400	410	400	390
668	665	660	679

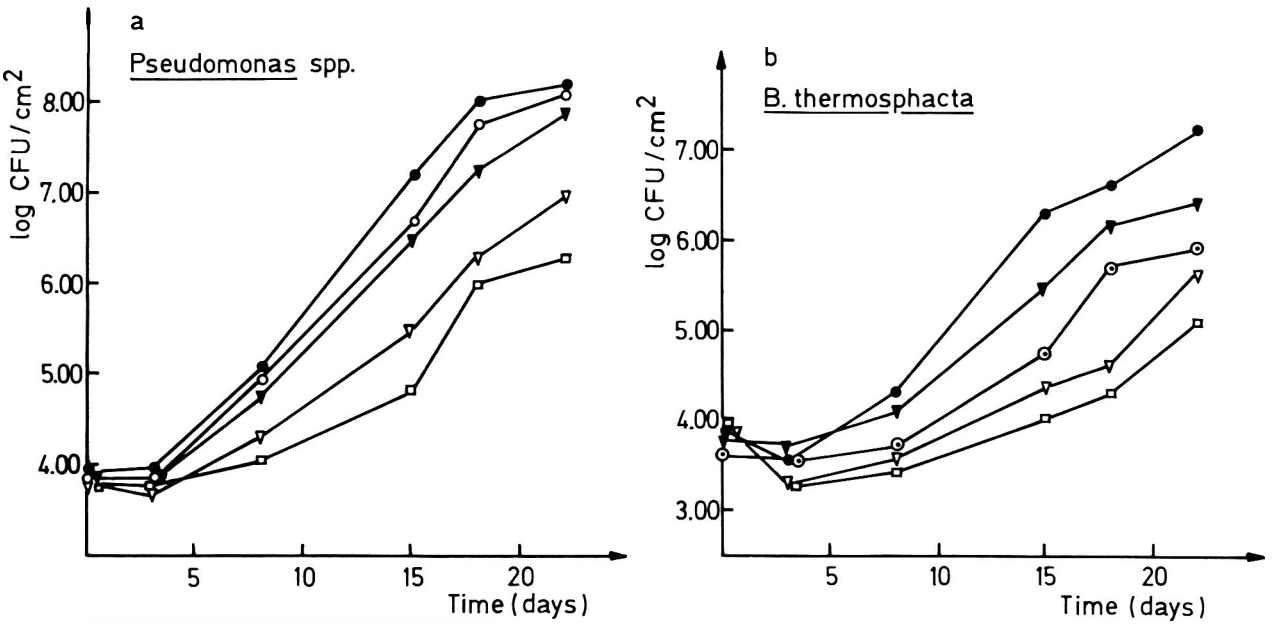

Fig. 1—Effect of residual sorbic acid (ppm) in polyethylene packaged beef (pH = 5.70) stored at 0°C on growth of (a) Pseudomonas spp.; (b) *B. thermosphacta.* ● control; ○ 60 ppm; ▼ 100 ppm; ○ 132 ppm; ▽ *360 ppm;* □ *400 ppm.*

beef samples with sorbic acid concentrations ranging from 0 to 60 ppm were not significant ($P > 0.05$).

The influence of the preservative on the lag phase of *B. thermosphacta* was more pronounced than on the growth rate, but a minimum level of preservative concentration (100 ppm) was necessary to observe a significant ($P < 0.05$) effect on θ; this effect of minimum concentration was not observable on the parameter μ.

Inhibition Index (Fig. 2) showed that for a residue of 100 ppm sorbic acid, *B. thermosphacta* and *Pseudomonas* spp. are similarly affected (I = 18% for both microorganisms) but, when the residue was 400 ppm, the inhibition of *B. thermosphacta* was 62% and that of *Pseudomonas* spp. only 37%.

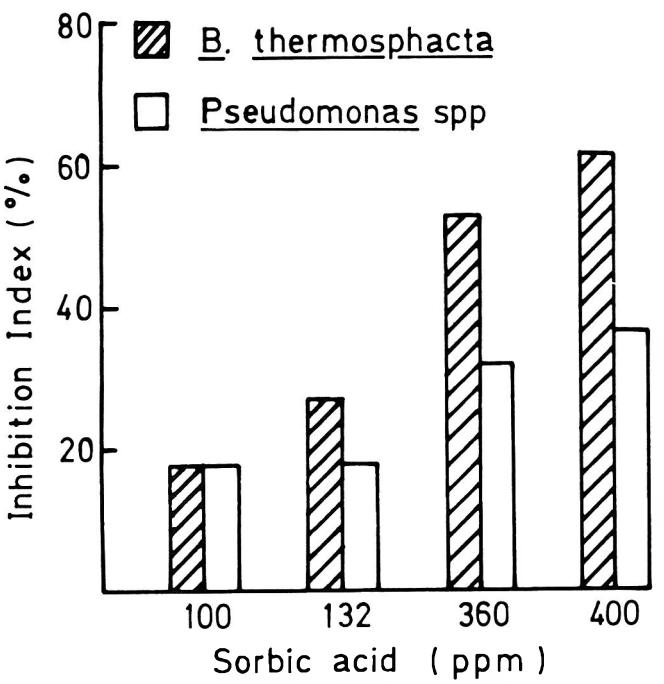

Fig. 2—Effect of sorbate concentration (as ppm sorbic acid) on percentage inhibition of Pseudomonas spp. and *B. thermosphacta* growing on beef wrapped in polyethylene and stored at 0°C (muscle pH = 5.70).

Inhibition Index indicated that *B. thermosphacta* was more sensitive to sorbate than *Pseudomonas* spp. when sorbic acid concentration increased. This effect could not be attributed to the different Gram reaction of the microorganisms because sorbic acid is a lipophilic acid preservative with a short chain length and this kind of substances inhibit both gram positive and gram negative bacteria (Sofos and Busta, 1981).

Aerobic counts (Fig. 3) showed that $t_{10}7$ increased from 14 days (control) to 17 days (100 ppm) and 22 days (360 ppm); there were no significant differences ($P > 0.05$) in the range between 0 and 70 ppm sorbic acid samples. Robach (1979) studied the effectiveness of potassium sorbate in poultry, finding that a dipping of 30 sec in a 5% solution (residue of 1200 to 1300 ppm of sorbic acid) prolonged the shelf-life of the product at 3°C from 10 days (control) to 19 days.

Colorimetric determinations in samples treated with 3% sor-

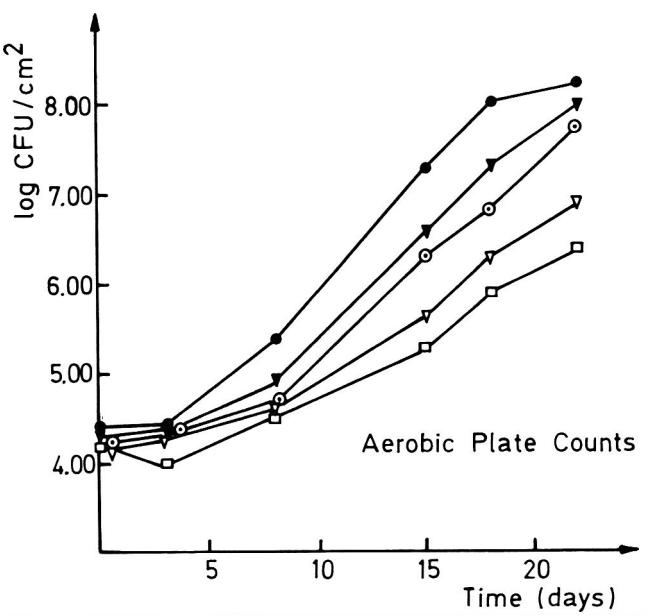

Fig. 3—Effect of residual sorbic acid (ppm) in polyethylene packaged beef (pH = 5.70) stored at 0°C on aerobic microbial counts: ● *control;* ▼ *100 ppm;* ○ *132 ppm;* ▽ *360 ppm;* □ *400 ppm.*

bate (400 ppm) showed that meat color highly deteriorated during an aerobic storage period of 21 days. This effect was observed by the marked reduction of the parameter "a" (redness) of the Hunter Lab scale that decreased from an initial value of 17.4 to a = 10.24 in control samples and to a = 12.0 in samples with 400 ppm sorbic acid during 21 days of storage. It must be considered that "a" values above 12 correspond to an acceptable red color; values between 10-12 are questionable and "a" values below 10 are not acceptable (Dean and Ball, 1960). The use of sorbic acid was not totally justified when oxygen permeable films were employed; even though microbial growth was retarded, color deteriorated before bacterial counts reached 10^7 CFU/cm^2.

Storage in low oxygen permeability films

The use of low oxygen permeability films combined with sorbate treatments (27–1325 ppm sorbic acid) retarded microbial growth. Results of aerobic counts on muscles with different pH (Fig. 4a, pH = 5.70 and Fig. 4b, pH = 5.90) wrapped in EVA-SARAN-EVA and stored at 4°C showed the influence of muscle pH and different concentrations of preservative. At pH = 5.70 a considerable extension of the lag phase was observed when sorbic acid concentration increased; comparatively growth rate was reduced in lower proportion. In samples with 1325 ppm sorbic acid, microbial counts remained in the range of the initial values during a period of 40 days of storage. At pH = 5.90, the extension of the lag phase was less pronounced than that obtained at pH = 5.70; no significant differences ($P > 0.05$) could be observed in the rate of bacterial growth at both muscle pH values. Microbial counts in samples with 27, 42 and 60 ppm sorbic acid (muscle pH = 5.70) were not significantly different ($P > 0.05$) from the control. When mucles pH was 5.90, no significant differences were detected up to 100 ppm sorbic acid.

Values of $t_{10}6.5$ (Fig. 5) were compared for different pH and preservative concentrations. Ratios of $t_{10}6.5$ between treated and control samples decreased from 1.9 to 1.4 in samples with

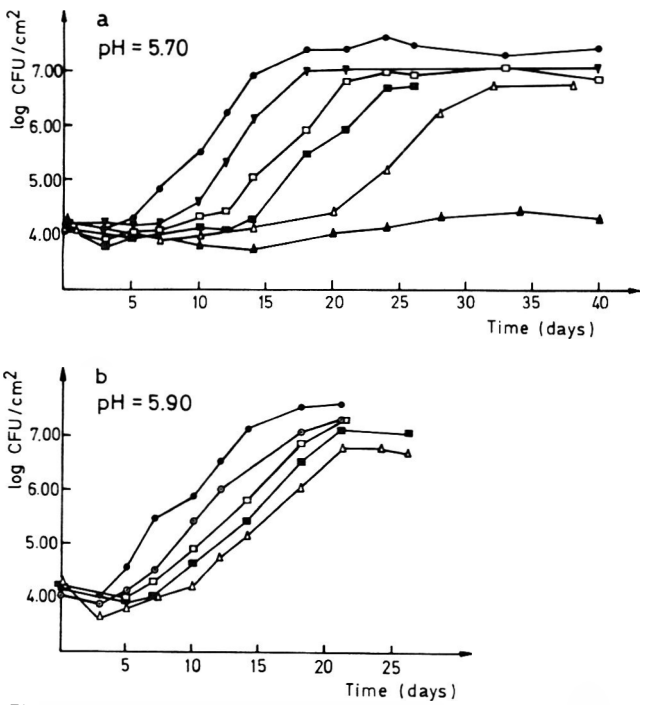

Fig. 4—Influence of muscle pH and sorbate concentration on Aerobic Plate counts in vacuum packaged beef stored at 4°C. (a) pH = 5.70, (b) pH = 5.90. ● control; ▼ 100 ppm; ○ 132 ppm; □ 400 ppm; ■ 530 ppm, △ 668 ppm, ▲ 1325 ppm (concentrations expressed as sorbic acid).

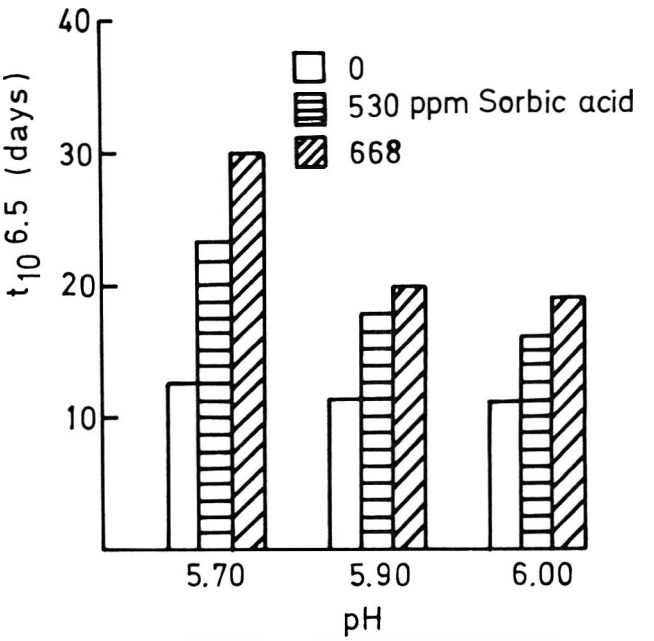

Fig. 5—Comparison of treatments to extend shelf life of vacuum packaged beef. Effect of muscle pH and sorbate concentration on the time to reach $10^{6.5}$ CFU/cm^2. Storage temperature = 4°C.

530 ppm sorbic acid and from 2.4 to 1.7 in samples with 668 ppm sorbic acid when beef pH increased from 5.70 to 6.00, respectively, showing the influence of muscle pH on sorbate effectiveness. Sorbate is more effective at pH approaching its dissociation constant (pka = 4.75); at this pH value, 50% of the acid is in the effective undissociated form being the maximum pH for sorbate activity 6.0 to 6.5 (Sofos and Busta, 1983).

The antimicrobial effect of sorbic acid on different microorganisms was detected on both parameters with θ the most affected. At pH = 5.70 and sorbic acid concentrations of 100, 400 and 530 ppm, the percentage increase of the lag phase (Table 3) was higher in the case of yeasts than of *B. thermosphacta*, *Enterobacteriaceae*, and *Pseudomonas* spp.; little effect on the lag phase of *Lactobacillus* spp. was observed. With respect to the growth rate decrease, *B. thermosphacta* showed the highest reduction with the yeasts being the less affected microorganisms.

Inhibitory Index for the different component of the microbial flora showed the influence of muscle pH on sorbate antimicrobial activity (Fig. 6a, b, c, d, e) in vacuum packaged samples stored at 4°C. At pH = 5.70 and residual sorbic acid concentrations of 400 and 530 ppm, *B. thermosphacta* showed the highest Inhibitory Index (I = 68 and 70%, respectively). At pH = 6.00, for the same sorbic acid concentrations antimicrobial effect was lower. Yeasts and *B. thermosphacta* were similarly affected (I = 47 and 50%, respectively), *Lactobacillus* spp. and *Enterobacteriaceae* showed comparable values (I = 37 and 43% respectively) with *Pseudomonas* spp. the less affected genus.

Sorbate improved superficial color; this effect was more pronounced when the concentration of preservative was higher (Table 4). Parameter "a" of Hunter Lab Scale after reoxygenation of vacuum packaged samples, remained approximately in the initial level for over 42 days at 4°C (a = 18.40) when the residue was 668 ppm sorbic acid. When it was 400 ppm, "a" value decreased to 11.15 and 8.21 for treated and control samples, respectively; in both cases, offensive odors were detected.

Aerobic microbial counts on treated beef sections (pH = 5.90), wrapped in EVA/SARAN/EVA and stored at 0°C (Fig.

Table 3—Effect of sorbic acid concentration on the growth of microorganisms in vacuum packaged beef (pH = 5.70) stored at 4°C

Sorbic acid conc (ppm)	Lactobacillus spp.		B. thermosphacta		Pseudomonas spp.		Enterobacteriaceae		Yeast	
	Δ θ%[a]	Δ μ%[b]	Δ θ%	Δ μ%	Δ θ%	Δ μ%	Δ θ%	Δ μ%	Δ θ%	Δ μ%
100	61.5	16.1	87.5	48.2	100.0	5.2	—	—	213.6	13.1
400	125.6	23.4	200.0	68.2	118.2	20.6	204.0	31.7	322.7	16.6
530	182.0	27.7	225.0	69.6	234.5	39.1	228.0	33.7	463.6	19.7

[a] Δ θ% = lag phase percentage increment ($\Delta\theta\% = \frac{\theta\text{treated} - \theta\text{control}}{\theta\text{control}} \times 100$)

[b] Δ μ% = growth rate percentage reduction ($\Delta\mu\% = \frac{\mu\text{control} - \mu\text{treated}}{\mu\text{control}} \times 100$)

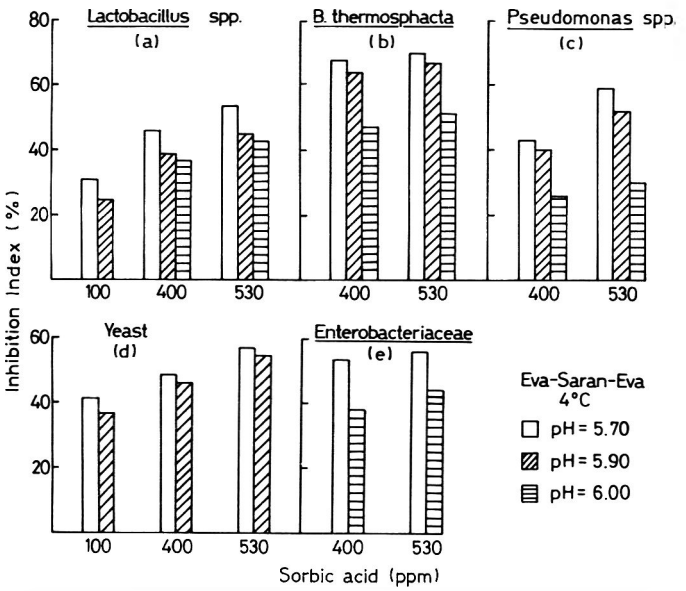

Fig. 6—Effect of muscle pH and sorbate concentration on percent inhibition of the different component of the microbial flora (a) Lactobacillus spp.; (b) *B. thermosphacta*; (c) *Pseudomonas* spp.; (d) Yeast; (e) *Enterobacteriaceae.*

Table 4—Superficial color (parameter a, Hunter Lab Scale) of beef samples treated with potassium sorbate and stores at 4°C in EVA-SARAN-EVA.

Days of storage	Parameter *a* Hunter Lab Scale		
	Residual sorbic acid (ppm)		
	0	400	668
0	18.10	18.15	18.20
33	14.30	16.06	18.50
42	8.21	11.15	18.40

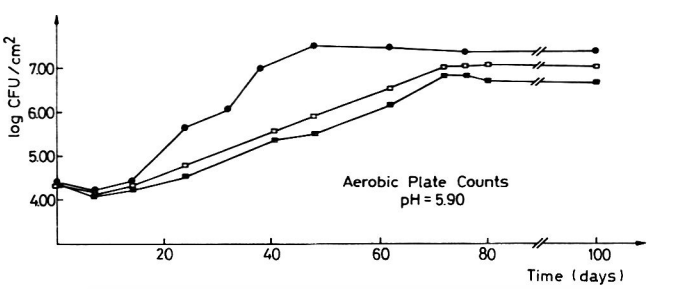

Fig. 7—Effect of potassium sorbate on aerobic microbial counts in beef packaged in EVA-SARAN-EVA and stored at 0°C: ● control; □ 400 ppm; ■ 530 ppm (concentrations expressed as sorbic acid).

7) showed the effect of lower temperatures on the inhibitory action with growth rate the most affected parameter.

Data of $t_{10}6.5$ in vacuum packaged treated muscles of pH = 5.90, stored at 4° and 0°C (Fig. 8), showed that in samples with 530 ppm, this time increased 97% with respect to the

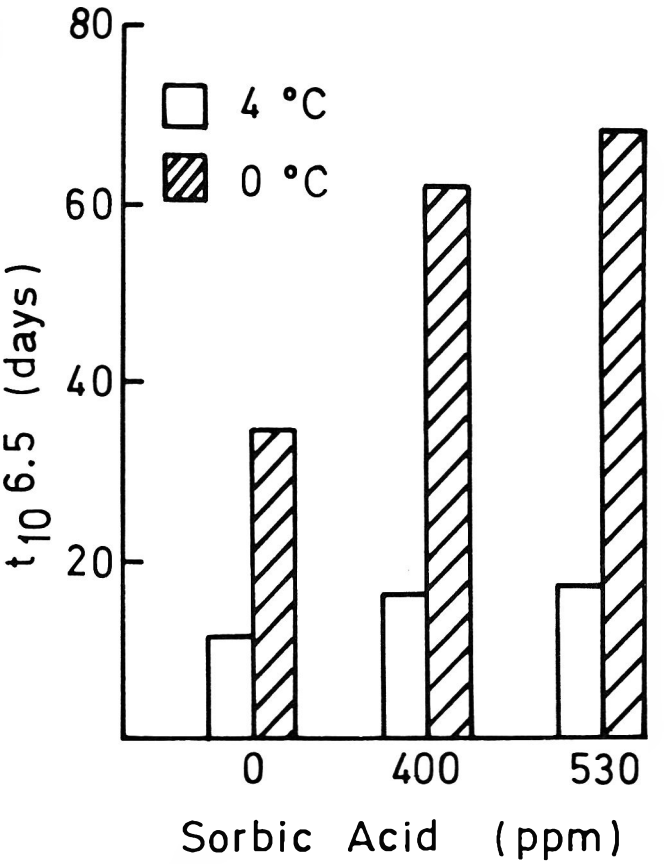

Fig. 8—Effect of storage temperature and sorbate concentration on time to reach $10^{6.5}$ CFU/cm² in beef samples packaged in EVA-SARAN-EVA (muscle pH = 5.90).

control when storage was at 0°C and only 54% when temperature was 4°C.

Color of treated samples (400 ppm sorbic acid) wrapped in EVA/SARAN/EVA and stored during 78 days at 0°C was unaltered. The "a" value changed from 19.75 (initial value) to 17 in comparison to the control sample (a = 11.50) which showed signs of deterioration. After 110 days of storage, "a" value was reduced to 9.92 and the color of the preservative containing samples was no longer acceptable even though microbial counts did not reach 10^7 CFU/cm².

Sorbic acid is considered to be a non-toxic compound for human beings, as it is metabolized like fatty acids, i.e., to carbon dioxide and water. Cunningham (1979) determined that the application of 3200 ppm sorbic acid in fresh poulty (10% potassium sorbate used as a dip for 60 sec) did not affect sensory characteristics of cooked poultry. An Admitted Daily Intake (ADI) of up to 1500 mg of sorbic acid for a 60 kg body weight was established by FAO/WHO (1974). Assuming an average daily consumption of beef of 150 g, and considering the maximum residue in the slices determined in the present study (1325 ppm sorbic acid), the mean daily intake resulted in 198.75 mg of sorbic acid; this value is well below the ADI.

Within the limits established by the results, the risk for the use of potassium sorbate in beef was negligible, because the amount ingested was quite low. The benefits included the accomplishment of an optimal quality product, with a longer shelf-life, which made marketing for longer periods of time possible.

REFERENCES

AOAC. 1980. "Official Methods of Analysis." Ed. W. Horwitz, Method 20: 104. Association of Official Analytical Chemists, Washington, DC.

Cunningham, F.E. 1979. Shelf-life and quality characteristics of poultry parts dipped in potassium sorbate. J. Food Sci. 44: 863.

Cunningham, F.E. 1981. Microbiology of poultry parts dipped in potassium sorbate. Poultry Sci. 60: 969.

Dean, R.W. and Ball, C.O. 1960. Pattern of redness loss and regeneration in prepackaged beef. Food Technol. 14(5): 222.

Elliot, P.H., Tomlins, R.I., and Gray, R.J.H. 1985. Control of microbial spoilage on fresh poultry using a combination potassium sorbate/carbon dioxide packaging system. J. Food Sci. 50: 1360.

FAO/WHO. 1974. Joint Expert Committee on Food Additives - 17th Report. WHO Techn. Rep. Ser., No. 539, World Health Organization, Geneva.

Gardner, G.A. 1966. A selective medium for the enumeration of *Microbacterium thermosphactum* in meat and meat products. J. Appl. Bacteriol. 29: 455.

Greer, G.G. 1982. Mechanism of beef shelf life extension by sorbate. J. Food Prot. 45: 82.

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

Masurovsky, E.B., Godblith, S.A., and Voss, J. 1963. Differential medium for selection and enumeration of members of the genus *Pseudomonas*. J. Bacteriol. 85: 722.

Mossel, D.A.A., Dijkmann, K.E., and Snijders, J.M.A. 1975. Microbial problems in handling and storage of fresh meats. In "Meat." (Ed.) D.J.A. Cole and E.A. Lawrie, p. 235. AVI Publishing Company, Westport, CT.

Myers, B.R., Edmondson, J.E., Anderson, M.E., and Marshall, R.T. 1983. Potassium sorbate and recovery of pectinolytic pychrotrophs from vacuum packaged pork. J. Food Prot. 46: 499.

Neter, J. and Wasserman, W. 1974. "Applied Linear Statistical Models." (Ed.) D. Pichard Irwin Inc., Homewood, IL.

Pierson, M.D., Smoot, L.A., and Stern, N.J. 1979. Effect of potassium sorbate on growth of *Staphylococcus aureus* in bacon. J. Food Prot. 42: 302.

Robach, M.C. 1978. Effect of potassium sorbate on the growth of *Pseudomonas fluorescens*. J. Food Sci. 43: 1886.

Robach, M.C. 1979. Influence of potassium sorbate on growth of *Pseudomonas putrefaciens*. J. Food Prot. 42: 312.

Robach, M.C. and Ivey, F.J. 1978. Antimicrobial efficacy of a potassium sorbate dip on freshly processed poultry. J. Food Prot. 41: 284.

Robach, M.C. and Sofos, J.N. 1982. Use of sorbates in meat products, fresh poultry and poultry products: A review. J. Food Prot. 45: 374.

Robach, M.C., To, E.C., Meydav, S., and Cook, C.F. 1980. Effect of sorbates on microbiological growth in cooked turkey products. J. Food Sci. 45: 638.

Sofos, J.N. and Busta, F.F. 1981. Antimicrobial activity of sorbate. J. Food Prot. 44: 614.

Sofos, J.N. and Busta, F.F. 1983. Sorbates. In "Antimicrobials in Foods." (Ed.) A.L. Branen and P.M. Davidson, p. 141. Marcel Dekker, Inc. New York.

Tompkin, R.B., Christiansen, L.N., Shaparis, A.B., and Bolin, H. 1974. Effect of potassium sorbate on salmonellae, *Staphylococcus aureus*, *Clostridium perfringens* and *Clostridium botulinum* in cooked, uncured sausage. Appl. Microbiol. 28: 262.

Torres, J.A., Motoki, M., and Karel, M. 1985. Microbial stabilization of intermediate moisture food surfaces. I. Control of surface preservative concentration. J. Food Proc. Preserv. 9: 75.

Vanderzant, C. and Nickelson, R. 1969. A microbiological examination of muscle tissue of beef, pork, and lamb carcasses. J. Milk Food Technol. 32: 357.

Weast, R.C. 1976. "Physical Constants of Organic Compounds, Handbook of Chemistry and Physics," p. C-327, 56th ed. CRC Press, Boca Raton, FL.

Zamora, M.C. and Zaritzky, N.E. 1985. Modeling of microbial growth in refrigerated packaged beef. J. Food Sci. 50: 1003.

Ms received 1/7/86; revised 10/6/86; accepted 10/7/86.

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ings," Lincoln, NB, Nov. 1-4. Natl. Live Stock and Meat Board, Chicago, IL.

Gill, C.O. 1983. Meat spoilage and evaluation of the potential storage life of fresh meat. J. Food Prot. 46: 444.

Greer, G.G. and Jeremiah, L.E. 1981. Proper control of retail case temperature improves beef shelf life. J. Food Prot. 44: 297.

Hull, C.H. and Nie, N.H. 1981. "SPSS update 7-9: New procedures and facilities for releases 7-9." McGraw-Hill Book Co., New York.

Ingram, M. and Dainty, R.H. 1971. Changes caused by microbes in spoilage of meats. J. Appl. Bacteriol. 34: 21.

Lazarus, C.F., West, R.L., Oblinger, J.L. and Palmer, A.Z. 1976. Evaluation of a calcium alginate coating and a protective plastic wrapping for the control of lamb carcass shrinkage. J. Food Sci. 41: 639.

Madril, M.T. and Sofos, J.N. 1984. Antimicrobial activity and functionality of polyphosphates in reduced NaCl comminuted meat products. European Meeting Meat Res. Workers Proc., Bristol, England, 9-14 Sept.

Mariott, N.G., Smith, G.C., Hoke, K.E., Carpenter, Z.L., and West, R.L. 1977. Long distance transoceanic shipments of fresh beef. J. Food Sci. 42: 316.

Means, W.J. 1985. Algin/calcium gel in structured meat products. Ph.D. Dissertation, Colorado State Univ., Fort Collins.

Means, W.J. and Schmidt, G.R. 1986a. Algin calcium gel as a raw and cooked binder in structured beef steaks. J. Food Sci. 51: 60.

Means, W.J. and Schmidt, G.R. 1986b. Restructured fresh meat without the use of salt or phosphate. Ch. 14, In "Advances in Meat Research," Vol. 3, A.M. Pearson and T.R. Dutson (Ed.). AVI Publishing Co., Inc., Westport, CT.

Nie, N.H., Hull, C.H., Jenkins. J.G., Steinbrenner, K., and Bent, D.H. 1975. In "Statistical Package for the Social Sciences," 2nd ed. McGraw-Hill Book Co., New York.

Ockerman, H.W. and Organisciak, C.S. 1979. Quality of restructured beef steaks after refrigerated and frozen storage. J. Food Prot. 42: 126.

Pearson, A.M., Love, J.D., and Shorland, F.B. 1977. Warmed-over flavor in meat, poultry and fish. Adv. Food Res. 23: 1.

Schmidt, G.R. and Trout, G.R. 1982. Chemistry of meat binding. In "Meat Science and Technology International Symposium Proceedings," Lincoln, NB, Nov. 1-4. Natl. Live Stock and Meat Board, Chicago, IL.

Simunovic, J., West, R.L., and Adams, J.P. 1985. Formulation of a pasteurized restructured beef product. J. Food Sci. 50: 693.

Snedecor, G.W. and Cochran, W.G. 1976. "Statistical Methods," 6th ed. The Iowa State University Press, Ames, IA.

Sofos, J.N. 1984. Antimicrobial effects of sodium and other ions in foods: A review. J. Food Safety 6: 45.

Souw, P. and Rehm, H.J. 1975. Microorganisms in thickening agents. IV. Microbial degradation of three plant exudates and two seaweed extracts. Zaitschrift fuer Lebensmittel Unterswhung and Forschung. 159(5): 297. [In Food Sci. Technol. Abstr. (1976) 8(12): 12T625].

Steel, R.G.D. and Torrie, J.H. 1980. "Principles and Procedures of Statistics," 2nd ed. McGraw-Hill Book Co., New York.

Taylor, A.A. and Shaw, B.G. 1977. The effect of meat pH and package permeability on putrefaction and greening in vacuum packed beef. J. Food Technol. 12: 515.

Wanstedt, K.G., Seideman, S.C., Donnelly, L.S., and Quenzer, N.M. 1981. Sensory attributes of precooked, calcium alginate-coated pork patties. J. Food Prot. 44: 732.

Williams, S.K., Oblinger, J.L., and West, R.L. 1978. Evaluation of a calcium alginate film for use on beef cuts. J. Food Sci. 43: 292.

Ms received 4/10/86; revised 10/10/86; accepted 10/17/86.

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Effects of Boning Time, Mechanical Tenderization and Partial Replacement of Sodium Chloride on the Quality and Microflora of Boneless Dry-Cured Ham

F.W. LEAK, J.D. KEMP, J.D. FOX, and B.E. LANGLOIS

ABSTRACT

SEVENTY-TWO HAMS were utilized to study the effects of KCl replacement level (100:0, 70:30, 50:50 ratios of NaCl and KCl), boning time and mechanical tenderization on the quality and microflora of boneless dry-cured hams. Boning time had no effect on palatability or final yield. Hotboned hames had higher levels of residual nitrite and slightly higher microbial counts. Mechanical tenderization improved tenderness and microbial quality. However, tenderized hams had lower flavor preference and overall satisfaction scores. The replacement of NaCl with KCl at a level of 30% had no effect on palatability or microbial quality. NaCl replacement at a level of 50% severely decreased palatability and increased microbial numbers.

INTRODUCTION

THE PRODUCTION of dry-cured hams in the Southeast has steadily increased over the past few years (AMI, 1984). Most hams are cured traditionally intact and until the last few years were sold as whole hams. Recently, however, marketing of dry-cured hams has shifted so that a higher percentage are sold as boneless vacuum packaged slices. This method of marketing is more desirable due to an increased interest in portioned-controlled meat products in the food industry and the desire for less preparation in the home. In response to these marketing changes studies have been conducted to investigate the possibility of producing boneless dry-cured hams (Abidoye, 1976; Kemp and Fox, 1977; Leak et al., 1983). These studies suggest that the production of a high quality boneless dry-cured ham is feasible. Curing rate is faster and aging time is shortened in these hams; however, some problems with internal mold, excessive drying and increased saltiness were incurred.

The use of new technology and the ability to alter processing procedures to meet changing consumer demands is necessary to remain competitive in today's food service industry. The production of a boneless dry-cured ham is a step in the right direction. However, the incorporation of some relatively new processing techniques, such as hot-processing, mechanical tenderization and the partial replacement of NaCl with KCl in the production of boneless dry-cured hams might further improve it's marketing advantages and enhance its quality and palatability.

The effects and advantages of hot-processing (Kastner, 1977; Cross and Tennent, 1980; Seideman and Cross, 1983), mechanical tenderization (Schwartz and Mandigo, 1974; Glover et al., 1977; Savell et al., 1977) and replacement of NaCl with KCl (Terrell and Olson, 1981; Keeton, 1984) have been well documented. However, these studies primarily utilized fresh beef or pork and brine cured pork products in their procedures. The information on the effects of these processing parameters on the quality and palatability of boneless dry-cured hams is limited.

Mechanical tenderization of beef and pork muscle has been shown to improve tenderness and enhance the extraction of salt soluble proteins (Glover et al., 1977; Savell et al., 1977; Huffman, 1979). The cellular disruption associated with mechanical tenderization facilitates the release of the salt soluble proteins which are essential to the integrity of restructured and reformed meat products.

Many meat processors are currently investigating ways to reduce the level of NaCl in meat products. These include absolute reduction of NaCl levels, and replacement of NaCl with other chloride salts containing K^-, Mg^{++}, or Ca^{++}. Of these substitute salts, KCl appears to be the best substitute for NaCl (Terrell and Olson, 1981). Unlike NaCl, excess dietary intake of KCl has not been linked to the development of hypertension. In fact, several studies suggest that deficiencies in dietary KCl may actually cause hypertension (McCarron et al., 1984). Thus, the replacement of NaCl with KCl is feasible from a physiological standpoint. However, KCl is a bitter chloride salt and the maximum level of replacement without deterimental palatability effects is not well defined for a boneless dry-cured ham product.

Therefore, the purposes of this research were to (1) determine if the use of pre-rigor muscle is a viable alternative to the use of post-rigor muscle in the production of boneless dry-cured hams, (2) evaluate the effects of mechanical tenderization on the sensory, physical and microbial qualities of boneless dry-cured hams and (3) examine the effects of various levels of KCl replacement for NaCl on the sensory, physical and microbial qualities of boneless dry-cured hams.

MATERIALS & METHODS

THIRTY-SIX market weight hogs (100–110 kg) of similar genetic background were separated into 3 groups of 12 and designated as salt treatment groups (Table 1), in which KCl was substituted for NaCl at levels of 0, 30 and 50%, on an equal molar basis. Hams from the right sides of each hog were removed from the carcass immediately after slaughter and designated as hot-processed hams. "Hot" hams were then fully boned, skinned and trimmed to approximately 1.0 cm fat. Hams from the left side of each carcass were allowed to remain on the carcass for 24 hr at 1°C. After chilling, "cold" hams were fabricated exactly as their hot counterparts. Hams were removed by a cut made perpendicular to the long axis of the shank bones 5.7 cm anterior to the tip of the aitch bone and the shank was removed slightly above the hock joint. Carcasses were held in a position similar to the rigor-fixed position of chilled carcasses during removal of hot hams. Ham weights were recorded for intact uncured hams and boneless

Table 1—Cure formulations

Ingredient	Curing mixtures[a,b] 100:0 (%)	70:30 (%)	50:50 (%)
NaCl	83.50	54.63	37.44
KCl	0.00	29.92	47.74
Sugar (white)	13.92	13.00	12.48
$NaNo_2$	0.87	0.81	0.78
$NaNo_3$	1.74	1.63	1.56
Application rate[c], %	5.0	5.35	5.57

[a] Based on a percentage of total cure mixture.
[b] NaCl:KCl (molar basis).
[c] Application rate adjusted for equal ionic strength.

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hams at each stage of processing. Weights of skin and fat, lean trim and bone were also recorded to determine the percentage of each constituent on a uncured ham basis.

Immediately before application of the appropriate cure mixture, half of the hams from each boning time were mechanically tenderized using a Ross Blade Tenderizer (Model #TC 700, Ross Industries, Midland, VA.). Hams were passed through the tenderizer twice, once with cushion side up and once with skin side of the ham up. Hams then received the appropriate amount of cure mixture (Table 1) and were tumbled for 3 hr continuously in a Roschermatic vacuum tumbler (Model #PM-130/1, Roscherwerke GMBH; Osmabruck, West Germany) operating at a rate of 9 rpm at 3°C and approximately 450 mm Hg. The maximum time between the beginning of the slaughtering process and the start of tumbling was 1 hr for hot-bones hams. After tumbling, all hams were tightly stuffed into Teepack fibrous casing and clipped using a Tipper Tie Clipper. All noticeable air pockets were removed by piercing the casings with a sharp shroud pin. Hams were then hung in a curing room for 2 weeks at 3°C. After curing, salt was allowed to equalize throughout the ham in a salt equalization room for 1 week at 13°C. Hams were then cool smoked (37.8° to 40.6°C) overnight and aged for 4 weeks at 23°C.

After aging, hams were sliced and evaluated for color, aroma and general appearance by two experienced dry-cured ham researchers. Color was scored as dark red (4), red (3), light red (2) and pink (1). The color score of hams with varying degrees of color uniformity was determined by taking the average of the various colors present. Aroma was scored as typically aged (4), moderately aged (3), slightly aged (2) and no aged aroma (1). General appearance was a visual appraisal of the lean surface based on the combination of color, firmness and freedom from defects and was scored as excellent (4), good (3), fair (2) and poor (1). Center slices (1.27 cm) were removed from each ham for subsequent chemical and sensory evaluation. Two 2.54 cm slices were also removed for Instron shear value evaluation and cooking loss determinations. Slices for chemical evaluation were vacuum packaged and frozen at − 22°C until analyzed. Slices used for sensory evaluation were vacuum packaged and stored at 1°C for no more than 2 weeks before evaluation. Center slices designated for chemical analysis were finely ground twice through a laboratory grinder using a 4.8 mm plate. Duplicate samples were analyzed for salt and nitrite using a Technicon Auto Analyzer II and moisture according to procedures from AOAC (1980). Sodium and potassium were determined using a Perkins-Elmer Model 560 atomic absorption spectrophotometer in the emission mode. A wet ashing procedure as outlined in section 18.038 of AOAC (1980) was used for sample preparation. Standards of 0.5 to 2.0 ppm Na^+ and 0.2 to 2.0 ppm K^- were used to calibrate the system. Readings were made at flame peak wavelengths of 589 (Na^+) and 766 nm (K^-) and calculated as mg/g of wet sample.

Two slices (1.27 cm thick) from each ham were broiled in a commercial type broiler for 5 min on one side and 3 min on the reverse side. Ham slices were sectioned and served, while warm, to a 10-member semi-trained sensory panel which was selected from a group of individuals who normally consumed dry-cured ham. Panel members were selected on the basis of their performance on previous dry-cured ham taste panels and received a short training session to sharpen their evaluation skills. At least 8 members were present for each session. The slices were number coded and served to panelists in duplicate in individual booths equipped with red lighting. Room temperature apple juice and water were used to rinse the mouth between samples. Apple juice was used to remove the saltiness and flavor of the ham sample while the water rinsed away the apple juice. Panelists rated samples on an 8 point descriptive scale for tenderness, flavor intensity and saltiness. An 8 point hedonic scale was utilized to evaluate flavor preference and overall satisfaction (an evaluation of eating quality based on a combination of the above factors). One 2.54 cm center slice was broiled for 7 min on one side and 5 min on the other and used for maximum shear value determination. After cooling, five 1.27 cm diameter cores were removed from each sample with a sharp coring device along the longitudinal center of the semimembranosus muscle. Cores were removed parallel to the muscle fibers. Each core was sheared twice on a Warner Bratzler device mounted on an Instron Universal Testing Machine and the peak values recorded in kilograms.

The surface swab rinse method (Marth, 1978) was used to determine the microbial population of the external surface of the hams. Three hams randomly selected from each subtreatment group were sampled after aging; this represented 50% of the hams in the group. Six areas 12 cm^2 from both the lean and fat portions were sampled per ham. After sampling, the swab was broken off below the line of haman contact and allowed to remain in the sample vial. Immediately prior to diluting and plating, 0.6 ml of a sterile 10% sodium citrate solution was added to the original buffered rinse solution to facilitate removal of organisms from the swab. This sample was then used to determine the following counts: (a) aerobic—one set of pour plates of Plate Count agar (PCA) was incubated at 35°C for 48 hr and a second set at 26°C for 72 hr (Read, 1978); (b) staphylococci—for each dilution plated, 1 mL diluent was equally distributed and spread with a sterile bent glass rod over 3 pre-poured and dried plates of Baird Parker agar (BP) and the plates were incubated at 35°C for 48 hr. Only black colonies with and without zones of clearing were counted as staphylococci (Read, 1978); (c) lactobacilli—pour plates of Rogosa SL agar were incubated at 35°C for 48 hr in BBL GasPak jars containing an activated BBL Gas Generator envelope which produced a CO_2 enriched atmosphere in the jar (Rogosa et al., 1951); (d) yeasts and molds—pour plates of Potato Dextrose Agar acidified with 10% tartaric acid were incubated at 26°C for 5 days (Marth, 1978). All counts were converted to $\log_{10}$ for statistical analysis and counts were reported as mean log counts.

A split plot repeated measures analysis (Gill, 1979) was performed to examine treatment differences and interactions. Multiple comparisons among the means were done using the Duncan REGWF procedures in SAS (1982).

RESULTS & DISCUSSION

Yield and chemical characteristics

Ham cut-out data (Table 2) are expressed as percentages of intact uncured ham weight to alleviate any difference due to average uncured ham weight between treatment groups. Skin and fat weight was higher ($P<0.05$) in the hot-boned hams, while lean trim and bone weights were both higher ($P<0.05$) in the cold-boned hams. Boneless ham weights before and after tumbling, and final ham weight were not affected by boning time. During the fabrication process it was noticed that external fat from "hot" hams was more difficult to trim while lean was more easily removed from the bone than in "cold" hams. These observations combined with the expected greater amount of evaporative loss through the skin of cold-boned hams during chilling probably accounted for most of the differences noted in the cut-out characteristics. Several previous studies have also noted these observations (Mandigo and Henrickson, 1966: Schmidt and Keman, 1974). Final yield based on boneless uncured ham weight (Table 3) was also not affected by boning time or tenderization treatment. However, final yield decreased as KCl replacement level increased. Hams in the 50:50 salt group had lower ($P<0.05$) final yields than both the 100:0 and 70:30 salt groups. These findings do not agree with those of Keeton (1984) who found no difference in final yield between 100% NaCl hams and hams cured with 33.3, 66.7 and 100% replacement levels of KCl.

Percent moisture was not affected by salt group, boning time or tenderization treatment (Table 3). Residual nitrite levels decreased as the replacement level of KCl increased. Nitrite levels for the 50:50 salt group were lower ($P<0.05$) than both the 100:0 and 70:30 salt groups. Residual nitrite levels were found to be higher ($P<0.05$) in hot-boned hams than in cold-boned hams and lower ($P<0.05$) in nontenderized hams than in tenderized hams. Percent chloride was not affected by bon-

Table 2—Effect of boning time on ham yield[a]

Item	Boning time	
	Hot-boned	Cold-boned
No.	36	36
Trait		
Skin and fat, %	14.8[x]	12.4[y]
Lean trim, %	5.6[x]	6.8[y]
Bone, %	9.8[x]	10.4[y]
Boneless ham, %	69.8	70.4
After tumbling ham, %	73.1	73.8
Final ham, %	56.5	56.4

[a] Values expressed as a percentage of uncured ham weight.
[xy] Means in rows with different superscripts are different ($P>0.05$).

Table 3—Means of lean composition, subjective evaluation and yield by treatment

Treatment	No.	Parameter								
		Moisture (%)	Nitrite (ppm)	Chloride (%)	Na^+ (mg/g)	K^+ (mg/g)	Aroma[a] score	Color[b] score	General appearance[c] score	Yield (%)
Salt ratio:[d]										
100:0	24	54.2	50.1[x]	3.7[x]	33.1[x]	9.0[x]	3.0	3.2	3.1[x]	81.6[x]
70:30	24	53.2	46.9[x]	3.1[y]	20.2[y]	21.9[y]	2.9	3.1	3.0[x]	80.7[x]
50:50	24	54.3	27.6[y]	3.5[x]	17.7[y]	36.9[z]	3.0	3.3	2.0[y]	79.3[y]
Boning time:										
Hot-boned	36	53.7	46.2[x]	3.4	24.4	21.6	3.0	3.2	2.7	80.9
Cold-boned	36	55.4	36.9[y]	3.5	22.9	23.6	2.9	3.1	2.7	80.2
Mechanical tenderization:										
Tenderized	36	54.1	51.2[x]	3.6	24.7	23.6[x]	3.0	3.1	2.6	80.5
Nontenderized	36	53.7	31.8[y]	3.4	22.7	21.6[y]	3.0	3.3	2.8	80.5

[a] 4 = typically aged; 3 = moderately aged; 2 = slightly aged.
[b] 4 = dark red; 3 = red; 2 = light red.
[c] 4 = excellent; 3 = good; 2 = fair.
[d] NaCl:KCl (molar basis).
[xyz] Means within a column within a main effect with different superscripts are different ($P<0.05$).

ing time or tenderization treatment but was lower ($P<0.05$) in the 70:30 salt group than in both the 100:0 and 50:50 salt groups.

As expected, sodium levels decreased and potassium levels increased as the level of KCl replacement increased. A reduction in sodium levels of 39.0% and 46.5% were noted as KCl was replaced at levels of 30 and 50%, respectively. Concurrently, potassium levels increased by 143.3% and 310%, respectively. Keeton (1984) found decreases in sodium levels of 27.6% and 67.4%, with KCl replacement levels of 33.3 and 66.7%, respectively. Sodium levels in the 70:30 and 50:50 salt groups were lower ($P<0.05$) than levels in the 100:0 salt group but were not significantly different from each other. Increases in potassium levels were different ($P<0.05$) at each increasing level of KCl replacement. Boning time and tenderization treatment had no effect on sodium levels in the final product. Tenderized hams had higher ($P<0.05$) potassium levels than nontenderized hams while boning time did not affect potassium level in the final product. An interaction ($P<0.05$) between boning time and salt group were noted for both sodium and potassium levels. However, differences within this interaction were inconsistent and small.

Subjective evaluations of color and general appearance were not affected by treatments (Table 3). General appearance scores were lower ($P<0.05$) in the 50:50 salt group than both the 100:0 and 70:30 salt groups but were not affected by boning time or tenderization treatment. Several hams had slight to moderate amounts of internal mold. These hams were scattered throughout treatment groups and thus, it is assumed that treatment did not affect the presence or amount of internal mold. Several hams also exhibited poor binding of the lean surfaces. This problem is also not believed to be affected by treatment, it is most likely the result of poor stuffing of the hams into the fibrous casing. From the standpoint of stuffing, tenderized hams were noted as being more pliable and were stuffed more easily.

Palatability traits

Hams that received the 50:50 salt cure mixture had higher ($P<0.05$) flavor intensity and lower ($P<0.05$) flavor preference and overall satisfaction scores than hams in the 100:0 and 70:30 salt groups (Table 4). Hams in the 50:50 salt group received a mean score of 4.1 for flavor preference and 4.0 for overall satisfaction, which are well into the unacceptable range. Hams in the 70:30 salt group were not rated different by ($P>0.05$) from hams in the 100:0 salt group for any palatability trait. These results agree with Keeton (1984) who found that an acceptable product can be produced with up to a 33.3% replacement of KCl for NaCl. However, this conclusion may only be valid for high salt products such as dry-cured hams. Products with lower salt similar to those evaluated by Whiting and Jenkins (1981) and Hand et al. (1982) may tolerate higher levels of KCl substitution. Instron shear values were also higher ($P<0.05$) for hams cured with the 50% KCl replacement mixture than hams from both the 100:0 and 70:30 salt groups.

Sensory panel flavor preference scores were higher ($P<0.05$) for CB than for HB hams. Since the difference was not great and no other sensory attributes were affected, the use of prerigor meat could be a viable alternative to the use of post-rigor muscle in the production of drypcured hams.

Sensory panel tenderness scores were higher ($P<0.05$) and Instron shear values were lower ($P<0.05$) in tenderized hams than non-tenderized hams. Although both differences are relatively small, their agreement and significance level indicate that a real increase in the tenderness of the product was achieved through blade tenderization. Numerous other studies (Goldner and Mandigo, 1974: Bowling et al., 1976; Savell et al., 1977) concur with these findings. Although tenderness was enhanced, sensory panel flavor preference and overall satisfaction scores were lower ($P<0.05$), and flavor intensity scores higher ($P<0.05$) in tenderized hams (Table 4). These differences of 0.2, 0.2 and 0.3 units, respectively, are very small. However, the fairly large number of samples (36) and a significance level of 0.05 did indicate that some aspect of the tenderization process adversely affected the flavor of the product.

Microbial counts

Surface aerobic (35°C) counts increased after aging as the KCl level of replacement increased. Aerobic (35°) counts were higher $P<0.05$) for the 50:50 salt group than for the 100:0 salt group. Counts for the 70:30 salt group were in between these two and did not differ ($P>0.05$) from either. All other microbial counts were not affected by salt treatment. The use of prerigor muscle had no affect onthe microbial quality of hams after aging. Although hot-boned hams did have slightly higher microbial counts than cold-boned hams, these differences were not significant ($P>0.05$).

Mechanical tenderization improved the microbial quality of the external surface of hams after aging. Tenderized hams had lower ($P<0.05$) aerobic (26, 35°C) and staphylococci counts than non-tenderized hams. These decreases in microbial counts may be due to the greater amount of surface drying noted in the tenderized hams.

CONCLUSIONS

IN THE PRODUCTION of boneless dry-cured hams, NaCl can be replaced with KCl up to a level of 30% (equal molar basis) with no detrimental effects on palatability or microbial quality. Use of this replacement level resulted in a decrease in Na of 39% and an increase in K content of 143%. Replacement of NaCl with KCl at a level of 50% (equal molar basis) resulted

Table 4—Means for palatability traits and instron shears by treatment

Treatment	No.	Trait: Tenderness[a] score	Flavor intensity[b] score	Flavor preference[c] score	Saltiness[d] score	Overall satisfaction[e] score	Instron shear kg/1.27 cm²
Salt ratio:[f]							
100:0	24	5.3	5.8[x]	5.4[x]	5.7	5.4[x]	3.2[x]
70:30	24	5.5	5.8[x]	5.4[x]	5.5	5.4[x]	3.2[x]
50:50	24	5.3	6.1[y]	4.1[y]	5.5	4.0[y]	3.9[y]
Boning time:							
Hot-boned	36	5.4	5.9	4.8[x]	5.5	5.0	3.4
Cold-boned	36	5.4	5.9	5.1[y]	5.6	4.8	3.5
Mechanical tenderization:							
Tenderized	36	5.5[x]	6.0[x]	4.8[x]	5.6	4.8[x]	3.2[x]
Nontenderized	36	5.3[y]	5.8[y]	5.1[y]	5.5	5.0[y]	3.7[y]

[a] 8 = mushy; 1 = extremely bland.
[b] 8 = extremely intense; 1 = extremely bland.
[c] 8 = like extremely; 1 = dislike extremely.
[d] 8 = extremely salty; 1 = extremely bland.
[e] 8 = like extremely; 1 dislike extremely.
[f] NaCl:KCl (molar basis).
[xy] Means within a column with a main effect with different superscripts are different (P>0.05).

Table 5—Microbiological results of surface swabs obtained after aging[a]

Treatment	Microbial counts, log_{10}/12 cm² AC26	AC35	SC	LC	YM
Salt ratio:[c]					
100:0	2.29	2.34[x]	1.89	1.04	1.64
70:30	2.72	2.66[xy]	1.87	1.08	1.46
50:50	2.60	3.33[y]	1.70	1.18	1.83
Boning time:					
Hot-boned	2.74	2.84	2.04	1.07	1.81
Cold-boned	2.33	2.71	1.60	1.14	1.48
Tenderization treatment:					
Tenderized	2.14[x]	2.43[x]	1.49[x]	1.16	1.57
Nontenderized	2.93[y]	3.12[y]	2.14[y]	1.04	1.71

Values are geometric means of three hams.
[b] AC26, 35 = Aerobic counts at 26°C and 35°C, respectively; SC = Staphylococci counts; LC = Lactobacilli counts; YM = Yeast and mold counts.
[c] NaCl:KCl (molar basis).
[xy] Means in columns within main effect with different superscripts are different (P<0.05)

in lower sensory panel flavor preference and overall satisfaction scores, and higher flavor intensity scores, Instron shear values and aerobic (35°C) counts. The use of pre-rigor muscle in the production of boneless dry-cured hams had no adverse effect on palatability or microbial parameters. Mechanical tenderization enhanced tenderness, improved microbial quality and slightly lowered flavor preference scores. The use of pre-rigor muscle and blade tenderization in the production of dry-cured hams could be advantageous. However, proper sanitation is essential when utilizing either of these processing parameters in a production system.

REFERENCES

Abidoye, D.F.O. 1976. The effect of curing ingredients, skinning and boning on yield and quality of dry-cured hams. M.S. Thesis, Univ. of Kentucky, Lexington, KY.
AMI. 1984. Meatfacts. 1984. American Meat Institute, Washington, DC.
AOAC. 1980. "Official Methods of Analytical Chemists." Association of Official Analytical Chemists. Washington, DC.
Bowling, R.A., Smith, G.C., Carpenter, Z.L., Marshall, W.H., and Shelton, M. 1976. Blade tenderization of ram lambs and kid goats. J. Anim. Sci. 43: 122.
Cross, H.R. and Tennent, I. 1980. Accelerated processing systems for USDA Choice and Good beef carcasses. J. Food Sci. 45: 765.
Gill, J.L. 1979. "Design and Analysis of Experiments in the Animal and Medical Sciences." Iowa State Univeristy Press, Ames, IA.
Glover, E.E., Forrest, J.C., Johnson, H.R., Bramblett, V.D., and Judge, M.D. 1977. Palatability and cooking characteristics of mechanically tenderized beef. J. Food Sci. 42: 871.
Goldner, W.J. and Mandigo, R.W. 1974. The effects of mechanical tenderization press/cleave portioning on boneless pork loins. J. Anim. Sci. 39: 971. (Abstr.).
Hand, L.W., Terrell, R.N., and Smith, G.C. 1982. Effects of chloride salts on physical and sensory properties of frankfurters . J. Food Sci. 47: 1800.
Huffman, D.L. 1979. Engineered steaks and chops. Proc. Meat Conf. 32: 41 Amer. Meat Sci. Assoc., Chicago, IL.
Kastner, C.L. 1977. Hot processing: Update on potential energy and related economics. Proc. of the Meat Ind. Res. Conf., p. 43. Amer. Meat Inst. Foundation, Chicago, IL.
Keeton, J.T. 1984. Effects of potassium chloride on properties of country-style hams. J. Food Sci. 49: 146.
Kemp, J.D. and Fox, J.D. 1977. Producing boneless drycured hams with different amounts of curing ingredients. J. Food Sci. 42: 1487.
Leak, F.W., Kemp, J.D., and Fox. J.D. 1983. The effect of boning time and sodium diacetate on the quality and palatability of tumbled dry-cured hams. Progress Report. No. 274. 1983. Swine Research Report. Ky. Agr. Expt. Sta.
Mandigo, R.W. and Henrickson, R.L. 1966. Influence of hot-processing pork carcasses on cured ham. Food Technol. 20: 538.
Marth, E.H. 1978. "Standard Methods for Enumeration of Dairy Products," 14th ed. American Health Assoc., Washington, DC.
McCarron, D.A., Morris, C.D., Henry, H.J., and Stanton, J.L. 1984. Blood pressure and nutrient intake in the United States. Science. 224: 1392.
Read, Jr., R.B. 1978. "Food and Drug Administration Bacteriological Analytical Manual," 5th ed. Association of Official Analytical Chemists, Washington, DC.
Rogosa, M., Mitchell, J.A., and Wiseman, R.F. 1951. A selective medium for the isolation and enumeration of oral and fecal *Lactobacilli*. J. Bacteriol. 62: 132.
SAS. 1982. "SAS User's Guide: Basics," 1982 ed. SAS Institute Inc., Cary, NC.
Savell, J.W., Smith, G.C., and Carpenter, Z.L. 1977. Blade tenderization of four muscles from three weight-grade groups of beef. J. Food Sci. 42: 866.
Schmidt, G.R. and Keman, S. 1974. Hot boning and vacuum packaging of eight major bovine muscles. J. Food Sci. 39: 140.
Schwartz, W.C. and Mandigo., R.W. 1974. Effect of conveyor speed on mechanical tenderization of beef inside rounds. J. Anim. Sci. 39: 174. (Abstr.).
Seideman, S.C. and Cross, H.R. 1983. The economics and palatability attributes of hot boned beef: A review (Advantages and Disadvantages) J. Food Qual. 5: 183.
Terrell, R.N. and Olson, D.G. 1981. Chloride salts and processed meats: Properties, sources, mechanisms of action, labeling. Proc. Meat. Ind. Res. Conf., p. 67. Amer. Meat Inst., Arlington, VA.
Whiting, R.C. and Jenkins, R.K. 1981. Partial substitutions of sodium chloride by potassium chloride in frankfurter formulations. J. Food Qual. 4: 259.

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Microstructural Comparisons of Meat Emulsions Prepared with Corn Protein Emulsified and Unemulsified Fat

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ABSTRACT

The microstructures of comminuted meat products containing 0, 1 and 2% of corn germ protein as a powder additive and as a stabilizer for pre-emulsified fat (PEF) were studied. A modified fixation technique and protein contrasting ingredient were used to improve the resolution of the microstructure. Meat products containing 2% protein additive exhibited a uniform distribution of fat globules and increased water and fat binding capacity. Corn germ protein used as a stabilizer in PEF preparation formed a more uniform distribution of fat globules than of additive product. These protein micellar structures may stabilize the larger size fat globules, resulting in less coalescence of the fat component during heat treatment.

INTRODUCTION

CONTRADICTORY HYPOTHESES of the protein-fat structural relationships have been proposed for comminuted meat products. The microstructure of sausage emulsions in relation to stability was first examined by Borchert et al. (1967). Using high resolution transmission electron microscopy (TEM), they observed a dense membrane-like layer surrounding the fat globule. The continuous phase of the emulsion was fibrous, but homogeneous. After thermal processing, the globule membranes were disrupted and the protein of the continuous phase was coagulated into dense, irregular zones (Carroll and Lee, 1981). In a sausage emulsion, the fat forms the discontinuous phase and the water-protein-salt solution represents the continuous phase. The dense membrane reported by Borchert et al. (1967) did not resemble a true bi-layer membrane. Kamat et al. (1977) published two micrographs of emulsion (soybean protein-phospholipid-water and soybean protein-phospholipid-sunflower oil) systems showing closed vesicles with one or more layers, which is different from sausage emulsions (batters). In a sausage emulsion, the amount of emulsified fat is rather low. This emulsion is not stable and coalesces during heat treatment.

The concept that the batter of comminuted meat products is related to true emulsions was not accepted (Terrell, 1980). Meat batters are more similar to materials with elastico-plastic properties, with fat globules distributed comparatively homogeneously in the structure. In batters of frankfurters, fat particles were mechanically fixed within the structures of the protein filaments (Hamm, 1973).

Carroll and Lee (1981) examined the thermal stability of meat emulsions by light, scanning electron, and transmission electron microscopy before and after the cooking process. Microstructures changed as a result of increasing chopping temperatures. The fat droplets changed from spherical to oval and irregular shapes. A weakening of the matrix was exhibited by the formation of channels within the protein matrix. Jones and Mandigo (1982) observed small pores in the protein membrane surrounding fat globules in the finished product. These factors appear to be directly related to the fat-binding and water-holding ability of the sausage emulsions.

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Protein substances are optimal stabilizers for fat emulsions, i.e. pre-emulsified fat (PEF). PEF may play an important role in improving sausage processing by stabilizing the fat component of comminuted meat products. PEF can be prepared by using water, fat and protein which are components of sausage ingredients. PEF prepared by ultrasonic emulsification of melted fat and water with protein additives (non-fat dry milk (NFDM), sodium caseinate, blood plasma proteins), made a superior quality finished product as indicated by higher water holding capacity, juiciness, tenderness and more uniform distribution of fat particles of finished product (Zayas, 1985).

No TEM micrograph has been published to show the microstructures of comminuted meat products containing pre-emulsified fat. Very few references can be found to help the interpretation and identification of protein, fat, and other components in heat-treated commuinuted meat products under high magnifications (17,000 – 36,000X) by the TEM method.

The goal of this research was to investigate the influence of the utilization of corn germ protein as the powder and stabilizer in PEF on the microstructure of comminuted meat products, dislocation of protein and fat components in the structure of the products.

MATERIALS & METHODS

Sample preparation

Fresh beef (beef trim and plate, 20% fat) obtained from the meat lab of the Kansas State University was ground through a 9.38 mm plate, mixed thoroughly, and reground three times through a 4.69 mm plate. The sample was divided randomly (300 g), sealed in vacuum packages, and stored at −12°C. Frankfurters were processed under laboratory conditions similar to Whiting and Richards (1978), differing from commercial practice (Sales et al., 1980). The frozen meat (263g) was thawed at 4°C for 12 hr, and chopped with 2% salt and 1/3 of total 39% added water (ice chips) in a prechilled food processor (General Electric, Model PF6) for 1 min. The temperature increased to an average of 6°–8°C. Another 1/3 of total water and 0.3% prague powder, containing 6.25% sodium nitrite (Griffith Lab., Alsip, IL), were added and chopped for 30 sec to 11–13°C average temperature. The whole batch was chilled in the freezer for 10 min to temper and lower the temperature of the batter.

Control samples were prepared without protein additive or PEF. Chopping time of 1 min was used to blend the melted PEF or unemulsified lard (35°C) with lean meat. Fat content in experimental and control samples was equal (25%).

Sausage batters were held another 10 min in the freezer for controlling temperature. A final 30 sec chopping time was used to add the remaining 1/3 of total water, sugar (1%), commercial frankfurter spice (0.5%; Baltimore spice, Weiner seasoning FF3118) and ascorbic acid (0.1%) to the end temperature of 14–15°C.

Corn germ protein was added as a powder additive (first experimental group) and in the liquid form as stabilizer of PEF (second experimental group) after 1 min blending process. Corn germ flour was obtained from the USDA Northern Regional Research Center (Peoria, IL) and processed by the method of Christianson et al. (1984). Hydrated protein solution was prepared by incubating a mixture of protein and water in an 85°C water bath for 1 hr and preblending at low speed (8,000 rpm) with Oster blender (Model 548-41A) for 1 min. Melted lard was emulsified with the stabilizer solution at high speed (20,000 rpm) for 4 min. The fat was added by drops within 1.5 min and allowed another 2.5 min emulsification. The composition of

PEF was: lard 37.5g; stabilizer, corn germ protein 3 or 6 g (1% or 2% in sausage batter formulation) and 45g water. The semi-liquid PEF was made fresh before addition to the batter.

Water-holding capacity

The Hamm press technique (Hamm, 1960) was used with modification to measure the water holding capacity (WHC) of batter. Batter (0.3g) was placed on filter paper (Whatman No. 1) which was placed between two plexiglas sheets and pressed for 20 min with a 1 kg standard weight. The areas were measured with a compensating polar planimeter and the WHC was calculated by the following equation:

$$\text{WHC} = \left[1 - \frac{\text{total area-meat film area}}{\text{meat film area}}\right] \times 100\%$$

Batter thermal stability

The method of Haq et al. (1972) and Saffle et al. (1967), with slight modifications, were used to test batter thermal stability. A 30g batter sample removed at the end of stuffing was placed in a centrifuge tube (27.5 mm × 110 mm) with screw cap, and heated for 30 min in a 70°C water bath. The tube was centrifuged for 3 min at 4,000 rpm. All the cookout liquids were drained and measured. Readings of water or fat were expressed as percentages (w/w).

The batters were stuffed into 24 mm diameter casings which were linked 11 cm in length and hung on cooking rack. An internal temperature of 70°C was obtained in the product after cooking in a rotating oven with relative humidity controlled at 45% at 87°C for 65 min. Stuffed weight and after-cooked weight were recorded to calculate the yields based on the original raw meat weight plus fat.

Electron microscopy

Sample preparation procedures followed the methods of Schiff and Gennaro (1979) and Mollenhauer (1964) with some modifications to preserve the fat component and improve osmium tetraoxide (OsO_4) penetration. Tannic acid was used to improve protein contrast (Hayat, 1981). Small blocks (less than 1 mm^3) were cut from the finished products (Carroll and Lee, 1981) in a pool of fixing solution (2.5% glutaraldehyde buffer with 0.1M PIPES (piperazine-N-N'bis 20-ethanol sulfonic acid), pH 7.4 and 4% tannic acid). Samples were fixed cold in the same buffer (4°C) for 3 hr. An ice bath was used for the following process up to the step of 95% acetone dehydration. The samples were washed three times for 30 min each in sucrose – PIPES buffer (0.1 M/0.1M). The postfixation was carried out in 1% OsO_4-PIPES buffer for 2 hr. The samples were washed four times for 5 min each with distilled water (4°C). Then uranyl acetate (1% in PIPES buffer) was used to stain the samples for 2 hr, after which they were rapidly dehydrated (10 min each) in increasing concentrations of acetone (acetone series 30, 50, 70, 85, 95 and 100%). The dehydrated samples were infiltrated through acetone: Mollenhauer plastic series (2:1, 1:1, 1:2) for a minimum of 2 hr each, then through 100% plastic for at least 8 hr. Vacuum was applied after infiltration for 30 min at 22 mm Hg at ambient temperature to remove all the air entrapped in the tissues. The samples were embedded in a flat BEEM mold and cured 48 hr at 65°C. Gray to silver (500A–700A) sections were cut with glass knives on a Reichert Om-2 ultramicrotome (Amer. Optical Corp., Buffalo, NY) and counterstained with uranyl acetate and lead citrate for 10 min each. A Philips 201 TEM operating at 60 kV was used to examine the sections.

Statistical analysis

Analysis of variance and least significant difference tests were conducted by the methods of Steel and Torrie (1980).

RESULTS & DISCUSSION

FUNCTIONAL PROPERTIES of batter due to corn protein additives are presented in Table 1. Higher water-holding capacity ($P<0.05$) was obtained with 2% of corn protein utilized as stabilizer for PEF. When 1% added corn protein was used, the trend was for WHC to increase, but there were no significant differences. There were no significant effects of 2% protein powder additive on WHC of batter. The trend of increasing thermal stability (less water loss) was shown by addition of 2% protein as powder additive and as stabilizer of PEF. A significant increase in yield of finished product was obtained with 2% corn germ protein with less water loss.

Table 1—Batter water-holding capacity, thermal stability and cook yield with corn protein additive[a]

Corn protein (%)	Water-holding capacity (%)	Thermal stability Fat loss (%)	Thermal stability H_2O loss (%)	Cook yield (%)
0	65.86[b]	4.77[b]	20.43[bc]	126.09[b]
Powder additive				
1	70.83[bc]	8.57[b]	21.00[bc]	126.78[bc]
2	69.72[bc]	4.57[b]	16.57[c]	128.13[b]
Stabilizer of emulsion				
1	70.52[bc]	9.00[b]	23.43[b]	127.39[bc]
2	77.73[c]	7.33[b]	16.30[c]	128.19[c]

[a] Means collected from three replications.
[b,c] Means in the same column with different superscripts are different ($P<0.05$).

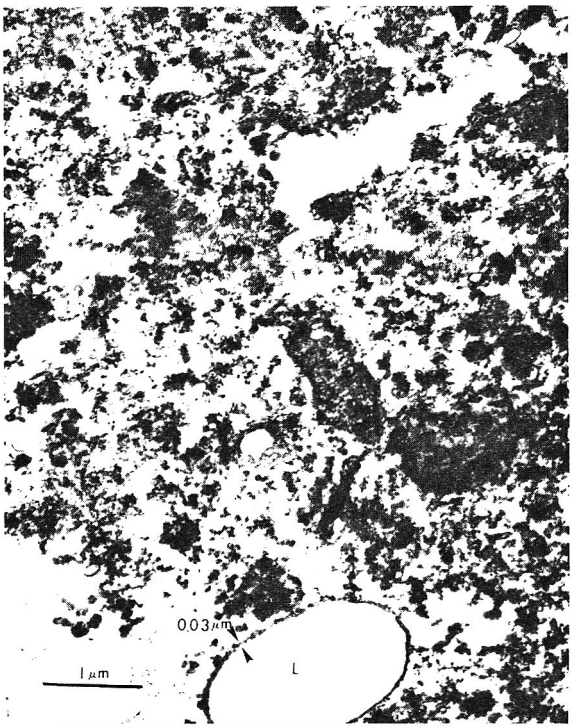

Fig. 1—Micrograph of comminuted meat product with 1% protein additive as powder. Fat droplet (L), diameter of membrane as indicated.

Results of control and experimental samples (protein powder additive and PEF) were presented in the micrographs. Magnifications were 16,200–36,300X. Microstructure of finished products containing PEF were more uniform with improved resolution of homogeneous particles.

Incorporation of 1% or 2% dry powder protein additive in comminuted meat product was evaluated by the micrographs of the samples (Fig. 1 and 2, respectively). The area of fat globules formed showed an elongated shape separated by protein film. Added 1% protein product showed no significant effect on the structures. Fig. 1 shows a large fat globule at bottom of the micrograph which has a thick membrane (0.03–0.05 μm); however, the membrane is not uniform in thickness. Dark areas represent the proteins in the meat batter, which have a similar structure as compared with the control (Fig. 6).

A different structure is seen in Fig. 2 due to increasing concentration of the corn germ protein to 2%. There are several small particles with a globular or elongated shape, which may represent the homogeneous structure of fat emulsion or the fat globules confined locally within the protein matrix. These pro-

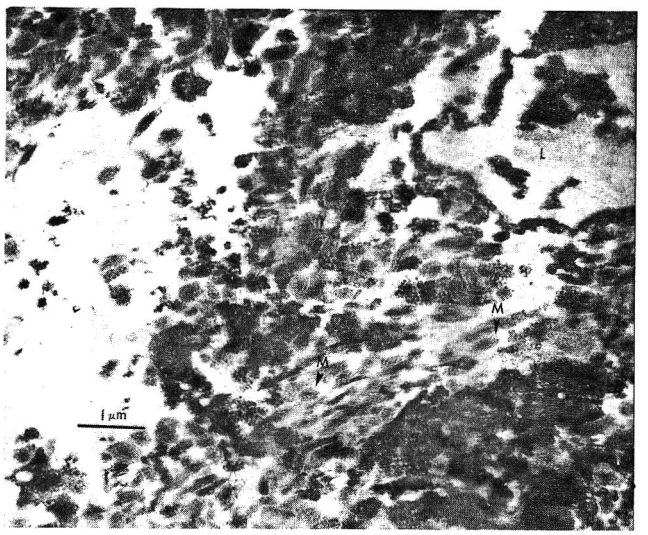

Fig. 2—Micrograph of comminuted meat product with 2% protein additive as powder. Fat droplet (L), Micellar structures (M).

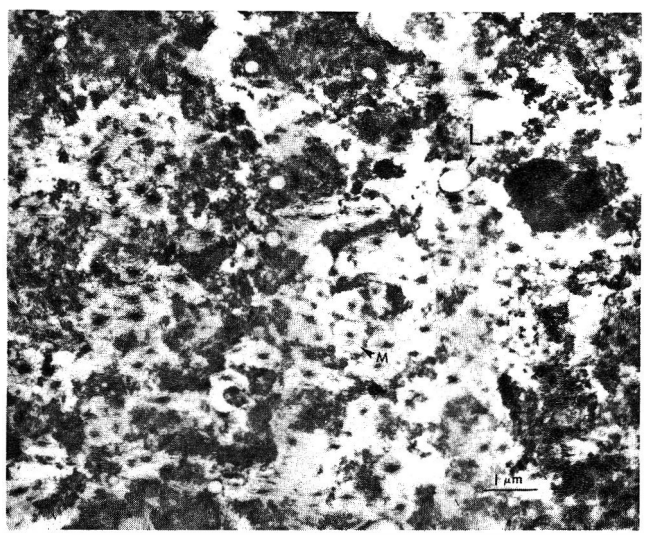

Fig. 3—Micrograph of comminuted meat product with 1% protein additive as stabilizer in the pre-emulsified fat. Fat droplet (L), Micellar structure (M).

tein micellar structures seem to have a thin discontinuous membrane and flow into a long shape. The coarse structures of the large fat globule do have thick membrane, which is in an irregular shape resulting from coalescence.

Differences in the protein matrix and fat globules were found in commercial frankfurters (Theno and Schmidt, 1978). The structures ranged from coarse to fine in terms of fat droplets and muscle components. The structure had small fat droplets distributed in the protein matrix.

Theno and Schmidt (1978) observed a true emulsion formed in the comminuted meat products. The level of fat emulsification depends on the properties of protein and fat components, temperature of emulsification, equipment for sausage batter comminution, and other factors (Gorbatov and Zayas, 1973; Zayas, 1985). It is possible to have partial, locally emulsified fat after heat treatment of the comminuted meat products.

Figures 3, 4, and 5 are the electron micrographs of the comminuted meat products with 1% or 2% corn protein additive used as stabilizer in PEF preparation. The lard was added as an emulsion with high dispersivity of both components, protein and fat. The homogeneous structures of experimental samples containing pre-emulsified fat can be considered as

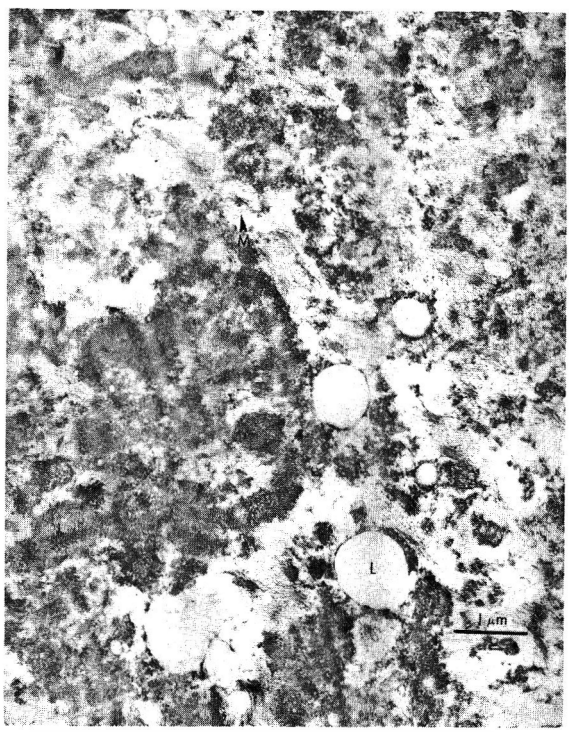

Fig. 4—Micrograph of comminuted meat product with 1% protein additive as stabilizer in the pre-emulsified fat. Fat droplet (L), Micellar structure (M).

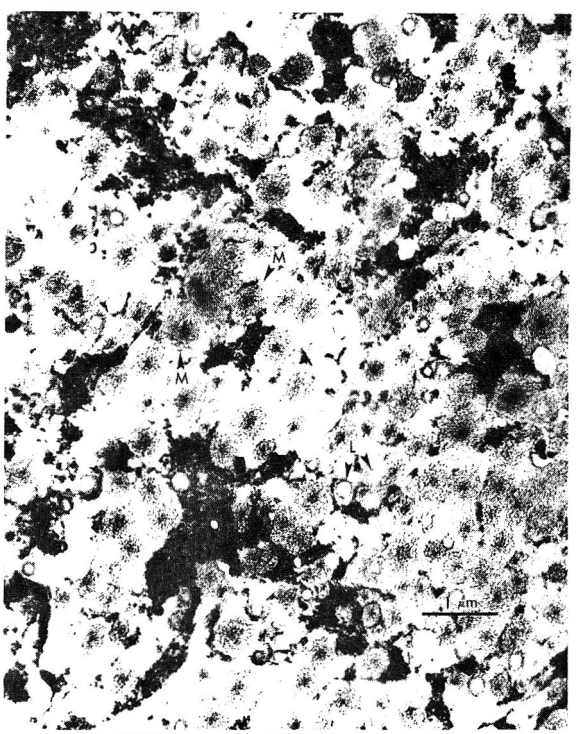

Fig. 5—Micrograph of comminuted meat product with 2% protein additive as stabilizer in the pre-emulsified fat. Fat droplets (L), Micellar structures (M).

having significant amount of emulsified fat. As shown in Fig. 3, some oval globules were found with sizes ranging from 0.31 μm to 0.43 μm. Several globules aggregated in a unique configuration which formed a dense area at the center, may resemble the thin protein film surrounding the small fat globules. Higher magnification (Fig. 4) shows uniform structure of fat globules and protein components. These micellar globules, 0.06

μm–0.18μm in diameter, may stabilize the larger sizes of fat globules (about 0.75 μm).

As shown in Fig. 5, in experimental samples containing 2% protein additive as stabilizer of pre-emulsified fat, fat droplets were evenly distributed in the structure. The fat component was stabilized as the medium size (0.15 μm to 0.18 μm) fat droplets throughout the emulsion without coalescence. These homogeneous structures of comminuted meat batters resulted in higher interaction between protein-fat-water components due to PEF preparation.

Electron micrograph of the control group is shown in Fig. 6, which shows a similar structure as Borchert et al. (1967) with the same dimension of fat droplets. Dark areas represent the protein components which in general were not evenly dispersed. This control group represents the limiting condition of comminution, therefore used as reference. Fat globules are surrounded by other particles with a round shape and a distinct protein layer. The large irregular clear areas represent voids resulting from trapped air or water. One fragment of myofibril in longintudinal section was observed in this micrograph (Fig. 6).

Formation of the more stable structures was shown by studies of the textural properties of sausage emulsions, containing sodium caseinate, NFDM and blood plasma proteins as stabilizer of PEF (Zayas, 1985), a conclusion supported by higher heating stability of those systems. PEF containing different protein stabilizers has a practical significance in the processing of comminuted meat products. Those large fat globules also have several small fat globules between them and may confirm the stabilizing effect of PEF.

Fat globules were visible in the light micrographs, published by Ray et al. (1979), and Theno and Schmidt (1978). However, using higher magnification obtained by transmission electron microscopy we can differentiate the structures of comminuted meat products. Generally, in cooked sausage emulsions, fat globules are elongated or coalesced providing the shape of the fat and protein components are not identical (Ray et al., 1979). The earliest paper (Borchert et al., 1967) showed globules as small as 0.1 μm and protein components in dense distribution. Compared with our work, the micrographs look very much alike. We obtained the same dimension fat droplets. However, the smaller block kept in fixation for a longer time in a cold environment (4°C) gave less fat separation in early stage of sample fixation.

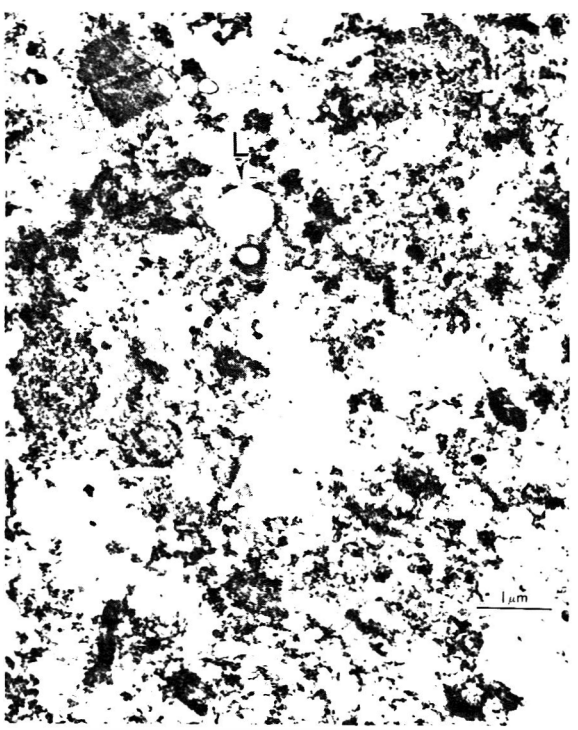

Fig. 6—Micrograph of comminuted meat product with no protein additive. Fat globule (L).

CONCLUSION

RESULTS showed that the addition of corn germ protein to the sausage formulation had a specific effect on the formation of the structure of comminuted meat products. Corn germ protein in the form of additive and stabilizer of fat emulsion can increase the degree of fat globule stabilization by formation of protein film on the surface of small fat droplets and to prevent coalescence of large fat globules during heat treatment. Pre-emulsified fat applied in the comminuted meat products can increase the amount of emulsified fat in the finished product and cause a more uniform distribution of the fat component in the structure of the product.

REFERENCES

Borchert, L.L., Greaser, M.L., Bard, J.C., Cassens, R.G., and Briskey, E.J. 1967. Electron microscopy of a meat emulsion. J. Food Sci. 32: 419.
Carroll, R.J. and Lee, C.M. 1981. Meat emulsions-fine structure relationships and stability. Scanning Electron Microscopy III: 447.
Christianson, D.D., Friedrich, J.P., List, G.R., Warner, K., Bagley, E.B., Stringfellow, A.C., and Inglett, G.E. 1984. Supercritical fluid extraction of dry-milled corn germ with carbon dioxide. J. Food Sci. 49: 229.
Gorbatov, V.M. and Zayas, J.F. 1973. Technological role of animal fats in the production of comminuted meat products. Proceedings of the 19th European Congress of Meat Research Workers, Paris. 2: 819.
Hamm, R. 1960. Biochemistry and meat hydration. In "Advances in Food Research," Vol. 10, p. 363. Academic Press, New York.
Hamm, R. 1973. The importance of the water binding capacity of meat when manufacturing frankfurter-type sausages. Die Fleischwirtschaft 1: 73.
Haq, A., Webb, N.B., Whitfield, J.K., and Morrison, G.S. 1972. Development of a prototype sausage emulsion preparation system. J. Food Sci. 37: 480.
Hayat, M.A. 1981. "Fixation for Electron Microscopy," p. 124. Academic Press, New York.
Jones, K.W. and Mandigo, R.W. 1982. Effects of chopping temperature on the microstructure of meat emulsions. J. Food Sci. 47: 1930.
Kamat, V.B., Graham, G.E., and Davis, M.A.F. 1977. Vegetable protein: lipid interactions. Cereal Chem. 55: 295.
Mollenhauer, H.H. 1964. Plastic embedding mixtures for use in electron microscopy. Stain Technology 39: 11.
Ray, F.K., Miller, B.G., Van Sickle, D.C., Aberle, E.D., Forrest, J.C., and Judge, M.D. 1979. Identification of fat and protein components in meat emulsions using SEM and light microscopy. Scanning Electron Microscopy III: 473.
Saffle, R.L., Christian, J.A., Carpenter, J.A., and Zirkle, S.B. 1967. Rapid method to determine stability of sausage emulsions and affects of processing temperature and humidities. Food Technol. 21: 101.
Sales, C.A., Bowers, J.A., and Kropf, D. 1980. Consumer acceptability of turkey frankfurters with 0, 40, and 100 ppm nitrate. J. Food Sci. 45: 1060.
Schiff, R.I. and Gennaro, J.F. Jr. 1979. The influence of the buffer on maintenance of tissue lipid in specimens for scanning electron microscopy. Scanning Electron Microscopy III: 449.
Steel, R.G. and Torrie, J.H. 1980. "Principles and Procedures of Statistics," 2nd ed. McGraw-Hill Book Co., New York.
Terrell, R.N. 1980. A meat scientist reviews what's known to date about the mysterious process that changes chopped-up meat to succulent sausage. Meat Industry 26: 57.
Theno, D.M. and Schmidt, G.R. 1978. Microstructural comparisons of three commercial frankfurters. J. Food Sci. 43: 845.
Whiting, R.C. and Richards, J.F. 1978. Influence of divalent cations on poultry meat emulsions and sausages. J. Food Sci. 43: 312.
Zayas, J.F. 1985. Structural and water binding properties of meat emulsions prepared with emulsified and unemulsified fat. J. Food Sci. 50: 689.

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Functionality of Six Nonmeat Proteins in Meat Emulsion Systems

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ABSTRACT

The emulsion stabilizing and textural effects of six nonmeat proteins, identified by brand name and manufacturers were investigated. Included were: Soy protein isolate (SPI), soy protein concentrate (SPC), soy flour (SF), milk protein hydrolysate (MPH), autolyzed yeast (AY), and spray dried nonfat dry milk (NFDM). SPI and NFDM were most effective, while SF and AY were least effective in emulsion stabilization. Bind value constants (Saffle system) were developed for each nonmeat protein. Mean compression values for all the nonmeat proteins except SPI decreased with increasing levels of the additives. The functional values derived in this study are specific for these nonmeat proteins and may not be applicable to other nonmeat proteins of a similar nature.

INTRODUCTION

NONMEAT PROTEINS derived from a variety of plant and animal sources are used extensively as fillers, binders, and extenders in meat systems. The functionality of these nonmeat proteins in meat emulsions has been estimated in model systems and in products such as wieners and bologna. An understanding of the functional effects of nonmeat proteins in relation to the functionality of salt soluble meat protein has long been sought.

Evidence suggesting that finely comminuted meat systems are emulsion systems was put forth by a number of workers (Hansen, 1960; Swift et al., 1961; Helmer and Saffle, 1963; Meyer et al., 1964; Borchert et al., 1967; Ivey et al., 1970; Theno and Schmidt, 1978). Emulsion theory holds that during chopping, salt soluble muscle proteins and water act as the continuous phase of the emulsion, coating fat globules in a two-phase system which is stabilized upon thermal processing. More recently, the role of the gelation of ingredients in the stabilization of emulsion-type sausage has been emphasized (Comer, 1979; Schmidt, 1981; Acton et al., 1983; Ziegeler and Acton, 1984).

Both emulsion theory and gelation theory have affected approaches to studying the functionality of nonmeat proteins in stabilizing finely comminuted meat systems. Model systems have often been utilized to determine such characteristics as emulsifying capacity, gelling ability, water-holding capacity, swelling ability, and various solubilities of nonmeat proteins (Hermansson, 1975; Torgersen and Toledo, 1977; Comer, 1979). Comer (1979) reported that nitrogen solubility index was a poor indicator of the functional effects of nonmeat proteins in meat products. Comer and Dempster (1981) reported that emulsifying capacity was likewise unreliable in predicting nonmeat protein functionality. Smith et al. (1973) reported no direct relationship between stability of cooked frankfurters with nonmeat proteins and stability of model system meat emulsions with nonmeat proteins. Thus, functional properties of nonmeat proteins are not predicted by currently used analyses.

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Emulsion stabilizing effects and textural effects of different extenders have also been studied extensively in meat products. Kinsella (1979) reported that soy products used in meat emulsions differ significantly in functionality, but generally bind water, improve overall yields, stabilize fat, and impart texture upon gelation. Soy isolates stabilize the emulsion, increase viscosity and form a strong gel upon heating (Schweiger, 1974). Comer and Dempster (1981) reported a relative functional ranking of eight different nonmeat ingredients based on cook stabilities and textural effects. Soy isolate, soy concentrate, and a textured soy protein ranked above wheat flour, sodium caseinate, skim milk powder and potato starch. Comer (1979) studied the functionality of textured soy protein, skim milk powder, wheat flour, potato starch, sodium caseinate and a vegetable protein and suggested that these extenders had positive effects upon cook stabilities, but a negative influence on texture. Smith et al. (1973) compared the functional performance of six different soy proteins, three cottonseed proteins, nonfat dry milk and a fish protein concentrate. These authors concluded that nonmeat proteins are inferior to muscle proteins as meat emulsion stabilizers. Several other workers have reported functional and textural effects of nonmeat proteins in meat emulsion systems, generally indicating superior performance of meat proteins (Rongey and Bratzler, 1966; Johnson, 1970; Lauck, 1975; Sofos et al., 1977; Sofos and Allen, 1977; Porteus and Quinn, 1979; Terrell et al., 1979).

Quantitative estimates of the performance of nonmeat proteins in meat emulsions have not been developed. Bind value constants which indicate the effects of different meat items in emulsion stabilization have been used extensively in least cost formulation for many years. Bind value constants were developed based primarily on emulsifying capacity and soluble protein of meat ingredients (Saffle, 1966, Porteus, 1979). These properties are, however, poor indicators of the effects of nonmeat proteins in meat emulsions. The development of bind value constants for nonmeat proteins requires characterization of their functional effects in meat products by other means.

The objectives of this study were to develop functional estimates of emulsion stabilization by six nonmeat proteins, to determine their textural effects, and to make comparisons of different methods of monitoring emulsion stability.

MATERIALS & METHODS

Meat and nonmeat protein sources

Meat sources used in this investigation included bull meat, pork cheek meat, beef spleens and pork back fat. Meat ingredients were coarse ground twice through a three hole (3.3 x 6.5 cm) plate, vacuum packaged in 2.7–5.5 kg lots, and frozen at −12°C and used within 3 months.

The six nonmeat proteins investigated were: "Purina Protein 500E," a soy protein isolate (SPI) from Ralston Purina (St. Louis, MO), "Promosoy 100," a soy protein concentrate (SPC) from Central Soya Co. (Ft. Wayne, IN); "Nutrisoy Flour," a soy flour (SF) from Archer Daniels Midland Corp. (Decatur, IL); "Zyest-FM," and autolyzed yeast (AY) from Pure Culture Products, Inc. (Chicago, IL); Milk Protein Hydrolysate 990 (MPH) from New Zealand Milk Products, Inc. (Petaluma, CA); and a nonfat dry milk (NFDM) from the John R. White Co. (Birmingham, AL).

Table 1—Proximate composition and formulated finished frankfurter content of nonmeat proteins

Ingredient	Proximate composition			Formulated finished frankfurter content (%)[b]		
	% Protein[a]	% Moisture	% Fat	20%	40%	60%
Soy protein isolate	90.3	6.1	0.18	1.0	1.9	2.9
Soy protein concentrate	71.2	7.6	0.18	1.2	2.5	3.7
Soy flour	53.2	7.7	8.50	1.7	3.4	5.2
Autolyzed yeast	52.8	7.5	0.26	1.7	3.4	5.2
Milk protein hydrolysate	87.5	6.6	0.02	1.0	1.9	2.9
Nonfat dry milk	36.9	4.5	0.12	2.5	4.9	7.4

[a] % Protein = Kjeldahl nitrogen × 6.25

[b] Percent of exchanged nonmeat protein for bull protein in frankfurters

Table 2—Smokehouse schedule

Time	Dry bulb temp (°C)	Wet bulb temp (°C)
15 min	60.0	0
15 min	71.1	53.9
to 68.3°C product internal temp	82.2	71.1

Experimental design

A weak but stable frankfurter formulation was developed with the meat ingredients used in this experiment. This was accomplished by substituting beef spleen for bull meat while keeping moisture, fat and protein constant in three replicates of ten different combinations of the meat ingredients. The subsequently described emulsion stability tests were used to monitor stability. The meat block of the chosen weak formula consisted of 24% bull meat, 20% pork cheek meat, 24% beef spleen and 32% pork back fat.

The six nonmeat proteins were substituted in the weak formulation at 20, 40 or 60% of the bull protein in four replicates of the experiment. Moisture, fat and protein were held constant by altering water and pork back fat levels. An all meat control consisting of the weak formulation determined by preliminary studies was also included in each replicate. The protein content of each nonmeat protein and formulated finished frankfurter content appear in Table 1.

Frankfurter production

A 2.3 kg meat block was used in formulating frankfurters to approximate commercial frankfurters in composition (fat = 30%; added water = 4 x protein % + 10%). After thawing 48 hr at 4°C, meat ingredients were ground twice through a 6.35 mm plate. Lean meat ingredients (bull meat, pork cheek meat) were combined with ice water, salt (63.0g), sugar (11.3g), frankfurter seasoning mix (11.3g), sodium erythorbate (1.0g) and sodium nitrite (0.35g) and chopped two min in a Hobart Model 8181D chopper. Beef spleen and pork back fat were added and chopping continued until the batters reached 16°C. When soy products were included, the soy protein was hydrated with ice water and chopped for two minutes before meat ingredients were chopped. Nonfat dry milk, autolyzed yeast, or milk protein hydrolysate were added as dry powders just prior to chopping the beef spleen and pork back fat. Batters were passed through a Stephan Microcot (type MCV 12B with 1.0 mm cutting ring), stuffed into 22 mm cellulose casings and linked with a "Tylinker," C. Benz Sohne Kg. Links were approximately 14.0 cm in length. After stuffing, the uncooked frankfurters were placed in an Alkar-Rasmussen Processing oven and cooked according to the schedule in Table 2. After cooking, the frankfurters were showered with cold water for 3 in and stored overnight in a 4°C cooler. The frankfurters were then reweighed to determine chilled yield. Frankfurters were peeled after chilling and five randomly selected frankfurters were packaged and frozen at −12°C and held for proximate analysis (within 4 wk). Additional frankfurters were randomly selected for texture analysis, a frankfurter stability test and for cook yield analysis. These tests were performed within 72 hr.

Batter samples from each treatment were collected from the stuffer and analyzed for stability using two different emulsion stability tests (within 24 hr).

Emulsion stability tests

The emulsion stability test reported by Saffle et al. (1967) as modified by Decker (1985), personal communication) was performed. Decker's modification included 30 sec of mixing samples with a Vortex-Genie Model K-550-G set at speed 4, immediately after cooking. Another emulsion stability test involved using a 50 mL plastic syringe with the needle end cut off to deliver 45–50g samples of batter into bags. Bags were prepared by cutting 16.51 x 20.32 cm polyester heat sealable pouches in half and heat sealing the open side created by cutting. Samples were placed in the bag, weighed, and the top was heat sealed. The bags with samples were cooked in a 70°C water bath for 60 min. Samples were removed from the water bath, the bags cut open, and the cook liquid poured into 10 ml graduated cylinders. The cooked samples were then reweighed. Total liquid released, fat released and water released were recorded.

A third test of emulsion stability was performed by peeling three chilled frankfurters from each treatment and immediately placing them on 18.5 cm Whatman #1 filter paper circles. These frankfurters were allowed to set 2 hr in a 25°C room (constant relative humidity). The area dampened by liquid released was immediately traced with a pencil and quantified using a compensating polar planimeter.

Cook yield of frankfurters was determined by the method of Tauber and Lloyd (1947).

Texture analysis

Firmness and rupture force were determined using the compression test described by Voisey et al. (1975). Two, 2 cm sections of three frankfurters from each treatment were cut. The skin from each of these six sections was removed using a 15 mm cork borer. Samples were allowed to equilibrate to room temperature (24°C) for 2 hr prior to the compression test.

Proximate analysis

Nonmeat proteins were analyzed for moisture, fat and protein according to AOAC (1975) procedures. Protein was determined as Kjeldahl nitrogen x 6.25. Meat ingredients were also subjected to proximate analysis prior to formulation of frankfurters.

Five frankfurters from each treatment were ground twice through a 0.31 cm plate and for moisture, fat and protein determined according to AOAC (1975) procedures.

Statistical analysis

Data were analyzed by the Statistical Analysis System using Goodnight's (1982) analysis of variance procedure and the mean separation technique of Duncan (1955).

RESULTS & DISCUSSION

THE PROXIMATE COMPOSITION of the nonmeat proteins studied varies considerably, particularly with regard to protein content. This resulted in variations in finished frankfurter content of the nonmeat proteins since the effects of these extenders were compared on a constant protein basis (Table 1). No significant differences in the proximate composition of the finished frankfurters existed despite the variations in the composition of the nonmeat proteins. Mean moisture, fat, and protein content of finished frankfurters was 54.4% 31.2%, and 11.2% respectively.

Mean values for chilled yields, cook yields, emulsion stability characteristics and compression values of frankfurters and batters appear in Table 3. Only the 40% autolyzed yeast treatment had chilled yields signifcantly lower ($P < 0.05$) than

*Table 3—Mean values for chilled yields, cooked yields, emulsion stability characteristics and compression values of frankfurters and batters**

Treatment	Chilled yield (%)	Fat released (mL)[a]	Fat released (mL)[b]	Liquid release (cm^2)	Cook yield (%)	Rupture force (g)	Slope (g/mm)
All meat	91.2^{d,e,f}	1.21^{g}	0.57^{f}	8.43^{g}	73.2^{d,e,f}	4518^{d}	533^{d}
20%[c] SPI	91.3^{d,e,f}	1.61^{f,g}	0.70^{f}	10.18^{f,g}	75.6^{d,e,f}	3795^{d,e,f}	458^{d,e}
4[illegible] [c] SPI	91.5^{d,e,f}	1.37^{f,g}	0.68^{f}	12.21^{e,f}	75.6^{d,e,f}	3198^{e,f}	370^{e,f}
60%[c] SPI	89.3^{e,f,g}	2.06^{f,g}	0.79^{f}	10.81^{f,g}	76.4^{d,e,f}	4055^{d,e}	460^{d,e}
20%[c] SPC	89.7^{d,e,f,g}	1.46^{f,g}	0.79^{f}	10.40^{f,g}	83.0^{d}	3710^{d,e,f}	436^{d,e,f}
40%[c] SPC	93.0^{d}	1.52^{f,g}	0.72^{f}	10.05^{f,g}	79.0^{d,e,f}	3210^{e,f}	385^{e,f}
60%[c] SPC	90.8^{d,e,f}	2.80^{d,e,f,g}	0.93^{e,f}	13.52^{d,e}	80.4^{d,e}	3135^{e,f}	351^{e,f,g}
20%[c] SF	90.7^{d,e,f}	2.11^{f,g}	0.91^{e,f}	10.05^{f,g}	74.3^{d,e,f}	4752^{d}	564^{d}
40%[c] SF	90.0^{d,e,f,g}	2.91^{d,e,f,g}	1.10^{d,e}	11.24^{e,f,g}	81.1^{d,e}	4082^{d,e}	468^{d,e}
60%[c] SF	88.4^{f,g}	4.78^{d}	1.73^{d,e}	15.45^{d}	76.4^{d,e,f}	2858^{f,g}	326^{f,g}
20%[c] MPH	90.9^{d,e,f}	3.61^{d,e,f}	1.80^{d,e}	8.69^{g}	73.4^{d,e,f}	3728^{d,e,f}	448^{d,e,f}
40%[c] MPH	92.0^{d,e}	1.90^{f,g}	0.72^{f}	10.31^{f,g}	71.8^{d,e,f}	3672^{d,e,f}	400^{e,f}
60%[c] MPH	90.7^{d,e,f}	2.29^{f,g}	0.97^{d,e}	10.26^{f,g}	71.4^{e,f}	3195^{e,f}	338^{e,f,g}
20%[c] AY	90.6^{d,e,f}	2.05^{f,g}	0.81^{f}	10.47^{f,g}	75.6^{d,e,f}	3185^{e,f}	374^{e,f}
40%[c] AY	87.3^{g}	1.32^{f,g}	0.62^{f}	11.32^{e,f}	74.1^{d,e,f}	2018^{g,h}	244^{g,h}
60%[c] AY	88.2^{f,g}	4.35^{d,e}	2.08^{d}	14.45^{d}	68.8^{f}	1408^{h}	157^{h}
20%[c] NFDM	92.3^{d,e}	2.11^{f,g}	0.82^{f}	10.74^{f,g}	80.6^{d,e}	4715^{d}	549^{d}
40%[c] NFDM	91.3^{d,e,f}	1.79^{f,g}	0.59^{f}	10.64^{f,g}	73.4^{d,e,f}	3858^{d,e,f}	456^{d,e}
60%[c] NFDM	90.4^{d,e,f,g}	1.32^{f,g}	0.45^{f}	10.84^{f,g}	78.4^{d,e,f}	3015^{f,g,h}	369^{e,f}

* SPI = soy protein isolate, SPC = soy protein concentrate, SF = soy flour, MPH = milk protein hydrolysate, AY = autolyzed yeast, NFDM = nonfat dry milk

[a] Modified Saffle method

[b] Cooking bag method

[c] % nonmeat protein exchanged for bull protein

[d,e,f,g,h] Means in the same column with different superscripts are significantly different ($P < 0.05$)

all meat controls. Most of the 19 treatments were grouped similarly with respect to chilled yield.

Few significant differences in the cooked yield of the frankfurters were determined. As Smith et al. (1973) reported, low cook yields are believed to result from low emulsion stability. The 60% AY treatment did have lower ($P < 0.05$) cook yield than the treatments with the highest cook yields (20% SPC, 60% SPC, 40% SF and 20% NFDM).

Voisey et al. (1975) compared a number of objective tests of weiner texture with sensory evaluation of the textural characteristics of weiners. These authors determined that compression test readings of rupture (behavior in shear) and slope (firmness) were consistently related to sensory data. Compression test data from this study suggests a number of differences in the textural effects of the nonmeat proteins in frankfurters. All meat controls were grouped with the highest mean rupture force values and were not significantly different from 20% SPI, 60% SPI, 20% SPC, 20% SF, 40% SF, 20% MPH, 40% MPH, 20% NFDM and 40% NFDM treatments. The correlation between rupture force (g) and slope (g/mm) in this study was 0.96 (Table 4). Not surprisingly then, mean slope values for all meat controls, 20% SPI, 60% SPI, 20% SPC, 20% SF, 40% SF, 20% MPH, 20% NFDM, and 60% NFDM were not significantly different. The frankfurters with 60% AY had the lowest mean slope values. For all the nonmeat proteins except SPI, mean rupture force values and slope values decreased with increasing levels of the protein indicating deterimental effects of the nonmeat proteins on frankfurter firmness and behavior in shear. Comer (1979) reported negative effects of six nonmeat ingredients on meat emulsion texture. Other workers have likewise reported adverse textural effects of nonmeat ingredients on meat emulsions (Smith et al., 1973; Comer and Dempster, 1981). Soy protein isolate may be an exception to this generalization in that increasing levels of SPI did not reduce rupture force and firmness. Sofos et al. (1977) reported that SPI improved the texture of weiners that contained high levels of textured soy protein. At some higher levels of incorporation, SPI may not have the adverse textural effects associated with other nonmeat proteins.

The effects of the nonmeat proteins on emulsion stability was monitored by the three different methods shown in Table 3. The quantities of fat released by treatments 60% SF, 60% AY and 20% MPH were significantly higher ($P < 0.05$) than all meat controls as measured by the modified Saffle technique. The amounts of fat released by treatments 40% SF, 60% SF, 20% MPH, 60% MPH and 60% AY were significantly higher ($P < 0.05$) than all meat controls as measured by the cooking bag technique. The third emulsion stability test indicated that all meat controls released significantly less ($P < 0.05$) liquid (more stable) than treatments 40% SPI, 60% SPC, 60% SF, 40% AY and 60% AY. All three methods suggested that the 60% AY and 60% SF treatments were the least stable. At the 40% protein exchange level, the cooking bag method indicated that the soy flour treatment was less stable than most other treatments while the filter paper method gave the same indication for autolyzed yeast. The protein additive most difficult

Table 4—Correlation coefficients of emulsion stability characteristics and compression values

	Liquid release (cm^2)	Fat re eased (mL)[a]	Fat released (mL)[b]	Water released (mL)[b]	Total Liquid released (mL)[b]	Rupture force (g)	Slope (g/mm)
Liquid released (cm^2)	1.00	0.33**	0.30**	0.04	0.13	−0.37***	−0.39***
Fat released (mL)[a]	0.33**	1.00	0.84***	−0.10	0.15	−0.12	−0.12
Fat released (mL)[b]	0.30**	0.84***	1.00	−0.01	0.28**	−0.21	−0.19
Water released (mL)[b]	0.04	−0.10	−0.01	1.00	0.95***	−0.19	−0.13
Total liquid (mL)[b]	0.13	0.15	0.28**	0.95***	1.00	−0.24*	−0.18
Rupture force force (g)	−0.37**	−0.12	−0.21	−0.19	−0.24*	1.00	0.96***
Slope (g/mm)	−0.39***	−0.12	−0.19	−0.13	−0.18	0.96***	1.00

[a] Modified Saffle method

[b] Cooking bag method

* Significant at $P < 0.05$ level; ** Significant at $P < 0.01$ level; *** Significant at $P < 0.001$ level

to characterize in terms of emulsion stabilizing effects is the MPH. Two of the stability tests showed that treatments containing MPH were less stable than all meat controls. The filter paper technique, however, indicated that treatments with MPH were among the most stable.

From the data in Table 3, it is apparent that soy flour and autolyzed yeast were least effective in emulsion stabilization. The NFDM and SPI appear to have the greatest emulsion stabilizing effects with SPC and MPH intermediate in their emulsion stabilizing performance. Rakosky (1974) suggested that SPI ranks above all other nonmeat proteins in its ability to stabilize fat in a cooked emulsion. Lauck (1975) reported the superior fat-binding performance of SPI, NFDM and whey proteins over meat protein. Holland (1984) also reported that NFDM increased cook stability. The superior performance of SPI in meat emulsions has been attributed to its significant gel-forming ability. SPC, on the other hand, has limited gel-forming ability due to its fiber content (Johnson, 1970). It seems likely that different protein additives affect different functional aspects of meat emulsion stabilization. Some proteins probably perform better than others due to superior gel-forming ability while others positively influence emulsifying capacity in meat-nonmeat protein mixtures.

Bind value constants for nonmeat proteins studied were calculated based on the Saffle (1966) bind value system. A percent emulsion stabilizing efficiency for each nonmeat protein was determined by comparing the ml of fat released by the all meat formulation to ml of fat released by the 60% protein replacement treatments for each additive (modified Saffle method). This percent emulsion stabilizing efficiency was multiplied by the bind value constant for bull meat (30.01) to arrive at constants for the nonmeat proteins. The bind value constants were: nonfat dry milk, 27.5; soy protein isolate, 17.6; milk protein hydrolysate, 15.8; soy protein concentrate, 13.0; autolyzed yeast, 8.3; soy flour, 7.6. These constants will vary with manufacturer of each protein.

The problem of estimating the functional contribution of nonmeat ingredients in meat emulsion stabilization exists in part due to a lack of reliable methods of testing their performance. Meat emulsions with additives are usually cooked in a container, resulting cook juices collected, and the quantity of fat and water-soluble protein quantified on a 100 g batter basis. The cooking bag method used in this study is an example of this and is similar to methods used by Townsend et al. (1968), Brown (1972) and others. Table 4 shows the correlation coefficients of the emulsion stability methods as well as the correlation coefficients of these methods with compression values. The cooking bag method, quantifying fat released, was highly correlated ($P < 0.001$) with the modified Saffle method of quantifying fat release. The cooking bag method was also highly correlated ($P < 0.01$) with the liquid released in the filter paper test though a relatively low R^2 value for the two methods would be obtained. No correlation between fat and water released in the cooking bag method existed. Thus, no relationship between water-holding and fat retention through cooking existed in the meat emulsions. This filter paper test may prove useful in monitoring the stability of finished frankfurters.

CONCLUSIONS

BIND VALUE CONSTANTS (Saffle system) for the six nonmeat proteins of specified brand names were calculated.

Those bind value constants were: nonfat dry milk, 27.5; isolated soy protein, 17.6; milk protein hydrolysate, 15.8; soy protein concentrate, 13.0; autolyzed yeast, 8.3; soy flour, 7.6. All the nonmeat proteins studied, with the exception of SPI, showed decreasing mean values for firmness and rupture force, with increasing levels of the additive. It should be recognized that the bind values for the nonmeat proteins reported in this research are for the particular proteins identified by brand names earlier in the paper. Nonmeat proteins, particularly milk protein, may vary in their ability to emulsify fat depending on the way they are manufactured. Their functional character may be considered an emulsifying characteristic of the protein (Tornberg and Hermansson, 1977). Milk proteins in their different forms are unique in the sense that they possess specify technological functionality (Hoogenkamp, 1986). This means that the values reported herein are specific for the proteins tested and may not apply to other similar proteins manufactured under different conditions. A method of objectively estimating the stability of finished frankfurters is presented which may prove useful in estimating the functional contributions of other nonmeat proteins.

REFERENCES

Acton, J.C., Ziegeler, G.R., and Burge, D.L. 1983. Functionality of muscle constituents in the processing of comminuted meat products. CRC Crit. Rev. Food Sci. Nutr. 18(2): 99.

AOAC. 1975. "Official Methods of Analysis," 12th ed., 2nd Suppl. Sec. 24. BO4. Association of Official Analytical Chemists, Washington, DC.

Borchert, L.L., Greaser, M.L., Bard, J.C., Cassens, R.G., and Briskey, E.J. 1967. Electron microscopy of a meat emulsion. J. Food Sci. 32: 419.

Brown, D.D. 1972. A study of factors affecting stability and quality characteristics in sausage systems. Ph.D. dissertation, Univ. of Georgia, Athens, GA.

Comer, F.W. 1979. Functionality of fillers in communited meat products. Can. Inst. Food Sci. Technol. J. 12(4): 157.

Comer, F.W. and Dempster, S. 1981. Functionality of fillers and meat ingredients in comminuted meat products. Can Inst. Food Sci. Technol. J 14(4): 295.

Decker, C.D. 1985. Personal communication, Ralston Purina, St. Louis, MO.

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

Goodnight, J.H. 1982. "SAS User's Guide: Statistics, 1982 Edition," SAS Institute, Inc., Cary, NC.

Hansen, L.J. 1960. Emulsion formation in a finely comminuted sausage. Food Technol. 14(11): 565.

Helmer, R.L. and Saffle, R.L. 1963. Effect of chopping temperature on the stability of sausage emulsions. Food Technol. 17(9) 115.

Hermansson, A.M. 1975. Functional properties of added proteins correlated with properties of meat systems: Effect of concentration and temperature on water-binding properties of model meat systems. J. Food Sci. 40: 595.

Holland, G.C. 1984. A meat industry perspective on the use of dairy ingredients. Proc. Conf. Canadian Dairy Ingredients in the Food Industry, Ottawa.

Hoogenkamp, H.W. 1986. "Milk Proteins in Meat and Poultry Products." De Melkindustrie Veghel by, P.O. Box 13, 5460 BH Veghel, The Netherlands.

Ivey, F.J., Webb, N.B., and Jones, V.A. 1970. The effect of disperse phase droplet size and interfacial film thickness on emulsifying capacity and stability of meat emulsions. Food Technol. 24(11): 91.

Johnson, D.W. 1970. Functional properties of oilseed proteins. J. Amer. Oil Chem. Soc. 47: 402.

Kinsella, J.E. 1979. Functional properties of soy proteins. J. Amer. Oil Chem. Soc. 56: 242.

Lauck, R.M. 1975. The functionality of binders in meat emulsions. J. Food Sci. 40: 736.

Meyer, J.A., Brown, W.L., Giltner, N.E., and Guinn, J.R. 1964. Effect of emulsifiers on the stability of sausage emulsions. Food Technol. 18: 1796.

Porteus, J.D. 1979. Some physico-chemical constants of various meats for optimum sausage formulation. Can. Inst. Food Sci. Technol. J. 12(3): 145.

Porteus, J.D. and Quinn, J.R. 1979. Functional property measurement of mixtures of meat and extender proteins. Can. Inst. Food Sci. Technol. J. 12(4): 203.

Rakosky, J. Jr. 1974. Soy grits, flour, concentrates and isolates in meat products. J. Amer. Oil Chem. Soc. 51: 123A.

Rongey, E.H. and Bratzler, L.J. 1966. The effect of various binders and meats on the palatability and processing characteristics of bologna. Food Technol. 20: 1228.

Saffle, R.L. 1966. Linear programming-meat blending. IBM Publication No. E20-0161-0. White Plains, New York.

Saffle, R.L., Christian, J.A., Carpenter, J.A., and Zirkle, S.b. 1967. Rapid method to determine stability of sausage emulsions and effects of processing temperatures and humidities. Food Technol. 21(5): 100.

Schmidt, R.H. 1981. Gelation and coagulation. In "Protein Functionality in Foods," (Ed.). Cherry, J.P. ACS Symposium Series 147. p. 131. Am. Chem. Soc., Washington, DC. p. 131.

Schweiger, R.G. 1974. Soy protein concentrates and isolates in comminuted meat systems. J. Amer. Oil Chem. Soc. 51: 192A.

Smith, G.C., Juhn, H., Carpenter, Z.L., Mattil, K.F., and Cater, C.M. 1973. Efficacy of protein additives as emulsion stabilizers in frankfurters. J. Food Sci. 38: 849.

Sofas, J.N. and Allen, C.E. 1977. Effects of lean meat source and levels of fat and soy protein on properties of weiner-type products. J. Food Sci. 42: 875.

Sofos, J.N., Noda, I., and Allen, C.E. 1977. Effects of soy proteins and their levels of incorporation on the properties of winer-type products. J. Food Sci. 42: 879.

Swift, C.E., Locker, C., and Frayar, A.J. 1961. Comminuted meat emulsions: the capacity of meats for emulsifying fat. Food Technol. 15: 468.

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Influence of Polyphosphate on Storage Stability of Restructured Beef and Pork Nuggets

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ABSTRACT

Influence of polyphosphate on sensory and chemical characteristics of battered and breaded, cooked, restructured beef and pork nuggets was evaluated over 20 wk storage. Beef and pork nuggets manufactured with polyphosphate had lower ($P<0.05$) initial thiobarbituric acid (TBA) values than those manufactured without polyphosphate (controls); and values remained lower ($P<0.05$) than control treatments over 20 wk of storage. Sensory panelists detected less ($P<0.05$) off-flavor in pork nuggets manufactured with polyphosphate than nuggets in control treatments. Detection of off-flavor in pork nugget control treatments may be attributed to higher TBA values (increased lipid oxidation) in the pork product. Addition of polyphosphate protected nuggets from lipid oxidation and subsequent off-flavor development.

INTRODUCTION

THE USE OF polyphosphates to increase the water-holding ability of meat has been documented (Hamm, 1960; Offer and Trinick, 1983). Offer and Trinick (1983) showed that polyphosphate helps increase water retention by decreasing the amount of salt needed to generate fiber swelling. They reported that cooking losses can be attributed to fiber shrinkage, but did not study effect of phosphate or salt on moisture loss during cooking. Trout and Schmidt (1984) showed that polyphosphate in combination with salt increased cook yield in a comminuted product.

Intrinsic factors that cause oxidative rancidity (lipid oxidation) can be controlled, but factors that occur naturally in meat are difficult to control; factors such as metal ions, heme pigments, and the ultimate low pH of meat promote lipid oxidation. Polyphosphates have been shown to protect meat lipids from oxidation (Watts, 1954; Tims and Watts, 1958; Matlock et al., 1984; Smith et al., 1984). Schwartz and Mandigo (1976) indicated that increased polyphosphate concentration (in products containing salt) did not have an increased beneficial effect on the protection of lipids from oxidation.

The number and type of breaded commercial meat products is rapidly increasing and the majority of these are precooked and frozen with breading material, prefried in oil, fully cooked and then stored in the frozen state. Brotsky (1976) reported that frozen, cooked, and breaded broiler parts were protected from the development of rancid flavors (as detected by sensory panel) by the use of polyphosphate. Oxidative deterioration and flavor stability are important considerations if these precooked products are to retain consumer acceptance. The new ruling by the USDA (1984) allows the addition of alkaline polyphosphate as a flavor protector in such meat products.

The objective of this study was to determine the effects of alkaline polyphosphate on sensory characteristics and lipid oxidation in restructured, battered and breaded, and precooked beef and pork nuggets.

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MATERIALS & METHODS

TWO EXPERIMENTS were conducted to determine the effect of alkaline polyphosphate addition and freezer storage on quality of battered and breaded, precooked meat products. The alkaline polyphosphate used for both studies was a commercially available polyphosphate (Kena), which contains approximately 85% sodium tripolyphosphate and 15% sodium hexametaphosphate. Treatments for each study consisted of the addition of 0.75% NaCl and either 0.0, 0.25, or 0.50% polyphosphate to the meat during the mixing stage of manufacture. Restructured beef and pork nuggets were manufactured for each treatment at a commercial processing company.

In Experiment one, restructured beef nuggets were manufactured from rounds and chucks from USDA Good graded carcasses. Rounds were deboned, trimmed free of fat and connective tissue and tenderized by passing twice through a reciprocating blade tenderizer (Bettcher, model TR-2) with a belt advance setting of 2.54 cm per advance. Meat was ground through a 2.54 cm plate and the ground product served as the lean component for the nuggets. Chucks were deboned and the deboned meat was passed through an Urschel Comitrol® equipped with a 270D head to form fine meat flakes. These meat flakes were then used for the fat component of the nuggets. Lean and fat meat components were combined to provide a product containing approximately 20% fat, mixed (approximately 3 min) to assure a homogenous mixture and then separated into three batches of 22.7 kg each. Salt and phosphate were added to meat batches at the level required for each treatment and then mixed in a Leland double action mixer until a tacky exudate was evident (approximately 8 min). Carbon dioxide snow was added to the mixture to reduce temperature to approximately 1°C.

Nuggets were formed using a forming machine (Formax 26) equipped with nugget shaped plates. Nuggets were then put through a four-phase batter/breader system (Specialty Products Div., General Mills Inc., Cleveland, OH), prefried, cooked in a counterflow oven to an internal temperature of 71.1°C, and individually quick frozen (−23°C). Nuggets were sealed in polyethylene bags and placed in wax-lined cartons, for storage (−23°C).

In Experiment two, fresh hams were used as the lean component and fresh picnics as the fat component in manufacturing pork nuggets, using the same procedure and formulation as for the beef nuggets in Experiment one. Treatments, manufacturing procedures, and storage time were identical to Experiment one.

Proximate composition

Frozen products (beef and pork nuggets) were placed in plastic foam shippers and transported via air to the Auburn University Meat Science Laboratory. Nuggets were stored at −23°C for the duration of the experiment (approximately 6 months). Coatings were removed from nuggets prior to proximate and expressible moisture analyses. Three nuggets per treatment were analyzed for moisture, fat, and protein following AOAC (1980) procedures. Expressible moisture of the cooked products was determined on 3 nuggets per treatment following the procedure of Wierbicki and Deatherage (1958).

Sensory evaluation

Twelve nuggets per treatment were examined every 4 wk for 20 wk of storage. Nuggets were heated for 12 min at 182°C in a Blodgett convection oven. Nuggets were evaluated by a trained (AMSA, 1978)

descriptive attribute panel for cohesiveness (1 = extremely uncohesive, 8 = extremely cohesive), juiciness (1 = extremely dry, 8 = extremely juicy), and off-flavors (1 = strong off-flavor, 8 = no off-flavor). Panelists were seated in a specially constructed room with a controlled atmosphere and free from noise and odors. Individual booths were lighted with low-intensity red light to mask possible cooked color differences. Nuggets were served warm (40°C) to 6 to 12 panelists.

Lipid oxidation

To determine lipid oxidation, three nuggets per treatment, per storage time, were ground in the frozen state through a 3.2 mm plate Kitchen Aid mixer (model # K45SS) with grinder attachments and analyzed by the thiobarbituric acid method (TBA) outlined by Tarladgis et al. (1960). Batter and breading were included from each nugget so that lipid oxidation of total nuggets were determined.

Statistical analysis

Both experiments were completely randomized designs that involved phosphate level and storage treatments in a factorial arrangement. Proximate composition and expressible moisture data were analyzed only before storing nuggets. Analysis of variance was performed on all data using the GLM procedure of SAS (1982). When the F-test was significant, differences between treatment means were determined using least significant difference (LSD) values (Cochran and Cox, 1966).

RESULTS & DISCUSSION

Proximate composition and expressible moisture

In Experiments one (beef nuggets) and two (pork nuggets), there were no differences ($P>0.05$) between treatments for moisture, fat, or protein percentage in the battered and breaded, cooked products (Table 1). Expressible moisture of the cooked products was not different ($P>0.05$) among treatments (Table 1). Polyphosphate had been shown to increase water retention and fiber swelling in raw meat (Offer and Trinick, 1983), but there were no differences in expressible moisture in the cooked products used in this study.

Sensory evaluation

Sensory panelists detected no differences ($P>0.05$) for off-flavor in beef nuggets between treatments (Table 2) or across storage time (Table 3). Thus, the concentration of polyphosphate had no effect on detectable off-flavors in beef nuggets.

Sensory panelists, however, detected differences ($P<0.05$) for off-flavor in pork nuggets between treatment (Table 2) and across storage time (Table 3). Smith et al. (1984) similarly showed that polyphosphate protected pork roasts from warmed-over flavor (off-flavor) as detected by a sensory panel. Analysis of variance procedures showed that there was an interaction ($P<0.05$) between the main effects, polyphosphate treatment and storage time, in the pork nugget experiment. In this study, significant differences were not detected between the control and polyphosphate treatments until after 16 weeks of storage (Table 4). Sensory panelists also detected increases in off-flavor ($P<0.05$) throughout storage time in the pork nugget control treatment (Table 4). Sensory panelist data for beef nuggets indicated that cohesiveness (texture) significantly increased ($P<0.05$) with the addition of polyphosphate (Table 2). Storage (−23 °C) of beef nuggets for 20 weeks had no detectable effect ($P>0.05$) on the texture (Table 3).

The addition of polyphosphate to pork nuggets increased ($P<0.05$) sensory panelists scores for cohesiveness (texture) of the cooked product, but there were no differences ($P>0.05$) between the 0.25% and 0.50% phosphate treatments (Table 2). These increases in cohesiveness for polyphosphate treated samples were similar to those of beef nuggets treated with polyphosphate, and were probably due to polyphosphate enhancement of myofibrillar protein extractability and the resultant increased bind (Romans et al., 1985).

Sensory panelists found no differences ($P>0.05$) in juiciness between treatments for beef nuggets (Table 2). However, there was a tendency for sensory panelists scores (beef nuggets) for juiciness to decrease ($P<0.05$) over storage time (Table 3). Sensory panelists detected differences in the pork nuggets between the control and the polyphosphate treatments for juiciness (Table 2). No differences ($P>0.05$) were found across storage time for cohesiveness or juiciness (Table 3).

Lipid oxidation

The addition of polyphosphates to beef nuggets protected lipids from oxidation, as indicated by lower TBA values ($P<0.05$), for polyphosphate treatments compared to the control treatment (Fig 1). This is in agreement with other researchers who have shown that the addition of phosphate to meat systems reduced lipid oxidation (Ramsey and Watts, 1963; Hayman et al. 1976; Keeton, 1983). There was no additional protection ($P>0.05$) of lipids from oxidation (lower TBA values) when the level of polyphosphate was increased from 0.25% to 0.50% (Fig 1). An observation of interest was the difference ($P<0.05$) in initial TBA values (Fig 1) between the control and the 0.25 and 0.50% polyphosphate treatments, indicating that polyphosphate may begin to protect cooked meat from lipid oxidation during the cooking process or very shortly thereafter. Younathan and Watts (1960) showed that cooking increased lipid oxidation. Other researchers showed that sodium chloride acts as a prooxidant in manufactured meat products and accelerates oxidative reactions of unsaturated lipids

Table 1—Proximate composition and expressible moisture of battered and breaded, cooked restructured beef and pork nuggets[a,b]

Nugget type	Polyphosphate (%) 0	0.25	0.50
Beef[c]			
Moisture (%)	51.86	50.07	49.42
Fat (%)	20.53	20.82	21.26
Protein (%)	14.03	12.72	14.93
Expressible moisture (ratio)[d]	0.82	0.85	0.81
Pork[c]			
Moisture (%)	48.43	48.34	51.31
Fat (%)	22.61	21.37	20.33
Protein (%)	14.23	13.37	13.63
Expressible moisture (ratio)[d]	0.72	0.76	0.76

[a] All values are expressed as means (n = 3).
[b] Coatings were removed from nuggets prior to analyses.
[c] No significant ($P>0.05$) differences between treatment means.
[d] Ratio of expressible free moisture to bound moisture.

Table 2—Influence of polyphosphate addition on the off-flavor, texture, and juiciness, as detected by sensory panelists, in battered and breaded, cooked, restructured beef and pork nuggets[a]

Nugget type	Polyphosphate (%) 0.00	0.25	0.50	LSD values[b]
Beef				
Off-flavor[c]	7.01	7.18	7.01	NS[h]
Texture[d]	5.10[f]	5.55[g]	5.96[g]	0.42
Juiciness[e]	5.17	5.80	5.70	NS[h]
Pork				
Off-flavor	6.05[f]	6.97[g]	7.26[g]	0.44
Texture	4.17[f]	5.24[g]	5.27[g]	0.46
Juiciness	6.17[f]	5.53[g]	5.64[g]	0.49

[a] All values are expressed as means.
[b] Least significant difference (LSD) values for the comparison of polyphosphate treatment means.
[c] Sensory panelists rated nuggets on a scale of 1 to 8 for off-flavor (1 = strong off-flavor, 8 = no off-flavor).
[d] Sensory panelists rated nuggets on a scale of 1 to 8 for texture (1 = extremely uncohesive, 8 = extremely cohesive).
[e] Sensory panelists rated nuggets on a scale of 1 to 8 for juiciness (1 = extremely dry, 8 = extremely juicy).
[f,g] Means in the same row bearing different superscripts differ ($P<0.05$).
[h] F-test not significant ($P>0.05$) for the main effect polyphosphate treatment.

Table 3—Influence of storage time on the off-flavor, texture and juiciness, as detected by sensory panelists, of battered and breaded, cooked, restructured beef and pork nuggets[a]

Nugget type	Weeks of storage						LSD values[b]
	0	4	8	12	16	20	
Beef							
Off-flavor[c]	7.36	6.87	6.83	7.19	7.14	7.00	NS[h]
Texture[d]	5.24	5.57	5.45	5.57	5.55	5.83	NS[h]
Juiciness[e]	5.91[f]	6.03[f]	6.13[f]	5.67[fg]	5.22[g]	5.50[fg]	0.64
Pork							
Off-flavor	7.18[f]	6.72[f]	6.86[f]	7.04[f]	6.89[f]	5.94[g]	0.57
Texture	4.81	5.05	4.97	4.72	5.17	4.61	NS[h]
Juiciness	6.03	5.82	6.07	5.85	5.40	5.50	NS[h]

[a] All values are expressed as means.
[b] Least significant difference (LSD) values for comparison of means by storage time.
[c] Sensory panelists rated nuggets on a scale of 1 to 8 for off-flavor (1 = strong off-flavor, 8 = no off-flavor).
[d] Sensory panelists rated nuggets on a scale of 1 to 8 for texture (1 = extremely uncohesive, 8 = extremely cohesive).
[e] Sensory panelists rated nuggets on a scale of 1 to 8 for juiciness (1 = extremely dry, 8 = extremely juicy).
[f,g] Means in the same row bearing different superscripts are significantly different ($P<0.05$).
[h] F-test not significant ($P>0.05$) for the main effect storage time.

Table 4—Influence of polyphosphate addition and storage time on the development of off-flavor, as detected by sensory panelists, in battered and breaded, cooked restructured beef and pork nuggets[a,b]

Nugget type	Polyphosphate (%)	Weeks of storage						LSD values[c]
		0	4	8	12	16	20	
Beef								
	0.00	7.91	7.20	6.75	6.85	7.33	6.00	
	0.25	7.18	7.00	6.88	7.43	7.11	7.50	NS[d]
	0.50	7.00	6.40	6.88	7.28	7.00	7.50	
LSD values[e]		NS[d]						
Pork								
	0.00	6.73	6.30	6.38	7.14	5.78	4.00	1.11
	0.25	7.18	6.60	6.88	7.14	7.22	6.83	1.11
	0.50	7.63	7.27	7.17	6.83	7.67	7.00	1.11
LSD values[e]		0.95	1.00	1.12	1.20	1.06	1.29	

[a] All values are expressed as means.
[b] Sensory panelists rated nuggests on a scale of 1 to 8 for off-flavor (1 = strong off-flavor, 8 = no off-flavor).
[c] Least significant difference (LSD) values for the comparisor of means by storage time within a given polyphosphate treatment and nugget type.
[d] F-test not significant ($P>0.05$) for the main effects, polyphosphate treatment and storage time.
[e] LSD values for the comparison of means between treatments for a given storage time within the nugget type.

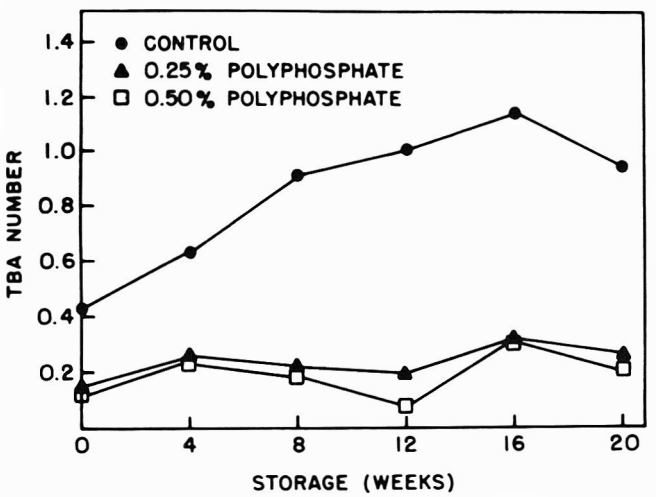

Fig. 1—Effect of polyphosphate on thiobarbituric acid (TBA) values (mg TBA reactive compounds/kg meat) of restructured beef nuggets during storage at −23°C. Each point is the mean of three samples. The least significant difference (LSD) value = 0.20.

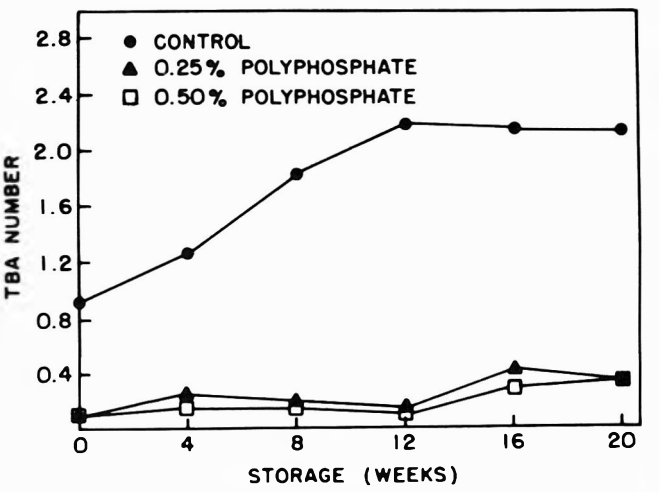

Fig. 2—Effect of polyphosphate on thiobarbituric acid (TBA) values (mg TBA reactive compounds/kg meat) of restructured pork nuggets during storage at −23°C. Each point is the mean of three samples. The least significant difference (LSD) value = 0.30.

and increases rancidity (Gray, 1978; Obann et al., 1980). Polyphosphate may protect lipids during the cooking processes from accelerated lipid oxidation due to salt and temperature.

Even though lipid oxidation (as determined by TBA values) was present in beef nuggets, sensory panelists could not detect off-flavors that would normally be correlated with lipid oxidation. The explanation for this is that there may be factors related to bovine muscle that prevent sensory detection or that the off-flavor components related to lipid oxidation in bovine muscle may have not been at high enough concentrations for the detection of differences.

TBA values for polyphosphate treated pork nuggets were lower ($P<0.05$) initially than the control and remained lower ($P<0.05$) throughout storage time (Fig. 2). TBA values for all treatments increased ($P<0.05$) over storage (−23 °C) time. This was in agreement with sensory panelist data (for pork

nuggets) where off-flavor differences ($P<0.05$) were detected between the control treatment and the two polyphosphate treatments after 16 weeks of frozen storage (Table 4).

Both sensory panelist data and TBA values (pork nuggets) indicated that the addition of polyphosphate protected lipids from oxidation. This was in agreement with results for beef nuggets, and also in agreement with several other studies where polyphosphates were shown to protect lipids from oxidation (Watts, 1954; Tims and Watts, 1958; Smith and Bowers, 1972; Hayman et al., 1976; Matlock et al., 1984; Smith et al., 1984).

SUMMARY & CONCLUSIONS

POLYPHOSPHATE ADDITION to restructured, cooked beef and pork nuggets had no effect on the total or expressible moisture in these experiments.

In beef and pork nuggets, thiobarbituric acid (TBA) values were initially lower ($P<0.05$) in the polyphosphate treatments than in control treatments and continued to remain lower over storage time. Sensory panelists detected less ($P<0.05$) off-flavor in pork nuggets manufactured with polyphosphate than the control treatment, but detected no differences ($P>0.05$) in the beef nuggets among treatments. The detection of off-flavor in the pork nugget control treatment may be attributed to the greater degree of lipid oxidation (higher TBA values) in the pork product. Addition of 0.25 or 0.50% polyphosphate to restructured, battered and bread, cooked, nugget products protected them from off-flavors and lipid oxidation. Therefore, it is recommended that polyphosphates be used in similar products to protect flavor. Even though polyphosphate addition had no effect on detectable off-flavors in beef, TBA values were lower in polyphosphate treated samples, which indicated that polyphosphates protected lipids from oxidation. The protective action, of polyphosphates may protect the flavor of similar beef products if stored frozen for longer periods of time.

REFERENCES

AMSA. 1978. "Guidelines for Cookery and Sensory Evaluation of Meat." American Meat Science Association, Chicago, IL.
AOAC. 1980. "Official Methods of Analysis," 13th ed. Association of Official Agricultural Chemists, Washington, DC.
Brotsky, E. 1976. Automatic injection of chicken parts with polyphosphate. Poultry Sci. 55: 653.
Cochran, W.G. and Cox, G.M. 1966. "Experimental Designs." John Wiley and Sons, Inc., New York.
Gray, J.I. 1978. Measurement of lipid oxidation: A review. J. Amer. Oil Chem. Soc. 55: 539.
Hamm, R. 1960. Biochemistry of meat hydration. Adv. Food Res. 10: 355.
Hayman, L.W., Brotsky, E., Danner, W.E., Everson, C.W., and Hammes, P.A. 1976. Frozen cooked meat antioxidant: Improved action of sodium tripolyphosphate with lemon juice concentrate. J. Food Sci. 41: 417.
Keeton, J.T. 1983. Effects of fat and NaCl/phosphate levels on the chemical and sensory properties of pork patties. J. Food Sci. 48: 878.
Matlock, R.G., Terrell, T.N., Savell, J.W., Rhee, K.S., and Dutson, T.R. 1984. Factors affecting properties of raw-frozen pork sausage patties made with various NaCl/phosphate combinations. J. Food Sci. 49: 1363.
Obann, Z.A., Ledward, D.A., and Lawrie, R.A. 1980. Lipid-protein interactions as agents of quality deterioration in intermediate moisture meats: an appraisal. Meat Sci. 4: 79.
Offer, G. and Trinick, J. 1983. On the mechanism of water holding in meat. The swelling and shrinking of myofibrils. Meat Sci. 8: 245.
Ramsey, M.B. and Watts, B.M. 1963. The antioxidant effects of sodium tripolyphosphate on pH, swelling and water-holding capacity of beef. J. Food Sci. 37: 360.
Romans, J.T., Costello, W.J., Jones, K.W., Carlson, C.W., and Ziegler, P.T. 1985. "The Meat We Eat," 11th ed. The Interstate Printers and Publishers, Inc., Danville, IL.
SAS User's Guide. 1982. SAS Inst. Inc., Cary, NC.
Schwartz, W.C. and Mandigo, R.W. 1976. Effect of salt, sodium tripolyphosphate and storage on restructured pork. J. Food Sci. 41: 1266.
Smith, M.L. and Bowers, J.A. 1972. Effects of a polyphosphate salt on eating quality of precooked-reheated and freshly cooked turkey roulades stored 4 and 8 weeks. J. Poultry Sci. 51: 998.
Smith, L.A., Simmons, S.L., McKeith, F.K., Bechtel, P.J., and Brady, P.L. 1984. Effects of sodium tripolyphosphate on physical and sensory properties of beef and pork roasts. J. Food Sci. 49: 1636.
Tarladgis, B.G., Watts, B.M., and Younathan, M.T. 1960. A distillation method for the quantitative determination of malonaldehyde in rancid foods. J. Amer. Oil Chem. Soc. 37: 44.
Tims, M. and Watts, B.M. 1958. The protection of cooked meat with phosphates. Food Technol. 12: 240.
Trout, G.R. and Schmidt, G.R. 1984. Effect of phosphate type and concentration, salt level and method of preparation on binding in restructured beef rolls. J. Food Sci. 49: 687.
USDA. 1984. "Meat and Poultry Inspection Regulations." United States Department of Agriculture, Washington, DC.
Watts, B.M. 1954. Oxidative rancidity and discoloration in meat. Adv. Food Res. 5: 1.
Wierbicki, E. and Deatherage, F.E. 1958. Determination of water-holding capacity of fresh meats. J. Agr. Food Chem. 6: 387.
Younathan, M.T. and Watts, B.M. 1960. Oxidation of tissue lipids in cooked pork. Food Res. 25: 538.
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Tauber, F.W. and Lloyd, J.H. 1947. Variations in composition of frankfurters with special references to cooking changes. Food Res 12: 158.
Terrell, R.N., Brown, J.A., Carpenter, Z.L., Mattil, K.F., and Monagle, C.W. 1979. Effects of oilseed proteins, at two replacement levels, on chemical, sensory and physical properties of frankfurters. J. Food Sci. 44: 865.
Theno, D.M. and Schmidt, G.R. 1978. Microstructural comparisons of three commercial frankfurters. J. Food Sci. 43: 845.
Torgersen, H. and Toledo, R.T. 1977. Physical properties of protein preparations related to their functional characteristics in comminuted meat systems. J. Food Sci. 42: 1615.
Tornberg, E. and Hermansson, A.M. 1977. Functional characterization of protein stabilized emulsions: effect of processing. J. Food Sci. 42: 468.
Townsend, W.E., Witnauer, L.P., Riloff, J.A., and Swift, C.E. 1968. Comminuted meat emulsions: Differential thermal analysis of fat transitions. Food Technol. 22: 319.
Voisey, P.W., Randall, C.J. and Larmond, E. 1975. Selection of an objective test of weiner texture by sensory analysis. Can. Inst. Food Sci. Technol. J. 8(1): 24.
Ziegler, G.R. and Acton, J.C. 1984. Mechanisms of gel formation by proteins of muscle tissue. Food Technol. 38(5): 77.
Ms received 11/6/86; revised 11/1/86; accepted 11/4/86.

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Palatability and Storage Characteristics of Precooked Pork Roasts

S. L. JONES, T. R. CARR, and F. K. McKEITH

ABSTRACT

Pairs of boneless pork loins (n = 10) were pumped to contain 0.5% tripolyphosphate or water and allotted to three treatments: PVC wrapped, convection oven cookery (CO); vacuum packaged, precooked and reheated in a water bath (PC); and vacuum packaged and cooked in a water bath (VP). Paired loins (n = 30) were injected with tripolyphosphate and allotted to the following treatments and storage (0, 14 or 28 days) conditions: Control (−20°C) [CO]; precooked (−20°C) [PCFR]; precooked (4°C) [PCRF]; vacuum packaged (−20°C) [VPFR]. VP and PC phosphate injected roasts were more tender and had lower cooking losses than CO roasts. After 28 days, al PC roasts had lower microbial counts than other treatments, and PC and VP roasts had improved palatability compared with CO roasts.

INTRODUCTION

THE DEMAND for quick, easy to prepare foods has increased in the last decade. Precooked meat products utilizing cook-in-the-bag technology may meet these criteria. Cook-in-the-bag processing using hot water bath cookery has numerous advantages over conventional oven cookery. Woodams and Nowery (1968) reported that the heat transfer coefficient between water and muscle tissue was approximately twenty times greater than between air and muscle tissue. Low temperature/long time cooking processes increase tenderness and decrease cooking losses (Bramblett et al., 1959; Woolsey and Paul, 1969; Bayne et al., 1969; Bouton and Harris, 1972, 1981; Harrison, 1975; Leander et al., 1980). Dinardo et al. (1984) demonstrated that beef samples prepared in a water bath and held for an additional 4 h past their temperature endpoint had lower cooking losses than those samples cooked to the same endpoint temperature in a conventional oven. Buck et al. (1979) reported more rapid heat penetration, decreased microbiological load, cost reduction, increased tenderness, increased yield and a more uniform degree of doneness in beef roasts cooked in a water bath. Miller et al. (1985) stated that precooked pork chops had improved tenderness and juiciness.

Precooked pork has been shown to develop warmed-over flavor when it is reheated (Tims and Watts, 1958). Smith et al. (1984) reported that the use of tripolyphosphates in pork roasts reduced warmed-over flavor and improved most sensory properties. Furthermore, it is well documented that tripolyphosphates possess antioxidant properties (Keeton, 1983) and reduce cooking losses (Mahon et al., 1970).

The present study was designed to evaluate cook-in-the-bag processing and the effect of tripolyphosphates on sensory properties and shelf life of fresh-frozen and precooked pork roasts for consumer use.

MATERIALS & METHODS

TEN PAIRED boneless pork loins of similar quality were selected from carcasses of market weight hogs. Loins were trimmed to approximately 0.6 cm of fat, wrapped in parchment paper and stored at 4°C.

Forty-eight hours prior to evaluation, one loin from each pair was pumped to 10% of its weight with a 5% tripolyphosphate solution using a Smith pokamat injector. The second loin of each pair was pumped to 10% of its weight with water. Each loin was then cut into three roasts, 15 cm in length, which were each allotted randomly to one of three cooking treatments: (1) convection oven cooking (CO); (2) vacuum packaged-water bath cookery (VP); (3) vacuum packaged-precooked and reheated water bath cookery (PC). Prior to vacuum packaging, a dial thermometer (Koch, 1 in. dial face) was placed approximately in the geometric center of each roast so that temperature could be monitored during cooking. The roasts were vacuum packaged in heat shrinkable Cryovac (CN 350) cooking bags using a heat seal vacuum packaging machine (Multivac AGW). The control roasts were placed on styrofoam trays and overwrapped with polyvinyl chloride film [(PVC) Goodyear Prime Wrap]. All roasts were stored for 24 hr at 4°C in an effort to allow equilibration of the phosphate solution.

Twenty-four hours prior to sensory evaluation, the roasts in the precooked treatment were heated in a 71°C water bath to an internal temperature of 65°C. They were then stored for 24 hr at 4°C.

Vacuum packaged roasts were cooked or reheated for sensory evaluation in a 100°C water bath to an internal temperature of 70°C. The control roasts were cooked conventionally in a 170°C South Bend Convection oven to an internal temperature of 70°C and monitored with copper constantan thermocouples and a Campbell Scientific CR5 recorder.

An experienced, six-member sensory evaluation panel evaluated the pork roasts for juiciness, tenderness, pork flavor intensity, and off-flavor using a continuous scale ranging from 0 – 15, where 0 = extremely tough, dry, bland or extreme off-flavor, and 15 = extremely tender, juicy, flavorful, or no off-flavor. Panelists consisted of staff and graduate students in the program who had sensory experience. Panelists were familiarized with the ballot prior to evaulation. Samples were prepared by cutting 1.3 × 1.3 × 1.3 cm cubes. Two cubes were served warm to panelists in individual booths with red lighting.

Total cooking loss was calculated using the pumped weight of the roast before packaging minus the cooked weight of the roast after it was removed from the cooking bag or the oven.

Determinations of Warner-Bratzler shear (WBS) values, proximate analysis, pH, and percent free water were made on the cooked samples after cooling to room temperature. A minimum of three cores (1.27 cm diameter) were removed from each roast for Warner-Bratzler shear value determinations and two shears were made per core. Moisture and pH determinations were made via the method described by Koniecko (1979). Fat determinations were made on the closely trimmed longissimus dorsi muscle via a modified soxhlet extraction utilizing chloroform:methanol (2:1) as described by Riss et al. (1983). Percent free water determinations were made using the procedure described by Wierbicki and Deathcrage (1958).

The second phase of this project involved a shelf life study.

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Table 1—Palatability attributes of pork roasts prepared using different cookery methods

	Treatment					
	CO[a]		PC[a]		VP[a]	
	Phosphate	Control	Phosphate	Control	Phosphate	Control
Tenderness[b]	10.92^{xyz}	10.00^{z}	12.26^{x}	11.80^{xy}	11.73^{xy}	10.86^{yz}
Juiciness[b]	8.74^{xy}	7.99^{y}	8.93^{xy}	7.90^{y}	9.99^{x}	8.76^{xy}
Pork flavor intensity[b]	10.14	9.57	9.89	9.16	10.01	9.63
Off-flavor intensity[c]	13.36	13.17	13.16	13.19	12.28	13.04
WBS (kg)	2.44^{xy}	2.56^{x}	1.91^{xy}	2.26^{xy}	1.80^{y}	1.91^{xy}

[a] CO = convection oven cookery; PC = vacuum packaged-precooked and reheated using water bath cookery; VP = vacuum packaged water bath cookery.
[b] Means derived from sensory panel scores with possible range from 0-15, where 0 = extremely tough, dry or bland, and 15 = extremely tender, juicy or flavorful.
[c] Means derived from sensory panel scores with possible ranges from 0-15, where 0 = extreme off-flavor and 15 = no off-flavor.
^{xyz} Mean values in same row bearing unlike superscripts differ significantly ($P<0.05$).

Thirty paired boneless pork loins were pumped to 10% of their weight with a 5% tripolyphosphate solution. Each loin was cut into two roast 18 cm in length. The four roasts from each pair of loins were randomly allotted to four treatments: (1) PVC wrapped, stored at −20°C and then conventionally cooked (CO); (2) precooked in a vacuum cooking bag, stored at 4°C and then warmed in the bag (PCRF); (3) fresh roast stored at −20°C in a vacuum cooking bag and cooked in the same bag (VPFR); (4) precooked in a vacuum cooking bag, stored at −20°C and then warmed in the bag (PCFR). Portions of the loins not cut into roasts were allotted to the above four treatments and used for microbiological evaluation (total plate counts). Roasts were stored for 0, 14 or 28 days and ten paired loins (N = 40 roasts) were represented per storage period.

As in Experiment 1, all roasts were evaluated by an experienced six-member taste panel for juiciness, tenderness, pork flavor intensity and off-flavor using the procedures previously discussed. Cooking loss, WBS and proximate analysis were also performed. The 2-thiobarbituric acid (TBA) reactive products from each roast were determined according to the method described by Witte et al. (1970).

For microbiological analysis, 10g samples were aseptically removed, weighed annd placed in sterile stomacher bags containing 90 mL sterile Lactose Broth. The samples were homogenized for 30 sec. Triplicate spread-plates of Plate Count Agar were made using appropriate dilutions and incubated at 35°C for 24 hr prior to enumeration of bacterial colonies.

Results of the sensory evaluation and the physical and chemical analysis were analyzed using the Statistical Analysis System (SAS, 1982) for analysis of variance and Duncan's mean separation.

RESULTS & DISCUSSION

MEANS for palatability attributes of roasts prepared using different cooking methods are presented in Table 1. No differences ($P>0.05$) in sensory characteristics were observed between the phosphate and control treatments within each cooking treatment; however, differences ($P<0.05$) were observed between cooking treatments. The PC roasts and the VP, phosphate treated roasts were more tender ($P<0.05$) than the CO, control roasts. Furthermore, the VP, phosphate roasts were more juicy ($P<0.05$) than both the CO controls and the PC controls and had lower WBS values ($P<0.05$) than the CO, control roasts. These results were similar to the results of Smith et al. (1984) who used tripolyphosphates on pork and Buck et al. (1979) who used cook-in-the-bag processing on beef roasts to achieve a more tender, juicy product. In addition, work done by Miller et al. (1985) concurred that precooked pork was more tender and juicy than conventionally prepared pork.

All phosphate-treated roasts had higher ($P<0.05$) pH's than the control roasts (Table 2) and the VP, phosphate roasts had a higher ($P<0.05$) percentage of free water than the PC, control roasts which corresponds to the difference noted by the sensory panel in perceived juiciness between the two treatments. The PC phosphate-treated roasts and both VP treatments had lower ($P<0.05$) cooking losses than the CO, control roasts. The reduced cooking losses in the phosphate treated roasts agreed with the results of Mahon et al. (1970). Phosphate addition has been shown to improve moisture retention which reduces cooking loss (Sherman, 1961; Hamm, 1970; Shults et al., 1972).

Palatability attributes of roasts stored for three different time periods are presented in Table 3. After 14 days of storage, the roasts which were vacuum packaged and then frozen were more juicy ($P<0.05$) than the conventional and precooked frozen roasts. However, after 28 days the PCFR treatment was less juicy than other treatments. PCRF roasts were more tender than control roasts at 14 days. PCFR tenderness decreased ($P<0.05$) from day 0 to 28. The PCRF roasts and the VPFR roasts had lower ($P<0.05$) pork flavor intensity scores after the 28 days of storage. However, no differences in off-flavor or WBS value were observed ($P>0.05$). These data agree with that of Miller et al. (1985) whose sensory data indicated that flavor scores decreased slightly in precooked pork chops stored for 28 days and that pork chops treated with antioxidants exhibited limited off-flavor. Results from the current study indicate that sensory characteristics are acceptable in precooked pork products treated with antioxidant after 28 days of refrigerated or frozen storage.

Mean of muscle characteristics of pork roasts stored for different time periods appear in Table 4. Cooking loss was higher ($P<0.05$) in control roasts stored for 28 days than in control roasts stored for 0 days. The amount of TBA reactive products did not change ($P>0.05$) over time indicating that oxidative rancidity and/or warmed over flavor was not a problem under these conditions. Matlock et al. (1984) reported similar results when storing precooked sausage patties treated with antioxidant for up to 8 wks. TBA values on vacuum packaged patties did not change over time, indicating that vacuum packaging and phosphate treatment are effective methods for controlling rancidity in precooked products. Differences between treatments followed the same trends that were observed in Experiment 1. Cooking losses of the precooked roasts were similar to the control roasts, while the VPFR roasts had less ($P<0.05$) cooking loss.

Total plate count means from microbiological analysis of the pork roasts over the three storage periods are presented in Table 5. Both precooked treatments had less than 100 colonies per gram tissue after 28 days storage. This advantage in the precooked roasts is due to the precooking of the roasts after being vacuum packaged. The majority of the microbes present were inactivated at the beginning of the storage period and no recontamination occurred.

CONCLUSION

PRECOOKING PORK ROASTS provides consumers with a fast, easy way to prepare pork product with tenderness, juiciness and flavor properties similar to those of a conventional, oven prepared, pork roast. Precooking did not increase cooking losses and the palatability attributes of the precooked roasts were as good or better than conventionally prepared roasts at all storage periods. Precooking pork roasts in a vacuum cook-

Table 2—Muscle characteristics of pork roasts prepared using different cookery methods

	Treatment					
	CO[a]		PC[a]		VP[a]	
	Phosphate	Control	Phosphate	Control	Phosphate	Control
pH	5.74[w]	5.52[x]	5.76[w]	5.56[x]	5.75[w]	5.48[x]
Free water (%)	35.75[wx]	35.91[wx]	38.59[wx]	34.55[x]	43.78[w]	41.52[wx]
Cooking loss (%)	26.06[wx]	28.22[w]	22.85[xy]	26.80[wx]	17.50[z]	20.80[yz]

[a] CO = convection oven cookery; PC = vacuum packaged-precooked and reheated using water bath cookery; VP = vacuum packaged water bath cookery.
[wxyz] Mean values in same row bearing unlike superscripts differ (P<0.05).

Table 3—Palatability attributes of pork roasts cooked using different methods and stored for 0, 14 and 28 days

		Cooking treatment			
	Day	CO[a]	PCRF[a]	PCFR[a]	VPFR[a]
Juiciness[b]	0	9.44	8.45	7.98	9.05
	14	8.36[ef]	9.04[de]	6.99[f]	10.04[d]
	28	8.64[d]	8.63[d]	6.64[e]	8.61[d]
Tenderness[b]	0	10.78	11.75	[x]11.63	10.98
	14	10.04[e]	11.69[d]	[xy]10.40[de]	11.42[de]
	28	10.86	10.61	[y] 9.94	10.22
Pork flavor intensity[b]	0	11.75	[x]11.81	11.13	[x]11.80
	14	11.32	[xy]11.20	10.70	[xy]11.16
	28	11.12	[y]10.76	10.10	[y]10.76
Off-flavor intensity[c]	0	14.02	14.06	14.17	13.96
	14	13.80	13.55	14.37	13.64
	28	13.50	13.82	13.52	13.69
WBS (kg)	0	2.53	2.39	2.32	2.34
	14	2.40	2.32	2.27	2.06
	28	2.52	2.83	2.53	2.32

[a] CO = PVC wrapped, −20°C storage, convection oven cookery. PCRF = Precooked in vacuum bag, 4°C storage, reheated in the bag. PCFR = Precooked in vacuum bag, −20°C storage, reheated in the bag. VPFR = Fresh roast vacuum bag, −20°C storage, cooked in the bag.
[b] Means derived from sensory panel scores with possible range from 0–15, where 0 = extremely tough, dry or bland, and 15 = extremely tender, juicy or flavorful.
[c] Means derived from sensory panel scores with possible range from 0–15, where 0 = extreme off-flavor and 15 = no off-flavor.
[def] Mean values in same row bearing unlike superscripts differ significantly (P<0.05).
[xy] Mean values in same column bearing unlike superscripts differ significantly (P<0.05).

Table 4—Muscle characteristics of pork roasts cocked using different cookery methods

		Cooking treatment			
	Day	CO[a]	PCRF[a]	PCFR[a]	VPFR[a]
Cooking loss (%)	0	[y]25.38[bc]	22.86[c]	[xy]27.41 [b]	18.24[d]
	14	[xy]28.47[b]	23.53[bc]	[y]24.22[bc]	19.58[c]
	28	[x]29.01[bc]	24.96[cd]	[x]31.36[b]	22.95[d]
TBA (mg/kg)	0	0.225[bc]	0.212[bc]	0.395[b]	0.096[c]
	14	0.202	0.209	0.385	0.253
	28	0.211	0.238	0.338	0.307
Water (%)	0	[xy]64.7	63.1	61.6	65.3
	14	[y]63.2	62.6	61.7	64.7
	28	[x]66.2[b]	64.7[bc]	63.4[c]	66.3[b]
Fat (%)	0	7.8	8.9	9.8	7.9
	14	9.9[b]	6.8[c]	8.7[bc]	7.4[bc]
	28	7.4	7.5	8.0	7.4

[a] CO = PVC wrapped, −20°C storage, convection oven cookery. PCRF = Precooked in vacuum bag, 4°C storage, reheated in the bag. PCFR = Precooked in vacuum bag, −20°C storage, reheated in the bag. VPFR = Fresh roast vacuum bag, −20°C storage, cooked in the bag.
[bcd] Mean values in same row bearing unlike superscripts differ significantly (P<0.05).
[xy] Mean values in same column bearing unlike superscripts differ significantly (P<0.05).

Table 5—Microbiological total plate counts (log/g) of pork roasts prepared using different cookery methods and stored for 0, 14 and 28 days

	Cooking treatment			
Day	CO[a]	PCRF[a]	PCFR[a]	VPFR[a]
0	[xy]5.13[b]	<2.00[c]	<2.00[c]	4.93[b]
14	[x]5.18[b]	<2.00[c]	<2.00[c]	5.17[b]
28	[y]4.76[c]	<2.00[d]	<2.00[d]	5.19[b]

[a]CO = PVC wrapped, −20°C storage, convection oven cookery. PCRF = Precooked in vacuum bag, 4°C storage, reheated in the bag. PCFR = Precooked in vacuum bag, −20°C storage, reheated in the bag. VPFR = Fresh roast vacuum bag, −20°C storage, cooked in the bag.
[bcd] Mean values in same row bearing unlike superscripts differ significantly (P<0.05).
[xy] Mean values in same column bearing unlike superscripts differ significantly (P<0.05).

ing bag limited microbial growth and it appears that these products would have a shelf life in excess of 28 days of refrigerated storage.

REFERENCES

Bayne, B.H., Meyer, B.H., and Cole, J.W. 1969. Response of beef roasts differing in finish, location and size to two rates of heat application. J. Anim. Sci. 29: 283.

Bouton, P.E. and Harris, P.V. 1972. The effects of cooking temperature and time on some mechanical properties of meat. J. Food Sci. 37: 140.

Bouton, P.E. and Harris, P.V. 1981. Changes in the tenderness of meat cooked at 40–65°C. J. Food Sci. 46: 475.

Bramblett, V.C., Hostetler, R.L., Vail, G.E., and Draudt, H.N. 1959. Qualities of beef as affected by cooking at very low temperatures for long periods of time. Food Technol. 13: 707.

Buck, E.M., Hickey, A.M., and Rosenau, J. 1979. Low-temperature air oven vs. a water bath for the preparation of rare beef. J. Food Sci. 44: 1602.

Dinardo, M., Buck. E.M., and Clydesdale, F.M. 1984. Effect of extended cook times on certain physical and chemical characteristics of beef prepared in a waterbath. J. Food Sci. 49: 844.

Hamm, R. 1970. Interactions between phosphates and meat proteins. Ch. 5. In "*Symposium: Phosphates for Food Processing*," p. 65. J.M. deMan and P. Melnchyn (Ed.). The AVI Publishing Co., Inc., Westport, CT.

Harrison, D.L. 1975. Selection of cooking method based on research objec-

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Restructured Mutton Roast Quality

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ABSTRACT

The influence of monosodium glutamate (MSG) and lamb on characteristics of restructured mutton roasts was evaluated. The status of pyridinoline (a trivalent collagen cross-link) in epimysial and muscle tissues of lambs and ewes was also investigated. Addition of MSG (0.2%) did not significantly alter restructured mutton roast flavor. Restructured roasts made with mutton had higher ($P<0.05$) cook losses and Warner-Bratzler shear values, and inferior binding characteristics when compared to roasts made with lamb, and roast characteristics improved linearly with increasing levels of lamb. Pyridinoline content was higher in mutton than lamb. The greater amounts of pyridinoline may be partially responsible for the lower quality restructured mutton roasts.

INTRODUCTION

MUTTON is a low value byproduct of the American sheep industry because of objectionable flavor, toughness, prejudices and lack of an effort directed toward developing added value products. High labor costs for slaughter and boning also reduce returns to sheep producers. A way to improve producer returns is to develop meat products which appeal to consumers. Breidenstein (1982) considered restructuring of meat as one avenue to add value. If an acceptable restructured mutton product were developed, processors could use that technology to increase returns to the sheep producer.

Williams (1962) claimed that mutton flavor could be masked by monosodium glutamate (MSG). Addition of MSG therefore, should improve the acceptability of restructured mutton roasts. According to Brewer et al. (1984), high quality restructured lamb roasts can be prepared but we found in preliminary studies that the same technology used by Brewer et al. for lamb did not produce high quality restructured mutton. Mutton roasts were tougher and possessed reduced binding characteristics when compared to lamb. Therefore, we theorized that addition of lamb at various levels could improve the quality of mutton roasts.

Meat from older animals is tough and chewy due to an increase in the tensile strength of collagen with age (Sims and Bailey, 1981). Nonreducible intermolecular cross-links in collagen of older animals are responsible for the increase in toughness with age (McClain, 1976). Pyridinoline is now known to be a prominent cross-linking residue that is present in most mature connective tissues (Eyre et al., 1984). Therefore, changes in pyridinoline content of collagen from epimysial and other muscle tissues might affect the texture of meat.

This study was designed to: (1) investigate the influence of MSG and various levels of lamb on objective and subjective characteristics of restructured mutton roasts; (2) determine the pyridinoline content of collagen from lambs and mutton.

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MATERIALS & METHODS

Meat Source

Nine 8 mo old Choice grade lambs and seven 6 yr old medium-conditioned ewes were slaughtered and processed in the University of Wyoming abattoir. The average weight of lambs was 50 kg and that of ewes was 65 kg. All carcasses were chilled for 24 hr at 0—1°C and then the fell and subcutaneous fat were stripped. Epimysium from M. longissimus was trimmed from both sides of lamb and ewe carcasses for further analysis. Muscles were removed from the leg, loin, rib and shoulder of each carcass. Pooled lean from lambs and from ewes (mutton) was ground separately through a grinder plate with three kidney shaped openings that were 3.8 cm long and 2.5 cm wide. Each of the separate ground lean segments was then mixed in a double action food mixer for 10 min. A 500-g composite sample was collected from each mixed lean for further analysis. The remainder of the lean from each segment was packed in 50 kg lots in cardboard boxes lined with a layer of freezer paper and frozen at −30°C for 1 to 6 mo.

Roast preparation

One day before roast preparation, frozen lean blocks were removed from the freezer, cut into 5-8 cm thick slices with a band saw and thawed in plastic lugs at 2–3°C for 24 hr. After thawing, each lean segment was mixed for 3 min in a food mixer and then weighed. Roasts were formulated with 1.5% salt, 0.3% phosphate (sodium tripolyphosphate and sodium hexametaphosphate) 0.2% MSG and 10% water. Control roasts contained the same levels of salt, phosphate and water but no MSG. Lamb was added to mutton at 25%, 50% and 75% of the meat portion. Roasts with 100% mutton or lamb were also prepared. Phosphate was dissolved in the water aliquot before it was added to the roasts. Total mixing time in the food mixer was 12 min. Temperature of the meat during mixing was 1–2°C. A 50-g sample from the roast mixture was collected before stuffing for pH estimation. Each roast mixture was stuffed into 100 mm fibrous casings to a final weight of approximately 4.5 kg. All roasts were cooked soon after stuffing in a smoke house at 63°C for 2 hr, 71°C for 2 hr and at 82°C to an internal temperature of 71°C. Roasts were then showered with cold water to an internal temperature of 32°C and chilled overnight at 0°–1°C. Roast preparation was repeated four times.

pH determination

Samples collected before stuffing were further cut into small pieces and 7g were dropped into 25 ml of a solution containing 5 mM sodium iodoacetate and 150 mM potassium chloride (pH 7.0) and homogenized in a blender (Bendall, 1973). pH measured using a combination glass electrode.

Cook Loss

Loss in weight due to cooking was recorded after the roasts were chilled overnight at 0–1°C. Cook loss was reported as percentage of weight before cooking.

Warner-Bratzler shear values

Three 2.54 cm thick slices were cut from each chilled roast and three 2.54 cm diameter cores were removed from each slice. Each core was sheared once using a Warner-Bratzler shear. Nine values thus obtained were averaged and reported as kg force required to shear across 2.54 cm diameter cores.

Instron measurements

An objective assessment of the degree with which meat chunks adhered to each other in a restructured roast was made by using an attachment to the Instron described by Field et al. (1984). The binding ability is reported as the average binding strength, elongation and energy from nine chilled 1.27 cm thick slices. Each slice was pressed onto tapered needles mounted 2 mm apart around the inner perimeter of a plexiglass ring 73 mm in diameter. A 1.27 cm diameter steel ball mounted on the cross-head of an Instron moving at a constant rate of 10 cm/min penetrated the slice. Peak load in kg required by the ball to penetrate the roast slice is reported as binding strength. Elongation is mm of distance traveled by the ball after contacting the roast slice until penetration occurs. Energy, in joules (J), is a measure of units of work done under the area of the peak load elongation curve at a constant rate of penetration. The values were obtained by converting the integrator units (Instron Corporation, 1967).

Sensory evaluation

Roasts containing mutton and lamb at varying levels were sliced 1.25 mm thick, coded, warmed in a microwave oven for 30 sec and presented to a 9–12 member trained sensory panel. The duo-trio test (Larmond, 1977) was used. Within each proportion of lamb to mutton roast, control roasts with no added MSG were compared to roasts containing MSG. Panelists were seated in individual booths illuminated with red lights. Apple juice was given for oral rinsing between samples. In the duo-trio comparison set-up, one sample was given first and then two samples were presented, one of which was identical to the first. Panelists were asked to identify the sample that was the same as the reference sample. They were also asked to mention whether their choice was based on color, flavor, texture or other. Each roast was replicated at three different sessions. Three samples were evaluated per session.

Hydroxyproline analysis

Moisture and lipid free epimysial and muscle samples were hydrolyzed with 6N HCl at 110–112°C for 24 hr. Hydroxyproline was estimated using the method of Woessner (1961).

Pyridinoline Analysis

Preliminary experiments with hydrolysates of muscle and crude epimysium gave unrepeatable results necessitating isolation and purification of connective tissue before hydrolysis. The procedures described below were used.

Epimysium. Adhering muscle tissue and fat were scraped off using a sharp knife. The tissue was then cut into strips approximately 0.5 cm wide and 2 cm long. The strips were stirred with 10 times the volume of acetone:hexane (1:1) solution for three 1.5 hr periods at 0°–1°C to remove any remaining lipid. Approximately 1 min elapsed between each stir period. The tissues were dried at 60°C for 4 hr to remove the solvent and then left in a dessicator for 12 hr. Dried tissues were analyzed for pyridinoline.

Muscle tissue. Each sample (500g) collected from the pooled lean of ewes or lambs was homogenized in a high speed homogenizer. About 10g homogenate was extracted at 0°–1°C for three 1.5 hr periods using 70 mL acetone:hexane (1:1) solution with filtrations in between. The lipid-free tissue was extracted as per the suggestions of Miller and Rhodes (1982) by stirring on a magnetic stirrer with 70 mL NaCl-Tris reagent (pH 7.5) for 12 hr. After filtration, the tissue was further extracted with 70 mL volumes of 0.1M phosphate buffer (pH 7.4) containing 0.6 M potassium iodide and 5 mM sodium thiosulfate for three 12 hr periods. The salts from the sample were washed by stirring with distilled water for three 1.5 hr periods. To remove any residual lipid and to hasten drying, the sample was stirred with 70 ml acetone:hexane solution for 1.5 hr. After filtration, and solvent evaporation, the extracted connective tissue was dried first in a 60°C oven for 4 hr and then over Drierite for 12 hr. The purified and isolated connective tissues were hydrolyzed in 6N HCl at 110–112°C for 24 hr.

Pyridinoline determination. The Pyridinoline standard was a generous gift from Dr. Daisaburo Fujimoto of Hamamatsu University School of Medicine (Handa Cho, Japan). Chromatographic isolation of pyridinoline was based on the method of Fujimoto and Moriguchi (1978) with some modifications. Hydrolysates of connective tissues were evaporated under reduced pressure in a rotary evaporator with water temperature at 40°C. The residue was dissolved in 7 ml distilled water, and 1 ml of this aqueous sample was eluted from a 2 cm × 30 cm column of Biogel P-2 (Bio-Rad Laboratories, Richmond, CA; Batch No. 268583) with 0.1M glacial acetic acid. Pyridinoline fractions detected by monitoring the fluorescence at 295 nm (Ex Max) and 395 nm (Em Max) using a Turner spectrofluorometer (Model 430; G. K. Turner Associates, Palo Alto, CA) were collected and pooled. The pooled fractions were concentrated under reduced pressure and residue was dissolved in 4.0 ml distilled water. Aliquots of this solution (0.5 mL in case of epimysial tissue and 1.0 ml in case of connective tissue from muscle) were eluted through 1 cm × 9 cm cation exchange column of Sephadex SP C-25 (Pharmacia Fine Chemicals, Piscataway, NJ; Lot No. GL 23360) using a linear gradient of 80 ml distilled water and 80 mL 1N HCl as suggested by Dr. Fujimoto (Personal Communication, 1984). Each 3 ml fraction was monitored for fluorescence at 295 nm and 395 nm. Fractions 20 to 30 contained fluorescent activity similar to that of the standard. These fractions were pooled and concentrated under reduced pressure. The residue was re-dissolved in 4.0 mL 0.1N HCl. Pyridinoline content was calculated using the value obtained for the 1 nM/mL standard. When calculating pyridinoline content as mol/mol collagen, it was assumed that collagen was 7.25 times the weight of hydroxyproline. A molecular weight of 300,000 was used for collagen.

Statistical analyses

Completely randomized two factor factorial analysis of variance was used to test the influence of MSG, the level of lamb, and their interaction on restructured roast characteristics Mean comparisons were made by Fisher's protected least significant difference test (Steel and Torrie, 1980). Statistical significance of the duo-trio test results was determined from the table of Roessler et al. (1978). Differences in pyridinoline content of lamb and ewe epimysial tissues were tested by the 't' test (Steel and Torrie, 1980).

RESULTS & DISCUSSION

PHYSICAL EFFECTS due to the addition of MSG and the interaction between MSG and lamb level were not significant ($P>0.05$) (Table 1).

Addition of lamb at various levels to mutton had a significant influence on objective characteristics of restructured mutton roasts at the $P<0.01$ or $P<0.005$ levels. Restructured roasts made with 100% lamb had significantly lower cook losses and Warner-Bratzler shear values and better muscle chunk binding ability than roasts made with 100% mutton (Table 2). Schmidt and Trout (1982) indicated that efficacy of muscle chunk binding in a restructured product is determined by the amount of protein extracted, mechanical treatment, presence and concentration of added salts and temperature of heating. Other workers (Acton, 1972; Randall and Voisey, 1977; Siegel and Schmidt, 1979 and Siegel et al., 1979) have also noted that an increase in the amount of salt-extracted myofibrillar proteins between meat surfaces produced a concomitant increase in the binding strength of sectioned and formed meat products and in model binding systems. As the processing conditions were similar for both lamb and mutton roasts, results of our study suggest that the amount of salt-extractable myofibrillar proteins in lamb and mutton differed. Hildebrand et al. (1977) and Turgut and Sink (1983) observed a higher proportion of salt-soluble protein in younger cattle than in older bulls and cows. Increased binding ability of muscle chunks with increased lamb levels in our study may also be due to higher amounts of salt-soluble protein in lambs or it may be due to lower amounts of less mature collagen.

MSG did not significantly influence the pH of the roast mixture, cooking losses, Warner-Bratzler shear values or muscle chunk binding characteristics (Table 2). In addition, panelists could not detect improvement in mutton flavor with the addition of MSG to any restructured roast, regardless of the proportion of lamb to mutton in the roast (data not shown).

Ewe tissues contained higher ($P<0.001$) levels of pyridinoline than tissue from lamb (Table 3) suggesting that the increased intermolecular cross linking which occurs with age (Hill, 1966; Bailey, 1972; Bailey and Shimokomaki, 1971;

Table 1—Analysis of variance for effect of lamb and MSG in restructured mutton roasts

Source of variation	df	Mean squares: pH at stuffing	Cook loss %	Warner-Bratzler Shear, kg	Binding strength kg	Elongation, mm	Energy, j
Mutton:Lamb (A)	4	0.013	2.569[a]	46.978[b]	0.555[b]	20.035	0.016[b]
MSG (B)	1	0.018	0.039	3.034	0.015	6.241	0.001
A × B	4	0.004	0.969	2.552	0.028	5.366	0.001
Residual	30	0.015	0.562	3.381	0.050	7.717	0.002

[a,b] Mean squares with superscripts show a significant difference (a = P<0.01, b = P<0.005) between restructured roasts made with lamb and mutton for the measurement listed. For mean squares without a superscript, source of variation was not significant (P>0.05).

Table 2—Effect of proportion of mutton to lamb and MSG on restructured roast characteristics

Traits	Proportion of mutton to lamb: 100:0	75:25	50:50	25:75	0:100	SE[a]
Number	4	4	4	4	4	
pH at stuffing						
MSG[b]	6.04	5.96	5.91	6.01	6.03	
Control	5.96	5.98	5.89	5.96	5.96	
Mean	6.00[c]	5.97[c]	5.90[c]	5.99[c]	6.00[c]	0.04
Cooking loss, %						
MSG	10.02	9.96	10.28	9.62	9.10	
Control	11.03	10.36	9.42	9.48	9.00	
Mean	10.52[c]	10.16[cc]	9.85[cd]	9.55[d]	9.05[e]	0.27
W-B Shear, kg						
MSG	10.12	8.50	8.53	5.77	4.46	
Control	11.98	10.73	8.18	5.72	3.95	
Mean	11.05[c]	9.62[cc]	8.36[d]	5.74[e]	4.21[e]	0.75
Binding strength, kg						
MSG	1.72	2.13	2.06	2.22	2.51	
Control	1.81	1.91	2.03	2.25	2.43	
Mean	1.76[c]	2.02[d]	2.04[de]	2.24[ef]	2.47[f]	0.08
Elongation, mm						
MSG	44.2	45.7	43.6	42.8	40.5	
Control	44.2	42.1	43.3	43.3	40.0	
Mean	44.2[c]	43.9[c]	43.4[c]	43.1[c]	40.2[c]	0.98
Energy, J						
MSG	0.30	0.36	0.35	0.38	0.44	
Control	0.32	0.33	0.34	0.38	0.42	
Mean	0.31[c]	0.35[cc]	0.35[cd]	0.38[d]	0.43[e]	0.01

[a] Standard error of the means.
[b] MSG = roasts contained 0.2% monosodium glutamate.
[c-f] Means in the same row with different superscripts are significantly (P<0.05) different.

Table 3—Means and standard errors of the means for hydroxyproline and pyridinoline contents in epimysium and means for meat of ewes and lambs

Tissue	Hydroxyproline[a] mg/g tissue	Pyridinoline content: nM/g tissue	nM/mg hydroxyproline	mol/mol collagen
Epimysium[b]				
Ewes	112.4 ± 9.15[e]	932 ± 75.7[e]	8 ± 0.4[e]	0.34 ± 0.02[e]
Lambs	109.9 ± 1.76[e]	354 ± 22.1[f]	3 ± 0.2[f]	0.13 ± 0.01[f]
Meat				
Ewes[c]	10.9	230	21	0.87
Lambs[d]	7.4	88	12	0.49

[a] Hydroxyproline was estimated on duplicate samples of fat free dry tissue.
[b] Epimysial tissue covering M. longissimus from six ewes and six lambs was used for analysis.
[c] One representative sample from pooled legs, loins, ribs and shoulders of seven 6-yr old ewes was analyzed.
[d] One representative sample from pooled legs, loins, ribs and shoulders of nine 8-mo old Choice grade lambs was analyzed.
[e,f] Means with different superscripts in a column are significantly (P<0.001) different.

Dutson, 1976) is at least partially due to this trivalent, nonreducible, cross-link. However, Robins and Duncan (1983) and Fujimoto (1984) believed that pyridinoline does not increase markedly after maturation. Collagen from epidmysial tissue could be more soluble than collagen from muscle because moles of pyridinoline per mole of collagen in epimysium was lower than it was for meat. Meat included muscle, epimysial tissue and other connective tissue; therefore, the pyridinoline values for intramuscular collagen in muscle alone would probably be higher than those in Table 3 for meat. Wu and Eyre (1985) reported that the concentration of 3-hydroxypyridinium cross-link residues in different tissue types varied considerably. They were not able to detect 3-hydroxylysyl pyridinoline in human or bovine skin but they reported values of 0.44, 0.41 and 0.68 moles per mole collagen for human tendon, ligament and fascia, respectively.

Although myofibrillar proteins are the most important in muscle chunk binding, collagen also contributes to the binding characteristics of meat and the ultimate texture of meat products (Randall and Voisey, 1977). Under appropriate conditions, collagen imparts texturizing, moisturizing, lubricating, viscoelastic and emulsifying properties to any food (Asghar and Henrickson, 1982). Wiley et al. (1979) showed that total collagen, insoluble collagen and percentage soluble collagen vary with the source of meat. They felt that soluble collagen, not total collagen, was related to binding characteristics of meat. More data on the pyridinoline content of collagen from different tissues in meat is obviously needed. The findings

could help explain some of the divergent views on the role of connective tissue in meat products.

Muscle chunk binding in a restructured product is a result of interactions between different proteins in meat (Schmidt and Trout, 1982). We believe that age related changes in collagen play an influential role in the process of muscle chunk binding. An increase in pyridinoline probably decreases the amount of salt-extracted proteins on the meat chunk surface, especially surfaces covered with connective tissue. The presence of more insoluble collagen in mutton than in lamb could also dilute extracted myofibrillar protein and weaken the matrix (Jones, 1983). We, therefore, agree with Secrist (1982) who considered connective tissue as the most important problem to be solved in restructured product manufacture, not only because it is involved in toughness of connective tissue strands, but because it may influence bind.

CONCLUSIONS

CHARACTERISTICS of restructured mutton and lamb roasts made with MSG (0.2%) were not distinguishable from control roasts. As 100% lamb roasts, and roasts with increased proportions of lamb had better bind than 100% mutton roasts, it is suggested that quantity of salt-extracted myofibrillar proteins could be different in lamb and mutton. Mutton used in product manufacture had more collagen and pyridinoline than lamb. Therefore, age related changes in collagen may influence muscle chunk binding by the mere presence of more insoluble collagen which dilutes extracted myofibrillar protein and weakens the matrix. The changes could also interfere with extraction of myofibrillar protein on the muscle surface.

REFERENCES

Acton, J.C. 1972. Effect of heat processing on extractability of salt-soluble protein, tissue binding strength and cooking loss in poultry meat loaves. J. Food Sci. 37: 244.

Asghar, A. and Henrickson, R.L. 1982. Chemical, biochemical, functional and nutritional characteristics of collagen in food systems. Adv. Food Res. 28: 231.

Bailey, A.J. 1972. The basis of meat texture. J. Sci. Food Agr. 23: 995.

Bailey, A.J. and Shimokomaki, M.S. 1971. Age related changes in the reducible cross-links of collagen. FEBS Lett. 16: 86.

Bendall, J.R. 1973. "The Structure and Function of Muscle," 2nd ed., Vol. 2. G.H. Bourne (Ed.), p. 243. Academic Press Nev' York.

Brewer, M.S., Field, R.A., Williams, J.C., Miller, G.J., Cross, H.R., and Secrist, J.L. 1984. Qualities of chunked and formed lamb roasts. J. Food Sci. 49: 1376.

Breidenstein, B.C. 1982. Industry's view - short-term research priorities in the restructured meat area. Proc. Intl. Symp. Meat Sci. Technol., Lincoln, NE Nov. 1-4, p. 311.

Dutson, T.R. 1976. Biosynthesis and structure of collagen. Proc. Recip. Meat Conf. 29: 336.

Eyre, D.R., Paz, M.A., and Gallop, P.M. 1984. Cross-linking in collagen and elastin. Ann. Rev. Biochem. 53: 717.

Field, R.A., Williams, J.C., Prasad, V.S., Cross, H.R., Secrist, J.L., and Brewer, M.S. 1984. An objective measurement for evaluation of bind in restructured lamb roasts. J. Texture Studies 15: 173.

Fujimoto, D. 1984. Human tendon collagen: Aging and crosslinking. Biomed. Res. 5: 279.

Fujimoto, D. and Moriguchi, T. 1978. Pyridinoline, a non-reducible cross-link of collagen. J. Biochem. 83: 863.

Hildebrand, C.A., Basey, D.R., and Jacobs, J.A. 1977. Effect of maturity on salt-soluble protein content of beef muscles. Proc. West. Sect. Am. Soc. Anim. Sci. 28: 74.

Hill, F. 1966. The solubility of intramuscular collagen in meat animals of various ages. J. Food Sci. 31: 161.

Instron Corporation. 1967. Instron Manual No. 10-1(B). Instron Corp., Canton, MA.

Jones, K.W. 1983. Collagen utilization in comminuted meat systems. Diss. Abstr. Int. B 43(1): 3522.

Larmond, E. 1977. "Laboratory Methods for Sensory Evaluation of Food." Publication 1637, Canada Department of Agriculture. Ottawa

McClain, P.E. 1976. Chemistry of collagen cross-linkings. Proc. Recip. Meat Conf. 29: 350.

Miller, E.J. and Rhodes, R.K. 1982. Preparation and characterization of the different types of collagen. Methods in Enzymol. 82: 33.

Randall, C.J. and Voisey, P.W.Q. 1977. Effect of meat protein fractions on textural characteristics of meat emulsions. J. Inst. Can. Sci. Technol. Aliment. 10(2): 88.

Robins, S.P. and Duncan, A. 1983. Cross-linking of collagen: Location of pyridinoline in bovine articular cartilage at two sites of the molecule. Biochem. J. 215: 175.

Roessler, E.B., Pangborn, R.M., Sidel, J.L. and Stone, H. 1978. Expanded statistical tables for extimating significance in paried-preference, paired-difference, duo-trio and triangle tests. J. Food Sci. 43: 940.

Schmidt, G.R. and Trout, G.R. 1982. Chemistry of meat binding. Proc. Intl. Symp. Meat Sci. Technol., Lincoln, NE, Nov. 1-4, p. 265.

Secrist, J.L. 1982. Long-term research needs and goals. Proc. Intl. Symp. Meat Sci. Technol., Lincoln, NE, Nov. 1-4, p. 299.

Siegel, D.G., Church, K.E., and Schmidt, G.R. 1979. Gel structure of non meat proteins as related to their ability to bind meat pieces. J. Food Sci. 44: 1276.

Siegel, D.G. and Schmidt, G.R. 1979. Crude myosin fractions as meat binders. J. Food Sci. 44: 1129.

Sims, T.J. and Bailey, A.J. 1981. Connective tissue. In "Developments in Meat Science-2," R.A. Lawrie (Ed), p. 29. Applied Science Publishers, London

Steel, R.G.D. and Torrie, J.H. 1980. "Principles and Procedures of Statistics." 2nd ed.; McGraw-Hill Book Co., New York, NY.

Turgut, H. and Sink, J.D. 1983. Factors affecting the emulsifying capacity of bovine muscle and muscle proteins. J. Food Sci. 48: 841.

Wiley, E.L., Reagan, J.O., Carpenter, J.A., and Campion, D.R. 1979. Connective tissue profiles of various sausage materials. J. Food Sci. 44: 918.

Williams, B.C. 1962. U.S. Patent 3, 016, 301. January 9, 1962-assigned to Hodges Research and Development Company.

Woessner, J.F. Jr. 1961. The determination of hydroxyproline in tissue and protein samples containing small proportions of this imino acid. Arch. Biochem. Biophys. 93: 440.

Wu, J. and Eyre, D.R. 1985. Studies on the distribution of hydroxypyridinium cross-links in different collagen types. In "Biology, Chemistry and Pathology of Collagen," R. Fleischmajer, B.R. Olsen, and K. Kuhn (Ed.), Ann. of the N.Y. Academy of Science 460: 520.

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tives. Proc. 28th Ann. Recip. Meat Conf., National Live Stock and Meat Board, Chicago, IL.

Keeton, J.T. 1983. Effect of fat and NaCl/phosphate levels on the chemical and sensory properties of pork patties. J. Food Sci. 48: 878.

Koniecko, E. 1979. "Handbook for Meat Chemists." Avery Publishing Groups, Inc., Wayne, NJ.

Leander, R.C., Hedrick, H.B., Brown, M.F., and White, J.A. 1980. Comparison of structural changes in bovine longissimus dorsi and semitendinosus muscles during cooking. J. Food Sci. 45: 1.

Mahon, J.H., Schlamb, K., and Brotsky, E. 1970. General concepts applicable to the use of phosphates in red meat, poultry and seafood processing. In "Phosphates in Food Processing," p. 153. AVI Publishing Co., Inc., Westport, CT.

Matlock, R.G., Terrell, R.N., Savell, J.W., Rhee, K.S., and Dutson, T.R. 1984. Factors affecting properties of precooked-frozen pork sausage patties made with various NaCl/phosphate combinations. J. Food Sci. 49: 1372.

Miller, L.F., Hedrick, H.B., and Bailey, M.E. 1985. Sensory and chemical characteristics of pork chops as affected by precooking, curing and frozen storage. J. Food Sci. 50: 478.

Riss, T.L., Bechtel, P.J., Forbes, R.M., Klein, B.P., and McKeith, F.K. 1983. Nutrient content of special fed veal ribeyes. J. Food Sci. 48: 1868.

SAS Institute, Inc. 1982. "SAS User's Guide: Statistics," 1982 ed. SAS Institute, Inc., Cary, NC.

Sherman, P. 1961. The water binding capacity of fresh pork. 1. The influence of sodium chloride, pyrophosphate and polyphosphate on water absorption. Food Technol. 15(2): 79.

Shults, G.W., Russell, D.R. and Wierbicki, E. 1972. Effect of condensed phosphate on pH, swelling and water-holding capacity of beef. J. Food Sci. 37: 860.

Smith, L.A., Simmons, S.L., McKeith, F.K., Bechtel, P.J., and Brady, P.L. 1984. Effect of sodium tripolyphosphates on physical and sensory properties of beef and pork roasts. J. Food Sci. 49: 1636.

Tims, M.J. and Watts, B.M. 1958. Protection of cooked meats with phosphates. Food Technol. 12: 240.

Wierbicki, E. and Deatherage, F.E. 1958. Determination of water-holding capacity of fresh meats. J. Agr. Food Chem. 6: 387.

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

Woodams, E.E. and Nowery, J.E. 1968. Literature values of thermal conductivities of foods. Food Technol. 22: 494.

Woolsey, A.P. and Paul, P.C. 1969. External fat cover influence on raw and cooked beef. 2. Cooking time, losses, press fluids and shear force values. J. Food Sci. 34: 568.

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Influence of Marinating on Weight Gain and Coating Characteristics of Broiler Drumsticks

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ABSTRACT

The weight gain of broiler drumsticks at 4°C was compared to those at 23°C after marinating in distilled water with agitation for 30 and 60 min. At 23°C there was significantly greater absorption ($P = 0.001$), with an average of 3.81%. The effect of different additives were studied in relation to weight gain, coating pickup, cooking loss, crumb loss, and overall yield of coated broiler drumsticks. Skinless drumsticks marinated in 4% Kena solution (90% tripolyphosphate and 10% hexametaphosphate blend) had significantly greater coating pickup, and a cooking loss was significantly reduced by increased marinating time in 4% Kena. The mean overall yield was highest for drumsticks soaked in distilled water (86.67%), and lowest for those treated with 0.5% ascorbic acid (80.01%) and 0.002% papain (78.30%).

INTRODUCTION

THE MANUFACTURING of batter and breading is a billion dollar industry (Anonymous, 1981). Commercial batters and breadings provide processors with their greatest value-added opportunity at the lowest cost (Jessup, 1981).

Batters and breadings add flavor and improve appearance (Elston, 1975), remove monotony (Jessup, 1981), contribute to the nutritional value of a food product (Elston, 1975), prevent oxidation and reinforce food structure (Morgan, 1971), and add crispness and browning to a food product.

Many factors that affect batter and breadings involve ingredient manipulation. Cunningham and Tiede (1981) found that increasing batter viscosity increased breading pickup and decreased cooking losses. A water:solids ratio of 1:2 was found to be best for breading adhesion. Suderman et al. (1981) studied the effect of protein and gum sources in relationship to coating adhesion. Protein sources that were studied included whey, soy, nonfat dry milk, egg albumen, and gelatin. Gelatin and egg albumen were the most effective in improving adhesion. Gums studied were sodium carboxymethyl cellulose (CMC), guar, tragacanth, and xanthan. CMC was the only one to improve adhesion significantly. Increasing the levels of gums and proteins used in the coating did not affect adhesion significantly. Baker et al. (1972) compared 15 predust materials and found proteins improved adhesion of batter and breaded coatings more than starches or gums. Hale and Goodwin (1968) combined gums and proteins, incorporating egg white and hydroxypropyl methyl-1- cellulose (HMC) with batters, and reported no significant adhesion differences.

Suderman and Cunningham (1979) used various additives to determine their effect on coating characteristics. Volume loss and losses due to baking were reduced as breading predip usage progressed from water to whole milk, to evaporated milk. Soy protein improved meat tenderness ($P<0.05$) and decreased baking loss ($P<0.05$). Single-cell protein (Schnell, 1976) and comminuted fish flesh (King et al., 1974) also have been used to improve coating adhesion.

Phosphates have been used in the meat industry for moisture retention, tenderization, to preserve color and flavor, and delay rancidity and spoilage (Cassidy, 1977). Phosphates and NaCl increase the solubility of proteins, causing more protein to be in the fluids on the surface of the meat (Vadehra and Baker, 1970), and increase binding. Bendall (1954) reported said the swelling effect on meat of inorganic pyrophosphate or tripolyphosphate can be traced to their ability to split actomysin into its component proteins, actin and myosin, resulting in the uptake of water, resembling the action of adenosine triphosphate.

Tenderness was increased in broiler breast muscle within 1 hr after slaughter by injecting the muscle with sodium polyphosphates, 20 min postmortem (Peterson, 1977).

Seeley (1981) also studied the coating adhesion qualities of various additives. Phosphates significantly decreased crumb loss as the concentration increased. A linear relationship was found with respect to concentrations of tripolyphosphate (Curafos, Calgon Corp. Granular 00-22), and sodium tripolyphosphate and sodium hexametaphosphate blend (Curafos, Calgon Corp., Formula 22-4). Kena (90% sodium tripolyphosphate and 10% sodium hexametaphosphate) did not show a linear relationship.

Landes (1972) found that percent cooked yield of broiler quarters increased as polyphosphate content increased. Phosphorus content of the cooked tissue also increased as the polyphosphate content of the marinade increased, with light meat absorbing more phosphate than dark meat. Sensory evaluations of the quarters indicated that chicken and meaty flavors decreased through the storage period with increased polyphosphate levels in the marinade. Warmed over flavor decreased with increased phosphate level and an objectionable baking soda flavor was present which increased in intensity with increased polyphosphate treatment.

The use of enzymes, including papain, to tenderize meat has been studied (Prusa et al., 1981; Fry et al., 1966), but no one has considered the effect of enzymes on batter and breading adhesion. Prusa et al. (1981) found off-flavor increased when papain was added (0.001 and 0.002% solutions), possibly due to the enzyme present or to the additional free amino acids liberated in the chicken muscle.

The purpose of this research was: (1) to determine the effect of NaCl, a phosphate blend, acids, and enzymes on weight gain and cooking loss, batter and breading adhesion, and flavor of broiler drumsticks; and (2) to determine the difference in the amount of marinade of distilled water absorbed by broiler drumsticks at room (23°C) and refrigerator temperature (4°C).

MATERIALS & METHODS

Water absorption

Twenty-four broiler drumsticks, bought frozen from a local supermarket, shipped directly from Tysons Foods, Inc., were tempered in a refrigerator at 4°C for 48 hrs along with two gallon jars containing 1900 ml distilled water. Twelve drumsticks were placed in each jar, one jar was agitated for 30 min and the other for 60 min, in a temperature-controlled water bath shaker (American Optical) set on the fastest speed of 10. The drumsticks were towel-dried and weighed before agitation, allowed to drain for 15 min after agitation, and towel-dried and weighed again to determine the amount of water absorbed. Forty-eight broiler drumsticks were tempered at room temperature

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Table 1—The % absorption of distilled water by broiler drumsticks while soaking at refrigerator temperature (4°C) or room temperature (23°C), for 30 or 60 min

Temp[e]	Time	Mean % absorption	Avg temp	P>F
23°C	30 min	3.17[b]	3.81	
	60 min	4.45[a]		0.0001
4°C	30 min	1.59[d]	1.75	
	60 min	1.91[c,d]		

[a-d] Means with the same letter are not significantly different at p = 0.05.
[e] Number of observations/time at 4°C = 12; 23°C = 24.

Table 2—The mean % weight gain of broiler drumsticks after soaking in various additives for 30 and 60 min

Additive	30 min	60 min	Avg.
Distilled water (control)	3.17	4.45*	3.81[a]
0.5% Ascorbic acid	3.17	4.29*	3.73[a,b]
4% Kena, skinless drumsticks	2.38	4.18*	3.28[b]
2% NaCl	2.46	2.97	2.71[c]
0.002% Papain	2.21	3.19*	2.70[c]
0.006% Papain	1.94	3.06*	2.50[c]
4% Kena	1.63	2.23	1.93[d]

[a-d] Means with the same letter are not significantly different at p = 0.05. Number of samples/treatment/time = 12.
* Percent weight gain within the additive treatment showed significant difference between soak times.

(23°C) along with four gallon jars containing 1900 ml distilled water each. Twenty-four drumsticks were agitated for 30 min and 24 for 60 min. Drumsticks were weighed before and after agitation, data were recorded, and analysis of variance was calculated (Snedecor and Cochran, 1976).

Additive absorption

Frozen broiler drumsticks, purchased from a local supermarket, were thawed overnight at room temperature, towel-dried, and weighed individually. Solutions of additives listed below were made and checked for pH initially and after agitation. Twenty-four drumsticks were used with each chemical additive tested. 12 soaked for 30 min and 12 for 60 min to measure differences due to time allowed for the additive to penetrate the drumsticks. Solutions were agitated and drained as explained above. The drumsticks were towel-dried, reweighed, and then battered and breaded. The controls for this part of the experiment were soaked in distilled water. To coat, the drumsticks were immersed in a whole milk predip for 10 sec; drained for 10 sec; placed in a 10" × 10" plastic bag containing 70g breading mix and shaken for 10 sec. The drumsticks were reweighed to determine coating pickup. The breading mix was composed of the following ingredients: wheat flour, partially hydrogenated soybean, cottonseed and palm oils, salt, malted barley, sugar, spices, paprika, yeast, cornstarch, dried garlic, monosodium glutamate (flavor enhancer), natural hickory smoke flavor, artificial color, and dried onion. The coated drumsticks were baked for 40 min in a commercial-sized Partlow oven (National Mfg. Co., Lincoln, Nebraska) at 204.5°C. After a 20 min cooling period, the drumsticks were reweighed and shaken for 1 min on a portable sieve shaker on a 1/4" grid (Suderman and Cunningham, 1979). The pieces were reweighed to determine crumb loss. Determinations were made of the solution uptake, coating pickup, cooking loss, crumb loss, and overall yield. Calculations were made as follows:

$$\% \text{ solution pickup} = \frac{\text{soaked wt-towel-dried wt}}{\text{towel-dried wt}} \times 100$$

$$\% \text{ coating pickup} = \frac{\text{coated wt-soaked wt}}{\text{towel-dried wt}} \times 100$$

$$\% \text{ cooking loss} = \frac{\text{coated wt-cooked wt}}{\text{towel-dried wt}} \times 100$$

$$\% \text{ crumb loss} = \frac{\text{cooked wt-shaken wt}}{\text{towel-dried wt}} \times 100$$

$$\% \text{ overall yield} = \frac{\text{shaken wt}}{\text{towel-dried wt}} \times 100$$

Two way analysis of variance (Snedecor and Cochran, 1976) was performed between each of the different additive solutions and the two soaking times of 30 and 60 min treatment for each of the variables calculated above at the 5% level. The additives and amounts used were: (1) distilled water (control); (2) 4% Kena (90% sodium tripolyphosphate, 10% sodium hexametaphosphate) provided by Stauffer Chemical Co., Westport, Connecticut, on drumsticks with and without skin; (3) 2% NaCl; (4) 0.5% ascorbic acid; (5) 0.002% papain (U.S. Biochemical Corp., Cleveland, OH 44128); (6) 0.006% papain.

Sensory evaluation

Although adhesive properties of the coating and effect on cooking loss were our main concerns, a 12 member, trained taste panel was used to determine how additives affected flavor. Drumsticks were soaked, battered and breaded, and baked exactly as outlined, and presented warm to the panel. Two samples were evaluated per seating. The taste panel scored the drumsticks for flavor, juiciness, tenderness, coating appearance and flavor, and overall acceptability, on an eight point hedonic scale from 1 (very undesirable), to 8 (very desirable). Analysis of variance (Snedecor and Cochran, 1976) was calculated to indicate significant differences between treatments at the 5% level. Individual differences between taste panel members were removed by statistical procedures.

RESULTS & DISCUSSION

Percent absorption of distilled water at 4°C or 23°C

The mean % absorption at 4°C and 23°C was significantly different (P=0.0001) as shown in Table 1. More distilled water was absorbed by broiler drumsticks at 23°C than at 4°C. The % absorption also was affected significantly by the time of marinating at 23°C, but not at 4°C. Chen (1982) found rotary-marinating at room temperature in a hexagonal shaped drum rotated at 31.5 rpm increased the rate of penetration of a salt solution over still-marinating at refrigerator temperatures. Equivalent salt marinade penetration into drumsticks took approximately 4 hr in still-marinating, compared to 25 min in rotary marinating. Marinade penetration equivalent to 4 hr of still-marinating of breasts, thighs, and wings could be accomplished by rotary-marinating in 10 min. Marinade solutions were agitated in the automatic water bath shaker to increase the rate of absorption of marinade into the drumsticks. Preliminary results found agitating-marinade times of 30 or 60 min to be adequate for marinade penetration of the wide variety of chemicals used. There was little increase in marinade penetration after 60 min of agitating-marinade agreeing with Chen (1982) who found little increase of marinade penetration in rotary-marinade after 10 min. Depending on the type of additive used in the marinade, the penetration time could be shortened.

Percent weight gain

The mean % weight gain of broiler drumsticks after soaking in various additives for 30 or 60 min is given in Table 2. The additive with the most absorption was distilled water, which was not significantly different from that of 0.5% ascorbic acid. Drumsticks after soaking in 0.5% ascorbic acid were pale, and the feather follicles had been denatured causing large "gooseBumps" on the skin surface. The absorption of 2% NaCl was significantly reduced from the above mentioned additives, possibly due to the ionic strength it gave to the poultry muscle. The addition of papain to the distilled water also reduced weight gain during marination significantly.

Four percent Kena solution in the marinade gave the lowest significant absorption. The skinless drumsticks absorbed more marinade because skin acts as a barrier to water absorption into the muscle. This was expected because one of the functions of polyphosphates in chill water of freshly killed eviscerated poultry is to reduce weepage during refrigerated storage or during thawing from frozen storage, which is accomplished partly by reducing water absorption during chilling (Mahon, 1983). Polyphosphates reduce moisture absorption and reduce weepage by the influence of pH, ionic strength effects, and

Table 3—The mean % coating pickup of broiler drumsticks after agitation for 30 and 60 min in different additive solutions

Additive	30 min	60 min	Avg.
4% Kena, skinless drumsticks	8.58	11.35*	9.96[a]
Distilled water (control)	7.02	7.58	7.30[b,c]
4% Kena	6.96	7.44	7.20[c]
0.006% Papain	6.71	6.43	6.57[c,d]
0.5% Ascorbic acid	6.41	6.61	6.51[d]
0.002% Papain	5.99	6.52	6.26[d]
2% NaCl	6.17	5.97	6.07[d]

[a-d] Means with the same letter are not significantly different at p = 0.05. Number of samples/treatment/time = 12.

* Percent coating pickup within the additive treatment showed significant difference between soak times.

Table 4—The mean % cooking loss of coated broiler drumsticks agitated for 30 and 60 min in different additive solutions

Additive	30 min	60 min	Avg.
4% Kena, skinless drumsticks	33.06	27.93*	30.49[a]
0.002% Papain	29.58	28.63	29.11[a]
0.5% Ascorbic acid	27.98	27.42	27.70[b]
0.006% Papain	25.47	26.96	26.21[b,c]
4% Kena	24.45	25.80	25.12[c]
2% NaCl	25.21	24.46	24.84[c,d]
Distilled Water (control)	22.07	24.84*	23.46[d]

[a-d] Means with the same letter are not significantly different at p = 0.05. Number of samples/treatment/time = 12.

* Percent cooking loss within the additive treatment showed significant difference between soak times.

specific phosphate anion interactions with divalent cations and myofibrillar proteins (Hamm, 1971).

The mean % weight gain of 2.41% for the broiler drumsticks after 30 min of soaking for all additives was significantly lower than those soaked for 60 min with a mean of 3.54% (P = 0.0001).

Percent coating pickup

Table 3 gives the mean % coating pickup of broiler drumsticks after agitation for 30 or 60 min in different additive solutions. The skinless drumsticks soaked in a 4% Kena solution had significantly higher coating pickup than drumsticks with any other treatment. Proctor and Cunningham (1983) found significantly increased coating pickup and decreased crumb loss of skinless drumsticks over those with the skin intact. The mean % coating pickup of skinless broiler drumsticks coated at 4°C without soaking was 8.08% (Proctor and Cunningham, 1983). In this experiment, the coating pickup for skinless drumsticks marinated in Kena solution was higher, 8.58% after 30 min and 11.35% after 60 min marinade. The skinless drumsticks were the only treatment where significant % coating pickup was found after 60 min of marinade time.

Percent cooking loss

The mean % cooking loss of coated broiler drumsticks agitated for 30 or 60 min in different additive solutions before coating is seen in Table 4. Skinless drumsticks soaked in 4% Kena solution had significantly more cooking loss ($P<0.05$) than drumsticks with other treatments except 0.002% papain. The soaking time of 60 min in a 4% Kena solution significantly reduced the amount of cooking loss over those soaked for only 30 min, from 33.06% to 27.93%, respectively. Proctor and Cunningham (1983) found the % cooking loss of non-marinated skinless drumsticks to be 29.20%. Increased penetration of Kena solution seemed to reduce the cooking loss. The cooking time of meats containing Kena solution can be reduced 5-10% according to Stauffer Chemical (Mahon, 1983), and this would reduce cooking loss. However, no adjustment of cooking time was made in this study. Intermediate cooking losses were found in drumsticks treated with 0.006% papain and 0.5% ascorbic acid. The lowest cooking losses were found in drumsticks treated with 2% NaCl, and distilled water. The amount of cooking loss was increased significantly from 30 to 60 min soaking time in distilled water.

Table 5—The mean % crumb loss of coated broiler drumsticks agitated for 30 and 60 min in different additive solutions

Additive[c]	30 min	60 min	Avg.
0.5 Ascorbic acid	2.33	2.49	2.41[a]
2% NaCl	1.95	1.91	1.93[b]
Distilled water (control)	2.16	1.59	1.88[b]
4% Kena	1.72	2.02	1.87[b]
0.006% Papain	1.54	2.04	1.76[b]
4% Kena, skinless drumsticks	1.46	1.99	1.72[b]
0.002% Papain	1.74	1.46	1.60[b]

[a-b] Means with the same letter are not significantly different at p = 0.05. Number of samples/treatment/time = 12.

[c] There were no significant differences between additive soak times.

Table 6—The mean % overall yield of coated broiler drumsticks agitated in different additive solutions for 30 and 60 min

Additive	30 min	60 min	Avg.
Distilled water (control)	86.40	86.92	86.67[a]
4% Kena	82.87	82.23	82.56[b]
2% NaCl	81.29	82.06	81.68[b,c]
4% Kena, skinless drumsticks	76.09	86.37*	81.23[b,c]
0.006% Papain	81.17	81.15	81.16[b,c]
0.5% Ascorbic acid	79.24	80.79	80.01[c,d]
0.002% Papain	76.78	79.84*	78.30[d]

[a-d] Means with the same letter are not significantly different at p = 0.05. Number of samples/treatment/time = 12.

* Percent overall yield within the additive treatment showed significant difference between soak times.

Table 7—The means of the variables for each of the chemical groups of additives

	Variables			
Groups[d]	% Coating pickup	% Cooking loss	% Crumb loss	% Overall yield
Distilled water (control)	7.30[b]	23.46[c]	1.88[b]	86.67[a]
Acids *1	6.51[c]	27.70[a]	2.41[a]	80.02[c]
Enzymes *2	6.41[c]	27.66[a]	1.63[c]	79.74[c]
Salts *3	7.74[a]	26.82[b]	1.84[b]	81.82[b]

[a-c] Means with the same letter are not significantly different within each variable at p = 0.05.

[d] alpha level = 0.05. *1 Acid group = 0.5% ascorbic acid. *2 Enzyme group = 0.002% papain and 0.006% papain. *3 Salts group = 2% NaCl, 4% Kena, 4% Kena, skinless drumsticks.

Table 8—The pH of solutions of each additive in which broiler drumsticks were soaked

	pH		
	0 min	30 min	60 min
Distilled water (control)	5.89	6.88	6.69
4% Kena	8.65	8.62	8.53
4% Kena, skinless drumsticks	8.65	8.57	8.46
2% NaCl	4.85	6.29	6.42
0.5% Ascorbic acid	3.05	3.49	3.51
0.002% Papain	5.72	6.50	6.54
0.006% Papain	5.76	6.59	6.53

Percent crumb loss

The mean % crumb loss of coated broiler drumsticks agitated for 30 and 60 min in different additive solutions is shown in Table 5. Percent crumb loss was significantly higher in drumsticks that had been treated with 0.5% ascorbic acid than in other treatments where no significant differences were found. The difference between the mean crumb loss for all additives after 30 and 60 min of soaking was not significant (P = 0.1982).

Percent yield

The mean % overall yield of coated broiler drumsticks agitated in different additive solutions for 30 or 60 min is shown in Table 6. The % overall yield average for the control was significantly higher ($P<0.05$) than all other drumsticks soaked in an additive solution. The skinless drumsticks soaked in 4% Kena had an overall yield mean of 86.37% after 60 min of

Table 9—The taste panel mean scores for flavor, juiciness, and tenderness of broiler drumsticks, flavor and texture of the coating, and acceptability of coated broiler drumsticks

Additive	Drumstick			Coating	Acceptability[c]
	flavor	juiciness	tenderness	flavor and texture	
2% NaCl	6.67[a]	6.29[a]	6.79[a]	6.96[a]	6.83[a]
0.006% papain	6.63[a]	6.50[a]	6.79[a]	6.79[a]	6.79[a]
0.05% ascorbic acid	6.41[a]	6.42[a]	6.71[a]	6.67[a]	6.58[a]
Distilled water (control)	6.75[a]	6.63[a]	6.83[a]	6.46[a]	6.54[a]
4% Kena	6.67[a]	6.33[a]	6.46[a]	6.54[a]	6.50[a]
0.002% papain	6.00[b]	6.00[a]	6.63[a]	6.67[a]	6.42[a]
4% Kena, skinless drumsticks	5.25[c]	4.91[b]	5.23[b]	4.97[b]	4.92[b]

[a-b] Means with the same letter are not significantly different within each variable tested at p = 0.05. Number of taste panel scores per each mean = 24.
[c] Arranged from highest to lowest acceptability score. Taste panel scale = (8) very desirable; (7) desirable; (6) moderately desirable; (5) slightly desirable; (4) slightly undesirable; (3) moderately undesirable; (2) undesirable; (1) very undesirable.

soaking, which was comparable to the control (86.92%). The extra 30 min of time in the marinade significantly increased the % overall yield.

Drumsticks treated with 4% Kena, and 2% NaCl were not significantly different from each other, but lower in yield than the control. Those drumsticks marinated in 0.5% ascorbic acid and 0.002% papain had the lowest % overall yield.

The additives, grouped according to their composition to determine if there were significant differences between them for the variables studied in this experiment, are shown in Table 7. The % coating pickup was greater for drumsticks marinated in salts. The acids and enzymes caused the greatest amount of cooking loss, and water marinating the least. Crumb loss was significantly higher for those drumsticks soaked in acid. The % overall yield was highest for the distilled water marinated control followed by salts, and then acids and enzymes.

pH

The pH of distilled water (when 12 drumsticks were soaked for 30 and 60 min) increased from 5.89 to 6.88 to 6.99 from 0, 30, and 60 min respectively (Table 8). Dodge and Peters (1960) reported that pH of postmortem poultry is around 5.8. A 4% Kena solution had a pH of 8.65. Little change in pH was observed after drumsticks were soaked for 30 and 60 min. The 0.5% ascorbic acid solution had a low initial pH of 3.05 and increased slightly after the poultry pieces had been soaked in it.

Taste panel results

Table 9 shows taste panel scores of coated baked broiler drumsticks treated with various additives. There were no significant differences due to soaking time of the drumsticks, so the data from 30 and 60 min was averaged to obtain table values. The lowest significantly different flavor scores were for drumsticks soaked in 0.002% papain and those skinned and soaked in 4% Kena solution. Prusa et al. (1981) reported an off-flavor due to papain injection. Landes (1972) found an objectionable baking soda flavor associated with high levels of polyphosphates in broiler meat. The lack of skin made it easier for the Kena solution to penetrate the muscle causing increased polyphosphate levels. The other scores were not significantly different from each other.

The lowest scores for juiciness of drumsticks were for poultry parts that had been skinned and soaked in 4% Kena. Possibly a shorter cooking time would help to correct this problem. The other scores were very similar and not significantly different.

Drumsticks skinned and soaked in a 4% Kena solution were significantly less tender than drumsticks treated with any other additive. The other scores given for drumsticks were not significantly different from the controls or other treatments.

Drumsticks skinned and soaked in a 4% Kena solution had significantly lower scores for flavor and texture of coating than drumsticks from any other treatment. The breading used in the experiment had a high salt content, and was the heaviest on the drumsticks skinned. The objectionable taste was due to the high salt content. Scores for flavor and texture of coating on drumsticks treated with other additives were not significantly different from one another.

The highest significant score for overall acceptability of broiler drumsticks when scores were statistically analyzed for 30 and 60 min times as an individual set of data, was for those soaked 60 min in a solution of 2% NaCl (7.33%). The values in Table 9 reflect an average of the 30 and 60 min soaking times. The difference in the score was significant from the controls and many of the other treated drumstick scores. The significantly lowest scores for overall acceptability were for the drumsticks that had been skinned and soaked in a 4% Kena solution.

CONCLUSIONS

THE ABSORPTION of distilled water into broiler drumsticks marinated for 30 and 60 min under agitation at room temperature (23°C) was significantly higher ($P = 0.0001$) than at refrigerator temperature (4°C).

Weight gain of broiler drumsticks after soaking for 30 and 60 min in distilled water and 0.5% ascorbic acid was significantly higher than in other additives. The drumsticks marinated in a phosphate blend (Kena) had the lowest weight gain.

Skinless drumsticks marinated in 4% Kena had a significantly greater coating pickup than drumsticks treated with other additives. These drumsticks also had a higher cooking loss than all other treatments except 0.002% papain because the skin was absent which acts as a moisture barrier. Crumb loss was significantly higher in acid treated drumsticks. The mean overall yield was significantly higher for drumsticks soaked in distilled water, and lowest for those treated with 0.5% ascorbic acid and 0.002% papain. The overall yield of skinless drumsticks soaked in a 4% Kena solution was significantly increased from 30 to 60 min soaking time, to give an overall yield close to that of distilled water.

When additives were grouped according to chemical composition, salts were responsible for the highest coating pickup, acids and enzymes had the highest cooking loss, and acids the greater crumb loss. Drumsticks marinated in distilled water had the highest overall yield.

The taste panel found no significant differences among drumsticks treated with various additives except the 4% Kena, skinless drumsticks which were scored lower due to a high salt concentration in the coating. These drumsticks also had a drier texture and a slight baking soda flavor.

REFERENCES

Anonymous. 1981. Frozen foods almanac. Quick Frozen Foods 44: 108.
Baker, R.C., Darfler, J.M., and Vadehra, D.V. 1972. Prebrowned fried chicken. 2. Evaluation of predust materials. Poultry Sci. 51: 1220.
Bendall, J.R. 1954. The swelling effect of polyphosphates on lean meat. J. Sci. Food Agr. 10: 468.
Cassidy, J.P. 1977. Phosphates in meat processing. Food Prod. Dev. 11: 74.
Chen, T.C. 1982. Studies on the marinating of chicken parts for deep-fat frying. J. Food Sci. 47: 1016.
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Effect of Fragmentation Method and Formulation on the Quality of Patties Made from Restructured Spent Layer Meat

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ABSTRACT

Poultry patties were prepared from spent layer meat fragmented by flake cutting or grinding (0.64 cm and 2.54 cm openings). The formulation consisted of either 100% breast meat, 50-50 breast and leg meat combination or 100% leg meat. Acceptability scores for both flavor and texture were highest for breast meat patties and lowest for leg meat patties. TBA (2-thiobarbituric acid) values showed increases for all treatments at all storage periods. Water-holding capacity (WHC) was highest for breast meat patties. Shear and resistance-to-tear (RTT) values showed that flaked-cut breast meat patties had the lowest shear values but the highest level of cohesiveness. The coarsely ground patties had higher shear values and lower RTT values.

INTRODUCTION

There are approximately 280 million laying hens in the United States that are eventually used in the food industry as a meat source after they are no longer useful as egg layers. The meat from these "spent layers" is less tender than meat from broilers, so much of it is used in soup, stews and chicken pies. A product of current interest to fast food chains and institutional feeding programs is the chicken patty. While many types of meat can be used to prepare patties, spent layer meat is a good starting material since it is an underutilized product.

A restructuring process has been used successfully with beef, pork and lamb trimmings in the production of steaks and patties having textural properties similar to intact muscle (Mandigo, 1974; Farrington, 1975; Ockerman and Organisciak, 1979; Randall and Larmond, 1977). The key to successful restructuring is adequate binding in the final product. Binding is enhanced by fragmentation and mixing, which releases the soluble protein required for the binding process (Anonymous, 1973).

The restructuring process has been investigated for use with spent layer meat (Roland et al., 1981; Siedman et al., 1982a,b,c). Siedman et al. (1982b) reported on the use of gluten to enhance the binding properties; while Roland et al. (1981) examined various proportions of breast and leg meat. It was found that 100% spent layer breast meat patties had a firmer texture and more acceptable flavor than patties containing varying amounts of leg meat. In preparing patties it is also more economical to include not only breast and leg meat but the natural proportions of skin.

This study was designed to determine the effects of fragmentation method and the formulation (breast, leg or both) of the starting material on the quality and acceptability of patties prepared from spent layer meat.

MATERIALS & METHODS

Preparation of patties

Hand-deboned spent layer breast and leg meat along with natural proportions of skin were obtained from a commercial poultry processing plant and shipped under refrigeration to a poultry further-processing plant. All materials were held for 24 hr at 4°C. The leg meat was separated into thigh and drumstick meat, with the latter being designed using a Beehive deboning machine (Beehive Machinery, Inc., Sandy, UT). The desinewed drumstick meat was added to the formulations without further fragmenting. The skin was partially frozen at −18°C (30 min) to facilitate grinding in a Hobart Grinder (Model 4046, Hobart Corp., Troy, OH) using a 0.64 cm grinding plate. The breast and thigh meat were fragmented (4°C) using an Urschel Comitrol (Model 3600, Urschel, Inc., Valpariso, IN, with head No. 2J0307500), a Hobart Grinder with a 0.64 cm grinding plate, and a Weiler Grinder (Model 1162, Weiler & Co. Inc., Inc., Whitewater, WI) with a 2.54 cm grinding plate to provide three types of fragmentation.

Table 1—Formulation of patties made from spent layer meat and skin

Treatment formulation	Breast (%)	Combination (%)	Leg meat (%)
Breast meat	82	41	0
Thigh meat	0	20.5	41
Drumstick meat	0	20.5	41
Skin	18	18	18

The formulation for each fragmentation method is presented in Table 1. The nine treatments were sampled using a Latin square design with the experiment being replicated.

Each treatment was mixed for 5 min in a Leland Mixer (Model 100DA, Leland Detroit Mfg. Co., Detroit, MI) prior to stuffing into fibrous moisture-proof casings using a Handtmann Stuffer (Model VF-20, Albert Handtmann Gmbl. & Co., West Germany) to form rolls 10.5 cm in diameter and 43.0 cm in length. The rolls, tempered to −5°C, were pressed in a Ross press at 352 kg/cm^2 and immediately sliced using a Ross slicer (Model 950, Ross Industries, Inc., Midland, VA). While still frozen the patties were breaded, packaged in Cryovac bags, evacuated, sealed and stored at −18°C until sampled at 0, 1, 3 and 6 months.

Samples for TBA, shear, resistance-to-tear (RTT), color and sensory evaluation were cooked from the frozen state in a deep fat fryer (Model 500KC, Henry Penny Corp., Eaton, OH) for 3 min at 177°C. Uncooked, unbreaded patties were used for water-holding capacity and proximate analysis.

Proximate analysis

AOAC (1980) procedures were used to determine moisture, protein, fat and ash.

2-Thiobarbituric acid (TBA) test

Treatment effects on oxidative rancidity were evaluated on a 10 g sample of patty using the method of Tarladgis et al. (1960).

Shear determination

Patties were trimmed to 1 cm x 6 cm, weighed, and placed in the shear cell (Model CS-1) of a Texturepress (Model TP-2, Food Tech. Corp., Rockville, MD). Shear values were obtained using peak force expressed as kg/g of meat (Burrill et al., 1962).

Resistance-to-tear (RTT)

The cohesiveness of the cooked patties was determined using a resistance-to-tear attachment (Model MS-1) for a texturepress (Model TP-2, Food Technology Corp., Rockville, MD). The attachment is similar to the one described by Gillett et al. (1978). The patties were

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Table 2—Proximate analyses of raw unbreaded restructured spent layer patties

Portion	Moisture (%)	Protein (%)	Fat (%)	Ash (%)
Breast	72.49a[a]	22.51a	6.71a	0.93a
Combination[b]	70.53b	21.75b	9.20b	0.86a
Leg	70.52b	20.57b	10.79b	0.87a

[a] Each value is a mean of 12 observations. Means in a column having the same letter are not significantly different (P>0.05).
[b] Contains equal portions of breast and leg meat.

Table 3—Water-holding capacity of restructured spent layer meat

Treatment	Storage, months (−18°C) 0	1	3	6
Type of meat		-----% Water Retained-----		
Breast	72.0a[a]	70.8a	68.3a	65.3a
Combination[b]	68.4b	64.4b	63.5b	62.0b
Leg	65.3c	64.4b	59.5c	58.7c
Method of fragmentation				
Flake-cut	69.8x	67.8x	63.4xy	62.4x
0.64 cm grind	68.0x	67.4x	66.2x	62.6x
2.54 cm grind	67.8x	64.3y	61.8y	61.0x

[a] Each value represents the mean of 12 observations. Within storage periods, means having the same letter (a-c) or (x-y) are not significantly different (P>0.05).
[b] Contains equal portions of breast and leg meat.

Table 4—Sensory evaluation of restructured spent layer patties: preference scaling and magnitude estimation

Treatment	Preference[a] Flavor	Texture	Magnitude estimation Chewiness
Type of meat			
Breast	8.45a[c]	7.33a	1.10a
Combination[b]	7.63b	6.41b	1.10a
Leg	6.66c	5.29c	1.06a
Method of fragmentation			
Flake-cut	7.72x	7.34x	0.98x
0.64 cm grind	7.72x	6.86y	1.04x
2.54 cm grind	7.23y	4.84z	1.27y

[a] Preference ratings were indicated by placing a verticle line on an ungraduated 12 cm scale, anchored with the terms poor flavor — good flavor, and poor texture — good texture.
[b] Contains equal portions of breast and leg meat.
[c] Within each parameter means having the same letter (a-c for type of meat and x-z for fragmentation) are not significantly different (P ≥ 0.05). The higher the value, the greater the preference or chewiness.

Table 5—Objective texture measurements of restructured spend layer patties

Treatment	Shear (kg/g sample)	RTT[a] (kg)
Type of meat (combined data)		
Breast	4.24a[c]	2.00a
Combination[b]	4.62b	1.67b
Leg	4.66b	1.30c
Method of fragmentation (combined data)		
Flake-cut	3.85x	2.00x
0.64 cm grind	4.50y	1.59y
2.54 cm grind	5.18z	1.38z

[a] Resistance-to-tear, a measurement for cohesiveness.
[b] Contains equal portions of breast and leg meat.
[c] Each value represents 48 observations; means having the same letter (a-c for composition and x-z for fragmenting) are not significantly different (P>0.05).

trimmed to 10 cm x 10 cm and placed on the split platform. As the platform pulls apart, the force necessary to separate the patty was recorded. Peak force required to tear the samples was reported in kg.

Water-holding capacity (WHC)

WHC was determined on a 25-g sample using the method described by Wierbicki et al. (1957). The following formula, reported by Mast et al. (1982), was used to calculate WHC.

$$\text{WHC or \% water retained} = 1 - \frac{\text{mL } H_2O \text{ in supernatant}}{\text{g } H_2O \text{ in raw sample}}$$

Color determination

Cooked patties were sliced and the interior was evaluated for color using a Gardner XL-10 color difference meter (Gardner Laboratory, Inc., Bethesda, MD). Patties were analyzed at the initial storage period. Each patty was split and placed directly over the aperture using the small beam and aperature. L (reflectance), $+a_L$ (redness), and $+b_L$ (yellowness) values were recorded. The instrument was standardized with a pink tile having the following values: L=67.4, $+a_L$=18.4, and $+b_L$=9.1.

Sensory evaluation

Twelve panelists were chosen from a pool of graduate students who were experienced sensory evaluators. Prior to the actual test, training sessions were conducted to acquaint the students with the chicken patties and to discuss the parameters of chewiness and texture.

A preference rating scale (12 cm anchored line with lower scores indicating poor texture and flavor) similar to that outlined by McGill (1980), was used to evaluate flavor and texture; magnitude estimation (Moskowitz, 1974) was used to measure chewiness. The flake-cut breast meat patty was used as a standard for the chewiness test, with each panelist assigning his/her own modulus for the standard. All taste sessions were conducted in individual cubicles in The Pennsylvania State University Sensory Testing Laboratory. Red lights were used to mask obvious visual differences among products.

Statistical analysis

Statistical analyses were conducted using SAS (Statistical Analysis System, Barr et al., 1979). Analysis of variance procedures were used to test the effects of meat formulation and type of grinding procedure. If analysis of variance showed a significant ($P < 0.05$) difference among treatments, then treatment means were separated using Duncan's multiple range test (Duncan, 1955).

RESULTS & DISCUSSION

PROXIMATE ANALYSES (Table 2) show that breast patties had significantly higher protein ($P < 0.05$) and lower fat ($P < 0.05$) than patties made from either all leg meat or a combination of leg and breast meat. Moisture levels of patties made from breast meat were significantly higher ($P < 0.05$) than those made from either the combination or all leg meat. No differences were observed in ash content in any of the treatments.

Water-holding capacity (WHC)

Breast meat patties had a significantly higher ($P < 0.05$) percentage of water retained than either the combination or leg meat patties (Table 3). With the exception of the 1 month storage period, the combination patties also had significantly higher ($P < 0.05$) percentages of water retained when compared to patties made from leg meat. This is due, in part, to the higher percentage of protein in breast meat with the potential for more binding sites for water. The pH of the material probably did not account for any of the increased WHC of breast meat patties since the pH of the breast meat was at 5.78 and leg meat was 6.37. With the pH of the breast meat closer to the isoelectric point of meat, one might expect a higher WHC in leg meat compared to breast meat if pH were the only factor considered. Similarly, as observed by Miller et al. (1980), there was a general decrease in WHC during the six month frozen storage period. This was probably caused by the fluctuations in temperature (−10°C to −18°C) during frozen storage which resulted in ice crystal formation in the meat.

Sensory evaluation

Flavor and texture preference scores for patties made from breast meat were rated significantly better ($P < 0.05$) than patties made from either the combination breast-leg meat or 100% leg meat (Table 4); in addition, combination patties were rated higher ($P < 0.05$) than leg meat patties. It should be pointed out that while differences in preference did exist among the patties made from different materials, all of the panelists indicated acceptable flavor scores for all treatments throughout the storage period. Panelists using magnitude estimation for chewiness did not indicate any significant differences ($P > 0.05$) between patties made from any of the three materials.

When the method of fragmentation was evaluated, panelists gave significantly lower values ($P < 0.05$) for flavor, texture and chewiness to patties made from the 2.54 cm grind. Panelists did not indicate any flavor differences in patties made from either flake-cut meat or the 0.64 cm grind, but did indicate significant differences in the texture of patties made from these two types of fragmented poultry meat (the flake-cut patties received the higher scores). No differences in chewiness were indicated between patties made from either flake-cut meat or a 0.64 grind, but as indicated earlier a significant difference ($P < 0.05$) did exist among patties made from the 2.54 grind, the flake-cut, and the 0.64 grind.

Table 6—Interior color of cooked restructured spent layer patties

Treatment	L^a	$+a_L{}^b$	$+b_L{}^c$
Breast flake-cut	75.6a[d]	1.4a	12.9a
Breast 0.64 cm grind	75.5a	1.1a	11.8a
Breast 2.54 cm grind	72.7a	1.6a	13.4a
Combination flake-cut	62.8b	4.1bc	12.4a
Combination 0.64 cm grind	62.5b	3.5b	13.0a
Combination 2.54 cm grind	61.0b	3.6b	12.8a
Leg flake-cut	54.8c	4.8cd	13.3a
Leg 0.64 cm grind	50.9d	5.4d	15.4b
Leg 2.54 cm grind	53.0cd	4.8cd	13.2a

[a] L value indicates light reflectance.
[b] $+a_L$ value indicates redness.
[c] $+b_L$ value indicates yellowness.
[d] Each value is a mean of two replications; each replicate consists of five observations. Within each column means with the same letter are not significantly different (P>0.05).

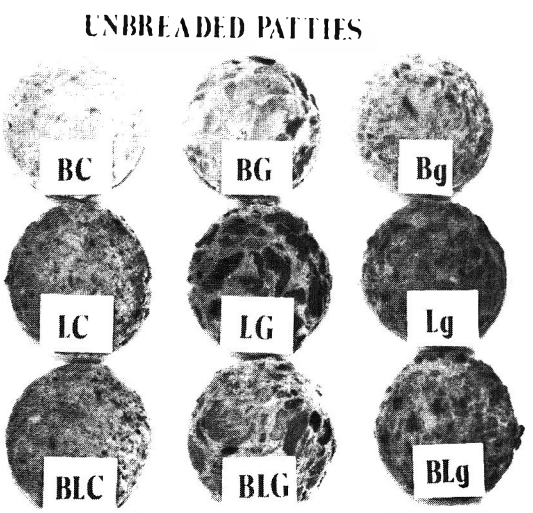

Fig. 1—Unbreaded spent-layer poultry patties: (BC) flake-cut breast meat, (BG) 2.54 cm ground breast meat, (Bg) 0.64 cm ground breast meat, (LC) flake-cut leg meat, (LG) 2.54 cm ground leg meat, (Lg) 0.64 cm ground leg meat, (BLC) flake-cut combination leg and breast meat, (BLG) 2.54 cm ground combination leg and breast meat, (BLg) 0.64 cm ground combination leg and breast meat.

Mechanical measurements

Patties made from breast meat had lower ($P < 0.05$) shear values than those made from either the combination or 100% leg meat (Table 5). The RTT cohesive measurement showed significant decreases ($P < 0.05$) as patty formulation changed from 100% breast meat to 100% leg meat. Of interest is the fact that panelists were reporting higher levels of chewiness (Table 4) in the coarser 2.54 cm grind while mechanical measurements showed a significant increase ($P < 0.05$) in shear values and a significant decrease ($P < 0.05$) in RTT values for these same products.

As expected, the method of fragmentation had a major effect on both shear and RTT values. Significant differences ($P < 0.05$) were observed in both shear and RTT values among all three types of fragmentation procedures (Table 5). Reported shear values indicated significant increases in this measurement as the formulation changed from flake-cut meat (3.85 kg/g) to 0.64 grind (4.50 kg/g) to the coarser 2.54 cm grind (5.18 kg/g). As the particle size in the patty increased, there was a significant decrease in RTT values from 2.00 kg for flake-cut patties, 1.59 kg for 0.64 grind patties, to 1.38 kg for the 2.54 grind patties.

The changes in the physical structure of the products as a result of either formulation or fragmentation can be seen in Fig. 1.

Color measurements

There were color differences (Table 6) due to formulation, but only minor differences associated with the fragmentation methods. Breast meat L values confirmed the lighter ($P < 0.05$) color of this material as compared to the darker leg meat or the combination product. The degree of redness, $+a_L$ values, indicated the lack of red pigment in the breast meat, with $+a_L$ values in the range of 1.1 – 1.6. Leg meat, with it's higher level of hemoglobin and myoglobin had $+a_L$ values of 4.8 – 5.4 while the combination meat patties had intermediate $+a_L$ values of 3.5 – 4.1. There were no significant trends ($p > 0.05$) in the $+b^L$ values for yellowness resulting from formulation or fragmentation methods used.

Table 7—TBA (2-thiobarbituric acid) values of restructured spent layer patties

Storage, months	Meat type	Fragmentation method: Flake-cut	0.64 cm grind	2.54 cm grind	Mean[a]
		mg malonaldehyde/1,000 g sample			
0	Breast	0.69a[b]	0.74a	0.82a	0.75r
	Combination	0.75a	0.79a	0.83a	0.80r
	Leg	1.12a	0.57a	0.82a	0.83r
	Mean[c]	0.85x	0.70x	0.82x	
1	Breast	0.98abc	0.81a	1.19abc	0.99r
	Combination	1.23bcd	1.64e	1.34cde	1.41s
	Leg	1.34cde	0.92ab	1.40de	1.22s
	Mean	1.18x	1.12x	1.32x	
3	Breast	1.31ab	0.95a	1.43ab	1.23r
	Combination	2.52d	1.99c	1.78bc	2.10s
	Leg	2.15cd	1.81bc	2.23cd	2.07s
	Mean	2.00x	1.58y	1.82xy	
6	Breast	2.05a	2.04a	2.43ab	2.17r
	Combination	3.32cd	3.47cd	2.85bc	3.21s
	Leg	3.82d	3.91d	3.76d	3.83t
	Mean	3.06x	3.14x	3.01x	

[a] Each value represents the mean of 12 observations. Within a storage period, summary means having the same letter (r-t) are not significantly different (P>0.05).
[b] Each value represents the mean of two replicates, each replicate consists of two observations. Within a storage period, means having the same letter (a-d) are not significantly different (P>0.05).
[c] Each value represents the mean of 12 observations within a storage period; summary means having the same letter (x-z) are not significantly different (P>0.05).

TBA values

TBA values generally increased during storage; however, patties made from breast meat maintained significantly lower ($P < 0.05$) values throughout the 6 month storage period than the other two formulations (Table 7). During this same period, only minor differences existed between the combination and 100% leg meat patties. The method of fragmentation did not influence TBA scores.

SUMMARY

THE RESULTS of this study indicate that spent layer meat can be used to make acceptable restructured products. The flavor, texture, cohesiveness, overall acceptability and functional properties of patties were affected by the kind of muscle tissue used, i.e., breast, thigh, or a combination of these. In addition, textural properties of patties were influenced by the fragmentation method used. Improving the textural properties of spent layer meat, as demonstrated in this study, should increase the demand for this underutilized material.

REFERENCES

Anonymous. 1973. Facts, Flakes and Fabricated Meats. Bull. 703. Urschel Laboratories Incorporated, Valparaisco, IN.
AOAC. 1980. "Official Methods of Analysis, 1980. 13th ed. Assoc. of Official Analytical Chemists, Washington, DC.
Barr, A.J., Goodnight, J.H., Sall, J.P., Blair, W.H., and Chilko, D.M. 1979. "A User's Guide to SAS 79." SAS Institute, Raleigh, NC.
Burrill, L.M., Deethardt, D., and Saffle, R.C. 1962. Two mechanical devices compared with taste-panel evaluation for measuring tenderness. Food Technol 16: 145.
Duncan, D.B. 1955. Multiple range and multiple F tests. Biometrics 11: 1.
Farrington, A.E. 1975. Flaked, formed and frozen lamb products. Proc. Recip. Meat Conf. 28: 140.
Gillett, T.A., Brown, C.L., Leutzinger, R.L., Cassidy, R.D., and Simon, S. 1978. Tensile strength of processed meats determined by an objective instron technique. J. Food Sci. 43: 1121.
Mandigo, R.W. 1974. Restructed meat products. Proc. Recip. Meat Conf. 27: 403.
Mast, M.G., Uijttenboogaart, Th.G., Gerrits, A.R., deVries, A.W. 1982. Effect of Auger- and Press-type Mechanical Deboning machines on Selected Characteristics of Mechanically Deboned Poultry. J. Food Sci. 47: 1757.
McGill, L.A. 1980. "Sensory Evaluation Methods for the Practicing Food Technologist," (Ed.) Johnston, M.R. p. 5. Institute of Food Technologists, Chicago, IL.
Miller, A.J., Acherman, S.A., and Palumbo, S.A. 1980. Effects of frozen storage on functionality of meat for processing. J. Food Sci. 45: 1466.
Moskowitz, H.R. 1974. Magnitude estimation: Notes on how, what, where and why to use it. J. Food Qual. 1: 195.
Ockerman, H.W. and Organisciak, C.S. 1979. Quality of restructured beef steaks after refrigerated and frozen storage. J. Food Protection 42: 126.
Randall, C.J. and Larmond, E. 1977. Effect of method of comminution (Flake-cutting and grinding) on the acceptability and quality of hamburger patties. J. Food Sci. 42: 728.
Roland, L.M., Seideman, S.C., Donnelly, L.S., and Quenzer, N.M. 1981. Physical and sensory properties of chicken patties made with varying proportions of white and dark spent fowl muscle. J. Food Sci. 46: 834.
Seideman, S.C., Durland, R.R., Quenzer, N.M., and Michels, J.J.D. 1982a. The effect of precooking and flake size on spent fowl restructured steaks. J. Food Protect. 45: 38.
Seideman, S.C., Durland, R.R., Quenzen, N.M., and Carlson, C.W. 1982b. Effect of added wheat gluten and mixing time on physical and sensory properties of spent fowl restructured steaks. J. Food Protection 45: 297.
Seideman, S.C., Durland, P.R., Quenzer, N.M., and Carlson, C.W. 1982c. Utilization of spent fowl muscle in the manufacture of restructured steaks. Poultry Sci. 61: 1087.
Tarladgis, B.G., Watts, B.N., Younathan, M.T., and Dugan, Jr., L.R. 1960. A distillation method for the quantitative determination of malonaldehyde in rancid foods. J. Am. Oil Chem. Soc. 37: 44.
Wierbicki, E., Kunkle, L.E., and Deatherage, F.E. 1957. Changes in the water-holding capacity and cationic shifts during the heating and freezing and thawing of meat as revealed by a simple centrifugal method for measuring shrinkage. Food Technol. 11: 69.
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MARINATING INFLUENCE ON BROILER DRUMSTICKS. . . From page 289

Cunningham, F.E., and Tiede, L.M. 1981. Influence of batter viscosity on breading of chicken drumsticks. J. Food Sci. 46: 1950.
Dodge, J.W., and Peters, F.E. 1960. Temperature and pH changes in poultry breast muscles at slaughter. Poultry Sci. 39: 765.
Elston, E. 1975. Why fish fingers top the market. Fishing News International 14: 30.
Fry, J.L., Waldroug, P.M., Ahmed, E.M., and Lychlich, H. 1966. Enzymatic tenderization of poultry meat. Food Technol. 20: 952.
Hale, K.K. Jr., and Goodwin, T.L. 1968. Breaded fried chicken: Effect of precooking, batter composition, and temperature of parts before breading. Poultry Sci. 47: 739.
Hamm, R. 1971. In "Symposium: Phosphates in Food Processing," p. 65. (Ed.) J.M. deMan and P. Melnychyn. AVI Publ. Westport, CT.
Jessup, L. 1981. Your lowest value-added cost. Quick Frozen Foods 44: 70.
King, F.J., Heilegman, F., and Wierbicki, E. 1974. Solubilized fish muscle as a food binding material. Marine Fisheries Review 36: 18.
Landes, D.R. 1972. The effect of polyphosphates on several organoleptic physical, and chemical properties of stored precooked frozen chickens. Poultry Sci. 51: 641.
Mahon, J.H. 1983. Effects of Kena treatment in poultry processing. Calgon Corp. Pittsburgh, PA, p. 30.
Morgan, B.H. 1971. Edible packaging update. Food Product Dev. 5: 75.
Peterson, K.W. 1977. Effect of polyphosphates on tenderness of hot cut chicken breast meat. J. Food Sci. 42: 100.
Proctor, V.A., and Cunningham, F.E. 1983. Effect of weight, temperature and skinning of broiler drumsticks on batter and breading adhesion. J. Food Quality 6: 315.
Prusa, K.J., Chambers, E. IV, Bowers, J.A., Cunningham, F.E., and Dayton, A.D. 1981. Thiamine content, texture, and sensory evaluation of postmortem papain-injected chicken. J. Food Sci. 46: 1984.
Schnell, P.G. 1976. Method to improve the physical organoleptical and functional properties of flour-based products through the use of yeast and protein of said method. U.S. Patent 3,997,683.
Seeley, L. 1981. The effect of chemical predips on adhesion of coating to broiler drumsticks. In Adhesion of Coating to Broiler Drumsticks. M.S. thesis, Kansas State University.
Snedecor, G.W. and Cochran, W.G. 1976. "Statistical Methods." The Iowa State University Press, Ames, IA.
Suderman, D.R. and Cunningham, F.E. 1979. Factors affecting the adhesion of coating to poultry skin. IV. The effect of various predips, protein sources, and particle size on breading pickup, volume loss, baking loss, tenderness, and water holding capacity of poultry parts. PhD dissertation, Kansas State Univ.
Suderman, D.R., Wiker, J., and Cunningham, F.E. 1981. Factors affecting adhesion of coating to poultry skin: Effects of various protein and gum sources in the coating composition. J. Food Sci. 46: 1010.
Vadehra, D.V., and Baker, R.C. 1970. The mechanism of heat initiated binding of poultry. Food Technol. 24: 775.
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Lipid Peroxidation and Phospholipid Hydrolysis in Fish Muscle Microsomes and Frozen Fish

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ABSTRACT

The interaction between lipid peroxidation and phospholipase A_2 was investigated in fish muscle microsomes and frozen fish muscle. Lysophosphatidylcholine (LPC), a phospholipid hydrolysis product produced by the activity of phospholipase A_2, was detected in the lipids extracted from microsomes peroxidized by incubation with NADH and ADP-Fe^{3+}. No detectable level of LPC was found in the microsomal lipids when NADH was withdrawn from the reaction mixture nor in the untreated microsomal lipids. LPC level in the lipids extracted from unheated microsomes was considerably higher than that from heat-treated microsomes when both were peroxidized by incubation with ascorbate and Fe^{3+}. Phospholipase A_2 was activated by either NADH-dependent or ascorbate-induced lipid peroxidation *in vitro*. The levels of lipid peroxidation in fish muscle were higher when fish were stored at higher frozen storage temperatures and so were the levels of LPC.

INTRODUCTION

A MICROSOMAL lipid peroxidation enzyme system has been identified in various fish species, such as red hake (McDonald et al., 1979), flounder (Shewfelt et al., 1981) and herring (Slabyj and Hultin, 1982). But the exact role of this enzyme system in fish quality deterioration postmortem is still not fully understood (Slabyj and Hultin, 1984). Enzymatic phospholipid hydrolysis in frozen fish has been recognized as a major cause of quality deterioration since the late 1950s (Shewfelt, 1981).

The peroxidation of phospholipids in rat liver microsomes was associated with increased phospholipase A_2 activity (Sevanian et al., 1981, 1983). Yasuda and Fujita (1977) reported that phospholipase A_2 in rat liver mitochondria was activated by approximately 60% after lipid peroxidation. Au et al. (1985) found that phospholipase A_2 was stimulated by introducing oxygen-derived free radicals to isolated rat brain capillaries. Phospholipase A_2 activity has been found in the microsomal fraction isolated from rainbow trout (Bilinski and Jonas, 1966) and flounder (Shewfelt et al., 1981).

Olley and Lovern (1960) suggested that phospholipase may be activated by freezing. Since the microsomal lipid peroxidation enzyme system is active at temperatures below the freezing point of fish tissue (Apgar and Hultin, 1982), it is possible that enzymatic lipid peroxidation activates phospholipase A_2 to initiate phospholipid hydrolysis in frozen fish muscle.

Both NADH-dependent enzymatic and ascorbate-induced nonenzymatic lipid peroxidation have been observed in microsomes isolated from rainbow trout muscle cells in our laboratory. The purpose of the present study was to investigate the interaction between lipid peroxidation and phospholipase A_2 in both microsomes and frozen minced fish muscle.

MATERIALS & METHODS

Fish

Rainbow trout (*Salmo gairdnerii*) weighing approximately 450–900g were obtained form the School of Fisheries Hatchery, University of Washington. Fish were killed by a blow to the head and placed immediately in crushed ice. The fish were transported to the laboratory where they were filleted and skinned. The red muscle was carefully removed. The white muscle was minced using an electric food grinder (Model 996, Oster Corp., Milwaukee, WI). Ground fish muscle was frozen and stored in Whirl-pack sterile sampling bags at −40°C for further use.

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Isotonic buffer solution

One-tenth molal KCl solution in 5 mM histidine buffer, pH 7.3, was used to prepare microsomes and various reagents for experiments.

Preparation of microsomes

Microsomes were prepared by differential centrifugation using a modification of the procedure described by McDonald et al. (1979).

Frozen fish muscle mince was thawed at room temperature (20°C) and homogenized with 4 volumes of cold isotonic buffer solution using a Waring blender at the maximum speed for 20 sec. Fish homogenate was then centrifuged at 22,000 xg (JA-14 rotor, 12,000 rpm, Beckman J2-21M high speed centrifuge, Beckman Instruments, Inc., Spinco Division, Palo Alto, CA) and 4°C for 30 min to remove mitochondria and fractions heavier than mitochondria. The post-mitochondrial supernatant was collected and centrifuged at 105,000 x *g* (Type 30 rotor, 30,000 rpm, Beckman L8-70 ultracentrifuge, Beckman Instruments, Inc.) and 4°C for 60 min. The microsomal pellet was collected and washed twice by suspending the microsomes in cold 0.60M KCl solution in 5 mM histidine buffer, pH 7.3, using a Potter-Elvehjem tissue grinder and by ultracentrifugation under the same conditions. Washed microsomes were dispersed in isotonic buffer solution. Protein concentration was adjusted to 10.0 mg/ml for experiments.

Protein concentration measurement

Protein concentration was measured using a modified Lowry method (Markwell et al., 1981).

Enzymatic peroxidation

Ten milliliters of a microsomal preparation with a protein concentration of 10.0 mg/mL were mixed with 10.0 mL of a cofactor solution containing 2.0 mM NADH (Grade III, Sigma Chemical Co., St. Louis, MO), 2.0 mM ADP (Grade X, Sigma Chemical Co., St. Louis, MO), and 0.3 mM $FeCl_3$ (J.T. Baker Chemical Co., Phillipsburg, NJ) in the isotonic buffer solution. This reaction mixture was incubated in an open 50 mL Erlenmeyer flask at 25°C for 2 hr in a water bath shaker (Aquatherm water bath shaker, New Brunswick Scientific Co., Inc., Edison, NJ) at 150 rpm/min. As a control, microsomes were incubated under the same conditions in a reaction mixture without NADH. Reactions were stopped by the addition of a lipid extracting solvent, chloroform/methanol (2:1, v/v) mixture. The original phospholipid composition was obtained by analyzing untreated microsomes suspended in the isotonic buffer solution.

Nonenzymatic peroxidation

Ten milliliters of the microsomal preparation with a protein concentration of 10.0 mg/mL were heated in a boiling water bath for 1 min, and 10.0 mL cofactor solution containing 2.0 mM sodium ascorbate (Sigma Chemical Co., St. Louis, MO) and 0.3 mM $FeCl_3$ in the isotonic buffer solution were added to both heat-treated and unheated microsomes. The reaction mixtures were incubated in open 50 mL Erlenmeyer flasks at 20°C for 2 hr in a water bath shaker at 150 rpm/min. Reactions were stopped by the addition of the lipid extracting solvent.

Lipid extraction from microsomal preparations

Microsomal lipids were extracted using a modification of the procedure of Folch et al. (1957) and Christie (1973). Twenty volumes of the extracting solvent were added to the microsomal preparation. Protein precipitates were removed by filtration through Whatman No. 1 filter paper. One quarter volume of the isotonic buffer solution was added to the filtrate to remove nonlipid contaminants. The organic layer containing lipids was washed twice by mixing with a one quarter volume of methanol/water (1:1, v/v) mixture.

The washed organic layer containing lipids was condensed to about 5 ml using a rotary vacuum evaporator (Buchi Rotavapor, R-110, VWR Scientific, San Francisco, CA.) at 30°C. The condensed lipid extract was transferred to a test tube with a teflon-lined screw cap and dried under a stream of nitrogen gas to a constant weight. Dried microsomal lipids were redissolved in chloroform to a concentration of approximately 100 mg/mL and stored at −20°C until ready for phospholipid analysis.

Lipid extraction from post-microsomal supernatant

Post-microsomal supernatant was lyophilized (Freezemobile 6 freeze dryer, Virtis Company, Inc., Gardiner, NY) before lipid extraction. Two hundred milliliters of the extracting solvent were used to extract lipids from the dried material. Nondissolvable solid was removed by filtration through Whatman No. 1 filter paper. Lipid extract was washed, condensed and dried using the same methods described in the previous section.

Phospholipid composition analysis

Two-dimensional thin-layer chromatography. The method described by Roelofsen and Ott (1981) was adapted for this study. Preparative silica gel TLC plates (0.5 mm, 20 × 20 cm, Adsorbosil plus 1, Alltech, Deerfield, IL) were activated at 130°C for at least 2 hr. Approximately 2 mg microsomal lipids were applied to the plate. TLC plates were developed in the following two solvent systems: Solvent I: chloroform/methanol/ammonium hydroxide/water (90:54:5.5:5.5, v/v); Solvent II: chloroform/methanol/acetic acid/water (90:40:12:2, v/v). The direction of the second development was perpendicular to that of the first development. After each development the plate was dried at 60°C in a forced air convection oven for 10 min. Each development took about 75 min. The separated lipid fractions on the developed plate were charred by using 50% sulfuric acid as a spraying reagent and heating at 130°C for 5 min.

Qualitative identification of phospholipids. Phospholipids were qualitatively identified using molybdenum blue reagent (Sigma Chemical Co., St. Louis, MO) for phosphorus-containing lipids; 0.2% ninhydrin spray for phospholipids containing free amino groups; and Dragendorff reagent (Christie, 1973) for choline-containing phospholipids. R_f values of various standards (Sigma Chemical Co., St. Louis, MO) were determined for phospholipid identification.

Quantitative analysis of phospholipids. Phospholipid spots were scraped from the plates and transferred to Pyrex test tubes. Silica gel blanks with areas of 100, 300 and 600 mm^2 were taken in duplicate from the sides along the two solvent fronts. Sodium phosphate monobasic (Mallinckrodt, Inc., Paris, KY) was used as a phosphorus standard. Standard curves were prepared with a range of 25–500 nmole phosphorus and new standard curves were prepared for each assay. Two aliquots of 3.0 μL lipid extract of each sample were used for total phospholipid measurement. The method used for phosphorus measurement was the procedure developed by Bartlett (1959) and modified by Broekhuyse (1968).

Calculation of phosphorus mole percentage (mole%)

The phosphorus level measured from each spot of a particular plate was corrected by substracting the phosphorus level of a silica gel blank which had the same area. The mole% of each phospholipid was calculated by dividing the corrected phosphorus level by the total phosphorus of that particular lipid sample.

Lipid peroxidation in frozen fish muscle

Two frozen storage experiments using fish muscle mince were carried out. In the first experiment, freshly excised fish muscle mince stored at −40°C for 18 hr or less was transferred to −40°, −20° and −10°C for a 3-month storage. In the second one, fish muscle mince having been stored at −40°C for 3 months was transferred to −40°, −20°, and −10°C for another 3-month storage.

At the end of storage, microsomes were quantitatively isolated from frozen muscles. Lipids were extracted from both microsomes and post-microsomal supernatant. Phospholipid composition of microsomes was analyzed using two-dimensional TLC.

Lipid peroxidation in post-mitochondrial supernatant was measured using the thiobarbituric acid assay described by Buege and Aust (1978). The level of thiobarbituric acid reactive substances (TBARS) was expressed in nmole malondialdehyde (MDA) per mg lipids extracted from both microsomes and post-microsomal supernatant.

RESULTS & DISCUSSION

THE RESULTS of quantitative analysis of microsomal lipids show that phosphatidylcholine (PC) and phosphatidylethanolamine (PE) are the major phospholipids in fish muscle microsomes (Table 1). PC and PE constitute about 60% and 20%, respectively, of total number of moles of phospholipids. Phosphatidylinositol (PI), cardiolipin (CL), sphingomyelin (SM) and an unidentified phospholipid (X) were the minor components. SM and X were not completely resolved by the solvent system employed in this study.

The appearance of cardiolipin (CL) in the microsomal preparations indicates contamination from mitochondria, since CL is found in substantial amounts in mitochondria but not in other animal cell membranes (Finean et al., 1978). However, the degree of contamination was low. Microsomes were prepared by differential centrifugation during which mitochondria fragments could have sedimented with sarcoplasmic reticulum fragments. Except for the occurrence of cardiolipin, the overall phospholipid composition of the microsomes isolated from rainbow trout white muscle in this study is similar to that of

Table 1—Phospholipid composition of untreated, enzymatically peroxidized and control microsomes (values are in mole%)[a]

Phospholipid	Untreated[b]	Enzymatically Peroxidized[b]	Control[b]
Phosphatidylcholine (PC)	61.8 ± 3.7	43.0 ± 1.0	62.0 ± 2.3
Phosphatidylethanolamine (PE)	20.2 ± 2.4	7.8 ± 1.0	19.1 ± 3.1
Sphingomyelin + X (SM + X)	3.4 ± 0.2	5.2 ± 0.5	3.6 ± 0.2
Phosphatidylinositol (PI)	3.7 ± 0.3	2.6 ± 0.4	4.2 ± 1.1
Cardiolipin (CL)	1.5 ± 0.2	0.6 ± 0.6	1.6 ± 0.2
Lysophosphatidylcholine (LPC)	nd[c]	5.0 ± 1.2	nd[c]
Peroxidation products (PP)	nd[c]	12.6 ± 0.4	0.1 ± 0.1

[a] Mean ± s.d. of 2 experiments in duplicate.
[b] For detail of reaction conditions refer to Materials & Methods Section.
[c] nd = not detected.

Table 2—Phospholipid composition of non-enzymatically peroxidized unheated and heat-treated microsomes (values are in mole%)[a]

Phospholipid	Unheated[b]	Heat-treated[b]
Phosphatidylcholine (PC)	43.6 ± 2.7	51.8 ± 3.0
Phosphatidylethanolamine (PE)	8.0 ± 0.3	12.1 ± 1.4
Sphingomyelin + X (SM + X)	5.1 ± 0.5	4.8 ± 0.3
Phosphatidylinositol (PI)	3.0 ± 0.4	3.8 ± 0.2
Cardiolipin (CL)	1.1 ± 0.1	1.5 ± 0.2
Lysophosphatidylcholine (LPC)	3.6 ± 0.8	1.1 ± 0.5
Peroxidation products (PP)	14.7 ± 1.1	10.0 ± 2.3

[a] Mean ± s.d. of 2 experiments in duplicate.
[b] For detail of treatments and reaction conditions refer to Materials & Methods Section.

Table 3—Lipid peroxidation in fresh and frozen fish muscle[a]

Storage temp (°C)	TBARS (nmole MDA mg lipids)[b] Experiment 1	Experiment 2
Fresh	---[c]	1.3
−40	2.2	1.6
−20	5.7	13.4
−10	16.0	13.3

[a] For storage conditions refer to Materials & Methods Section.
[b] Average of duplicate measurements: TBARS = thiobarbituric acid reactive substances; MDA = malondialdehyde.
[c] not measured.

Table 4—Phospholipid composition of microsomal lipids isolated from fresh and frozen fish muscle[a] (values are in mole %)[b]

Phospholipid	Fresh	Storage temp (°C) −40 Expt. 1	−40 Expt. 2	−20 Expt. 1	−20 Expt. 2	−10 Expt. 1	−10 Expt. 2
Phosphatidylcholine (PC)	65.7	60.6	65.0	55.4	51.0	42.8	42.7
Phosphatidylethanolamine (PE)	20.0	16.7	20.3	17.2	15.4	12.2	12.4
Sphingomyelin + X (SM + X)	2.8	4.3	3.6	5.9	5.2	6.4	5.9
Phosphatidylinositol (PI)	4.7	4.3	4.4	5.2	4.0	5.8	4.6
Cardiolipin (CL)	1.5	1.9	2.3	1.3	1.0	0.1	0.3
Lysophosphatidylcholine (LPC)	nd[c]	nd[c]	0.1	0.3	3.6	2.6	4.1
Peroxidation products (PP)	0.1	0.4	0.2	0.5	0.6	4.8	2.0

[a] For storage conditions refer to Materials & Methods Section.
[b] Average of duplicate measurements.
[c] nd = not detected.

Table 5—Recovery of microsomes and total post-mitochondrial lipids from fresh and frozen fish muscle

Storage temp (°C)	Experiment 1 Microsomes[a]	Experiment 1 Total lipids[b]	Experiment 2 Microsomes[a]	Experiment 2 Total lipids[b]
Fresh	—[c]	—[c]	7.4	0.3644
−40	3.8	0.2345	4.6	0.2870
−20	1.1	0.0985	2.1	0.0662
−10	0.5	0.0455	2.0	0.0717

[a] Microsomes were measured after the first ultracentrifugation. Values are in g/100 muscle.
[b] Total lipids were extracted from both microsomes and post-microsomal supernatant. Values are in g/100 g muscle.
[c] not measured.

sarcoplasmic reticulum vesicles isolated from flounder muscle by gradient centrifugation reported by Borhan et al. (1984).

The phospholipid composition of peroxidized microsomes (Table 1) changed considerably after 2-hr incubation. Two extra phosphorus-containing components were found in peroxidized microsomes. One was identified as lysophosphatidylcholine (LPC), which was a product of phospholipid hydrolysis catalyzed by phospholipase A_2. The second one was peroxidation products (PP), a band lying across the plate along the starting lie of the second solvent development. No LPC and PP were detected in untreated microsomes or control microsomes which were incubated under the same reaction conditions used for peroxidized microsomes except that NADH was omitted. This indicated that the formation of PP and LPC appeared to be dependent on lipid peroxidation in microsomes. The formation of LPC was a result of the activation of phospholipase A_2 by the lipid peroxidation being initiated by NADH and ADP-Fe^3 in microsomal preparations. This observation agrees with those reported for rat liver microsomes (Sevanian et al., 1981, 1983), rat liver mitochondria (Yasuda and Fujita, 1977) and rat brain capillaries (Au et al., 1985).

The involvement of phospholipase A_2 in the phospholipid hydrolysis was further tested by inducing lipid peroxidation in both unheated and heat-treated microsomes using ascorbate and Fe^{3+} (Table 2). After 2 hr incubation, the level of peroxidation products (PP) was approximately the same for both microsomal preparations, suggesting both had undergone peroxidation to the same extent. In a similar experiment of ascorbate-induced nonenzymatic lipid peroxidation, TBA measurements showed this reaction reached a maximal level of peroxidation prior to 30 min. The level of LPC in the heat-treated microsomes in this experiment was considerably lower than that in the unheated microsomes indicating that its formation may be linked to a heat labile enzyme. The results of these experiments using fish muscle microsomes suggested that phospholipid hydrolysis due to the activity of phospholipase A_2 could be triggered by either enzymatic or nonenzymatic lipid peroxidation *in vitro*.

The extent of mitochondrial contamination of microsomal preparations was low, as indicated by an average cardiolipin (CL) content of only 1.0 to 2.0 mole% (Table 1 and 2). The level of CL in mitochondria isolated from different tissues has been reported to be approximately 19% (Tzagoloff, 1982). The major membranal constituent in our microsomal preparations was sarcoplasmic reticulum vesicles. Bilinski and Jonas (1966) have investigated the distribution of phospholipase A_2 (lecithinase) in the subcellular fractions of rainbow trout lateral line muscle. They found that the specific activity of phospholipase A_2 in the microsomal fraction was approximately 6 times as high as that in the mitochondrial fraction. This suggested that the activity of phospholipase A_2 of mitochondrial origin in our microsomal preparation was very minor.

If the reaction pathway in sarcoplasmic reticulum in fish muscle cells during frozen storage is similar to that found in the microsomal preparations, we would expect an increased level of lipid peroxidation correlating with an increasing level of phospholipid hydrolysis in frozen fish muscle. Lipid peroxidation measured in the post-mitochondrial supernatant isolated from frozen fish muscle in both experiments showed increasing values corresponding to storage at −40°, −20° and −10°C (Table 3).

The phospholipid composition of microsomes isolated from freshly excised muscle and muscle stored at −40°, −20° and −10°C is shown in Table 4. Freshly excised muscle and muscle stored at −40°C had very similar phospholipid composition. These samples had the highest level of phosphatidylcholine, but the lowest lysophosphatidylcholine among all the muscle samples.

The level of lysophosphatidylcholine in various fish muscle samples was proportional to lipid peroxidation (Table 3 and 4). Thus, phospholipid hydrolysis occurred in frozen fish muscle tissue and appeared to correlate with lipid peroxidation.

The large difference in level of lipid peroxidation and phospholipid hydrolysis in −20°C frozen fish muscle between experiment 1 and 2 (Table 3 and 4) was probably due to the different prior treatment of the fish muscle samples used in these two storage experiments. Freshly excised fish muscle was used for experiment 1, but for experiment 2, fish muscle that had been stored at −40°C for 3 months was used. Nevertheless the direction of change was the same in both cases.

The level of peroxidation products (PP) in whole muscle preparations was quite low even when lysophosphatidylcholine was relatively high (Table 4). This may be explained by the hydrophilic characteristics of these compounds and their probable diffusion into the cytosol due to the action of phospholipase A_2.

The recovery of microsomes and associated lipids from the fresh and frozen fish muscle decreased as fish was stored at higher frozen storage temperatures (Table 5). Lipid peroxidation expressed as TBARS per unit weight of total post-mitochondrial lipids was inversely correlated with the recovery of both microsomes and lipids. This suggested a link between peroxidation and destruction of lipids by hydrolysis.

The results of this study indicated that in isolated microsomal preparations phospholipid hydrolysis was dependent on peroxidation and the results from stored frozen fish muscle supported the hypothesis that this relationship also existed in whole muscle. Experiments are underway to determine whether

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Influence of Frozen Storage and Phosphate Predips on Coating Adhesion in Breaded Fish Portions

M. L. COREY, D. L. GERDES, and R. M. GRODNER

ABSTRACT

The influence of freeze thaw-cycles and the presence of phosphate on the adhesion of two commercially produced batterfry coatings to frozen fish portions were investigated. Prior to coating, one-half of the samples were treated with a 10% sodium tripolyphosphate predip. After coating, the samples were pre-fried and frozen. The storage conditions consisted of 1, 2 or 3 freeze-thaw cycles during a three-month period and storage at a constant temperature. Freeze-thaw cycles resulted in moisture loss from the breading, but did not influence coating loss. The presence of phosphates did not influence adhesion of batter fry coating. The type of batter (Tempura and Batter Fry) did not influence coating adhesion.

INTRODUCTION

IN THE MID-1960's, the concept of batter frying was introduced. In this process the food is usually pre-dusted with flour or dry batter mix, conveyed through a special batter applicator; and then pre-fried to set the batter and impart the desired frying oil content for enhanced texture and quality (Suderman and Cunningham, 1983). Batter and breading technology is still considered an art but is slowly becoming a science. Most current developmental work is centered on improving batters and the manufacturing processes. However, many other problems exist. One of the main problems is that of adhesion.

Adhesion in this context is defined as the chemical and physical binding of a coating, both with itself and with the food product it coats (Suderman and Cunningham, 1979). Loss of coating may occur during application, processing, transportation, frozen storage and/or handling during consumption. Cooking method, cooking ingredients, thickening agents, skin ultra-structure, holding temperature, and chemical predips have all been found to influence adhesion (Hansen and Fletcher, 1965; Hale and Goodwin, 1968; Baker et al., 1976; Suderman and Cunningham, 1983). Various phosphate forms such as sodium tripolyphosphate may affect cooking and freezing times. Holding temperature was also found to influence coating adhesion (Seeley, 1981). In current distribution and marketing practices the possibility of fluctuating storage temperature exists. The objectives of this study were to investigate the influence of freeze-thaw cycles on the adhesion of batterfry coatings to pre-fried fish portions; to determine if the presence of phosphates would influence coating adhesion during freeze-thaw cycles; and to evaluate the adhesive qualities of two commercially available batterfry coatings.

MATERIALS & METHODS

Sample materials

Frozen cod fillets (*Gadus* sp.) were obtained from a local distributor in Baton Rouge, LA. The frozen fillets were purchased in 10 lb. blocks and stored at −20°C. The coatings applied to the fish were composed of either a Tempura Batter Fry Mix #2108 or Batter Fry Mix #2079. A predust (#3149) and Japanese style bread crumb (#6010) were also used to make the coatings. These products were purchased from Newly Wed Foods, Chicago, IL.

Sample preparation

The fillets were tempered at 2–4°C for 12 hr and cut with an electric knife into portions weighing approximately 56g. Samples were randomly assigned to the type of coating applied and then subdivided on the basis of a predip application or no predipping. A 10% sodium tripolyphosphate (NaTPP) solution was used as the predip. The fish portion was dipped in the NaTPP solution for one minute (Ellinger, 1980). The procedures used for preparation batter and application to the samples closely simulated commercial procedures for the production of crispy, crunchy batter fry products. After pretreatment, the portions were predusted, dipped into a batter, rolled in bread crumbs and prefried at 204°C in soybean oil for 30 sec to set the coating. The first portions were air cooled, frozen, hand packed into waxed lined paper board cartons and stored at −20°C. The total storage period was three months, with three freeze-thaw cycles occurring at three-week intervals. The freeze-thaw cycle was accomplished by removing the fillets from the freezer, thawing at room temperature (25°C) for 8 hr and refreezing to −20°C. Samples were removed from storage and reconstituted by baking for 20 min at 204°C after 3, 6, 9, and 12 weeks of storage.

Moisture

Moisture content was determined for the breading, fish and whole product by drying in a precision gravity convection oven for 2 hr at 130°C (AOAC, 1980).

Crude fat

The percent crude fat (dry basis) was determined by the Goldfish method (AOAC, 1980).

Color

Coating color of the baked product was measured using a Model D-25 Hunter Lab Color Difference Meter (Francis and Clydesdale, 1975). L, a and b values were reported.

Adhesion

Batter adhesion was measured by the mechanical sieve shaker method as described by Suderman and Cunningham (1979). The percent breading loss was calculated by the following formula:

$$\% \text{ Breading loss} = \text{BCL/WBA} \times 100$$

BCL = weight of bread crumbs shaken from the fillets by mechanical shaking; WBA = weight of breading adhering to a control fish stick after cooking. A product that maintains 85% of its coating after shaking in a No. 30 sieve is considered to have good adhesion (Suderman and Cunningham, 1979).

Statistical analysis

Statistical analysis of the data was carried out using the Statistical Analysis System (SAS). The data were analyzed using the Generalized Linear Model (GLM) procedure and means were separated using Duncan's Multiple Range Test.

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RESULTS & DISCUSSION

Coating adhesion

There was no significant difference in coating loss based on batter composition (Table 1). The Batterfry coating contained a greater variety of materials (i.e. starches, proteins) which affect adhesion as compared to the Tempura batter (Anon., 1984). Researchers have found that predusting with breadings containing modified starches and various proteins improves adhesion (Baker et al., 1976). In this study the additional starches and proteins in the Batterfry coating did not greatly enhance its adhesive properties when compared to the Tempura batter.

The samples which were dipped in a 10% sodium tripolyphosphate (NaTPP) solution for 1 min prior to coating showed no significant difference ($P>0.05$) in coating loss when compared to the nondipped fillets (Table 1). Phosphate compounds are utilized in seafood processing to retain moisture, increase tenderness and preserve color (Ellinger, 1980; Seeley, 1981). MacCallum (1969) reported that polyphosphates minimized spattering and breading blow-off by controlling moisture loss that could occur during the flash-frying step. In the present study neither the phosphate dip nor lack of phosphate dip caused the typical losses due to the "blow-off" phenomenon.

The results from the shaker method indicated a significant ($P < 0.05$) increase in breading loss due to storage time at constant temperature. There was no significant change in crumb loss after 3 or 6 wk of storage; however, there was a significant increase in crumb loss from 6 to 9 wk and from 9 to 12 wk. This loss was expected as a result of the overall dehydration noted after 6 weeks of storage. Suderman and Cunningham (1979) noted the same trend in breading loss due to dehydration in breaded drumsticks. The lowest mean crumb loss was 1.65% after 3 wk at constant temperature. Samples which were held at constant storage temperature for 12 wk had the highest mean crumb value.

Exposing the Cod fillets to three freeze-thaw cycles affected the crumb loss. The increase in crumb loss between cycle 1 and cycle 2 was not significant, while the increase from cycle 2 to cycle 3 was significant ($P < 0.05$).

Coating moisture and fat content

The moisture and fat content of the breaded Cod fillet did not differ significantly due to batter composition (Table 2) or the presence of phosphates (Table 3). However, it was observed that significant dehydration of the breading occurred during storage at constant temperature ($P < 0.05$). The loss of moisture in the breaded fillets over storage time indicates that freeze-dehydration (freezer-burn) of the fillet surface was occurring. This is common in frozen breaded products. The decrease in moisture content of the breading caused by the freeze-thaw cycling was partially due to freeze-dehydration of the surface and drip loss during the thawing cycle.

Higher fat contents were observed in frozen and thawed samples (Table 3). During the baking process some of the oil was released from the coating into the pan in which it was baked. The coating hardened during the freeze-thaw cycles releasing less oil as the samples were baked.

Table 1—Crumb loss value (% crumb loss) for cod fillets stored at constant temperature and cod fillets treated with various freeze-thaw cycles

Treatment	n	% Crumb loss
Batter type		
Tempura	42	2.33[a]
Batterfry	42	2.41[a]
Phosphate dip		
0% NaTPP	42	2.36[a]
10% NaTPP	42	2.37[a]
Storage time (wk)		
3	12	1.65[c]
6	12	1.90[c]
9	12	2.56[b]
12	12	3.27[a]
Freeze-thaw (wk)[d]		
6 - 1 cycle	12	1.77[c]
9 - 2 cycles	12	2.12[bc]
12- - 3 cycles	12	3.29[a]

[a c] Means with the same letter within treatments are not significant at the 5% level according to Duncan's Multiple Range Test.
[d] Storage time values and freeze-thaw values were analyzed as one treatment.

Table 2—Moisture content (%) of breaded frozen Cod fillets

Treatment	n	Breading	Fish	Breading and fish
Batter type				
Tempura	28	34.49[a]	65.57[a]	51.18[a]
Batterfry	28	32.76[a]	63.91[a]	48.81[a]
Phosphate dip				
0% NaTPP	28	34.52[a]	64.58[a]	51.21[a]
10% NaTPP	28	32.74[a]	64.90[a]	48.78[a]
Storage time (wk)				
3	8	39.12[a]	69.73[a]	54.96[a]
6	8	35.80[ab]	60.57[b]	52.33[ab]
9	8	31.62[bc]	65.52[ab]	48.75[b]
12	8	34.07[abc]	67.81[a]	50.07[ab]
Freeze-thaw (wk)[d]				
6 - 1 cycle	8	34.15[abc]	60.06[b]	48.86[b]
9 - 2 cycles	8	32.57[bc]	65.15[ab]	48.47[b]
12 - 3 cycles	8	28.16[c]	64.35[ab]	48.53[b]

[a c] Means with same letter within treatments are not significantly different at the 5% level according to Duncan's Multiple Range Test.
[d] Storage time values and freeze-thaw values were analyzed as one treatment.

Table 3—Crude fat content (%) of breaded frozen Cod fillets

Treatment	n	Breading	Fish	Breading and Fish
Batter type				
Tempura	28	10.28[a]	3.72[a]	8.04[a]
Batterfry	28	11.06[a]	3.84[a]	8.32[a]
Phosphate dip				
0% NaTPP	28	11.33[a]	4.05[a]	8.49[a]
10% NaTPP	28	10.01[b]	3.51[a]	7.88[a]
Storage time (wk)				
3	8	11.35[ab]	2.36[b]	7.84[ab]
6	8	8.49[c]	3.41[ab]	6.10[b]
9	8	10.64[bc]	5.59[a]	8.98[ab]
12	8	10.32[bc]	1.91[b]	6.66[b]
Freeze-thaw (wk)[d]				
6 - 1 cycle	8	9.62[bc]	4.27[ab]	7.95[ab]
9 - 2 cycles	8	10.85[b]	4.50[ab]	8.71[ab]
12 - 3 cycles	8	13.43[a]	4.42[ab]	11.04[a]

[a c] Means with same letter within treatments are not significantly different at the 5% level according to Duncan's Multiple Range Test.
[d] Storage time values and freeze-thaw values were analyzed as one treatment.

Coating color

The color of the batters differed significantly due to the presence of phosphates ($P < 0.05$). The HunterLab color difference meter "a" values were lower for samples treated with NaTPP (Table 4). Thus, phosphates caused the cooked color to be lighter. Storage time (3–12 wk) and freeze-thaw cycling (1–3 cycles) had no significant effect on the Hunter color values. There was a general increase in "L" values from 41.37 (3 wk constant temperature storage) to 47.07 (12 wk constant temperature storage). This follows the findings of Suderman and Cunningham (1983). As storage time and fat content increases and the moisture content decreases the product is susceptible to browning therefore lower "L" (lightness) values. The "L" values of the freeze-thawed product gave the same trend. The same trends were observed for the "a" (red) and "b" (yellow) color values.

CONCLUSION

KNOWLEDGE of certain time-temperature relations in regard to product quality may be beneficial to the seafood industry.

The results of this study indicated that storage time alone had the greatest affect on adhesion. The longer the storage

Table 4—Hunter color difference meter "L", "a" and "b" values for breaded cod fillets

Treatment	n	L	a	b
Batter type				
Tempura	234	43.98[a]	5.57[a]	12.13[a]
Batterfry	234	43.52[a]	5.28[a]	16.14[a]
Phosphate dip				
0% NaTPP	234	42.30[a]	5.74[a]	16.76[a]
10% NaTPP	234	45.27[a]	5.10[b]	11.33[a]
Storage time (wk)				
3	64	41.44[a]	5.36[a]	11.12[a]
6	64	52.15[a]	5.43[a]	13.15[a]
9	64	44.70[a]	5.50[a]	11.36[a]
12	64	47.07[a]	6.17[a]	14.70[a]
Freeze-thaw (wk)*				
6 - 1 cycle	64	41.48[a]	5.22[a]	11.21[a]
9 - 2 cycles	64	45.00[a]	4.88[a]	11.19[a]
12 - 3 cycles	64	46.17[a]	5.66[a]	13.21[a]

[a,b] Means with same letter within treatments are not significantly different at the 5% level according to Duncan's Multiple Range Test.

[c] Storage time values and freeze-thaw values were analyzed as one treatment.

time the greater the breading loss. Coating loss was attributed to dehydration of the breading with age. Batterfry coatings, whether traditional or a tempura, have good adhesive properties. In this study, differences in the ingredient composition of the coatings, the presence of phosphates, and freeze-thaw cycling did not significantly affect adhesion. However, there was a significant increase in breading loss with each freeze-thaw cycle.

REFERENCES

Anonymous. 1984. Batterfry coating and Tempura batter ingredient statement. Newly Wed Foods, Inc. Chicago, IL.

AOAC. 1980. "Official Methods of Analysis," 13th ed. Association of Official Analytical Chemists. Washington, DC.

Baker, R., Darfler, J., Mulnix, E., and Nath, K. 1976. Palatability and other characteristics of repeatedly frozen chicken broiler. J. Food Sci. 41: 43.

Ellinger, R. 1980. "Phosphates as Food Ingredients." CRC Press, Cleveland, OH.

Francis, F. and Clydesdale, F. 1975. "Food Colorimetry: Theory and Applications." AVI Publishing Co., Westport, CT.

Hale, K. and Goodwin, T. 1968. Breaded fried chicken: Effects of precooking, batter composition and temperature of parts before breading. Poultry Sci. 47: 739.

Hansen, H. and Fletcher, L. 1965. Preparation of pre-cooked frozen poultry products, U.S. Patent 3,169,069.

MacCallum, W. 1969. Freezing and Irradiation of Fish. Fishing News, LTD., London.

Seeley, F. 1981. Adhesion of coatings of broiler drumsticks. M.S. thesis, Kansas State Univ., Manhattan, KS.

Suderman, D. 1979. Factors affecting adhesion of coating to poultry skin. Ph.D. dissertation, Kansas State Univ., Manhattan, KS.

Suderman, D. and Cunningham, F. 1979. New portable sieve shaker tags breading adhesion. Broiler Industry 42: 66.

Suderman, D. and Cunningham, F. 1981. The effect of freezing broiler drumsticks on breading adhesion. J. Food Sci. 46: 1953.

Suderman, D. and Cunningham, F. 1983. "Batters and Breadings." AVI Publishing Co., Westport, CT.

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inhibition of peroxidation in stored frozen fish muscle will reduce or prevent phospholipid hydrolysis.

REFERENCES

Apgar, M.E. and Hultin, H.O. 1982. Lipid peroxidation in fish muscle microsomes in the frozen state. Cryobiology 19: 154.

Au, A.M., Chan, P.H., and Fishman, R.A. 1985. Stimulation of phospholipase A_2 activity by oxygen-derived free radicals in isolated brain capillaries. J. Cell. Biochem. 27: 449.

Bartlett, G.R. 1959. Phosphorus assay in column chromatography. J. Biol. Chem. 234: 466.

Bilinski, E. and Jonas, R.E.E. 1966. Distribution of lecithinase in the subcellular fractions of rainbow trout (*Salmo gairdneri*) lateral line muscle. J. Fish. Res. Bd. Canada. 23: 1811.

Borhan, M., Shewfelt, R.L., and Hultin, H.O. 1984. Sarcoplasmic reticulum from flounder muscle having improved lipid peroxidative activity. Anal. Biochem. 137: 58.

Broekhuyse, R.M. 1968. Phospholipids in tissues of the eye. I. Isolation, characterization and quantitative analysis by two-dimensional thin-layer chromatography of diacyl and vinyl-ether phospholipids. Biochim. Biophys. Acta. 152: 307.

Buege, J.A. and Aust, S.D. 1978. Microsomal lipid peroxidation. Methods Enzymol. 52: 302.

Christie, W.W. 1973. The isolation of lipids from tissues. In "Lipid Analysis-Isolation, Separation, Identification and Structural Analysis of Lipids," W.W. Christie (Ed.), Ch. 2, p. 30. Pergamon Press Ltd., Oxford, England.

Finean, J.B., Coleman, R., and Michell, R.H. 1978. Composition and structure. In "Membranes and their Cellular Functions," 2nd ed. J.B. Finean, R. Coleman, and R.H. Michell (Ed.), Ch. 2, p. 30. John Wiley and Sons, New York.

Folch, J., Lees, M., and Stanley, G.H.S. 1957. A simple method for the isolation and purification of total lipides from animal tissues. J. Biol. Chem. 226: 497.

Markwell, M.A.K., Haas, S.M., Tolbert, N.E., and Bieber, L.L. 1981. Protein determination in membrane and lipoprotein samples: manual and automated procedures. Methods Enzymol. 72: 296.

McDonald, R.E., Kelleher, S.D., and Hultin, H.O. 1979. Membrane lipid peroxidation in a microsomal fraction of red hake muscle. J. Food Biochem. 3: 125.

Olley, J. and Lovern, J.A. 1960. Phospholipid hydrolysis in cod flesh stored at various temperatures. J. Sci. Food Agric. 11: 644.

Roelofsen, B. and Ott, P. 1981. Two-dimensional thin layer chromatography. Separation of lipids extracted from native and phospholipase A_2 treated human erythrocyte ghosts. In "Membrane Proteins-A Laboratory Manual," A. Azzi, U. Brodbeck and P. Zahler (Ed.), p. 41. Springer-Verlag Berlin Heidelberg, Germany.

Sevanian, A., Muakkassah-Kelly, S.F., and Montestruque, S. 1983. The influence of phospholipase A_2 and glutathione peroxidase on the elimination of membrane lipid peroxides. Arch. Biochem. Biophys. 223: 441.

Sevanian, A., Stein, R.A., and Mead, J.F. 1981. Metabolism of epoxidized phosphatidylcholine by phospholipase A_2 and epoxide hydrolase. Lipids 16:781.

Shewfelt, R.L. 1981. Fish muscle lipolysis-a review. J. Food Biochem. 5: 79.

Shewfelt, R.L., McDonald, R.E., and Hultin, H.O. 1981. Effect of phospholipid hydrolysis on lipid peroxidation in flounder muscle microsomes. J. Food Sci. 46: 1297.

Slabyj, B.M. and Hultin, H.O. 1982. Lipid peroxidation by microsomal fractions isolated from light and dark muscles of herring (*Clupea harengus*). J. Food Sci. 47: 1395.

Slabyj, B.M. and Hultin, H.O. 1984. Oxidation of a lipid emulsion by a peroxidizing microsomal fraction from herring muscle. J. Food Sci. 49: 1392.

Tzagoloff, A. 1982. Mitochondrial structure and compartmentalization. In "Mitochondia," A. Tzagoloff (Ed.), Ch. 2, p. 30. Plenum Press, New York.

Yasuda, M. and Fujita, T. 1977. Effect of lipid peroxidation on phospholipase A_2 activity of rat liver mitochondria. Jap. J. Pharmacol. 27: 429.

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Prediction of Shelf-Life of Frozen Minced Fish in Terms of Oxidative Rancidity as Measured by TBARS Number

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ABSTRACT

The lipid, iron, myoglobin, and hemoglobin contents and the time for extracted lipids to gain 1% weight (a measure of autoxidation potential) were measured in 12 samples of six fish species. These were taken singly and in combination to prepare models to predict the shelf life of frozen minced fish, based on thiobarbituric acid reactive substance levels. By using only the potential for the extracted lipids to autoxidize, a reasonably accurate model was obtained (R^2 = 0.83). Incorporating some of the compositional factors into the models improved the simple model. These models could be useful in providing fairly rapid estimation of the TBA number based shelf-life of frozen minced fish.

INTRODUCTION

A MAJOR PROBLEM with minced fish products is the loss attributed to rancidity (Deng et al., 1977). This problem is becoming especially important with the advent of mechanically separated fish (Silberstein and Lillard, 1978). The large proportion of highly unsaturated fats in many fish (Standal et al., 1975) contributes to the ease with which fish products undergo oxidative rancidity and develop off flavors. Freezing and frozen storage do not completely arrest all possible quality changes, and reactions that lead to oxidative changes can proceed at low temperatures, although slowly.

The characteristic features of lipid oxidation in fish flesh are influenced by factors such as the amount of lipids, their susceptibility to autoxidation (Ke et al., 1982), the level of microsomal associated lipid oxidation system present (McDonald et al., 1979; Slabyj and Hultin, 1982), the level of heme compounds (Zipser and Watts, 1961; Castell and Bishop, 1969; Fischer and Deng, 1977) and the presence of metal ions (Allen et al., 1979).

In theory, it should be possible to combine the influence of some of these factors into a model or formula to predict actual frozen shelf-life of a particular fish product. Recently, a model to predict the shelf-life of frozen fish was developed based solely on autoxidation of extracted fish lipids (Ke et al., 1982). Since this technique gives an average deviation of 17% from the measured shelf-life, a more accurate model or formula for the prediction of stable shelf-life would be desirable. The purpose of this study then was to determine if, by including certain intrinsic factors of the muscle composition, models could be obtained to give a more accurate prediction of thiobarbituric acid reactive substances (TBARS)-based shelf-life from measurements made over a relatively short period of time.

MATERIALS AND METHODS

ONE FILLET from each of six commercial species was obtained from a local seafood market on two different occasions. The species used in this study were skipjack tuna (*Katsuwonus pelamis*), yellowfin tuna (*Thunnus albacares*), wahoo (*Acanthocybium solandri*), jack (*Caranx* species), dolphin fish (*Coryphaena hippurus*) and broadbill swordfish (*Xiphias gladius*). These were transported to the laboratory, the skin was removed, with no fat attached, and the fillet was ground for 2 min in a Cuisinart Food Processor. Each fillet was kept separate from the others and treated as a single sample. Most of the minced fish was packed tightly in small (ca. 10g) packets in polyethylene sandwich bags to avoid excessive air, frozen and stored at −20 °C.

All measurements throughout this work were made in duplicate. Total lipids were extracted from the freshly minced flesh samples by the method of Bligh and Dyer (1959). The potential for autoxidation of these extracted lipids was estimated by determining the time needed for a 1% weight gain to occur during 60 °C storage (Ke et al., 1982). Total iron was determined by flame atomic absorption spectrophotometry (Model 303, Perkin-Elmer Corp., Norwalk, CT) after ashing according to the procedure of Rowe (1973).

Heme pigments were extracted from the fish and total pigments, myoglobin and hemoglobin contents of the minced fish were measured as their cyanmet-derivatives (Rickansrud and Henrickson, 1967) after reacting with cyanmethemoglobin reagent (Data Medical Associates, Inc., Arlington, TX, 1982). The results for total pigments, myoglobin and hemoglobin were expressed in % hemoglobin ($\times 10^3$) of minced fish. Hemoglobin concentration was calculated by subtracting the myoglobin content from that of the total pigment content.

Rancidity (as indicated by TBARS number) in the stored frozen minced fish samples was measured weekly (Tarladgis et al., 1960 as modified by Robles-Martinez et al., 1982), using 1, 1, 3, 3, tetraethoxypropane (TEP) as a stable malonaldehyde standard. While the TBARS number of a fish sample may not be a good indicator of shelf-life, Robles-Martinez et al. (1982) proposed a TBARS content of 18 μmole/kg as a general indicator of rancidity in frozen fish.

The "time for 1% lipid weight gain," total iron content, myoglobin content, hemoglobin content and lipid content of each sample were taken separately and in combination for development of a model to predict the shelf-life of the minced

TABLE 1—Components of fish which affect oxidative stability: total iron, lipid (%), and "time for a 1% weight increase of extracted lipids"

Sample	Iron (mg/100g)	Lipid (%)	Time for 1% Lipid wt gain (days)
Skipjack tuna	2.15[a]	1.34	9.0
	1.85[b]	1.26	9.5
Yellowfin tuna	0.80	7.48	3.6
	0.86	6.61	3.6
Wahoo	0.44	2.07	10.0
	0.42	2.35	8.2
Jack	0.60	2.98	5.4
	0.65	2.05	7.2
Dolphin fish	0.72	0.76	11.0
	0.69	0.55	12.0
Swordfish	0.69	5.76	4.3
	0.71	6.11	3.8

[a] First lot
[b] Second lot

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Table 2—Total pigment, myoglobin and hemoglobin content (% × 10^3) in minced fish

Sample	Total Pigment (A)	Myoglobin (B)	Hemoglobin (A-B)	Hgb:Myo
Skipjack tuna	6.30[a]	3.80	2.50	0.66:1
	8.26[b]	5.00	3.25	0.65:1
Yellowfin tuna	2.72	1.49	1.23	0.83:1
	3.25	1.65	1.60	0.97:1
Wahoo	2.10	0.97	1.13	1.16:1
	2.58	1.20	1.38	1.15:1
Jack	3.42	1.61	1.81	1.12:1
	2.64	1.63	1.01	0.62:1
Dolfin fish	0.20	0.13	0.07	0.54:1
	3.28	2.08	1.20	0.58:1
Swordfish	3.51	1.36	2.15	1.58:1
	2.74	1.16	1.58	1.36:1

[a] First lot
[b] Second lot

Table 3—Time (weeks) for TBARS levels to reach 18 μmol/kg minced fish

Sample	Time (wk) First lot	Second lot
Skipjack tuna	6.55	6.10
Yellowfin tuna	3.75	3.10
Wahoo	5.90	5.90
Jack	4.60	5.20
Dolfin fish	9.10	8.10
Swordfish	4.80	4.30

fish, assuming a frozen sample to be rancid when the TBARS content reached 18 μmoles/kg. Each fish was taken as a separate data set, so that the data from 12 samples (6 fish types in duplicate) were used to develop the model. This was accomplished using a BMDP Statistical Software Package—9R option (BMDP9R) (Dept. of Biomathematics, Univ. of California, Los Angeles, CA) run on an IBM 3081 computer system at the University of Hawaii Computing Center. The fit of the predicted shelf-life to the actual shelf-life was determined by using Linear Multiple Regression (Neter et al., 1983). The BMDP9R was also used to select possible subsets of independent variables as well as the best subset choosing Mallow's Cp as the criterion.

RESULTS & DISCUSSION

A NUMBER of components of the fish muscle were examined for their role in lipid oxidation. These included lipid content, hemoglobin content, myoglobin content, total iron content, and the susceptibility of the isolated lipids to autoxidation. Results of these analyses were correlated into a multiple regression formula to predict the shelf-life of the frozen minced fish

Table 4—Summary of regression equations (all significant at P = 0.01) for variables used in the prediction of the TBARS based shelf life of frozen, minced fish

Regression equation[a]	R^2
Weeks[b] = 1.86 + 0.5′ × T	0.83
Weeks = 1.85 + 0.53 × T + 1.40 × I − 0.72 × M	0.91
Weeks = 1.54 + 0.55 × T + 1.46 × I − 0.89 × M + 0.25 × H	0.91
Weeks = 2.21 + 0.50 × T + 1.48 × I − 0.90 × M + 0.23 × H − 0.07 × L	0.91

[a] T = "time for 1% lipid weight gain" (days); I = total iron content (mg/100g); M = myoglobin content (% × 10^3); H = hemoglobin content (% × 10^3); L = lipid (%).
[b] Weeks of TBARS based shelf life at −20 °C.

samples based on the formation of thiobarbituric acid reactive substances (TBARS).

The time (days) required for a 1% increase in isolated fish lipid weight has been used to determine the potential for oxidative rancidity in fish flesh (Ke et al., 1982). In the first lot, lipids extracted from yellowfin tuna were the most unstable, followed by swordfish, jack, skipjack tuna, wahoo and dolphin fish (Table 1). Similar results were found for the second lot, except that wahoo was slightly less stable than skipjack tuna.

Ke et al. (1982), in experiments related to oxidative rancidity potential, considered fish with extracted lipid reaction times greater than 7 days to be very stable. In a similar context, one can consider skipjack tuna, wahoo, and dolphin fish to be stable in terms of oxidative rancidity, while jack could be marginally placed in that category.

The total iron content of the minced samples is shown in Table 1. Watt and Merrill (1963) reported that skipjack tuna had 0.7 mg/100g, yellowfin tuna 1.1 mg/100g, dolphin fish 1.7 mg/100, and swordfish 1.1 mg/100g. Comparitive values for wahoo and jack could not be found. Skipjack tuna in this study had a higher iron content than has been reported previously. The reverse was observed for dolphin fish, i.e. iron values were almost half of what has been reported. The remainder of samples had relatively lower iron contents than reported earlier (Watt and Merrill, 1963) which may reflect slight losses due to volatilization during ashing, although care was taken to minimize losses of evolved ferric chloride by careful temperature control during ashing, or may be a result of fish-to-fish variation.

Table 1 also indicates the percent lipid present in the fish samples. Yellowfin tuna had the most lipid, followed closely by swordfish, while skipjack tuna had only 1.2-1.4%. Wahoo

Table 5—Actual vs predicted TBARS based shelf life of frozen, minced fish (weeks)

		Number of independent variables used in model[a]							
		1		3		4		5	
Sample	Actual	Predicted	% Dev.	Predicted	% Dev.	Predicted	% Dev.	Predicted	% Dev.
Skipjack tuna	6.55	6.49	0.93	6.87	4.66	7.01	6.56	6.93	5.48
	6.10	6.75	9.63	5.85	4.27	6.01	1.50	5.84	4.45
Yellowfin tuna	3.75	3.71	1.08	3.80	1.32	3.71	1.08	3.59	4.46
	3.10	3.71	16.44	3.77	17.77	3.77	17.77	3.68	15.76
Wahoo	5.90	7.01	15.08	7.06	16.43	7.15	17.48	7.07	16.55
	5.90	6.08	2.96	5.91	0.17	5.99	1.50	5.33	10.69
Jack	4.60	4.64	0.86	4.38	5.02	4.47	2.91	4.54	1.32
	5.20	5.57	6.64	5.39	3.53	5.32	2.26	5.37	3.17
Dolfin Fish	9.10	7.52	21.01	8.59	5.94	8.57	6.18	8.58	6.06
	8.10	8.04	0.75	7.66	5.74	7.68	5.47	7.56	7.14
Swordfish	4.80	4.07	17.94	4.11	16.79	4.30	11.63	4.23	13.48
	4.30	3.82	12.57	4.02	6.97	4.08	5.89	4.03	6.70
Average deviation:			8.82 ± 7.59[b]		7.38 ± 6.10		6.69 ± 5.92		7.94 ± 5.01

[a] For formulae, see Table 4
[b] Standard deviation

and jack had 2-3% whereas dolphin fish had extremely low lipid levels. These results were comparable to those of Walker et al. (1958), who found that yellowfin tuna had 7.8% lipid, skipjack tuna 1.0-2.4%, dolphin fish 0.7% and wahoo 0.8–3.8%. Watt and Merrill (1963) reported that yellowfin tuna had 3% lipid and swordfish 4%. These variations from previously reported studies could have been due to any of several intrinsic factors of the fish involved, such as sex, age, size, etc. Such variation also could be found within a given fish depending on the location in the fish from which the fillet was taken.

The total pigment and individual hemoprotein content of the fish samples are shown in Table 2. Variations between lots may have been due to the fish samples having variable amounts of dark lateral line muscle. The amount of myoglobin varied from 0.00013% to 0.005%, while hemoglobin varied from 0.00007% to 0.00325%. Brown et al. (1957) found that the range of myoglobin concentration for 9 species of fish, including tuna, was from 0.002% in cod to 0.09% in pilchard. The values in Table 2 may be lower than the actual content however, as Fischer and Deng (1977) reported iron in the extraction residue (18-19% of total iron) on the filter paper, although the form of this iron was not clarified.

Except for jack, the ratios of hemoglobin to myoglobin were similar for the two lots. Silberstein and Lillard (1978) stated that in addition to the concentration of total heme pigments, the hemoglobin-to-myoglobin ratio affected the oxidative stability of deboned fish. In the present study, it appeared that the amount of total pigments accounted for the differences observed in oxidative rancidity rates, while the hemoglobin-to-myoglobin ratio had little or no effect on "rancidity," as all models prepared with this ratio had an R^2 <0.90. Some variation in the pigment concentration can be found between different fish as well as within different muscle samples of the same fish (Brown, 1962). The high hemoprotein content of skipjack tuna was consistent with an observed higher content of dark muscle in those samples compared with that of minced samples from other fish.

The time needed for TBARS to reach 18 μmole/kg in the frozen stored fish samples are presented in Table 3. Yellowfin tuna showed the highest rate of TBARS development and dolphin fish the lowest. These respective rates would depend on the concentration and type of unsaturated fatty acids present and the concentration of any pro-oxidants. A similar pattern was observed between fish in both sets of samples.

The regression equations found for the relationship of fillet paramaters to "shelf life" (TBARS = 18 μmole/kg) for frozen, minced fish using 1, 4 or 5 sets of independent variables are summarized in Table 4. It also gives the equation using the Cp criterion which employs the "best" subset from all possible subsets of independent variables.

Generally, all the equations gave R^2 of 0.9 and above, although "time for 1% lipid weight gain" correlated with observed shelf life had a slightly lower R^2 of 0.83. This was the best single factor model generated from the data. The next best single factor model was based on the % lipid in the sample (R^2 = 0.71), while other single factor models had no correlation. These equations could be used to estimate the "shelf-life" of frozen minced fish depending on the independent variables available. The equation with only one independent variable ("time for 1% lipid weight gain") could be used with considerable accuracy for prediction and would eliminate lengthy compositional analyses with respect to total iron, hemoproteins and percent lipid, the independent variables which are mainly responsible for initiating randicidity.

A model prepared from one independent variable, "time for 1% lipid weight gain," resulted in predicted "shelf-life" deviations of 0.75% to 21% from experimentally obtained values (Table 5). The average deviation for this model was 8.88%, the highest observed in this study. Ke et al. (1982) calculated the quality index with a mackerel skin sample as a reference standard based on only one variable, viz: the potential for the extracted lipids to autoxidize. By their method, the frozen "shelf-life" could be estimated satisfactorily with a variation from the actual "shelf-life" of less than 17%, and an average of 13.9%.

When four independent variables were used in preparing the model, i.e. "time for 1% lipid weight gain", total iron and hemoproteins, the model was more precise (Table 5). Overall deviations ranged from 1.08% to 17.48% with an average of 6.69%. Using all five independent variables to prepare the model, deviations of 1.32% to 16.55% from the actual "shelf-life" were found (Table 5), with an average of 7.94%. While this was an increase in average deviation over the four factor model, the standard deviation was less than for the other three models.

When Mallows Cp was used as a criterion for selecting the best subset of independent variables, it chose only 3, i.e. "time for 1% lipid weight gain", total iron and myoglobin content. Overall deviations ranged from 0.17% to 17.77%, with an average of 7.38%.

This study showed that models can be used to predict the frozen "shelf-life" of minced fishes with considerable accuracy. The average percent deviation for the equations generated were 8.88%, 7.38%, 6.69%, and 7.94%, when 1, 3, 4, or 5 independent variables, respectively, were used to produce a model. These equations could be valuable in predicting frozen "shelf-life" by employing tests that could be done within a reasonably short period, depending on the number of independent variables under consideration. Further work in this area may include incorporation of endogenous oxidation activities and/or naturally occurring antioxidants into the models, as well as the use of complex, non-linear modeling.

REFERENCES

Allen, J.C., Farag, R.S., and Crook, E.M. 1979. The metal-catalyzed oxidation of aqueous emulsions of linoleic acid and trilinolein. J. Appl. Biochem. 1: 1.

Bligh, E.G. and Dyer, W.J. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37: 911.

Brown, W.D., Venolia, A.W., Tappel, A.L., Olcott, H.S., and Stansby, M.E. 1957. Oxidative deterioration of fish and fishery products. II. Progress on studies concerning mechanism of oxidation of oil in fish tissue. Com. Fish. Rev. 19: 27.

Brown, W.D. 1962. The concentration of myoglobin and hemoglobin in tuna flesh. J. Food Sci. 27: 26.

Castell, C.H. and Bishop, D.M. 1969. Effect of hematin compounds on the development of rancidity in muscle of cod, flounder, scallop and lobster. J. Fish. Res. Bd. Can. 26: 2299.

Deng, J.C., Matthews, R.F., and Watson, C.M. 1977. Effect of chemical and physical treatments on rancidity development of frozen mullet (*Mugil cephalus*) fillets. J. Food Sci. 42: 344.

Fischer, J. and Deng, J.C. 1977. Catalysis of lipid oxidation: a study of mullet (*Mugil cephalus*) dark flesh and emulsion model system. J. Food Sci. 42: 610.

Ke, P.J., Linke, B.A., and Smith-Lall, B. 1982. Quality preservation and shelf-life estimation of frozen fish in terms of oxidative rancidity development. Lebensm-Wiss. u.-Technol. 15: 203.

McDonald, R.E., Kelleher, S.D., and Hultin, H.O. 1979. Membrane lipid oxidation in a microsomal fraction of red hake muscle. J. Food Biochem. 3: 125.

Neter, J., Wasserman, W., and Kutner, M.H. 1983. "Applied Linear Regression Models," Ch 7. Multiple regression-1, p.226. Richard D. Irwin, Inc., Homewood, IL.

Rickansrud, D.A. and Henrickson, R.L. 1967. Total pigments and myoglobin concentration in four bovine muscles. J.Food Sci. 32: 57.

Robles-Martinez, C., Cervantes, E., and Ke, P.J. 1982. Recommended method for testing the objective rancidity development in fish based on TBARS formation. Can. Tech. Rpt. Fish. Aquatic Sci. No. 1089.

Rowe, C.J. 1973. Food Analysis by Atomic Absorption Spectroscopy. Varian Techtron, Springvale, Vic., Australia. p. 8-9.

Silberstein, D.A. and Lillard, D.A. 1978. Factors affecting the autoxidation of lipids in mechanically deboned fish. J. Food Sci. 43: 764.

Slabyj, B.M. and Hultin, H.O. 1982. Lipid peroxidation by microsomal fraction isolated from light and dark muscles of herring (*Clupea harengus*). J. Food Sci. 47: 1395.

Standal, B.R., Bassett, D.R., Policar, P.B., and Thom, M. 1975. Fatty acids, cholesterol, and proximate composition of certain prepared and unprepared foods in Hawaii. Hi. Agri. Exp. Stn. Res. Bull. No 146.

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

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Assessment of Cheddar Cheese Quality by Chromatographic Analysis of Free Amino Acids and Biogenic Amines

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ABSTRACT

The free amino acids and biogenic amines extracted from normal and late-gassing Cheddar cheeses were derivatized with heptafluorobutyric anhydride and trifluoroacetic anhydride, respectively, before quantification by gas-liquid chromatography. On a microgram scale, twenty amino acids were positively identified in both types of cheese, but only high levels of γ-amino acid butyrate (0.3 to 19.4 mg/g) and small quantities of arginate were found to be associated with "poorly aged" Cheddar cheeses. Histamine (1.54 and 1.22 mg/g) and tyramine (0.32 and 0.43 mg/g) were the bioamines present in highest concentrations in both cheeses.

INTRODUCTION

CHEESES are among those high-protein-containing foodstuffs in which enzymatic and microbial activities cause the formation of amino acids and biogenic amines. Their contents in cheese have been investigated in several studies (Hickey et al., 1983; Silverman and Kosikowski, 1956; Bullock and Irvine, 1956; Zee et al., 1981).

The ripening of Cheddar cheese is a complex process involving the fermentation of lactose, degradation of proteins and fats, production of volatile fatty acids, and other changes. Amino acids are produced during cheese ripening as a result of the proteolytic action of starter bacteria (Reiter et al., 1969). The extent of this proteolysis has been used as an indication of the degree of maturity of ripening cheese (Bullock and Irvine, 1956; Ney, 1971).

The amino acids and biogenic amines are a group of simple compounds that can be easily identified and measured. Several methods have been used in the past to determine these compounds in cheeses. For quantitative determination, free amino acids and biogenic amines are usually separated by paper chromatography (Bullock and Irvine, 1956), thin-layer chromatography (Askar et al., 1972) and ion-exchange chromatography (Zee et al., 1981).

These methods are generally time-consuming, semi-quantitative and not very sensitive. To overcome these disadvantages, more specific and sensitive gas-liquid chromatographic methods have been developed. Several workers have reported that quantitative analysis of free amino acids by GLC offers three major advantages over ion-exchange chromatography: (1) higher sensitivity, (2) more rapid analysis, and (3) lower initial cost (Golan-Goldhirsh and Wolfe, 1979; Vovan et al., 1985a).

The derivatization conditions are very important in amino acid analysis by GLC. The use of isopropanol as an esterification reagent in amino acid analysis has given excellent results (Golan-Goldhirsh and Wolfe, 1979). In this report the N-heptafluorobutyryl-isopropyl (N-HFB) ester derivative of amino acids, as introduced by Golan-Goldhirsh and Wolfe (1979) and modified by Vovan et al. (1985a,b), was used for the GLC analysis.

The purpose of this work was to apply these recent methods to the analysis of free amino acids extracted from high-quality aged Cheddar cheeses and late-gassing Cheddar cheeses. Moreover, the method of trifluoroacetylation was developed to quantify biogenic amines on a microgram scale.

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MATERIALS & METHODS

Apparatus

A Hewlett-Packard (HP) gas chromatograph (model 5890A) equipped with a flame ionization detector was coupled to a Hewlett-Packard integrator (model 3392A multi-function, digital processor) which provides peak integration data as well as the final computation of results. The column was purchased from Hewlett-Packard (Kirkland, Qc) and was an "Ultra Performance" crosslinked 5% phenylmethyl silicone (SE 54, 0.52 μm thickness, 25 m × 0.31 mm i.d.) flexible capillary column.

The single-column mode of operation was used with hydrogen as carrier gas at 55 kPa with split vent at 50 mL/min and septum purge at 3 mL/min. The injection and the detector port temperatures were maintained at 225° and 275°C, respectively. A one-level temperature programming of the column was utilized: the initial temperature of 75°C was immediately increased to a final temperature of 225°C at a rate of 10°C/min. With this program, 24 amino acids were completely separated in 18 min.

One-level temperature programming of the column was also utilized for the separation of biogenic amines: the initial temperature of 125°C was immediately increased to the final temperature of 300°C at a rate of 10°C/min. Injection and detector port temperatures were maintained at 310° and 325°C, respectively. A flame ionization detector (F.I.D.) was used.

Preparation of amino acid standards

For derivatization, a 1 mg/mL solution of each of 24 amino acids (Sigma Chemical Co., St. Louis, MO) was prepared in 2N NH_4OH. From this stock solution aliquots of different concentrations were dried under a stream of dry nitrogen in 3 mL reactivials (Pierce Chemical Co., Rockford, IL) held in a heating block (Reacti-Therm, Pierce Chemical Co., Rockford, IL) at room temperature. Residual traces of water were removed azeotropically using dichloromethane (BDH, Ville St-Laurent, Qc). The derivatization was composed of two steps: (a) esterification and (b) acylation according to Golan-Goldhirsh and Wolfe (1979) as modified by Vovan et al. (1985a).

The dried residue was dissolved in 200 μL of the esterification reagent prepared according to the quick method proposed by Pearce (1977); acetylchloride (5 mL) was slowly added to 25 mL isopropanol held at 0°C with agitation. The esterification was done in the reactivials placed in the heating block and heated at 100°C for 10 min with continuous agitation. After the reaction, the vials were cooled in an ice bath for 7 min (Vovan et al., 1985a), then the esterification solvent was evaporated under a stream of dry nitrogen.

The dried residue was dissolved in 100 μL ethyl acetate plus 200 μL heptafluorobutyric anhydride (HFBA) (Chromatographic Specialties Ltd, Brockville, Ont.) and the vials heated on the reacti-therm at 100°C for 10 min with continuous agitation. The vials were then cooled in an ice bath for 7 min. The reagent was evaporated and the

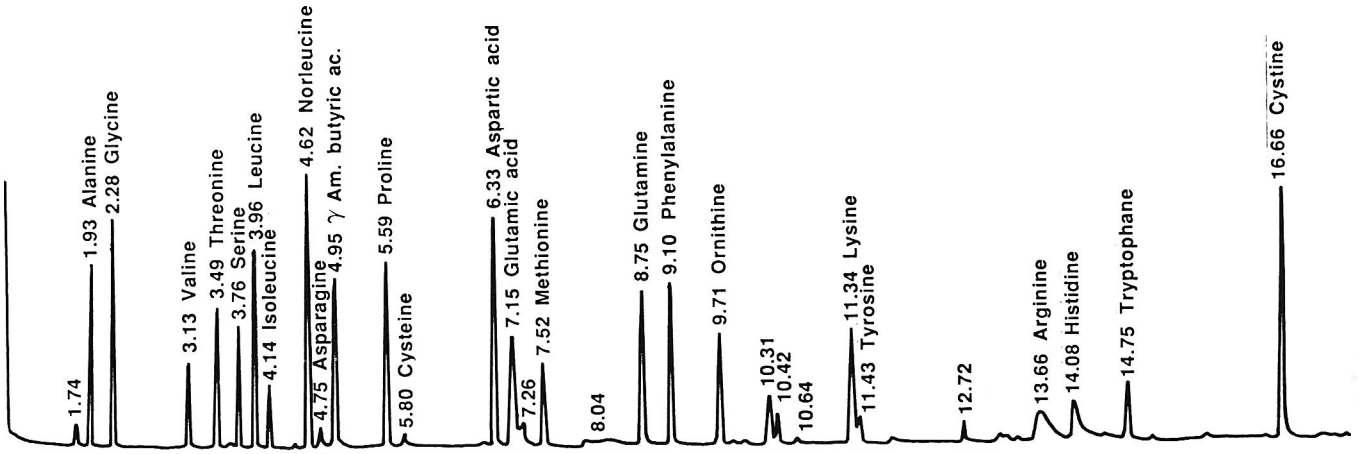

Fig. 1—Gas chromatogram of N-HFB-isopropyl ester derivatives of the standard amino acid mixture.

Table 1—Linear regression equation[a] and correlation coefficients for amino acid standards

Amino acids	Linear regression coefficients a	b	Correlation coefficients
Alanine	− 0.10	0.001	0.98
Glycine	− 0.11	0.001	0.95
Valine	3.434	8.569	0.93
Threonine	0.02	0.001	0.94
Serine	0.004	0.001	0.96
Leucine	0.008	0.001	0.99
Isoleucine	0.02	0.0001	0.84
Norleucine (I.S.)[b]	0.0003	0.001	0.99
Asparagine	8.410	− 33.04	0.98
γ-Am. buty. ac.	− 0.11	0.001	0.92
Proline	− 0.11	0.002	0.93
Cystine	0.03	0.0007	0.84
Cysteine	2.782	− 21.30	0.87
Aspartic acid	0.024	0.0006	0.94
Glutamic acid	− 0.016	0.001	0.99
Methionine	0.011	0.0006	0.98
Glutamine	14.210	−42.72	0.99
Phenylalanine	− 0.002	0.001	0.99
Ornithine	− 0.020	0.001	0.99
Lysine	− 0.019	0.001	0.99
Tyrosine	− 0.007	0.001	0.99
Arginine	− 0.021	0.0007	0.98
Histidine	1.69	4.82	0.65
Tryptophane	− 0.030	0.0004	0.92

[a] $\gamma = ax + b$, where Y = relative peak area and x concentration of amino acid.
[b] I.S. Internal standard.

residue dissolved in 500 μL ethyl acetate. Duplicate samples (2 μL) were injected into the GLC.

Preparation of biogenic amine standards

A total of 12 amines were dissolved in 2N HCl to give a stock solution containing 1 mg/mL of each amine. Different aliquots (5 μL to 100 μL) of the stock solution of biogenic amines were dried under a stream of dry nitrogen in 1.0 mL reactivials held in a heating block at room temperature. The dried residue was dissolved in 200 μL 0.05 M pyridine in benzene and 100 μL trifluoroacetic anhydride (TFAA) (Chromatographic Specialties Ltd, Brockville, Ont.). The vials were heated on the reacti-therm at 60–65°C for 30 min with continuous agitation. After cooling at room temperature, 200 μL benzene was added to the acylation reagent and the vials were shaken for 1 min. The vials were then placed on the automatic sampler injector (model HP 7671A) and 2 μL of the aliquot was injected into the GLC. Duplicate analyses and injections were done.

Extraction and purification of free amino acids and biogenic amines from cheddar cheese

Twenty samples of high-quality (grade 1) Cheddar cheese aged more than 12 months (Cracker Barrel brand, 227g retail-packaged) and fifteen samples of blown and/or late-gassing cheese aged from 1 to 27 months (20-kg blocks) were obtained from Agropur (Coopérative agro-alimentaire, Granby, Qc.).

The extraction method of Nakamura et al. (1979) was used with modification. The Cheddar cheese sample (30g) was homogenized with 60 mL 5.0% TCA at 4°C in a Virtis homogenizer (model 45, Research Equipment, Gardiner, NY). Thirty mg norleucine and 30 mg dopamine (Sigma Chemical Co., St Louis, MO) were added to each sample as internal standards prior to homogenization. To obtain separation of fat, the slurry was heated at 60°C for 20–30 min and stored at 4°C for 15–20 min. The supernatant fat was carefully removed with a spatula. The remaining slurry was then centrifuged at 9000 *g* for 10 min at 4°C (Sorvall Centrifuge, model RC-5B, Du Pont Co., Wilmington, DE). The residue was re-extracted with 60 mL 5.0% TCA and centrifuged at 12,000 *g* for 10 min at 4°C. The combined supernatant fluids were centrifuged at 20,000 *g* for 20 min at 4°C. This clear solution was extracted three times with 100 mL portions of diethyl ether. The extract was lyophilized to obtain a whitish powder which was kept in a desiccator until analyzed.

The method of sample purification was similar to that of Golan-Goldhirsh et al. (1982). For purification of biogenic amines, AG50W-X8 cation exchange resin columns (1 × 5 cm, 750 ± 2 mg of resin, 200/400 mesh, Bio-Rad Lab., Mississauga, Ont.) were regenerated by washing sequentially with deionized water (5 mL), NaOH 1 N (5 mL; pH 10–14), deionized water (5 mL), HCl 2 N (5 mL; pH 2.5) and then deionized water (15–20 mL; pH 5.0–6.0). Powder (200–250 mg) extracted previously was dissolved in 1 mL 25% glacial acetic acid in a 5 mL vial, transferred onto the column resin using a Pasteur pipette, and passed through the column at about 1 drop/sec. The sample vial was washed with 1 mL deionized water and the washing solution was transferred to the column. The biogenic amines were eluted by passing 30 mL 2 N HCl through the column at about 1 drop/sec.

The eluate was evaporated to 2–3 mL in a vacuum oven at 30–35°C. Aliquots of the concentrated solution (200, 300 and 400 μL) were transferred to 1 mL reactivials, evaporated to dryness and analyzed by the trifluoroacetylation reaction in the same manner as the standards. Complete elution of biogenic amines was verified by treating a standard biogenic amine mixture identically.

RESULTS & DISCUSSION

Standard amino acid mixture

In preliminary trials, different combinations of holding times, initial oven temperature and oven temperature programming rate revealed that the best resolution was obtained at a rate of 10°C/min with a capillary column (Vovan et al., 1985a). However, a chromatogram of the mixture of 24 amino acids in Fig. 1 showed that complete resolution could be obtained in 10 min with a temperature rate of 20°C/min, but maximum relative peak areas were less accurate.

Table 1 provides the best-fit linear regression equation and correlation coefficients for concentration and peak area for each

Table 2—Free amino acids extracted from aged high-quality (grade 1) Cheddar cheeses

Sample N.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
Age (month)	1	>12	>12	>12	>12	>12	>12	>12	>12	>12	>12	>12	>12	>12	>12	>12	>12	>12	>12	>12	
Grade N.	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
Amino acids (mg/g cheese)																					$\bar{X}$ ± S.E.[a]
Alanine	0.3	2.4	2.1	1.7	1.7	2.2	3.9	0.7	1.4	1.4	2.4	2.0	2.8	2.0	1.9	1.9	1.8	2.2	2.4	2.4	2.0 ± 0.4
Valine	0.5	4.4	5.6	3.5	4.8	4.9	8.8	7.8	5.2	4.2	5.1	2.1	6.5	4.5	3.8	7.8	6.6	5.3	8.9	6.2	5.6 ± 1.8
Glycine	0.1	1.7	1.4	1.2	1.1	1.7	2.3	0.7	1.0	1.2	1.6	1.5	1.3	1.3	1.2	1.3	1.6	1.4	1.9	2.0	1.4 ± 0.4
Threonine	0.3	3.6	3.8	3.0	6.7	3.3	4.0	1.0	1.8	1.0	2.9	2.4	3.6	4.0	3.1	3.9	5.0	3.2	2.4	3.7	3.3 ± 1.3
Serine	0.2	3.3	2.3	2.9	2.6	4.9	5.2	1.6	2.5	1.1	2.2	2.4	2.9	2.6	2.2	2.5	3.2	3.4	3.2	0.9	2.7 ± 1.1
Leucine	1.1	11.3	10.0	11.3	10.4	11.8	10.7	7.9	7.9	9.4	7.0	8.9	6.9	11.3	8.9	8.3	9.8	13.0	12.4	6.1	9.6 ± 2.0
Isoleucine	0.2	1.8	0.8	1.7	1.9	3.3	6.6	—[b]	0.4	—	0.8	1.0	1.6	1.6	1.8	1.1	0.8	2.4	1.2	—	1.8 ± 1.5
γ-Amino butyric acid	0.1	—	—	0.1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.1 ± 0.0
Glutamic acid	0.9	11.2	8.2	11.8	9.8	12.0	14.0	10.3	10.9	12.9	6.9	7.5	11.7	10.8	9.5	9.6	13.6	10.9	12.3	6.7	10.6 ± 2.1
Proline	0.3	0.9	—	—	0.6	1.0	1.7	0.8	0.8	0.9	0.9	0.6	0.5	0.7	0.7	0.9	0.8	0.7	1.2	1.8	0.9 ± 0.4
Phenylalanine	0.7	3.2	3.7	3.7	3.7	6.9	7.0	3.9	2.7	3.1	2.6	2.8	3.2	3.2	3.1	3.4	3.5	4.2	4.7	3.2	3.8 ± 1.2
Ornithine	0.1	3.1	1.2	1.4	0.3	1.0	—	0.8	0.9	2.2	—	0.4	0.5	3.3	1.3	0.7	1.5	1.5	3.3	—	1.5 ± 1.0
Lysine	1.3	6.9	6.4	6.6	5.9	7.0	6.9	4.9	3.5	6.1	4.4	4.8	5.9	7.1	5.5	4.6	6.9	6.9	9.2	4.2	6.0 ± 1.4
Tyrosine	0.2	1.5	1.6	1.6	1.5	2.6	4.0	1.4	1.2	1.8	1.1	1.1	1.4	1.8	1.3	1.3	1.7	2.0	1.2	1.0	1.6 ± 0.7
Arginine	1.4	1.4	5.3	3.4	6.0	5.1	3.9	2.7	1.6	1.3	3.2	4.0	4.5	1.1	2.6	2.9	3.9	3.0	4.7	3.3	3.4 ± 1.4
Methionine	—	1.5	—	0.4	0.9	—	—	0.8	0.5	0.5	—	—	0.3	0.3	1.9	1.7	1.4	—	1.0	1.2	1.0 ± 0.6
Tryptophane	—	—	—	—	0.6	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.6 ± 0.0
Cystine	—	—	—	—	0.7	—	—	—	0.2	—	—	—	—	—	—	—	—	—	—	—	0.5 ± 0.4
Aspartic acid	—	—	—	—	—	—	—	1.1	—	—	—	—	—	—	—	—	—	—	—	—	1.1 ± 0.0
Histidine	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	— —
Total amino acids	7.7	58.2	52.4	54.3	59.2	67.7	79.0	46.4	42.5	47.1	41.1	41.5	52.6	55.6	48.8	51.9	62.1	60.1	70.0	42.7	54.4 ± 10.4

[a] $\bar{X}$ ± S.E. = mean ± standard error; n = 19 samples over 12 months aged.
[b] — = trace.

Table 3—Free amino acids extracted from blown and/or late-gassing Cheddar cheeses

Sample N.	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	
Age (month)	1	2	4	4	8	12	12	12	12	13	13	13	13	13	27	
Grade N.	2	3	3	3	3	3	3	2	2	3	2	2	G.F.	G.F.	N.G.	
Code[a]	F_4L_2	F_3L_2	F_3L_2	F_3L_6	$F_{39}L_3$	F_3L_2	$F_{59}L_2$	F_4L_2	F_3L_2	F_3L_2	F_3L_2	F_3L_2	F_{60}	F_{60}	$F_{40}L_2$	
Amino acids (mg/g cheese)																$\bar{X}$ ± S.E.[b]
Alanine	0.4	0.8	0.9	1.4	2.1	2.0	1.1	1.5	0.9	1.8	1.1	1.5	2.6	2.4	5.0	2.0 ± 1.2
Valine	0.5	0.5	1.8	3.5	7.8	4.5	5.2	4.1	2.2	5.0	1.5	1.8	4.5	3.2	5.2	3.7 ± 1.4
Glycine	0.2	0.7	0.7	1.4	1.8	1.6	1.0	1.4	1.0	1.7	0.9	1.6	2.0	1.7	0.9	1.4 ± 0.4
Threonine	1.3	0.6	2.5	2.1	3.0	4.3	1.6	2.2	—	2.7	1.1	1.7	1.6	2.6	1.6	2.2 ± 1.0
Serine	0.3	—	0.7	1.3	2.6	3.1	—	2.6	1.3	1.9	0.8	2.8	2.8	2.3	0.4	2.0 ± 0.1
Leucine	1.4	4.4	5.6	5.2	10.0	10.8	7.4	10.6	5.8	10.5	0.6	10.6	9.9	11.3	12.5	9.0 ± 3.5
Isoleucine	—	—	0.1	3.5	4.6	6.1	0.9	3.6	0.3	2.8	—	1.6	4.5	5.2	7.1	3.4 ± 2.4
γ-amino butyric acid	—	—	—	0.3	4.4	2.1	3.3	5.1	7.2	1.2	6.1	5.2	1.1	5.2	19.4	5.6 ± 5.3
Glutamic acid	1.7	4.2	4.8	4.3	12.4	12.0	11.6	10.0	8.8	9.7	11.5	12.8	12.0	13.1	3.0	10.5 ± 3.0
Proline	0.5	0.9	0.6	1.4	2.7	1.6	1.4	1.8	1.3	1.5	0.9	1.8	1.4	1.3	1.3	1.4 ± 0.3
Phenylalanine	0.8	2.0	2.3	2.5	4.2	3.7	3.2	3.5	2.5	2.8	3.4	3.3	3.4	3.4	3.6	3.3 ± 0.4
Ornithine	—	3.5	2.1	3.7	5.9	5.7	6.4	4.3	1.2	2.8	1.4	1.8	1.1	1.6	1.7	2.8 ± 2.0
Lysine	1.1	1.7	1.6	1.8	4.1	8.3	6.9	8.5	3.7	5.4	5.2	7.0	5.6	5.1	7.2	6.3 ± 1.5
Tyrosine	—	0.3	0.6	0.6	2.2	1.5	0.8	1.2	1.0	0.2	1.1	1.9	1.6	1.6	2.7	1.4 ± 0.7
Arginine	0.9	—	—	—	0.3	—	—	1.1	1.3	0.4	1.2	0.4	3.3	2.6	—	1.5 ± 1.1
Methionine	—	—	—	0.4	0.8	1.0	2.7	2.2	1.0	—	1.1	2.6	1.6	1.9	2.2	1.8 ± 0.7
Tryptophane	—	—	—	—	0.8	—	0.8	0.8	0.8	—	—	—	—	—	—	0.8 ± 0.0
Crystine	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	— ± —
Aspartic acid	—	—	—	—	—	—	—	—	—	—	—	—	—	1.0	0.9	1.0 ± 0.1
Histidine	—	—	—	1.4	—	—	—	1.1	—	—	—	—	—	—	1.4	1.5 ± 0.6
Total amino acids	9.1	19.6	24.3	34.8	69.7	68.3	54.3	65.6	40.3	50.4	37.9	58.4	59.0	65.5	76.1	57.6 ± 12.2

[a]Code	Flavor	Closeness
F_3	Slightly unclean flavor	L_2 open
F_4	Unclean flavor	
F_{39}	Slightly objectionable flavor	
		L_3 very open
F_{40}	Objectionable flavor	L_6 slight gas or pin holes
F_{59}	Slightly off flavor	
F_{60}	Off flavor	

[b] $\bar{X}$ ± S.E. = mean ± standard error; n = 10 samples 12 to 27 months aged.
[c] — = trace
G.F.: Garlic flavor
N.G.: Not graded

standard amino acid. The correlation coefficients ranged from 0.84 to 0.99 except for histidine which had a value of 0.65. Vovan et al. (1985a) also reported a weak correlation coefficient for histidine (0.71).

Free amino acids in Cheddar cheeses

The results of free amino acids extracted from aged grade 1 Cheddar cheeses are shown in Table 2. Glutamic acid content

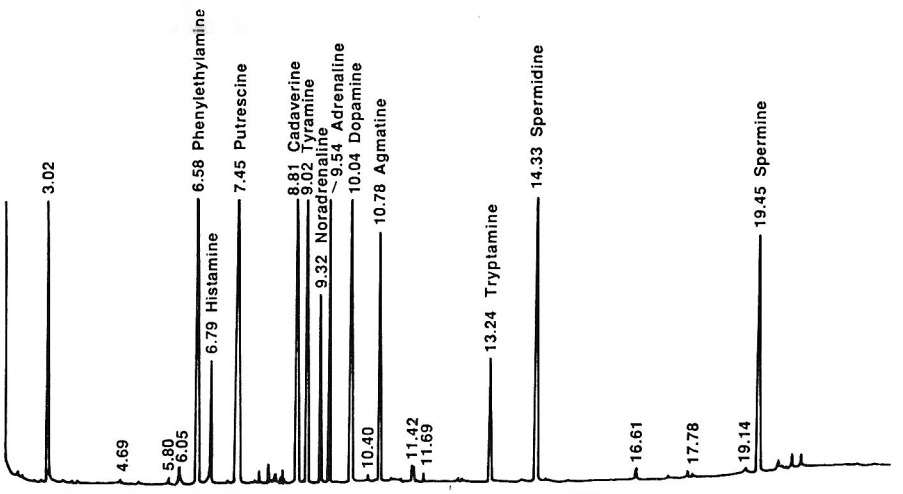

Fig. 2—Gas chromatogram of TFAA ester derivatives of the standard biogenic amine mixture.

Table 4—Linear regression equation[a] and correlation coefficients for biogenic amine standard

Biogenic amines	Linear regression coefficients a	b	Correlation coefficients
Histamine	0.38	86.4	0.99
Putrescine	−7.54	340.9	0.99
Cadaverine	−6.14	212.2	0.99
Tyramine	−1.28	350.1	0.99
Noradrenaline	1.57	10.0	0.81
Phenylethylamine	−2.29	436.9	0.99
Tryptamine	0.12	1.78	0.96
Adrenaline	0.64	28.39	0.91
Dopamine (I.S.)[b]	1.17	− 0.98	0.99
Agmatine	0.38	84.1	0.96
Spermidine	1.31	− 18.37	0.99
Spermine	1.49	− 6.69	0.99

Y = ax + b, where Y = relative peak area and x concentration of biogenic amines
[b] I.S. Internal standard

of grade 1 aged cheese was high compared to cheese aged one month. The results obtained in the present study are in agreement with earlier works on Cheddar cheese by Bullock and Irvine (1956) and Weaver et al. (1978) who found that glutamate increased steadily throughout the eight-month period of ripening. On the other hand, the content of glutamate varied greatly among late-gassing Cheddar cheese as shown in Table 3. For example, sample no. 35, an off-flavored late-gassing cheese aged 27 months, contained only 3.0 mg/g.

It has been reported (Agropur, 1984) that there is a linear correlation for "properly aged" Cheddar cheese between glutamic aicd content and ripening period. This finding agrees with that of Bullock and Irvine (1956) who reported that glutamic acid, valine, lysine, leucine and methionine continued to increase with the age of cheese. This relationship does not appear to exist for late-gassing cheeses.

The following amino acids were detected in all cheese samples: glutamic acid, aspartic acid, leucine, methionine, lysine, alanine, glycine, valine, phenylalanine, threonine, tyrosine, serine, isoleucine, γ-amino butyric acid, proline, ornithine, arginine, tryptophane, cystine and histidine. In addition, some peaks not corresponding to any component of our standard mixture were observed.

There was no consistent difference in total free amino acids between aged grade 1 and late-gassing Cheddar cheeses. However, Cheddar cheese with a strong unclean, fruity and garlic flavor, contained higher total free amino acids than properly aged cheese. It appeared that more total free amino acids were produced in blown and gassy cheeses as the age of cheeses increased. Data in Table 2 show that the γ-amino butyric acid content of grade 1 cheese was low compared to late-gassing cheeses (Table 3). The concentration determined for this amino acid may indicate intensive abnormal fermentation reactions resulting in unclean flavor (Silverman and Kosikowski, 1956). An inverse relationship does appear to exist to some degree between γ-amino butyric acid and the amount of glutamic acid in late-gassing cheeses. Reduced amounts of glutamic acid and increased γ-amino butyric acid were observed in late gassing cheeses aged 12 to 27 months. However, arginate was significantly higher in grade 1 Cheddar cheeses than in late gassing cheeses, indicating poorly aged cheeses. It is known that arginine is a precursor of some biogenic amines (Silverman and Kosikowski, 1958); the relatively small amounts of arginine in late-gassing cheeses may be explained by this conversion.

Among the amino acids not detected with accuracy are histidine, because the derivatization method caused its decomposition, and asparagine, because derivatization caused its partial

Table 5—Biogenic amines in aged high-quality (grade 1) Cheddar cheese

Sample N.	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
Age (month)	>12	>12	>12	>12	>12	>12	>12	>12	>12	>12	>12	>12	>12	>12	>12	>12	>12	>12	
Grade N.	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
Biogenic amines (mg/g cheese)																			$\bar{X}$ ± S.E.[a]
Histamine	0.75	1.75	1.53	1.61	1.78	1.77	1.55	0.96	1.74	1.30	1.79	1.68	2.12	1.85	1.19	1.65	1.09	1.56	1.54 ± 0.35
Tyramine	0.15	0.34	0.31	0.26	0.37	0.39	0.35	0.39	0.33	0.17	0.29	0.32	0.14	0.34	0.16	0.58	0.32	0.57	0.32 ± 0.12
Putrescine	—[b]	—	—	—	—	—	—	—	—	—	—	—	0.22	0.14	0.15	0.26	0.33	0.23	0.22 ± 0.07
Cadaverine	—	—	0.14	—	—	—	—	—	—	—	—	—	0.25	—	—	—	0.23	—	0.21 ± 0.06
Adrenaline	—	—	0.10	0.17	0.31	—	—	—	—	—	0.13	—	—	—	—	0.21	—	—	0.18 ± 0.08
Tryptamine	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	— —
Total biogenic amines	0.90	2.09	2.08	2.04	2.46	2.16	1.90	1.35	2.07	1.47	2.21	2.00	2.73	2.33	1.50	2.70	1.97	2.36	2.02 ± 0.47

[a] $\bar{X}$ ± S.E. = mean ± standard error; n = 18 samples over 12 months aged.
[b] — = trace.

Table 6—Biogenic amines in blown and/or late-gassing Cheddar cheese

Sample No.	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	
Age (month)	1	2	4	4	8	12	12	12	12	13	13	13	13	13	27	
Grade N.	2	3	3	3	3	3	3	2	2	3	2	2	G.F.	G.F.	N.G.	
Code[a]	F_4L_2	F_3L_2	F_3L_2	F_3L_6	$F_{39}L_3$	F_3L_2	$F_{59}L_2$	F_4L_2	F_3L_2	F_3L_2	F_3L_2	F_3L_2	F_{60}	F_{60}	$F_{40}L_2$	
Biogenic amines (mg/g cheese)																$\bar{X} \pm$ S.E.[b]
Histamine	0.10	0.89	1.48	1.64	0.82	0.51	1.85	1.15	1.53	0.69	1.69	1.79	1.21	0.81	1.00	1.22 ± 0.48
Tyramine	0.05	0.22	0.28	0.72	0.29	0.26	0.85	0.49	0.30	0.39	0.81	0.40	0.33	0.27	0.23	0.43 ± 0.22
Putrescine	—[c]	0.16	0.41	0.16	—	—	0.52	0.18	0.21	0.10	0.28	0.24	—	—	0.26	0.26 ± 0.13
Cadaverine	—	—	—	0.25	—	—	0.21	—	0.31	—	0.30	0.35	—	—	0.35	0.30 ± 0.06
Adrenaline	—	—	—	—	—	—	0.13	—	—	—	—	—	0.15	0.17	0.07	0.13 ± 0.04
Tryptamine	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	— —
Total biogenic amines	0.15	1.27	2.17	2.77	1.11	0.77	3.56	1.82	2.35	1.18	3.08	2.78	1.69	1.25	1.91	2.04 ± 0.90

[a]Code	Flavor	Closeness
F_3	Slightly unclean flavor	L_2 open
F_4	Unclean flavor	L_3 very open
F_{39}	Slightly objectionable flavor	L_6 slight gas or pin holes
F_{40}	Objectionable flavor	
F_{59}	Slightly off flavor	
F_{60}	Off flavor	

[b]$\bar{x} \pm$ S.E. = mean ± standard error; n = 10 samples 12 to 27 months aged.
[c]— = trace.
G.F.: Garlic flavor
N.G.: Not graded.

conversion into the free amino acid, preventing direct quantitative determination by the GLC method. Traces of asparagine were found in aged and late-gassing Cheddar cheese but these were not quantitative.

Biogenic amine standards

A chromatogram of the mixture of 12 biogenic amines is presented in Fig. 2. Retention time of the slowest moving amine, spermine was 19.45 min with the oven temperature increased by 10°C/min from 125° to 300°C. It should be noted that these peaks can be identified with absolute certainty only by using a second, confirmatory method. However, because the fundamentals of gas chromatography theory were employed in this initial identification, we have confidence in the identification of the biogenic amines.

Trifluoroacetylated biogenic amines had high correlation coefficients (Table 4). Noradrenaline showed the lowest coefficient (Table 4). Simard and L'Heureux (1982) also reported a weak correlation coefficient for noradrenaline (R = 78) using a modified automated ion-exchange chromatography method. This method of acylation was reliable, permitting sharp separation and quantification of 12 biogenic amines usually present in cheeses. Application of this method to the determination of biogenic amines in aged cheese was therefore investigated. Most workers have used organic solvents to extract amines from fermented foods. We found, however, that no simple solvent system could extract all the amines adequately. For the simple and rapid separation of free biogenic amines from Cheddar cheeses, a cation-exchange column purification stage was devised. Interfering substances such as amino acids were excluded and satisfactory chromatograms were then obtained.

As shown in Tables 5 and 6, aged grade 1 and late-gassing Cheddar cheeses contained histamine, tyramine, putrescine, cadaverine and adrenaline. No tryptamine was detected. Histamine and tyramine were among the amines found in highest concentrations. Significant levels of putrescine and cadaverine were present in the majority of late-gassing Cheddar cheeses, but putrescine was present at relatively high levels. There was no consistent difference in total biogenic amine content between aged high-quality grade 1 and late-gassing Cheddar cheeses. The content of histamine and adrenaline in grade 1 cheeses was slightly higher than in gassed Cheddar cheeses.

As reported by other workers, histamine and tyramine concentrations were found to be in the range of 0.037 to 0.272 mg/g and 0.353 to 1.085 mg/g, respectively, in all Cheddar cheeses (De Vuyst et al., 1976). Simard and L'Heureux (1982) also found histamine (0.002 and 4.61 mg/g) and tyramine (0.002 and 0.09 mg/g) in mild and aged Cheddar cheeses, respectively. Using ion-exchange chromatography, they reported that adrenaline, noradrenaline, spermine and tyramine were present in very mild Cheddar cheeses, while adrenaline, noradrenaline, putrescine, histamine, cadaverine, spermine and tyramine were identified in old Cheddar cheeses. The concentrations of histamine measured in all our samples were higher than those values reported by other workers (Silverman and Kosikowski, 1956; De Vuyst et al., 1976).

CONCLUSION

THE USE OF N-HFB isopropyl ester derivatives of free amino acids gave excellent results for quantitative determination of free amino acids by GLC during Cheddar cheese ripening. Most of the available data on this subject were generated in the early 1950's using less sensitive methods. Further work is being conducted to use N-methyl-N(ter-butyldimethylsilyl) trifluoroacetamide (MTBSTFA) to increase the stability of the derivatives and the sensitivity of the method.

The combined use of ion-exchange resin and capillary gas chromatography permitted the determination of at least 6 biogenic amines in Cheddar cheese with high sensitivity, precision and specificity. Results obtained here agree well with those obtained for automated ion-exchange chromatography. The presence of sub-microgram quantities of other biogenic amines in late-gassing Cheddar cheese is probable and investigation is currently underway to develop an NPD (nitrogen and phosphorus) detector in order to quantify these trace amounts.

REFERENCES

Agropur, Coopérative Agro-alimentaire. 1984. Rapport de recherche. Centre de recherche et de contrôle de qualité, Granby, Québec.

Askar, A., Rubach, K. and Schormuller, J. 1972. Dünnschichtchromatographische Trennung der in Bananen vorkommenden Amine-Fraktion. Chem. Mikrobiol. Technol. Lebensm., 1: 187.

Bullock, D.H. and Irvine, O.R. 1956. A chromatographic study of Cheddar cheese ripening. J. Dairy Sci., 39: 1229.

De Vuyst, A., Vervack, W., and Foulon, W. 1976. Détection d'amines non volatiles dans quelques fromages. Le Lait 56(557): 414.

Golan-Goldhirsh A. and Wolfe, F.H. 1979. Gas chromatographic analysis of protein amino acid N-heptafluorobutyryl-isopropyl esters. Can. Inst. Food Sci. Technol. J. 12: 123.

Golan-Goldhirsh, A., Hogg, A.M., and Wolfe, F.H. 1982. Gas chromatographic analysis of the free amino acid pool of the potato and gas chromatography-mass spectrometry identification of γ-amino butyric acid and ornithine. J. Agri. Food Chem. 30: 320.

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Mass Transfer During Ripening of Cuartirolo Argentino Cheese

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ABSTRACT

The mass transfer occurring during ripening of Cuartirolo Argentino cheese was mathematically modelled as an effective diffusion process. The model contained the final NaCl concentration profile resulting from the brining step as starting point and used the same effective diffusivity of NaCl as for brining. The equation developed allowed the calculation of the equilibrium salt concentration resulting from different brining times. Theoretical results were compared against experimental data showing very good agreement.

INTRODUCTION

SALT AND MOISTURE are important in determining microbial and enzyme activity during ripening of cheese. This activity, in turn, influences the quality of the final product. Salt distribution in several types of cheese was studied by different authors. Geurts et al. (1974) presented data on the time required to obtain a uniformly distributed salt concentration over the whole mass of cheese. Sutherland (1977) and Gilles (1977) studied salt and moisture gradients whereas Godinho and Fox (1982) and Hardy (1984) analyzed the influence of salt and moisture on the ripening process. Fundamental knowledge of the underlying principles of salt diffusion in soft cheese during ripening has not yet been reported.

The objective of this work was to design a model to describe the evolution, during ripening, of the salt concentration profiles (moisture gradients can be neglected) resulting from the brining step. The influence of the effective mass diffusivity of NaCl on the profiles as well as the equilibrium concentration of salt as a function of brining time was also analyzed.

THEORY

THE SYSTEM under consideration consists of a cheese (packed under vacuum in a impermeable plastic bag) kept in a cool room at constant temperature during the entire ripening period. The cheese is assumed to be a finite rigid slab and as stated previously (Luna and Bressan, 1985, 1986), it is assumed that there are no chemical reactions in the system and that there is no convective mass transfer into the pores but only diffusion.

During ripening, the spatial salt profile originated in the brining process tends toward equilibrium. The mathematical boundary value problem describing this process can be stated as follows. The governing equation for the diffusion of NaCl into the cheese is:

$$\frac{\partial\theta(x,y,z,t)}{\partial t} = D_{eff}\,\nabla^2\,\theta(x,y,z,t) \qquad (1)$$

where

$$0 < x < 2R_1, \qquad 0 < y < 2R_2, \qquad 0 < z < 2R_3$$

For the case under consideration theoretical and experimental results show that the initial NaCl concentration profile is a function of the spatial coordinates (Luna and Bressan, 1986). Then:

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Initial Condition: At $t = 0$, at any position

$$\theta(x,y,z,0) = \frac{C - C_o}{C_e - C_o} = 1 - \sum_{i=1}^{\infty}\sum_{j=1}^{\infty}\sum_{k=1}^{\infty} A_{i,1}\cdot A_{j,2}\cdot A_{k,3} \cdot \cos\mu_{i,1}\frac{(x - R_1)}{R_1}\cdot\cos\mu_{j,2}\frac{(y - R_2)}{R_2}\cdot\cos\mu_{k,3}\frac{(z - R_3)}{R_3} \cdot \exp\left[-(\mu_{i,1}^2 K_1^2 + \mu_{j,2}^2 K_2^2 + \mu_{k,3}^2 K_3^2)\,Fo\right] \qquad (2)$$

where:

$$A_p = (-1)^{(i,j,k)+1}\cdot\frac{2}{\mu_p};\quad \mu_p = (2p - 1)\,\pi/2$$

$$\frac{1}{R^2} = \frac{1}{R_1^2} + \frac{1}{R_2^2} + \frac{1}{R_3^2}; \qquad K_p = \frac{R}{R_p}; \qquad p = i,j,k;$$

$$Fo = \frac{D_{eff}\,t_b}{R^2}$$

The boundary condition for this case corresponds to a impermeable surface (i.e.: there is no mass flow through the sides of the cheese). Then:

Boundary Condition:

$$\frac{\partial\theta\left(\begin{matrix}x = 2R_1\\ x = 0\end{matrix},y,z,t\right)}{\partial x} = 0$$

$$\frac{\partial\theta\left(x,\begin{matrix}y = 2R_2\\ y = 0\end{matrix},z,t\right)}{\partial y} = 0 \qquad (3)$$

$$\frac{\partial\theta\left(x,y,\begin{matrix}z = 2R_3\\ z = 0\end{matrix},t\right)}{\partial z} = 0$$

The solution to the problem (i.e.: the salt concentration profile into the cheese during the ripening period) obtained by integrating a product of three one-dimensional solutions is (Carslaw and Jaeger, 1959; Crank, 1975; and Ozisik, 1980):

$$\theta(x,y,z,t) = 1 - \left\{\frac{8}{\pi^2}\sum_{i=1}^{\infty}\frac{1}{(2i-1)^2}\cdot\exp\left(-Fo\,\mu_{i,1}^2 K_1^2\right) + \frac{16}{\pi^2}\sum_{n=1}^{\infty}\cos\frac{n\pi x}{R_1}\cdot\exp\left(-D_{eff}\frac{n^2\pi^2}{R_1^2}t\right)\cdot\sum_{i=1}^{\infty}\frac{1}{(2i-1-2n)(2i-1+2n)}\cdot\exp\left(-Fo\,\mu_{i,1}^2 K_1^2\right)\right\} \cdot \{\text{idem y-coordiante}\}\cdot\{\text{idem z-coordinate}\} \qquad (4)$$

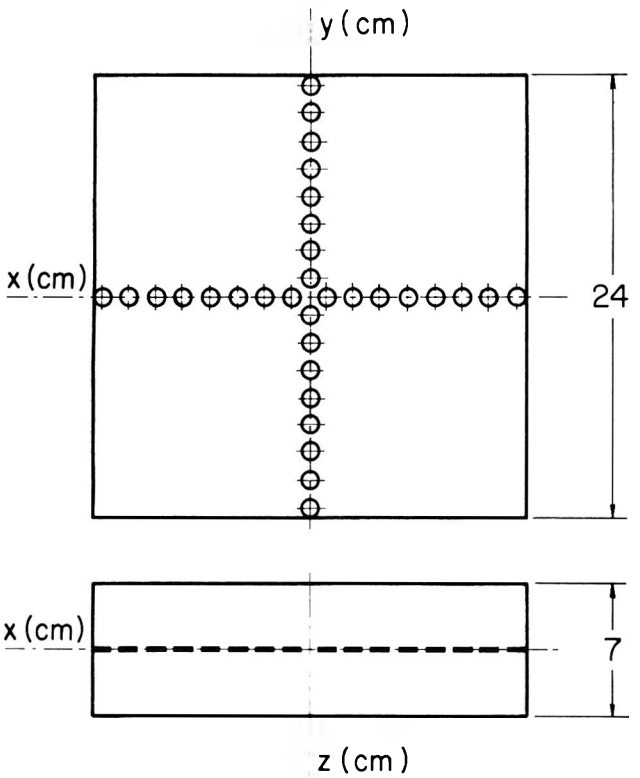

Fig. 1—Sampling points. From each cheese, 33 samples (ϕ = 1.0 cm, h = 0.2 cm) were obtained for determining NaCl content.

At equilibrium ($t \rightarrow \infty$) the uniform salt concentration profile is:

$$\theta(t \rightarrow \infty) = 1 - \left\{ \frac{8^3}{\pi^6} \sum_{i=1}^{\infty} \sum_{j=1}^{\infty} \sum_{k=1}^{\infty} \frac{1}{(2i-1)^2 (2j-1)^2 (2k-1)^2} \cdot \exp\left[-Fo\left(\mu_{i,1}^2 k_1^2 + \mu_{j,2}^2 K_2^2 + \mu_{k,3}^2 K_3^2\right)\right] \right\} \quad (5)$$

MATERIALS & METHODS

THE CUARTIROLO Argentino cheese used in this study and NaCl determination were described previously (Luna and Bressan, 1985, 1986).

Sampling and ripening experiments

For a 6-month period, seven molded cheeses per month were taken from a commercial factory and transported to the pilot plant. After the cheeses reached the desired acidity, one was used to measure the initial NaCl concentration profile. The remaining six cheeses were brined for 5 hr in a 20.5° Be well agitated brine kept at 7.5°C. After brining the cheese was packed under vacuum in heat shrinkable plastic bag (trade name Cryovac Vacuum Process) and put into the ripening room kept at 7.5°C. The cheeses were sampled at t = 5, 10, 15, 20, 25, and 30 days. From each cheese thirty-three samples (ϕ = 10 mm, h = 2 mm) were obtained as indicated in Fig. 1.

RESULTS & DISCUSSION

THE EFFECTIVE mass diffusivity of NaCl used during ripening was the same as calculated for the brining process (Luna and Bressan, 1986). By replacing this $D_{eff} = 0.31$ cm²/day value in Eq. (4), the theoretical spatial concentration profiles can be obtained. Figure 2 shows the theoretical profiles on the

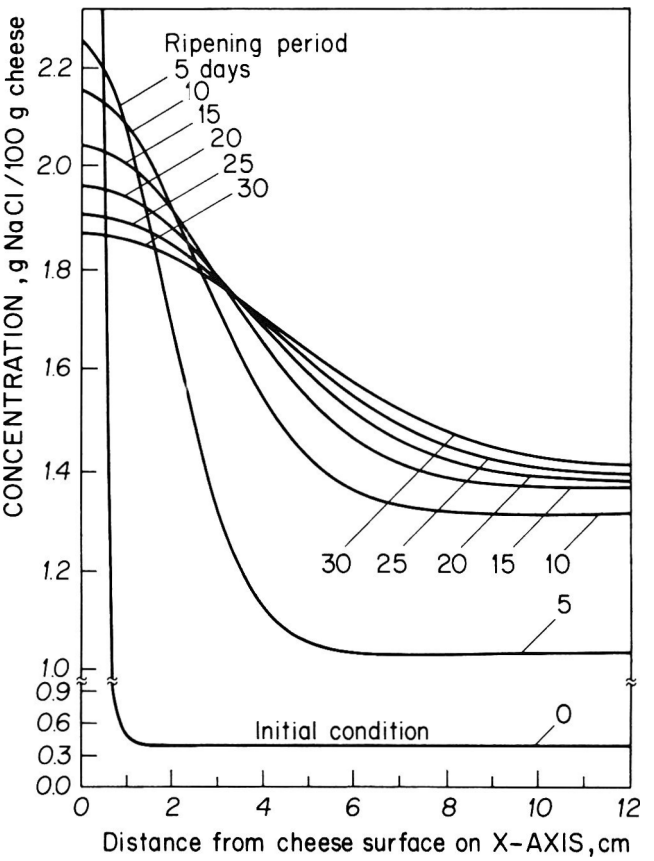

Fig. 2—Theoretical spatial concentration profiles during ripening of Cuartirolo Argentino cheese. X-axis belongs to the domain $0 < x < 2R_1$; $0 < y < 2R_2$; $0 < z < 2R_3$.

x-axis during the overall ripening period corresponding to a previous 5 hr brining process. From Fig. 2 it is important to realize that at 30 days ripening the NaCl concentration profile into the cheese is still not flat.

Table 1 shows the comparison of the theoretical and experimental NaCl concentration values by means of the percent deviation values (Heldman, 1974) according to

$$\% \text{ Deviation} = \sqrt{\sum_{n=1}^{N} \frac{\left(\frac{VT - VE}{VE}\right)_n^2}{N - 1}} \times 100 \quad (6)$$

where VT is the theoretical concentration value, VE is the experimental concentration value and N is the number of points considered. The obtained percent deviation values showed that theoretical and experimental data for several ripening times agreed closely validating the proposed theoretical model. A further check of the model is presented in Fig. 3 where comparison is made of the theoretical and experimental evolutions at the cheese center and at 0.5 cm and 5.0 cm from the cheese surface on the x-axis.

The proposed model can be used for any type of soft cheese with similar geometry provided the mass transport parameter is known. Figure 4 shows the uniform concentration in the cheese (at $t \rightarrow \infty$) for ripening period given by Eq. (5) vs brining time at several effective diffusivity values.

CONCLUSIONS

THE EVOLUTION with time of the NaCl concentration profiles in the whole mass of cheese can be accurately predicted with the proposed mathematical model. The model contained

Table 1—Comparison of experimental and theoretical NaCl concentration data (g NaCl/100 g cheese) by means of the percent deviation values.

Ripening period (days)		Distance from cheese surface on x-axis (cm)									% DEVIATION[c]
		0.5	2	3.5	5	6.5	8	9.5	11	Center	
	VE[a]:	2.25 ± 0.11	1.73 ± 0.09	1.22 ± 0.05	1.03 ± 0.06	0.98 ± 0.04	1.03 ± 0.06	1.05 ± 0.01	1.05 ± 0.10	0.97 ± 0.05	
5											3.13
	VT[b]:	2.21	1.68	1.20	1.05	1.02	1.02	1.02	1.02	1.02	
	VE:	2.20 ± 0.10	2.03 ± 0.15	1.55 ± 0.01	1.38 ± 0.07	1.32 ± 0.03	1.33 ± 0.01	1.32 ± 0.00	1.30 ± 0.02	1.31 ± 0.03	
10											2.99
	VT:	2.14	1.92	1.62	1.42	1.33	1.31	1.30	1.30	1.30	
	VE:	2.07 ± 0.15	2.04 ± 0.14	1.72 ± 0.14	1.51 ± 0.14	1.39 ± 0.08	1.34 ± 0.02	1.39 ± 0.04	1.36 ± 0.06	1.35 ± 0.06	
15											2.73
	VT:	2.04	1.91	1.71	1.53	1.42	1.37	1.36	1.35	1.35	
	VE:	1.98 ± 0.02	1.84 ± 0.02	1.66 ± 0.09	1.60 ± 0.12	1.47 ± 0.12	1.37 ± 0.06	1.36 ± 0.05	1.39 ± 0.04	1.38 ± 0.05	
20											2.19
	VT:	1.96	1.88	1.73	1.58	1.47	1.41	1.38	1.37	1.37	
	VE:	1.97 ± 0.05	1.82 ± 0.04	1.67 ± 0.08	1.58 ± 0.06	1.54 ± 0.06	1.37 ± 0.04	1.34 ± 0.03	1.34 ± 0.04	1.35 ± 0.04	
25											3.43
	VT:	1.90	1.84	1.73	1.61	1.51	1.44	1.40	1.38	1.38	
	VE:	1.92 ± 0.05	1.87 ± 0.01	1.71 ± 0.06	1.63 ± 0.05	1.55 ± 0.10	1.48 ± 0.01	1.41 ± 0.03	1.37 ± 0.02	1.41 ± 0.05	
30											1.71
	VT:	1.87	1.82	1.73	1.63	1.54	1.47	1.43	1.40	1.40	

[a] Experimental concentration value ± pooled sample standard deviation (A Bartlett test (Himmelblau, 1970) was used to check homogeneity of variances).
[b] Theoretical concentration value.
[c] From Heldman (1974).

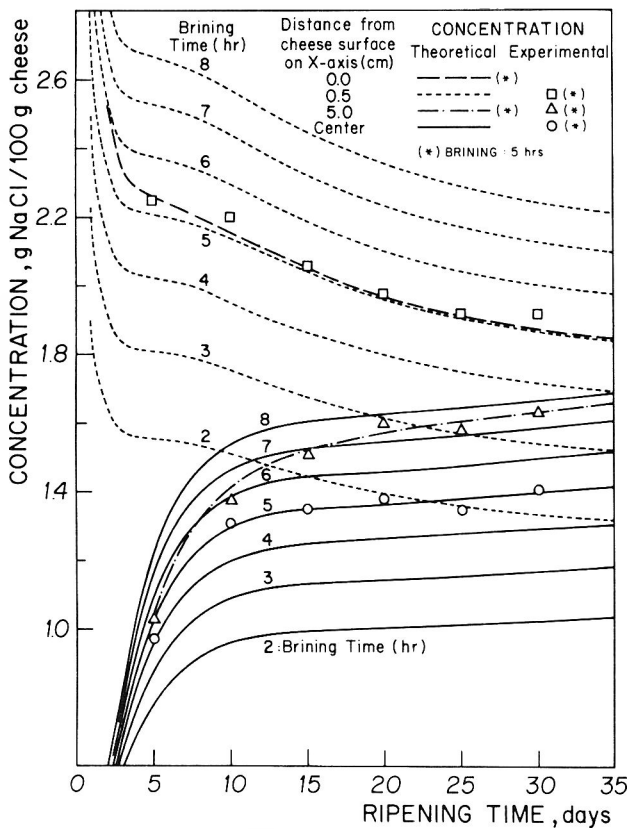

Fig. 3—Theoretical (lines) and experimental (points) NaCl concentration at several distances from cheese surface (0.0, 0.5, 5.0 cm and center) on X-axis during ripening period of Cuartirolo Argentino cheese. Points represent experimental concentration values with 5 hr of brining; pooled sample standard deviation are in Table 1.

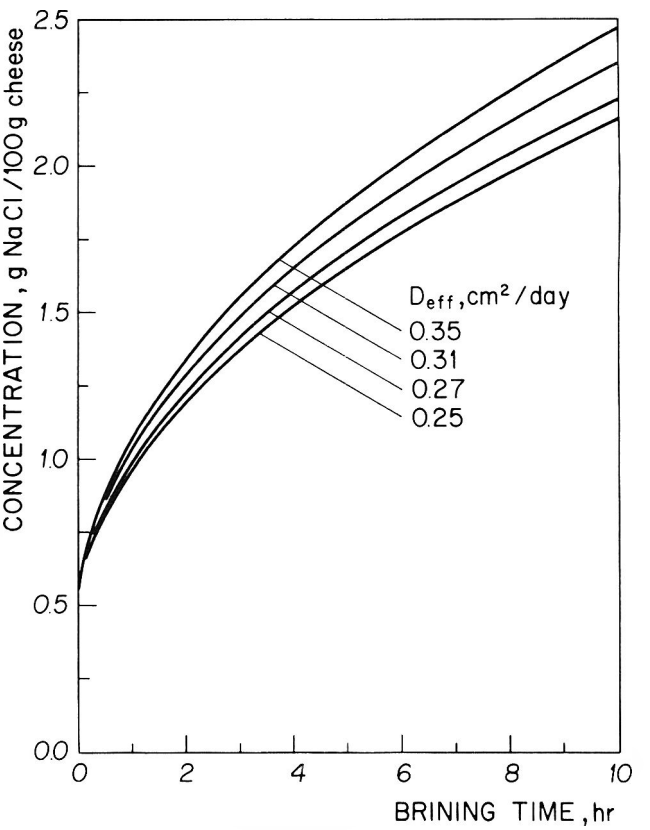

Fig. 4—Uniform concentration of NaCl in the cheese (at $t \rightarrow \infty$) for ripening period vs brining time at several effective diffusivity values.

the final concentration profile resulting from the brining step as starting point and used the same D_{eff} as for brining.

The equation developed allowed the calculation of the equilibrium salt concentration resulting from different brining times and was very useful in predicting the NaCl concentration values at any time and position which in turn influenced the microbial and enzyme activity, and, therefore, the quality of the cheese. This model could be a significant contribution for the study of textural properties and evolution, because the salt profiles will influence the rate of proteolysis during ripening.

NOMENCLATURE

C	NaCl specific mass concentration, m_{NaCl}/m_o
D_{eff}	effective diffusivity, cm^2/day
Fo	Fourier number
h	height, cm
m_o	mass of dry body, g
m_{NaCl}	mass of NaCl, g

R	characteristic length, cm
t	time, days
t_b	brining time
x,y,z	spatial coordinates, cm
ϕ	diameter, cm
θ	dimensionless concentration

Subscripts

o	initial state
c	surrounding medium

REFERENCES

Carslaw, H.S. and Jaeger, J.C. 1959. "Conduction of Heat in Solids." Oxford University Press, Oxford.
Crank, J. 1975. "The Mathematics of Diffusion." Oxford University Press, London.
Geurts, T., Walstra, P., and Mulder, H. 1974. Transport of salt and water during salting of cheese. 1. Analysis of the processes involved. Neth. Milk Dairy J. 28: 102.
Gilles, J. 1977. Moisture and salt distribution in Gouda cheese. New Zealand J. Dairy Sci. Technol. 12: 203.
Godinho, M. and Fox, P.F. 1982. Ripening of Blue cheese. Influence of salting rate on proteolysis. Milchwissenschaft 37: 72.
Hardy, J. 1984. "Le Fromage." Technique et Documentation (Lavoisier), Paris.
Heldman, D.R. 1974. Predicting the relationships between unfrozen water fraction and temperature during food freezing using freezing point depression. Trans. ASAE 17: 63.
Himmelblau, D.M. 1970. "Process Analysis by Statistical Methods." J. Wiley and Sons, Inc., New York.
Luna, J.A. and Bressan, J.A. 1985. Heat transfer during brining of Cuartirolo Argentino cheese. J. Food Sci. 50: 858.
Luna, J.A. and Bressan, J.A. 1986. Mass transfer during brining of Cuartirolo Argentino cheese. J. Food Sci. 51: 829.
Ozisik, M. 1980. "Heat Conduction." J. Wiley and Sons, Inc., New York.
Sutherland, B.J. 1977. Some observations on salt and moisture concentration gradients in Cheddar cheese. Aust. J. Dairy Technol. 3: 17.

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SHELF LIFE PREDICTION OF FROZEN MINCED FISH. . . From page 302

Walker, M., Wenkam, N.S. and Miller, C.D. 1958. Composition of some Hawaii fishes. Hi. Med. J. 18: 144.
Watt, B.K. and Merrill, A.L. 1963. In "Composition of Foods," Agric. Handbook No. 8. U.S. Govt. Printing Office, Washington, DC.
Zipser, M.W. and Watts, B.M. 1961. Oxidative rancidity in cooked mullet. Food Technol. 15: 318.

Ms received 2/24/86; revised 7/25/86; accepted 11/29/86.

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This is journal series 3081 of the Hawaii Institute of Tropical Agriculture and Human Resources, Honolulu, HI 96822.

ASSESSMENT OF CHEDDAR CHEESE QUALITY. . . From page 307

Hickey, M.W., Van Leeuwen, H., Hillier A.J., and Jago, G.R. 1983. Amino acid accumulation in Cheddar cheese manufactured from normal and ultrafiltered milk. Aust. Dairy Technol. 38: 110.
Nakamura, M., Samaya, Y., and Kawabata, H. 1979. Polyamine content in fresh and processed pork. J. Food Sci. 44: 515.
Ney, K.H. 1971. Bestimmung des Reifungsgrades von Kase mittels einfacher Kennzahlen. Milchwissenschaft 26: 269.
Pearce, R.J. 1977. Amino acid analysis by gas-liquid chromatography of N-heptafluorobutyril isobutyl esters. J. Chromatogr. 136: 113.
Reiter, B. Sorokin, Y., Pickering, A., and Hall, A.J. 1969. Hydrolysis of fat and protein in small cheeses made under aseptic conditions. J. Dairy Res., 36: 65.
Silverman, G.J. and Kosikowski, F.V. 1956. Amines in Cheddar cheese. J. Dairy Sci. 39: 1134.
Simard, R.E. and L'Heureux, L. 1982. Les amines biogènes dans les produits laitiers fermentés. Rapport de recherche. Centre de recherche en nutrition et Département de sciences et technologie des aliments, Université Laval, Sainte-Foy, Québec.
Vovan, X., Boulet, M., Simard, R.E., and Gosselin, C. 1985a. Analyse des acides aminés par GLC. I- Séparation des esters de N-heptafluorobutyryl-isopropyl des acides aminés par la colonne capillaire. Submitted for publication.
Vovan, X., Boulet, M., and Simard, R.E. 1985b. Analyse des acides aminés par GLC. II-Influence des temps et température sur la formation des dérivés des esters de N-heptafluorobutyryl-isopropyl. Submitted for publication.
Weaver, J.C., Kroger, M., and Thompson, M.P. 1978. Free amino acid and rheological measurements on hydrolyzed lactose Cheddar Cheese during ripening. J. Food Sci. 43: 579.
Zee, J.A., Simard, R.E., and Roy, A. 1981. A modified automated ion-exchange method for the separation and quantitation of biogenic amines. Can. Inst. Food Sci. Technol. 14: 71.

Ms received 6/12/86; revised 9/15/86; accepted 11/21/86.

The authors thank the Canadian Dairy Commission, Agropur (Cooperative Agro-alimentaire) and the Natural Sciences and Engineering Research Council of Canada for financial assistance. Contribution No 42 (Centre de recherche alimentaire de St-Hyacinthe, Agriculture Canada).

Effect of HTST Pasteurization of Milk, Cheese Whey and Cheese Whey UF Retentate upon the Composition, Physicochemical and Functional Properties of Whey Protein Concentrates

C. V. MORR

ABSTRACT

The effect of high temperature-short time (HTST) pasteurization of milk, Cheddar cheese whey and cheddar cheese whey ultrafiltration (UF) retentate upon the composition, physicochemical and functional properties of whey protein concentrates (WPC) was investigated. HTST pasteurization (72°C–15 sec) of milk, whey and UF retentate caused no significant differences in chemical composition of resulting WPCs. HTST pasteurization of milk and whey had no significant effect upon WPC solubility, whereas, heating UF retentate caused significant loss of WPC solubility. HTST pasteurization of milk caused a significant lowering ($P<0.10$) of maximum foam expansion of WPC dispersions, but HTST pasteurization of whey and UF retentate had no significant effect upon this latter parameter.

INTRODUCTION

THE DAIRY INDUSTRY has the capacity to greatly increase its production of whey protein concentrates (WPC) from the vast surplus of whey for use as functional and nutritional ingredients in formulated food products (Morr, 1984). However, the somewhat disappointing performance of WPC as ingredient in key food product applications is a major factor responsible for its poor acceptance by the industry. The processing conditions used in the manufacture of whey and for recovering the whey proteins influence the compositional, physicochemical and functional properties of the WPC (Morr, 1979, 1982; Marshall, 1982).

Additional research is needed to determine the effects of whey processing and fractionation treatments upon the physicochemical properties of WPC as a means for improving their functional properties and utilization. WPC produced from pasteurized and nonpasteurized acid and Cheddar cheese whey by ultrafiltration (UF) and spray drying had generally similar compositional and physico-chemical properties, but had noticeably different foaming properties (Morr, 1985). The present study was conducted to compare the effects of high temperature-short time (HTST) pasteurization of milk, Cheddar cheese whey and UF retentate from Cheddar cheese whey upon the compositional, physicochemical and functional properties of WPC.

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MATERIALS & METHODS

Whey protein concentrate

Eight WPCs were produced from different lots of Cheddar cheese whey at the New Zealand Dairy Research Institute, Palmerston North, New Zealand. The cheese milk was preheated and clarified at 32°C or 55°C and HTST pasteurized at 72°C - 15 sec or nonpasteurized (Table 1). Cheddar cheese whey was prepared from the milks using *Streptococcus cremoris* starter culture and rennet as coagulant (Morr, 1985). The curd was cooked and drained at 50°C. Aliquots of whey were preheated and clarified at 37°C or 55°C and HTST pasteurized or nonpasteurized (Table 1). The pH of the wheys ranged from 6.39 to 6.59 and their total solids contents ranged from 6.42 to 6.54%. Each whey was concentrated 20:1 (v/v) by ultrafiltration and subsequently diafiltered using a water to retentate volume ratio of 2.37 (Morr, 1985). UF retentates were HTST pasteurized or nonpasteurized (Table 1), stored at 5°C and warmed to 50°C before spray drying in an Anhydro spray dryer at 205°C inlet and 92°C outlet air temperatures.

Analytical

The composition of the WPCs was determined by the New Zealand Dairy Research Institute as previously described (Morr, 1985). Moisture was by oven drying, ash by ignition in an electric muffle furnace, lactose by ferricyanide reduction, protein by micro-Kjeldahl using trichloroacetic acid treatment to correct for nonprotein nitrogen compounds and milkfat by Mojonnier modification of the Roese-Gottlieb ether extraction method after hydrolysis with heat and HCl.

Physicochemical and functional properties

Soluble proteins from the WPCs were examined and characterized by polyacrylamide gel electrophoresis, Sephadex G-150 gel filtration and reversed phase high performance liquid chromatography (HPLC) as previously described (Morr, 1985). WPC soluble proteins were also examined by size exclusion HPLC using a 7.5 mm × 30 cm TSK-3000 SW column (Altex Scientific, Berkeley, CA): pH 6, 0.1M phosphate buffer prepared in 0.1 M sodium nitrate for elution and 280 ηm effluent monitoring. The concentration of the fractionated proteins was estimated from peak height to concentration ratio determined with

Table 1—Preheat and pasteurization treatments used for manufacturing whey protein concentrates[a]

	Milk		Whey		UF retentate
WPC[b]	Preheat temp (°C)	Past. or nonpast.	Preheat temp (°C)	Past. or nonpast.	Past. or nonpast.
1 (− + +)	32	NP	55	P	P
2 (+ + −)	55	P	55	P	NP
3 (− + −)	32	NP	55	P	NP
4 (+ − −)	55	P	37	NP	NP
5 (+ + +)	55	P	55	P	P
6 (− − +)	32	NP	37	NP	P
7 (+ − +)	55	P	37	NP	P
8 (− − −)	32	NP	37	NP	NP

[a] P = pasteurization at 72°C – 15 sec; NP = nonpasteurized.

[b] Symbols within brackets denote whether milk, whey or UF retentate, respectively, were pasteurized (+) or nonpasteurized (−).

Table 2—Composition of whey protein concentrates

WPC[a]	Moisture	Ash	Protein	Lactose	Milkfat
1 (− + +)	5.3	2.7	73.9	7.2	4.8
2 (+ + −)	3.6	2.6	74.7	8.4	2.1
3 (− + −)	6.7	2.5	77.0	6.7	4.9
4 (+ − −)	3.3	2.9	73.1	10.1	4.2
5 (+ + +)	3.1	2.7	74.8	8.4	4.2
6 (− − +)	3.1	2.9	72.3	11.1	4.2
7 (+ − +)	1.8	2.8	73.7	9.6	3.9
8 (− − −)	2.8	2.7	73.8	9.7	4.1

[a] See Table 1 for pasteurization/nonpasteurization conditions. All data are means for duplicate values.

reference whey proteins. Reference whey proteins were purchased from Sigma Chemical Company (St. Louis, MO) except immunoglobulin IgG, which was from Ross Laboratories (Columbus, OH). Fresh Blue cheese whey was produced by the university dairy plant and used as a source of whey proteins for size exclusion HPLC.

WPC solubility was determined by both micro-Kjeldahl and Biuret modifications of the reference procedure (Morr et al., 1985). The foaming properties of the WPCs were studied by the method of Morr (1985) using 6% protein concentration (w/v) in distilled water adjusted to pH 4.5 or 7 with 1.5N HCl or NaOH. The WPC dispersions were pretreated 30 min at 25° or 55°C just prior to whipping with a Sunbeam Mixmaster (Sunbeam Appliance Company, Chicago) at a speed setting of 13 times for up to 20 min. Foam expansion, viscosity and stability were determined after whipping 20 min by previously reported procedures (Morr, 1985), except that foam stability was the volume (mL) of liquid drainage collected from 100 mL of foam within a 30 min time period.

For statistical purposes the experiment was a completely random design with a 2 × 2 × 2 factorial arrangement between heating or nonheating of milk, whey and UF retentate, respectively. Differences between main effect means were tested by analysis of variance (Steel and Torrie, 1980).

RESULTS & DISCUSSION

Chemical composition

The eight WPCs (Table 1) were generally similar in composition, e.g., moisture ranged from 1.8–6.7%, ash from 2.5–2.9%, protein from about 72–77%, lactose from 6.7–11.1% and milkfat from 2.1–4.9% (Table 2). These values were generally in good agreement with those from the previous study (Morr, 1985). The HTST pasteurization treatment caused no statistically significant differences in WPC composition, except that heating whey caused a lower ($p<0.05$) ash and lactose content than from nonheated whey.

Protein solubility

WPC solubility data in Table 3 ranged from about 87–102% mat pH 3 and from about 90–102% at pH 7 where soluble protein was determined by micro-Kjeldahl. Corresponding val-

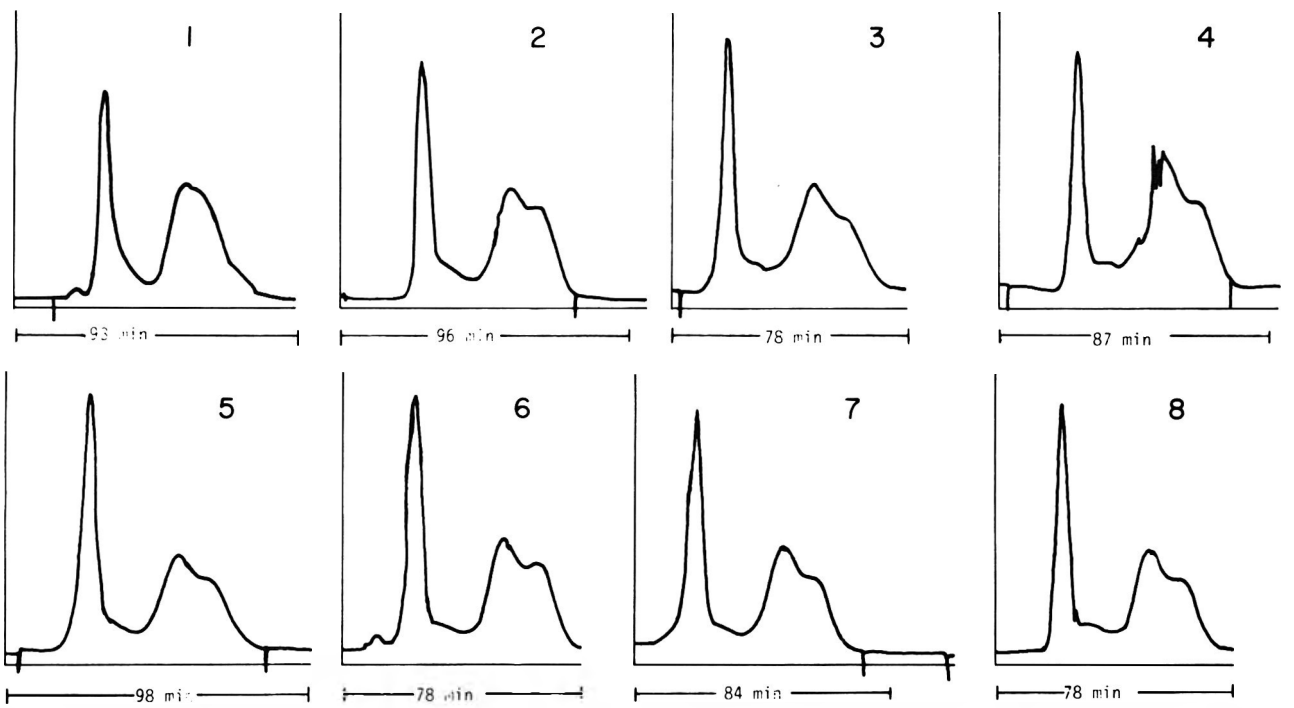

Fig. 1—Sephadex G-150 gel filtration patterns for soluble proteins from whey protein concentrates (WPC) eluted with 0.1M, pH 7 phosphate buffer and monitored at 280 ηm. See Table 1 for identification of WPCs 1-8.

Table 3—Solubility of whey protein concentrates

	Protein solubility, %[a]			
	micro-Kjeldahl		Biuret	
WPC[b]	pH 3	pH 7	pH 3	pH 7
1 (− + +)	86.8 ± 1.03	93.0 ± 5.96	74.0 ± 2.26	83.5 ± 2.35
2 (+ + −)	98.5 ± 3.60	100.6 ± 3.13	85.2 ± 0.40	95.7 ± 7.70
3 (− + −)	94.2 ± 3.93	95.9 ± 3.83	81.6 ± 1.68	90.4 ± 1.24
4 (+ − −)	99.7 ± 1.43	100.9 ± 3.37	86.4 ± 0.76	89.7 ± 1.98
5 (+ + +)	87.0 ± 2.01	89.7 ± 1.20	68.9 ± 2.07	78.6 ± 0.45
6 (− − +)	88.4 ± 3.91	90.9 ± 3.70	77.9 ± 0.80	83.0 ± 0.90
7 (+ − +)	92.3 ± 3.19	94.5 ± 3.41	79.8 ± 0.40	87.4 ± 1.17
8 (− − −)	102.1 ± 1.84	102.1 ± 2.08	87.8 ± 1.96	94.6 ± 1.27

[a] Means of three replicate determinations with standard deviation values.
[b] See Table 1 for pasteurization/nonpasteurization conditions.

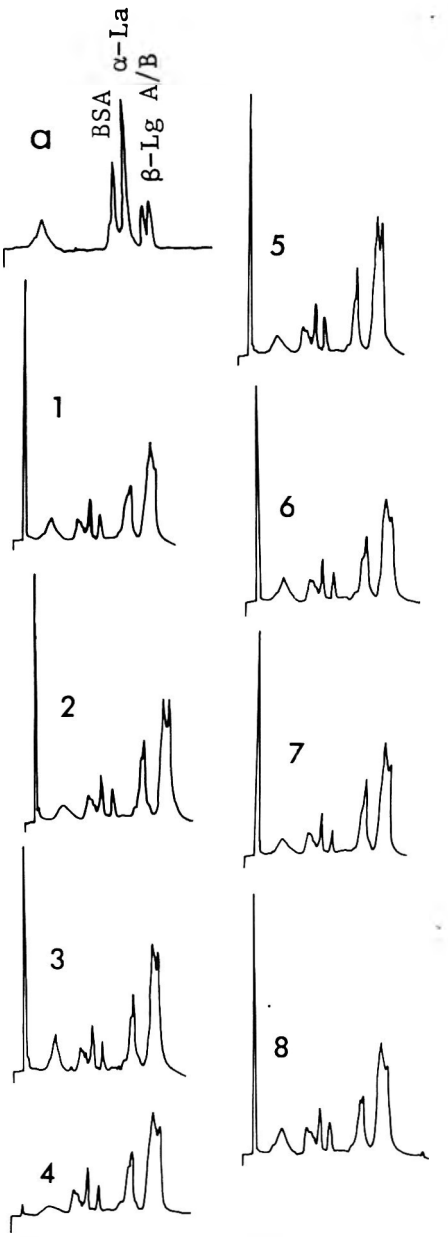

Fig. 2—Reversed phase HPLC patterns for reference whey proteins (a) and soluble proteins from whey protein concentrates (WPC) eluted with pH 2.1 NaCl-HCl: acetonitrile gradient and monitored at 210 ηm. Protein standards were: bovine serum albumin (BSA), α-lactalbumin (α-La), and β-lactoglobulin A/B (β-Lg). See Table 1 for identification of WPCs 1-8.

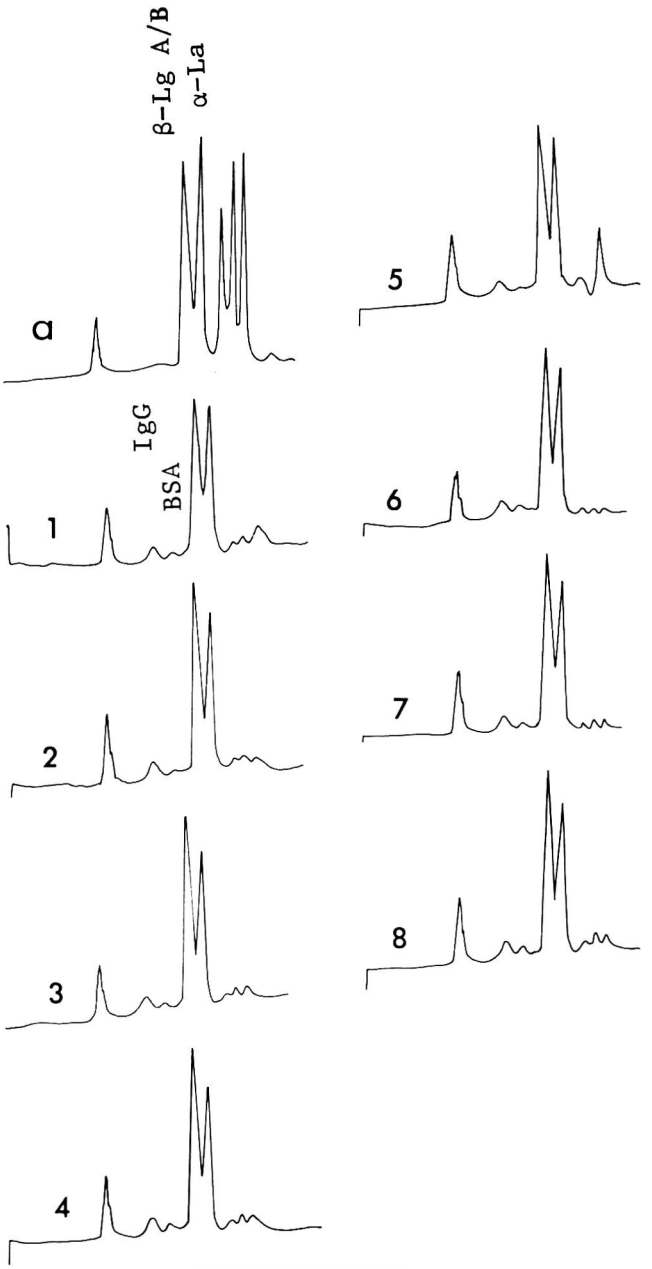

Fig. 3—Size exclusion HPLC patterns for fresh cheese whey (a) and soluble proteins from whey protein concentrates (WPC) eluted with 0.1 M, pH 6 phosphate buffer in 0.1M sodium nitrate solution and monitored at 280 ηm. Protein standards were: bovine serum albumin (BSA), α-lactaibumin (α-La), β-lactoglobulin A/B (β-Lg A/B), and immunoglobulin G (IgG). See Table 1 for identification of WPCs 1-8.

ues ranged from about 69–88% at pH 3 and from 79–96% at pH 7 where soluble protein was determined by Biuret. Although these values were generally similar to those previously reported for acid and Cheddar cheese WPCs (Morr, 1985), statistical analysis indicated that heating milk had no effect on WPC solubility, heating whey caused a reduction of WPC solubility ($P<0.05$) when determined at pH 3 and heating UF retentate caused significant solubility reduction ($P<0.01$) when determined by micro-Kjeldahl at pH 3 and 7 and by Biuret at pH 3 and also when determined by Biuret at pH 7 ($P<0.05$).

Sephadex gel filtration

Gel filtration elution patterns for soluble proteins recovered from WPC dispersions (Fig. 1) revealed generally similar protein molecular weight-size distributions that were not influenced by variations in heat processing. The relative proportion of the larger sized protein aggregates eluted in the void peak was generally similar for all WPCs. Thus, the different heat treatments failed to induce detectable differences in the state of protein aggregation of WPC proteins by this method.

Reversed phase HPLC

HPLC elution patterns (Fig. 2) and peak area data (Table 4)were generally similar for all WPCs. BSA and α-La were coeluted in Peak 5 that accounted for about 23% of the total absorbance pattern area. β-Lg A & B were also coeluted in Peak 6 that accounted for about 56-58% of total absorbance pattern area. These results generally agree with those of the

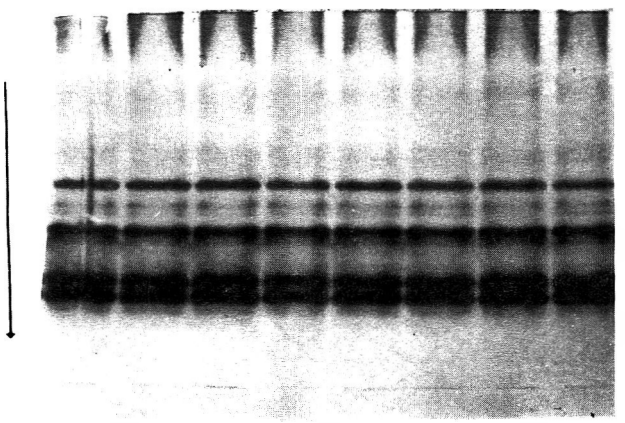

Fig. 4—Polyacrylamide gel electrophoresis patterns for soluble proteins from whey protein concentrates (WPC). See Table 1 for identification of WPCs 1-8.

Table 4—Reversed phase HPLC patterns for whey protein concentrates

Peak number	1	2	3	4	5[a]	6[b]
Retention time (Min)	5.8	6.9	7.8	8.5	10.9	12-13
WPC[c]	Peak area percentage[d]					
1 (− + +)	8.6	7.1	4.2	—	23.7	56.3
2 (+ + −)	7.6	6.5	4.1	1.4	23.4	57.1
3 (− + −)	8.5	5.9	4.1	—	23.6	57.8
4 (+ − −)	7.8	6.4	4.5	—	23.3	58.0
5 (+ + +)	9.4	7.0	4.5	—	22.8	56.2
6 (− − +)	8.6	7.0	4.4	2.3	23.2	54.4
7 (+ − +)	8.4	6.6	4.6	1.7	23.0	55.6
8 (− − −)	8.5	6.3	3.6	1.9	23.5	56.0

[a] BSA and α-La.
[b] β-Lg A & B.
[c] See Table 1 for pasteurization/nonpasteurization conditions.
[d] Peak areas determined by integrator.

Table 5—Molecular exclusion HPLC fractionation of whey protein concentrates

Protein component	IgG	BSA	β-Lg	α-La	Total
Retention time (min)	19.5	22.0	25.4	26.8	—
WPC[a]	Protein concentration, % of total[b]				
1 (− + +)	3.9	7.6	45.5	9.7	66.7
2 (+ + −)	4.1	10.4	55.7	10.4	80.6
3 (− + −)	3.8	10.4	55.0	10.4	79.6
4 (+ − −)	4.8	14.3	54.3	9.5	82.9
5 (+ + +)	3.3	8.0	44.6	9.8	65.9
6 (− − +)	3.9	11.0	47.7	9.2	71.8
7 (+ − +)	3.5	9.7	46.9	8.9	69.0
8 (− − −)	3.8	14.3	54.3	9.5	81.9

[a] See Table 1 for pasteurization/nonpasteurization conditions.
[b] Percentage of total protein contained in 20 μL sample.

previous study (Morr, 1985) with respect to Cheddar cheese WPC proteins and confirm that the different heat treatments used in this study failed to significantly alter the hydrophobicity of the whey proteins. It is perhaps worth noting, however, that the low pH and organic solvent conditions employed for this HPLC procedure may tend to minimize the conformational differences induced in the major whey proteins by the different heat treatments.

Size exclusion HPLC

HPLC elution patterns (Fig. 3) and protein percentage data (Table 5) reveal that although there was considerable similarity in the patterns, there was a general relationship between percentages of BSA and β-Lg and pasteurization of UF retentate. For example, WPCs prepared from HTST pasteurized retentates (1, 5, 6, and 7) had smaller percentages of β-Lg ranging

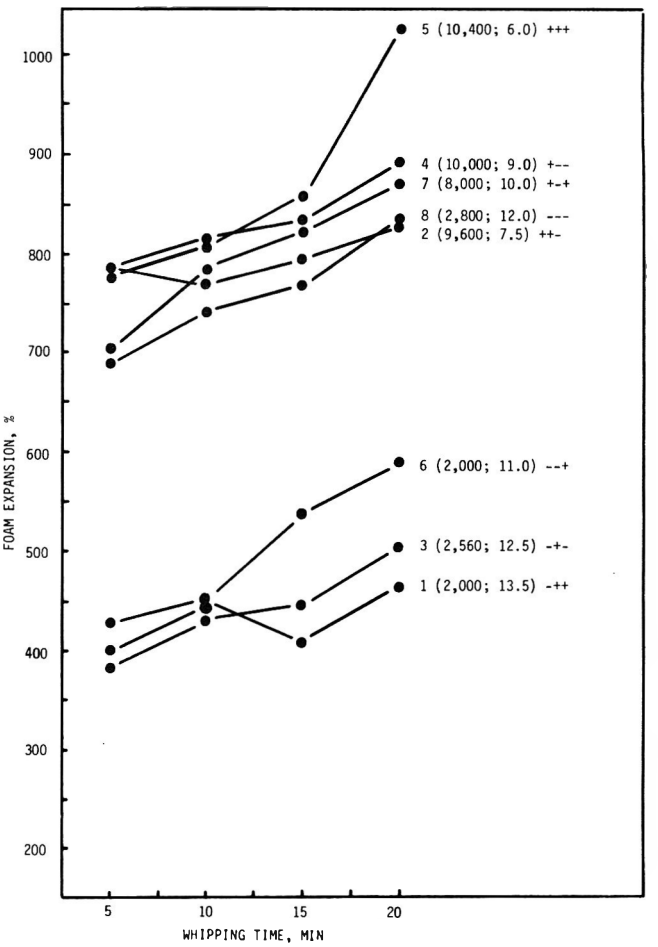

Fig. 5—Foaming properties of pH 4.5, 25°C pretreated whey protein concentrate dispersions. Numbers within brackets indicate relative viscosity (cp) and stability (ml drainage in 30 min) for 20 min whipped foams. See Table 1 for identification of + and − symbols.

from 45 to 48%, smaller percentages of BSA ranging from 7.6–9.7% and smaller percentages of total pattern area for the four major proteins ranging from 67–72% than corresponding values for the other four WPCs produced without HTST pasteurization of UF retentates. These results, although merely estimates of protein molecular weight distributions, tend to indicate that size exclusion HPLC is more sensitive than the other analytical methods used here to detect minor alterations of protein molecular weight. They also revealed that HTST pasteurization of UF retentates with their high protein concentrations causes minor aggregation of whey proteins that may explain the observed differences in functionality of these WPCs.

Gel electrophoresis

Polyacrylamide gel electrophoresis patterns in Fig. 4 appeared identical. These findings confirm those of above and previously reported (Morr, 1985) that HTST pasteurization causes no major alterations of whey proteins in WPC. They also indicate that this method is not as sensitive as size exclusion HPLC for studying the minor alterations of whey proteins in WPCs.

Foaming properties

Foam expansion, viscosity and stability data in Figs. 5-8 and Table 6 reveal that the different WPCs had major differences in foaming properties as a function of pH and pretreat-

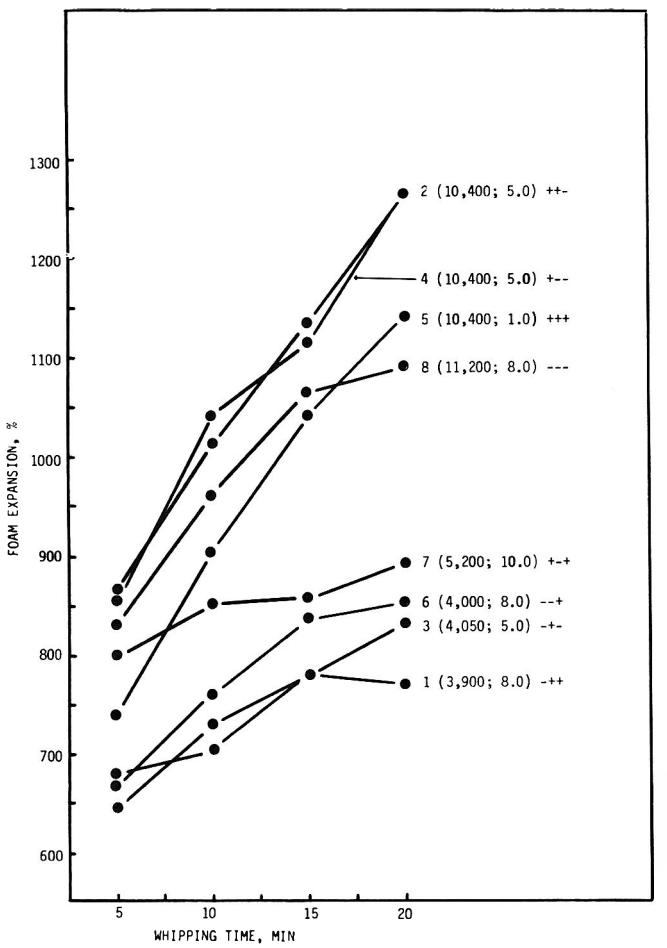

Fig. 6—Foaming properties of pH 4.5, 55°C pretreated whey protein concentrate dispersions. Numbers within brackets indicate relative viscosity (cp) and stability (ml drainage in 30 min) for 20 min whipped foams. See Table 1 for identification of + and 1 symbols.

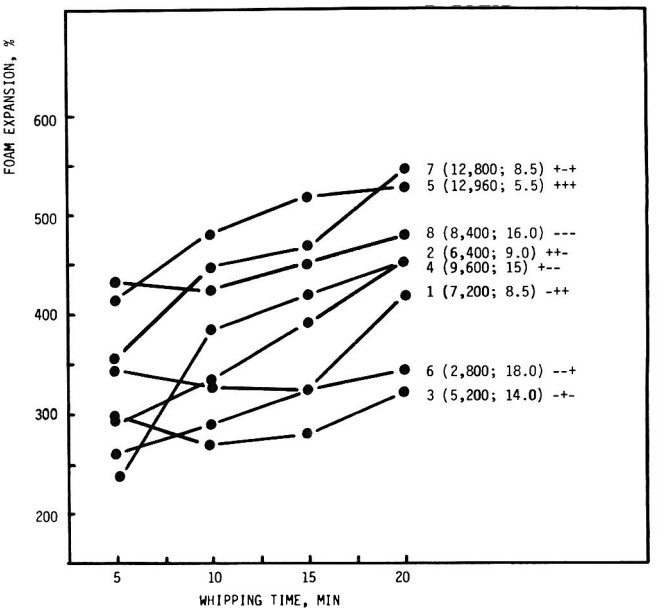

Fig. 7—Foaming properties of pH 9, 25°C pretreated whey protein concentrate dispersions. Numbers within brackets indicate relative viscosity (cp) and stability (mL drainage in 30 min) for 20 min whipped foams. See Table 1 for identification of + and − symbols.

Table 6—Maximum foam expansion after 20 min whipping

	Foam expansion, %			
WPCA[a]	pH 4.5/25°C	pH 4.5/55°C	pH 9.0/25°C	pH 9.0/55°C
1 (− + +)	460	780	420	750
2 (+ + −)	825	1275	450	900
3 (− + −)	500	820	320	800
4 (+ − −)	900	1275	450	1000
5 (+ + +)	1050	1150	525	800
6 (− − +)	600	850	340	840
7 (+ − +)	870	900	550	1100
8 (− − −)	830	1100	475	910

[a] See Table 1 for pasteurization/nonpasteurization conditions.

ment temperature. For example, maximum foam expansion data in Table 6 reveal values ranging from 460–1050% for pH 4.5 and 25°C pretreated WPC, 780–1275% for pH 4.5 and 55°C pretreated WPC, and 750–1100% for pH 9 and 55°C pretreated WPC. Poorest foam expansion values were provided by WPCs given the pH 9 and 25°C pretreatment. The slopes for foam expansion versus whipping time curves (Fig. 5–8) were generally steeper for 55°C than for 25°C pretreated WPC dispersions, indicating that the higher temperature treatment may have modified the proteins conformational state such that they were better able to aid air entrapment during whipping. There also appeared to be a general relationship between foam viscosity and maximum expansion after 20 min whipping (Fig. 5-8), but there was no apparent relationship between foam

Table 7—Ranking of whey protein concentrates for composition, solubility and foaming properties[a]

	Composition (%)					Solubility (%)				Foam expansion (%)			
WPC[b]	Moisture	Ash	Protein	Lactose	milkfat	pH 3, micro-Kjeldahl	pH 7, micro-Kjeldahl	pH 3, Biuret	pH 7, Biuret	pH 4.5/25°C	pH 4.5/55°C	pH 9.0/25°C	pH 9.0/55°C
1 (− + +)	2	3	4	6	2	8	6	7	6	8	8	6	8
2 (+ + −)	3	4	3	5	6	3	3	3	1	5	1	4	4
3 (− + −)	1	5	1	7	1	4	4	4	3	7	7	8	7
4 (+ − −)	4	1	7	2	3	2	2	2	4	2	2	5	2
5 (+ + +)	5	3	2	5	3	7	8	8	8	1	3	2	6
6 (− − +)	5	1	8	1	3	6	7	6	7	6	6	7	5
7 (+ − +)	7	2	6	4	5	5	5	5	5	3	5	1	1
8 (− − −)	6	3	5	3	4	1	1	1	2	4	4	3	3

[a] Ranking in descending order; largest (1) to smallest (8).
[b] See Table 1 for pasteurization nonpasteurization conditions.

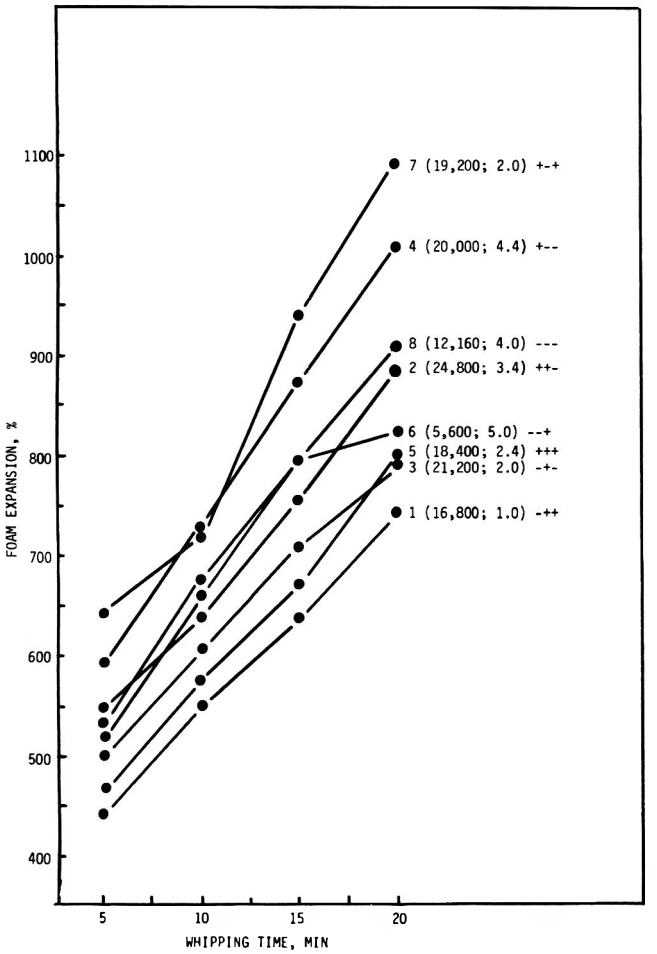

Fig. 8—Foaming properties of pH 9, 55°C pretreated whey protein concentrate dispersions. Numbers within brackets indicate relative viscosity (cp) and stability (mL drainage in 30 min) for 20 min whipped foams. See Table 1 for identification of + and − symbols.

stability and maximum foam expansion values for whipped WPC dispersions.

Statistical analysis of the data revealed that HTST pasteurization of milk significantly increased ($P<0.10$) maximum foam expansion values of WPCs. Heating whey generally had no effect on maximum foam expansion of WPC dispersions, except at pH 9 and 55°C pretreatment, where maximum foam expansion was significantly lowered ($P<0.05$). Heating UF retentates had no significant effect upon WPC dispersion maximum foam expansion values.

Comparison of the ranking of WPCs with respect to chemical composition, protein solubility and maximum foam expansion values (Table 7) confirm the above statistical analysis. For example, WPCs 1, 5, 6 and 7 exhibited maximum foam expansion rankings ranging from 1 to 8 indicating little correlation of these two factors. However, ranking values for these WPCs with respect to protein solubility ranged from 5 to 8 indicating good correlation of this parameter with HTST pasteurization of UF retentate. Similarly, WPCs 1, 2, 3 and 5 produced from HTST pasteurized whey and WPCs 2, 4, 5 and 7 produced from HTST pasteurized milk had protein solubility rankings ranging from 1 to 8, confirming that these treatments had little effect upon WPC protein solubility.

CONCLUSIONS

THE HTST PASTEURIZATION treatments used in this study represent the minimum allowable time and temperature conditions for producing high quality WPC with adequate microbial safety and shelf life stability. Results confirm those of the previous study (Morr, 1985) that although this mild heat treatment did not produce detectable alterations in the composition and physicochemical properties of the WPCs, it nevertheless resulted in significantly altered protein solubility and functionality. Whereas, heating UF retentate with its relatively high protein concentration caused the greatest loss of WPC protein solubility, it unexpectedly had no significant effect upon WPC foaming properties. It is thus obvious that only minor changes in protein composition and conformational state are necessary to significantly alter WPC solubility and functionality. From this it appears that solubility and functionality testing are more sensitive than the present compositional and physicochemical parameters for detecting the minor protein conformational state changes produced by HTST pasteurization of milk, whey and UF retentate.

REFERENCES

Marshall, K.R. 1982. Industrial isolation of milk proteins: whey proteins. In "Developments in Dairy Chemistry-1." Applied Science Publishers, New York.
Morr, C.V. 1979. Functionality of whey protein products. New Zealand J. Dairy Sci. Technol. 14: 185.
Morr, C.V. 1982. Functional properties of milk proteins and their use as food ingredients. In "Developments in Dairy Chemistry-1." Applied Science Publishers, New York.
Morr, C.V. 1984. Production and use of milk proteins. Food Technol. 38(7): 39.
Morr, C.V. 1985. Composition, physico-chemical and functional properties of reference whey protein concentrates. J. Food Sci. 50: 1406.
Morr, C.V., German, B. Kinsella, J.E., Regenstein, J.M., Van Buren, J.P., Kilara, A., Lewis, B.A., and Mangino, M.E. 1985. A Collaborative study to develop a standardized food protein solubility procedure. J. Food Sci. 50: 1715.
Pearce, R.J. 1983. Analysis of whey proteins by high performance liquid chromatography. Australian J. Dairy Technol. 38: 114.
Steele, R.G.D. and Torre, J.H. 1980. "Principles and Procedures of Statistics." McGraw-Hill Publishers, New York.
Ms received 7/28/86; revised 9/22/86; accepted 10/22/86.

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Flow Properties of Tomato Concentrates: Effect of Serum Viscosity and Pulp Content

T. TANGLERTPAIBUL and M. A. RAO

ABSTRACT

Shear rate-shear stress data were obtained on tomato serum samples at 5.6, 10, 15, and 20 °Brix, and on 28 concentrates prepared from the serum samples and containing different amounts of pulp: +30%, +20%, +10%, normal, −10%, −20%, and −30%. Serum samples were Newtonian fluids while concentrates were shear-thinning non-Newtonian fluids. Apparent viscosities of concentrates increased linearly with pulp content expressed as weight %, volume %, or the weight ratio of pulp to serum. Apparent viscosity of the concentrates (η_{100} conc) was related to the viscosity of serum (η serum) and pulp content (P) by the expression: η_{100} conc = η serum + A * P ** B. The contribution of pulp content to η_{100} conc was much more than that of serum. η serum was well correlated with pectin content by the expression: η serum = A_1 + B_1 (Pectin) ** C_1.

INTRODUCTION

CONSISTENCY of tomato concentrates is measured by various instruments such as the Bostwick consistometer, the Adams consistometer, the Stormer viscosimeter, the Efflux-tube viscometer, and the Blotter test. The details fo some of the methods and comparison with other instruments can be found in various articles (Davis et al., 1954; Rao and Bourne, 1977; Elder and Smith, 1969). However, these instruments perform only a single point measurement and the consistencies are not in terms of the fundamental units of mass, length, and time so that they cannot be used with well established methods for designing handling systems (Rao, 1986a) and for the assessment of stimuli associated with oral and non-oral evaluation of viscosity (Shama et al., 1973; Shama and Sherman, 1973). Another limitation of Bostwick consistency is that its magnitude decreases exponentially with concentration (Marsh et al., 1977) so that data cannot be obtained on viscous concentrates with more than about 15% total solids. Nevertheless, consistencies have proved to be adequate for quality control and numerous studies have been conducted on the Bostwick consistency of tomato concentrates as affected by changes in soluble and insoluble solids (Sornsrivichai and Rao, 1986).

Tomato juice consists of a dispersing medium (serum) and suspended particles (pulp) that can be separated by centrifugation. The clear serum contains low and high molecular-weight solutes of pectic substances, sugars, salts, and organic acids. Therefore, tomato juice is a special type of dispersion (suspension) in which pulp is suspended in a colloidal medium. Harper and El Sahrigi (1965) showed that concentrated tomato serum (65 °Brix) was a shear thinning fluid. The non-Newtonian behavior of concentrated fruit serums can be attributed to the presence of high molecular-weight solutes such as pectins (Mizrahi and Berk, 1970; Vitali and Rao, 1984).

Takada and Nelson (1983) developed a new method for evaluations of the consistency of tomato products. They found that precipitate weight ratio (PPT), which refers to the weight ratio of precipitate after centrifugation of a sample at 12,800 × g to the initial sample weight, was highly correlated with the Bostwick consistency and efflux viscosity of tomato products. Therefore they proposed that PPT may be a reliable parameter to evaluate the consistency of tomato products.

There are few published articles about rheology of tomato concentrates using well designed viscometers and these have been reviewed by Sornsrivichai and Rao (1987).

The primary objective of the present study was to determine and model the flow properties of tomato concentrates with different serum concentrations and amounts of pulp.

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MATERIALS & METHODS

CANNED TOMATO JUICE that was prepared as described earlier (Sornsrivichai and Rao, 1986) was used. Briefly, hot break juice was prepared by heating crushed tomatoes to 97.8 °C and holding at the temperature for 4 min and 5 sec. The hot macerate was passed through a finisher operated at 1,000 rpm and with a screen having holes 0.033 in diameter. The juice from the finisher was boiled in a kettle and hot-filled into #303 cans. The cans were rolled for 3 min and cooled in cold water.

Preparation of tomato concentrates with various serum concentrations and pulp contents

The canned juice was centrifuged at 11,700 × g at 20 °C for 45 min in a Sorvall RC-5 centrifuge (Ivan Sorvall, Inc., Norwalk, CT), and the clear serum was transferred to a breaker after its volume was measured; it was then stored in a refrigerator. The pulp was scraped from the centrifuge tubes and transferred to a plastic bottle and stored in a refrigerator. The serum was concentrated in a steam-jacketed kettle to various °Brix concentrations.

In addition to proportionally combining the concentrated serum with the pulp as was done in an earlier study (Sornsrivichai and Rao, 1986), the amounts of pulp and serum at different °Brix were combined in a manner to give concentrates with various pulp contents: +30%, +20%, +10%, −10%, −20%, −30%. In the +30% sample, the amount of pulp combined with concentrated serum of a specified °Brix was 30% more than that in the normal sample in which proportional combination was employed. In contrast, in the −30% sample the amount of pulp combined with the concentrated serum was 30% less than that in the normal sample. Serum samples at 4 levels of concentration (°Brix) were prepared: 5.6, 10, 15 and 20. The serum at each concentration was used to make 7 concentrates with +30%, +20%, +10%, normal, −10%, −20%, and −30% pulp content. Therefore, 28 concentrates were prepared, each with a volume of about 100 ml.

The amount of pulp added was expressed as % volume pulp (VP), as % wet weight pulp (WWP), and as pulp:serum weight ratio (PS).

Determination of rheological properties of tomato concentrates and juices

Flow properties of the concentrates were determined at 25°C with a concentric cylinder viscometer (Haake, RV2, System MVI) and those of serum samples were determined with measuring system NV as described earlier (Vitali and Rao, 1984). The torque was converted to shear stress and the rpm was converted to shear rate. The method of Krieger was employed to correct Newtonian shear rates for the non-Newtonian nature of the test fluids (Van Wazer et al., 1963; Vitali and Rao, 1984).

Determination of total pectic substances

Total pectic substances were determined using the method of Kintner and Van Buren (1982). This method requires that the sample be diluted to contain pectic substances equivalent to galacturonic acid between 0 to 75 μg.

RESULTS & DISCUSSION

Percept pulp centrifuged at high g

The % volumetric pulp (VP) and % wet weight pulp (WWP) in tomato concentrates were found to depend on the centrifugation force, centrifugation time, and the concentration of tomato concentrates. Takada and Nelson (1983) also found that precipitate weight ratio (PPT) defined as the weight of precipitate after centrifugation to total sample weight was dependent on the centrifugation force. It is theoretically possible to compare VP or WWP at any given centrifugation force. However, Takada and Nelson (1983) found that if centrifugal forces were less than 2,240 × *g* the precipitate was loosely packed and sometimes collapsed when the supernatant was removed. In the present study, the centrifugal force of 11,700 × *g* and the centrifugation time of 45 min were selected so that the precipitate was tightly packed with only a very small amount of serum incorporated.

Because the volume concentration of solids is employed in studies on the rheological properties of non-food suspensions (Metzner, 1985), VP and WWP were employed as measures of the amount of pulp that was added. They were not used as a measure of the concentration of insoluble solids in the tomato concentrates because it was very difficult to obtain by centrifugation precipitates at the same degree of packing from tomato concentrates of different concentrations.

Flow properties of serum

At 25 °C, tomato serum samples were nearly Newtonian fluids over the concentration range: 5.6 to 20 °Brix. Magnitudes of their flow behavior index (n) of the power law model (Eq. 1) ranged between 0.91 and 1.0.

$$\tau = K\dot{\gamma} \qquad (1)$$

In Eq. (1), τ is the shear stress (N/m^2), $\dot{\gamma}$ is the shear rate (s^{-1}), n is the flow behavior index (−), and K is the consistency index ($N\ s^n/m^2$). The nearly Newtonian behavior can be attributed to the relatively small magnitudes of pectic substances in the serum samples that ranged from 0.16% in the 5.6 °Brix sample to 0.80% for the 20 °Brix sample. The mild non-Newtonian nature of serum samples has been observed also for samples obtained from apple sauce (Rao, 1986b) and concentrated orange juice (Vitali and Rao, 1984). In the present study, the tomato serum samples will be treated as Newtonian fluids over the studied concentration range; their viscosities at 25 °C ranged from 6.02 × 10^{-3} Pa·s for the 5.6 °Brix sample to 0.016 Pa·s for the 20.0 °Brix sample.

Effect of pulp content

A plot of pulp added (%) versus the apparent viscosity (η_{100}) of tomato concentrates as a function of concentration (°Brix) of serum is shown in Fig. 1. At each concentration level of serum, the apparent viscosity of the concentrates increased with increase in the amount of added pulp. However, when serum concentration was high (e.g., 20 °Brix) added pulp had a more pronounced effect on the apparent viscosity of the concentrates than when the serum concentration was low (e.g., 5.6 °Brix). This can be seen from the difference in the slopes of the lines in Fig. 1.

When apparent viscosities were plotted against total solids content, there were no patterns with respect to the effect of added pulp and the effect of added pulp on apparent viscosity could not be explained in terms of total solids content (Sornsrivichai, 1986). However, it is interesting to note that plots of apparent viscosities against WWP, VP, and PS ratio of all concentrates containing serum of different °Brix fall on single lines as seen in Figures 2, 3, and 4, respectively. The respective regression equations are:

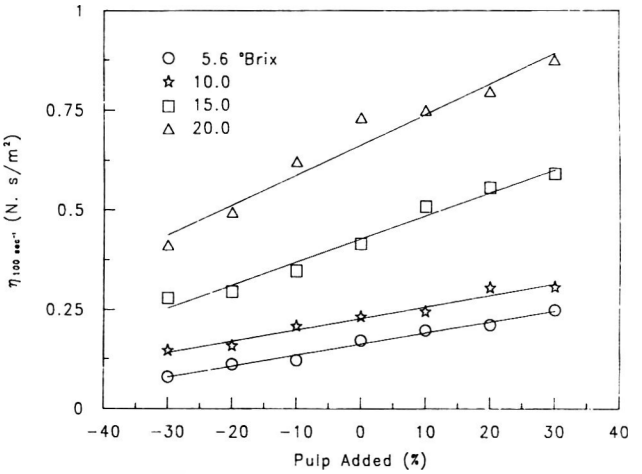

Fig. 1—Apparent viscosities (η_{100}) of tomato concentrates versus pulp content at different serum concentrations.

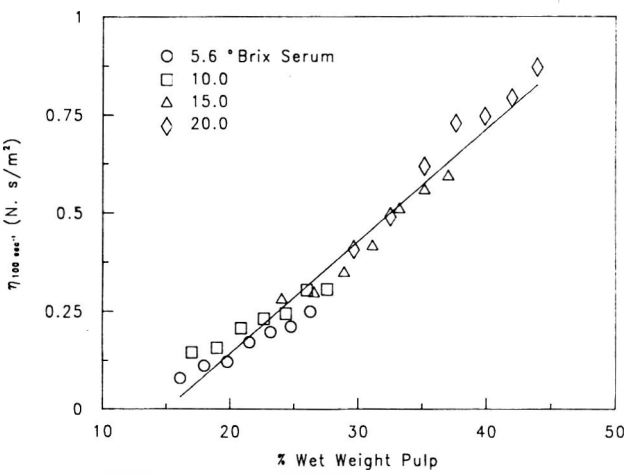

Fig. 2—Apparent viscosities (η_{100}) of tomato concentrates versus % wet weight pulp (WWP).

$$\eta_{100}\ \text{conc} = -0.4299 + 0.0286(\text{WWP});\ R^2 = 0.964 \qquad (2)$$

$$\eta_{100}\ \text{conc} = -0.4022 + 0.0303(\text{VP});\ R^2 = 0.979 \qquad (3)$$

$$\eta_{100}\ \text{conc} = -0.1934 + 1.3923(\text{PS});\ R^2 = 0.966 \qquad (4)$$

Because the magnitudes of R^2 for the three measures of pulp content are nearly equal, each of the three measures can be used to describe quantitatively the effect of pulp content on η_{100} of tomato concentrates in applications such as evaluation of different tomato cultivars.

From Fig. 2, 3, and 4, it is seen that adding pulp to tomato concentrates having low magnitudes of soluble solids (°Brix), produces concentrates that have the same viscosity as those prepared from direct concentration to higher °Brix. A major advantage is that it is not necessary to employ the concentration step and thus avoid the concomitant changes in the quality of the tomato concentrates.

Model for viscosity of tomato concentrates

Even though Eq. (2), (3), and (4) correlate well the apparent viscosity of tomato concentrates with pulp content, they do not indicate explicitly the role of serum viscosity. In addition, the

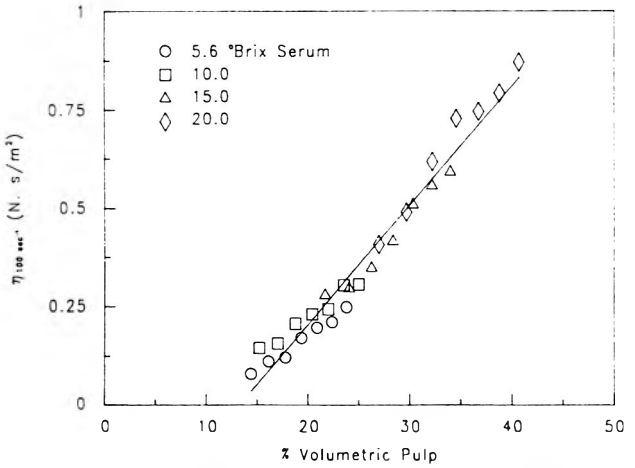

Fig. 3—Apparent viscosities (η_{100}) of tomato concentrates versus % volumetric pulp (VP).

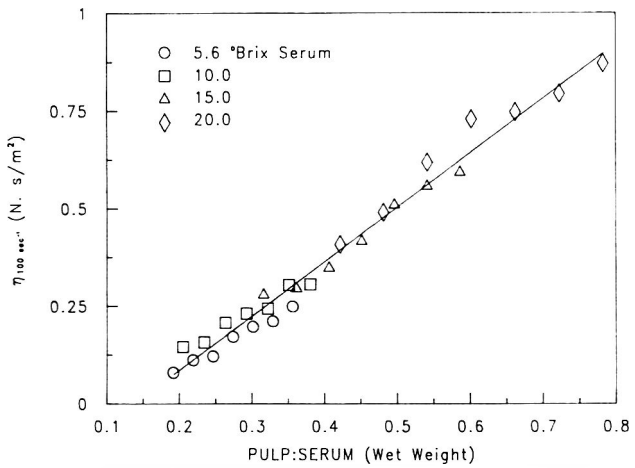

Fig. 4—Apparent viscosities (η_{100}) of tomato concentrates versus pulp:serum weight ratio (PS).

negative intercepts of the equations cannot be explained because they imply negative magnitudes of serum viscosities.

It would be desirable to develop relationships that will allow estimation of the viscosity of tomato concentrates from knowledge of the viscosity of serum and the amount of pulp. Such a relationship must be consistent with the structure of a tomato concentrate being a dispersion of insoluble matter (pulp) in a fluid medium (serum). Based on previous studies on concentrated orange juice (Rao, 1986b) and apple sauce (Rao et al., 1986) a suitable form for the relationship is:

$$\eta_{100}\ \text{conc} = \eta\ \text{serum} + A\ (\text{pulp content})^B \qquad (5)$$

In Eq. (5), pulp content refers to VP, WWP or PS ratio. Equation (5) is simple and it satisfies the requirement that when the pulp content is zero the viscosity obtained must be that of the serum. Further, it allows the calculation of η_{100} of a concentrate from the η of the serum and the amount of pulp content. It also can be used to adjust the magnitude of the apparent viscosity of a concentrate by adjusting either η serum or pulp content so that samples having a specific viscosity can be produced with either low or high pulp content. The coefficient A reflects the contribution to viscosity by a unit amount of pulp and the coefficient B can be used to compare the influence of pulp content on viscosity of concentrates from different cultivars and/or processes.

The magnitudes of the coefficients A and B in Eq. (5) determined by nonlinear regression analysis employing VP, WWP, and PS ratio for the pulp content are in Table 1 along with the magnitudes of R^2. Based on the magnitudes of R^2, the three

Table 1—Coefficients in the relationship η_{100} conc = η_{serum} + A (Pulp content) **B[a]*

	VP	WWP	Pulp:Serum ratio
A	3.430×10^{-4}	1.971×10^{-4}	1.320
B	2.122	2.228	1.485
R^2	0.975	0.974	0.966

[a] η_{100} conc is apparent viscosity (Pa·s of tomato concentrate at 100 sec^{-1}, η_{serum} is viscosity of serum (Pa·s), and A anc B are coefficients. Measures of pulp content employed were: % volumetric pulp (VP), % wet weight pulp (WWP), and pulp:serum weight ratio.

measures of pulp content are capable equally of describing quantitatively the influence of pulp content. The nearly equal magnitudes of the exponent B—2.12 for VP and 2.23 for WWP—indicate that VP and WWP are proportional to each other. The PPT concept has been developed by Takada and Nelson (1983) for modeling consistency of tomato concentrates. It can be shown that PPT and PS ratio are related by: PPT = 1/(PS + 1). Therefore, it appears that PPT can be used also for modeling the apparent viscosity of tomato concentrates.

The viscosity of serum can be expected to depend on the dissolved solids content, viz., sugars and pectins. For this reason, attempts were made to model the viscosity of serum in terms of °Brix and pectin content:

$$\eta\ \text{serum} = A_1 + B_1\ (\text{Brix})^{C1}(\text{Pectin})^{D1} \qquad (6)$$

The magnitudes of A_1, B_1, C_1, and D_1 were determined by nonlinear regression analysis to be 0.00492, 0.0141, 0.0242, and 1.465, respectively. The magnitude of R^2 for the correlation was 0.999. Because °Brix did not play an important role in the modeling for viscosity of serum from apple sauce (Rao et al., 1986), a simpler relationship was tested for the tomato serum samples:

$$\eta\ \text{serum} = A_2 + B_2\ (\text{Pectin})^{C2} \qquad (7)$$

The magnitudes of the coefficients A_2, B_2 and C_2 were found to be 0.00492, 0.0153, and 1.483 respectively, and the R^2 was 0.999. The coefficients A_1 and A_2 represent the viscosity of the aqueous medium containing dissolved organic acids and salts and not surprisingly their magnitudes are equal. Also of interest is that the exponents of pectin content in both the equations (D_1 and C_2) are also nearly equal.

It is emphasized that the forms of Eq. (5) to (7) will be applicable to all tomato concentrates, but the magnitudes of the various coefficients in the equations will depend on factors such as the cultivar and the processing methods employed. The coefficients presented here can be used to make order of magnitude calculations of the apparent viscosity of tomato concentrates. For this purpose, either Eq. (6) or Eq. (7) can be used first for estimating serum viscosity and this can be substituted in Eq. (5) for calculating the apparent viscosity of a tomato concentrate. These calculations can be performed from knowledge of the pectin and the pulp content of a concentrate.

In order to illustrate the contributions of serum and pulp content expressed as PS ratio, Eq. 5 was used to calculate the magnitudes of η_{100} of tomato concentrates over the range of magnitudes of η serum and PS ratio employed in the experiments. From a three dimensional plot of the respective quantities (Fig. 5) it can be seen that the contribution of serum viscosity is small compared to that of the pulp content.

CONCLUSIONS

UNDERSTANDING THE ROLE of soluble solids in serum and that of insoluble solids (pulp) on apparent viscosities of tomato concentrates will be useful in the manufacture of concentrates with desired flow characteristics. In this study, by varying the pulp content between +30% and −30% of the normal amount of pulp and the serum concentration from 5.6

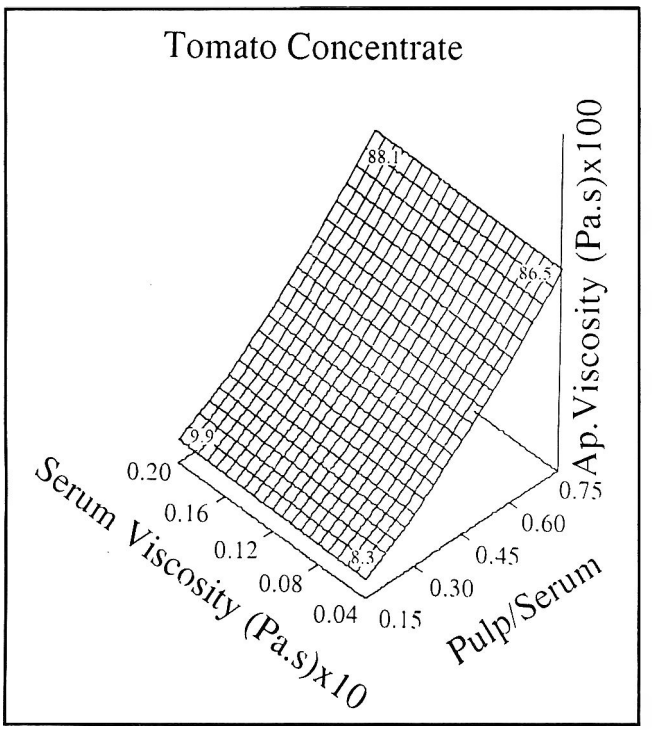

Fig. 5—Three dimensional plot of apparent viscosity (η_{100}) of tomato concentrates as a function of serum viscosity and pulp content expressed as pulp:serum weight ratio.

to 20°Brix, the relative roles of soluble and insoluble solids on rheological properties of tomato concentrates was established. The insoluble solids or the pulp content can be expressed by WWP, VP, or PS ratio. Further, PS and PPT introduced by Takada and Nelson (1983) are related so that PPT can also be used to model apparent viscosities of tomato concentrates.

The successful modeling of the apparent viscosity of tomato concentrates in terms of the serum apparent viscosity and pulp content will be useful in several applications: (1) for producing tomato concentrates of a desired viscosity with the desired amount of pulp, (2) for evaluating the relative contribution of soluble and insoluble solids to flow properties of tomato concentrates, and (3) for comparing and understanding the flow properties of different food suspensions such as apple sauce (Rao et al. 1986) and concentrated orange juice (Vitali and Rao, 1984).

REFERENCES

Davis, R.B., DeWesse, D., and Gould, W.A. 1954. Consistency measurements of tomato puree. Food Technol. 8: 330.

Elder, A.L. and Smith, R.J. 1969. Properties of foods, instruments and people determine the state of food rheology today. Food Technol. 23: 629.

Harper, J.C. and El Sahrigi, A.F. 1965. Viscometric behavior of tomato concentrates. J. Food Sci. 30: 470.

Kintner, P.K., III and Van Buren, J.P. 1982. Carbohydrate interference and its correction in pectin analysis using the m-hydroxydiphenyl method. J. Food Sci. 47(3): 756.

Marsh, G.L., Buhlert, J., and Leonard, S. 1977. Effect of degree of concentration and of heat treatment on consistency of tomato pastes after dilution. J. Food Proc. and Preserv. 1: 340.

Metzner, A.B. 1985. Rheology of suspensions in polymeric liquids. J. Rheology 29: 739.

Mizrahi, S. and Berk, Z. 1970. Flow behavior of concentrated orange juice. J. Text. Stud. 1: 342.

Rao, M.A. 1986a. Flow properties of fluid foods. In "Engineering Properties of Foods," (Ed.) M.A. Rao and S.S.H. Rizvi. Marcel Dekker, Inc., New York.

Rao, M.A. 1986b. Flow properties of plant food suspensions. Paper presented at the 46th Annual Meeting, Institute of Food Technologists, June 15–18, Dallas, TX.

Rao, M.A. and Bourne, M.C. 1977. Analysis of the plastometer and correlation of Bostwick consistometer data. J. Food Sci. 42: 261.

Rao, M.A., Bourne, M.C., and Cooley, H.J. 1981. Flow properties of tomato concentrates. J. Texture Studies 12: 521.

Rao, M.A., Cooley, H.J., Nogueira, J.N., and McLellan, M.R. 1986. Rheology of apple sauce: effect of apple cultivar, firmness, and processing parameters. J. Food Sci. 51: 176.

Shama, F. and Sherman, P. 1973. Identification of stimuli controlling the sensory evaluation of viscosity. II. Oral methods. J. Texture Studies 4: 111.

Shama, F., Parkinson, C., and Sherman, P. 1973. Identification of stimuli controlling the sensory evaluation of viscosity. I. Non-oral methods. J. Texture Studies 4: 102.

Sornsrivichai, T. 1986. A study on rheological properties of tomato concentrates as affected by concentration methods, processing conditions, and pulp content. Ph.D. thesis, Cornell Univ., Ithaca, NY.

Sornsrivichai, T. and Rao, M.A. 1987. Rheological properties of tomato concentrates as affected by particle size and methods of concentration. J. Food Sci. 52: 141.

Takada, N. and Nelson, P.E. 1983. A new consistency method for tomato products: The precipitate weight ratio. J. Food Sci. 48: 1460.

Van Wazer, J.R., Lyons, J.W., Kim, K.Y., and Colwell, R.E. 1963. "Viscosity and Flow Measurement, A Laboratory Handbook of Rheology." Interscience Pub., NY.

Vitali, A.A. and Rao, M.A. 1984. Flow properties of low-pulp concentrated orange juice: serum viscosity and effect of pulp content. J. Food Sci. 49(3): 876.

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Effect of Corn Varieties on Ogi Quality

I. A. ADEYEMI, A. T. OSUNSAMI, and M. A. B. FAKOREDE

ABSTRACT

Six Nigerian corn varieties were assessed for kernel weight and proximate composition. The quality of ogi processed from the corn varieties was evaluated on a Brabender amylograph and an Adams consistometer. While kernel weight of the corn samples varied between 23.1 and 28.5g, the yield of ogi ranged from 51–60%, with FARZ 27 and TZSR-W giving the highest yield. TZE-4 recorded the highest amylograph peak viscosity of 615 B.U. while FARZ 27 was the most stable with a value of 115 B.U. with TZESR-W recording the highest value of 980 B.U. for its index of gelatinization. The range of 6–14 obtained for Adams consistometer value suggested its possible use in ogi quality evaluation.

INTRODUCTION

CORN (*Zea mays L*) accounts for about 13% of cereal harvest and between 80 and 90% of the total cereal consumed in Nigeria and some other tropical countries (Olatunji et al., 1980; Ekpeyong, 1980). Consumption is in various processed forms such as roasting, boiling or fermentation (Banigo and Muller, 1972a). A common form of consumption of corn in Nigeria is as ogi, for which corn is steeped in water for 2–3 days, wet-milled, wet-sieved and soured for at least 12–48 hr (Akinrele, 1970). The final product is a white mash which, when cooked, produces a thin gruel (porridge) which is used as a weaning food for infants and major breakfast cereal for adults (Banigo and Muller, 1972a; Eka, 1978). Ogi is also cooked and turned into a stiff gel, called eko or agidi, to make a meal (Akinrele, 1970; Umoh and Fields, 1981). Although there is some information on the manufacture of ogi from corn, most of these studies did not relate ogi quality with corn variety (Akinrele, 1970; Banigo and Muller, 1972a, b; Adeniji and Potter, 1978; Umoh and Fields, 1981; Akobundu and Hoskins, 1982). Earlier investigations had shown that corn variety affected yield and amylograph pasting viscosity of ogi (Banigo and Adeyemi, 1975; Banigo et al., 1974). However, in the above studies only two varieties, high lysine corn (HLC) and a normal corn were compared. This study, therefore, investigates the yield of ogi from six Nigerian corn varieties and evaluates the pasting viscosity and consistency of the ogi samples.

MATERIALS & METHODS

Corn Samples

Six corn varieties viz:- FARZ 27, FARZ 34, TZSR-W, TZESR-W, TZE-4 and a 'local' variety were used for this study. While the 'local' variety was purchased from a farmer in Abeokuta, the other five varieties were obtained from the National Seed Service Centre, Ibadan, and the Corn Improvement Program of the University of Ife, Ile-Ife, Nigeria. Both TZSR-W and TZESR-W are resistant to the streak virus disease and so can be grown in the late season when the disease is a problem. TZE-4 and TZESR-W are 15-20 days earlier maturing than the other varieties. All of the varieties are white except TZE-4 which is yellow, and are widely grown by farmers in Nigeria. Furthermore, inbred lines for subsequent hybrid production are being developed from these varieties. The yield/hectare is given in Table 1 for all the corn samples.

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Kernel weight

One hundred corn grains were counted and weighed (Hilliard and Daynard, 1974). Determination was carried out in quadruplicate.

Ogi manufacture

Ogi was produced from the corn varieties by the traditional wet-miling process which is still the commercial practice of ogi manufacture in Nigeria. Two kilograms cleaned corn grains were steeped in 4L water for 48 hr, wet-milled in a Premier mill (No. 1A), a plate disc mill, wet-sieved using a 300 μm sieve (B.S. No. 410). The ogi slurry was subjected to further fermentation (souring) by standing at room temperature (27 ± 1°C) for 18 hr while the ogi sedimented. Ogi was recovered as described earlier (Adeyemi, 1983) and dried in a cabinet dryer at 35°C to obtain ogi powder (flour). Material balance was calculated as described by Banigo and Muller (1972a). Determinations were carried out for dry matter content of fermented grains, overtails (bran and germ) and ogi, and total solids in steeping water and souring water (ogi wash water), respectively. Each of these was then expressed as a percentage of the dry matter content of cleaned corn grains to obtain the material balance.

Analyses

pH of steeping and souring water was measured with a pH meter attached to a Spectroplus-D (MSE). Titratable acidity (% lactic acid) was determined as described by Olatunji (1977). Moisture, protein, fat, ash and crude fiber contents were determined using the AOAC methods (AOAC, 1975). Starch was determined by the polarimetric method of Fraser et al. (1956) as modified at the Institute for Cereals, Flour and Bread TNO (Adeyemi, 1980). Analyses were carried out in triplicate for all samples.

Viscosity and consistency measurements

Amylograms of ogi samples were recorded on a Brabender amylograph using slurries at 8% concentration on a dry basis (Adeyemi, 1983). The amylograms were evaluated as described by Banigo et al. (1974). An Adams consistometer was used to compare the consistency of ogi porridge after recording the amylograms at 6% concentration (d.b.). The cooled paste (50°C) was poured into the consistometer cone, the cone was lifted and readings obtained on the plate after 30 s (Szczesniak, 1973).

Statistical analysis

Data were analyzed by analysis of variance and means were separated by Duncan Multiple Range test (Duncan, 1955). Correlation coefficients (r) between Adams consistometer values and amylograph data were also calculated (Hayslett, 1974).

RESULTS & DISCUSSION

Kernel weight and material balance

TZSR-W had the highest value for its kernel weight while FARZ 27 had the least value (Table 1). Hilliard and Daynard (1974) reportd a range of 21–25.8g for the kernel weight of some Canadian corn varieties. The highest loss of materials was in the overtails followed by souring water (ogi wash water) and steeping water (Table 1). Similar losses of materials have been reported in the manufacture of ogi from either corn or sorghum (Banigo and Muller, 1972a; Akingbala et al., 1981; Adeyemi, 1983). The highest loss in overtails is not unex-

Table 1—Effect of corn variety on kernel weight and yield of ogi

Variety	Yield (ton/hectare)	100 Kernel weight (g)	Material balance[a] Corn grain (%)	Steeping water (%)	Steeped grain (%)	Overtails (%)	Souring water (Ogi wash water) (%)	Ogi (uncooked) (%)
FARZ 27	7.7	23.1	100	0.4	97.3	17.2	4.2	60.6
FARZ 34	7.3	25.8	100	0.4	97.6	16.4	4.3	57.4
TZSR-W	6.3	28.5	100	0.5	98.7	20.5	4.5	60.2
TZESR-W	5.0	26.9	100	0.5	98.3	21.2	4.5	51.8
TZE-4	5.8	25.3	100	0.5	97.3	16.5	6.3	55.4
'Local'	3.2	23.5	100	0.4	97.5	24.1	5.9	51.2

[a] Data are expressed as percentage of the original cereal grain, on dry weight basis.

pected since the germ and bran fractions constitute a greater proportion of overtails. Varietal differences affected yield of ogi with FARZ 27 recording the highest yield. However, between 15 and 20% of materials were lost during milling and sieving and could not be recovered either as overtails or ogi (Table 1). Banigo and Adeyemi (1975) had earlier reported a range of 63.7–69.4% and 65.8–74.2% for the yield of ogi obtained from high lysine corn (HLC) and normal corn, respectively, depending on the method of ogi manufacture from the traditional process. A yield of 50% has been reported for an industrial pilot process (Olatunji, 1977). It is possible that the aperture of sieve used in wet-sieving would affect the yield of ogi from the traditional process. Kernel weight had no correlation with yield of ogi from corn, thus confirming the earlier results of Hilliard and Daynard (1974) that kernel weight and starch yield were not correlated.

pH and titratable acidity

Corn variety had no effect on the pH of the steeping and souring water (Table 2). The lower pH values of souring water is attributed to the activities of microorganisms such as *Lactobacillus plantarum* and *Cephalosporium fusarium* which are responsible for the production of carboxylic acids during souring (Akinrele, 1970). Variation in the titratable acidity of ogi samples (Table 2) was not unexpected since the effective microorganisms were not controlled in the natural fermentation and souring stages (Banigo and Muller, 1972b).

Proximate composition of corn, overtails and ogi

The results (Table 3) showed that starch, protein, fat, ash, and crude fiber contents of corn samples were within the range earlier reported for some Nigerian corn varieties (Olatunji, 1977). The overtail fraction, which is often used for livestock feed, was higher in protein, fat and crude fiber contents than either the corn grain or ogi (Table 3). Higher values for protein and fat contents of overtail fractions than in sorghum ogi was also reported by Akingbala et al. (1981). This is attributed to a higher proportion of bran and germ in the overtails. The relative increase in starch content (9–18%) of ogi compared with corn (Table 3) was due to the separation of bran and germ from the endosperm during wet-sieving. Protein contents of ogi showed a loss of between 34 and 47%. Losses in protein content during ogi manufacture could vary from 5–50% (Oke, 1967; Muller, 1980; Akobundu and Hoskins, 1982; Adeyemi, 1983). However, the exact method of ogi manufacture would affect its proximate composition and nutrient losses (Banigo et al., 1974; Muller, 1980).

Table 2—pH and titratable acidity[a] of steeping water and ogi samples

Variety	pH Steeping water	pH Souring water[b]	Titratable acidity (% lactic acid)
FARZ 27	5.08a	3.49a	0.59a
FARZ 34	4.38a	3.49a	0.63a
TZSR-W	4.33a	3.33a	0.59a
TZESR-W	4.49a	3.35a	0.54ab
TZE 4	4.24a	3.63a	0.45b
'Local'	4.45a	3.5 a	0.52ab

[a] Means in a column with same letters are not significantly different (P>0.05).
[b] Ogi wash water.

Table 3—Chemical composition[a,b] of maize, overtails and ogi samples

Variety		Starch %	Protein %	Fat %	Ash %	Crude fiber %
FARZ 27	Grain	66.09a	8.23a	5.01a	1.39a	1.93ab
	Overtails	—	16.71b	6.07a	0.61b	32.58c
	Ogi	78.54b	5.45c	3.79b	0.28c	1.19a
FARZ 34	Grain	67.67a	8.89a	5.70a	1.37a	1.77a
	Overtails	—	16.96b	6.12a	0.63b	27.35d
	Ogi	77.81b	5.19c	4.75b	0.38c	1.19a
TZSR-W	Grain	69.65a	9.82a	4.81a	1.61a	1.65a
	Overtails	—	23.24	5.92a	0.53b	31.36c
	Ogi	76.72b	5.70c	3.73b	0.38c	1.04a
TZESR-W	Grain	69.01a	11.19a	4.27a	1.61a	1.50a
	Overtails	—	18.57b	5.35a	0.58b	25.69d
	Ogi	75.34b	6.11c	3.57b	0.38c	1.04a
TZE 4	Grain	67.03a	10.08a	5.46a	1.27a	2.40b
	Overtails	—	14.83b	5.60a	0.55b	34.98c
	Ogi	79.41b	5.32c	4.34b	0.27c	1.76ab
'Local'	Grain	70.40a	11.25a	6.10a	1.56a	2.05a
	Overtails	—	15.5 b	5.8 a	0.52b	30.05c
	Ogi	78.85b	6.20c	3.32b	0.32c	1.20a

[a] All data are means of three replicates, expressed on dry-weight basis.
[b] Means in a column with same letters are not significantly different (P>0.05).

Brabender amylograph pasting viscosity

Gelatinization temperature, and the time taken to reach it, were similar for all the ogi samples except for the 'local' variety (Table 4). The maximum viscosity (V_m) recorded byTZE-4 was higher than that for other ogi samples while the ease of cooking (M_n-M_g) was lower for the improved varieties than that of the 'local' variety (Table 4). Banigo et al. (1974) had earlier reported 18 and 24 min for the ease of cooking of high lysine and normal corn ogi samples. It would appear, therefore, that both the variety and method of ogi manufacture would affect its ease of cooking. FARZ 27 ogi was the most stable (V_m-V_r) followed by the 'local' corn, TZESR-W, TZSR-W, FARZ 34, and TZE-4 ogi samples, respectively. The index of gelatinization (V_e-V_r) or consistency values (Juliano, 1982) for TZESR-W, FARZ 27, TZSR-W, TZE-4, FARZ 34, and 'local' corn ogi (Table 4) indicated in decreasing order the agidi-making quality of the ogi samples (Banigo et al., 1974).

Adams consistometer value

Ogi paste when prepared in the amylograph at 8% concentration and cooled to 50°C, did not flow on the Adams consistometer plate. Ogi porridge is usually described as a "thin gruel" and therefore, unlike agidi, must exhibit some flow properties. Our observation from this investigation showed that corn ogi porridge prepared at 6% concentration appeared to satisfy this definition. Data and correlation coefficients (r) between Adams consistometer value and some amylograph data are presented in Table 5. From our experience an ogi porridge

Table 4—Brabender amylograph pasting viscosity of ogi made from six varieties of corn[a]

Variety	T_g (°C)	M_g (min)	V_m (B.U.)	M_n (min)	V_r (B.U.)	V_e (B.U.)	M_n-M_g (min)	V_m-V_r (B.U.)	V_e-V_m (B.U.)	V_e-V_r (B.U.)
FARZ 27	78.6	32.4	530	38.6	415	1,260	6.2	115	730	845
FARZ 34	78.6	32.4	565	37.0	350	740	4.6	215	175	390
TZSR-W	78.6	32.4	555	37.4	385	1,085	5.0	170	530	700
TZESR-W	78.3	32.2	530	37.8	365	1,345	5.6	165	815	980
TZE-4	77.1	31.4	615	37.8	375	965	6.4	240	350	590
'Local'	66	24	575	34.4	420	600	10.4	155	25	180

T_g = temperature of gelatinization; M_g = time to reach gelatinization temperature;
V_m = maximum viscosity during heating; M_n = time to obtain V_m;
V_r = viscosity after 15 min at 92°C; V_e = viscosity on cooling to 50°C;
M_n-M_g = ease of cooking; V_m-V_r = stability of the starch;
V_e-V_m = setback value; V_e-V_r = index of gelatinization

[a] Viscosity was expressed in Brabender Units (B.U.)

Table 5—Adams consistometer value and Brabender amylograph pasting viscosity of ogi from six corn varieties[a]

Sample	Adams consistometer value	Peak viscosity (B.U.)	Viscosity at 50°C (B.U.)	Stability (B.U.)	Setback value (B.U.)	Index of gelatinization (B.U.)
FARZ 27	14	165	390	10	125	135
FARZ 34	7	180	180	40	0	40
TZSR-W	12	185	360	5	175	180
TZESR-W	12	165	320	15	155	170
TZE-4	11	220	260	50	40	90
'Local'	6	200	220	30	20	50
Correlation coefficient (r)	—	−0.39	0.92	−0.57	0.81	0.84

[a] Viscosity was expressed in Brabender Units (B.U.)

must not be so "thin" as to record Adams consistometer value of < 10 as obtained for 'local' corn and FARZ 34 ogi samples (Table 5). A "thin" ogi porridge of this type is usually prepared for babies, the aged and the sick while a slightly "thick" ogi porridge with consistometer values ≥10 would be preferred by normal adults. Adams consistometer value was highly correlated with viscosity at 50°C, setback value and index of gelatinization (Table 5).

In conclusion, the data obtained appear to show that the quality of corn ogi porridge could be evaluated on the amylograph at 6% dry weight basis. Previous workers have assessed quality of corn or sorghum ogi on the amylograph at between 8 and 12% flour concentration (Banigo et al., 1974; Akingbala et al., 1981; Adeyemi, 1983). However, for agidi manufacture the test would have to be conducted at a concentration much higher than 6%. Further work would, therefore, aim at correlating Adams consistometer values with data that might be obtained from other objective instruments such as Gel tester, Gelometer and the Instron Universal Testing machine which could be suitable for evaluating quality of agidi or closely related products such as tô (Da et al., 1981; Cagampang et al., 1982, 1984).

REFERENCES

Adeniji, A.O. and Potter, N.N. 1978. Properties of ogi powders made from normal, fortified and opaque-2 corn. J. Food Sci. 43: 1571.

Adeyemi, I.A. 1980. Training in Cereal Technology. Report No.: 8-792. Institute for Cereals, Flour and Bread TNO. Wageningen, The Netherlands.

Adeyemi, I.A. 1983. Dry-milling of sorghum for ogi manufacture. J. Cereal Sci. 1: 221.

Akingbala, J.O., Rooney, L.W. and Faubion, J.M. 1981. Physical, chemical and sensory evaluation of ogi from sorghum of differing kernel characteristics. J. Food Sci. 46: 1532.

Akinrele, I.A. 1970. Fermentation studies on maize during the preparation of a traditional African starch-cake food. J. Sci. Food Agric. 21: 619

Akobundu, E.N.T. and Hoskins, F.H. 1982. Protein losses in traditional agidi paste production. J. Food Sci. 47: 1728.

AOAC. 1975. "Methods of Analysis." Association of Official Analytical Chemists, Washington, DC.

Banigo, E.O.I. and Adeyemi, I.A. 1975. A comparative study of the commercial practice of traditional ogi manufacture using High-Lysine (Opaque-2) corn (HLC) and normal corn. Proceedings of the 10th International Congree of Nutrition, Victory-Sha Press, Kyoto, Japan, 402.

Banigo, E.O.I., DeMan, J.M. and Duitschaever, C.L. 1974. Utilization of high-lysine corn for the manufacture of ogi using a new, improved processing system. Cereal Chem 51: 559

Banigo, E.O.I. and Muller, H.G. 1972a. Manufacture of ogi (a Nigerian fermented cereal porridge): Comparative evaluation of corn, sorghum and millet. Can. Inst. Food Sci. Technol. J. 5: 217.

Banigo, E.O.I. and Muller, H.G. (1972b). Carboxylic acid patterns in ogi fermentation. J. Sci. Food Agric. 23: 101.

Cagampang, G.B., Griffith, J.E., and Kirleis, A.W. 1982. Note on the modified adhesion test for measuring stickiness of sorghum porridges. Cereal Chem. 59: 234.

Cagampang, G.B., Kirleis, A.W., and Marks, J.S. 1984. Application of small sample back extrusion test for measuring texture of cooked sorghum grain. J. Food Sci. 49: 278.

Da, S., Akingbala, J.O., Rooney, L.W., Scheuring, J.F., and Miller, F.R. 1981. Evaluation of tô quality in a sorghum breeding program. Proceedings of the International Symposium on sorghum grain quality. ICRISAT Centre, Patancheru, India, 11.

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

Eka, O.U. 1978. Chemical evaluation of nutritive value of soya paps and porridges, the Nigerian weaning foods. Food Chem. 3: 199.

Ekpeyong, T.E. 1980. Biological evaluation of improved local maize hybrids using rats. Food Chem. 6: 133.

Fraser, J.R., Brandon-Bravo, M., and Holmes, D.C. 1956. The proximate analysis of wheat flour carbohydrates. I. - Methods and scheme of analysis. J. Sci. Food Agric. 7: 577.

Hayslett, H.T. 1974. "Statistics Made Simple." W.H. Allen, London.

Hilliard, J.H. and Daynard, T.T. 1974. Starch content, test weight and other quality parameters of corn produced in different areas of Ontario. Crop Sci. 14: 546.

Juliano, B.O. 1982. An international survey method used for evaluation of the cooking and eating qualities of milled rice. IRRI Research paper series No. 77, Manila, Phillipines.

Muller, H.G. 1980. Fermented cereal products of Tropical Africa. Proceedings of the 6th International Symposium on Fermentation, Ontario, Canada, 541.

Oke, O.L. 1967. Chemical studies on the Nigerian foodstuff. Food Technol. 21: 202.

Olatunji, O. 1977. Production and utilization of local dry corn milled products in Nigeria. Nutr. Rep. Int'l. 16: 595.

Olatunji, O., Edwards, C., and Koleoso, O.A. 1980. Processing of maize and sorghum in Nigeria for human consumption. J. Food Technol. 15: 85.

Szczesniak, A.S. 1973. Instrumental methods of texture measurements. In "Texture Measurements of Foods." A. Kramer and A.S. Szczesniak (Ed.), p. 71. D. Reidel Publishing Co., Dordrecht-Holland.

Umoh, V. and Fields, M.J. 1981. Fermentation of corn for Nigerian agidi. J. Food Sci. 46: 903.

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In Vitro Digestibility of Phytate-Reduced and Phenolics-Reduced Soy Protein Isolates

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ABSTRACT

The effect of removal of phytate and phenolic compounds upon the *in vitro* digestibility of soy protein isolates by trypsin and pronase enzymes at pH 8.0 and by pepsin at pH 2.0 was studied by pH stat and dialysis equilibrium methods, respectively. Phenolics-reduced (PRSPI) and phytate and phenolics-reduced (PPRSPI) soy protein isolates were both slightly more digestible than control (CSPI). 11S soy-protein with 0.07% phytate was slightly more digestible than 7S soy protein with 1.41% phytate. Kinetic studies indicated that differences in *in vitro* digestibility of soy protein isolates was probably due to accumulation of end products rather than stearic hindrance at enzyme-substrate reaction sites.

INTRODUCTION

LARGE AMOUNTS of soy protein isolates and concentrates are used as functional and nutritional ingredients by the food industry (Wolf and Cowan, 1975). A high degree of digestibility is desirable in providing a protein with acceptable nutritional bioavailability. Enzymatic modification of soy proteins is also useful for improving their solubility and functionality for certain food applications (Sekul and Ory, 1977).

Numerous procedures have been proposed for determining *in vitro* digestibility of food proteins (Ritter, 1985). Enzymes used include pepsin, pancreatin, papain, trypsin, chymotrypsin and peptidase. Methodologies include the multi-enzyme method of Hsu et al. (1977), diafiltration (Knuckles et al., 1985) and the pH stat method (Rothenbuhler and Kinsella, 1985; Stinson and Snyder, 1980).

Soy protein isolates commonly contain 1.5–2.0% phytate (Brooks and Morr, 1982) and 1–1.5 mg phenolics/g (Seo and Morr, 1984) that are capable of inhibiting protein digestibility (Kratzer, 1965; Sharma et al., 1978; Singh and Krikorian, 1982; Abdul-Kadir, 1980). Processes have been developed for removing major portions of phytate (Brooks and Morr, 1982) and phenolic compounds (How and Morr, 1982) from soy protein isolates.

This study was conducted to determine (1) the important qualitative and mechanistic effects of phytate and phenolic compound removal upon the *in vitro* digestibility of soy protein isolates, and (2) the *in vitro* digestibility of 7S and 11S soy protein fractions which contain significantly different phytate concentrations.

MATERIALS & METHODS

Materials

Commercially defatted soy flakes were obtained from Ralston Purina Company (St. Louis, MO). Ion exchanger media were Rexyn 101 and 202 from Fisher Scientific. Type CPG 12 × 40 activated carbon pellets were from Calgon Corporation (Pittsburgh, PA). Enzymes were T-0134 crystallized porcine pancreas trypsin (10,000–13,000 units/mg protein); P-5147 pronase E nonspecific protease from *Streptomyces griseus* (4 units/mg); and P-7000 pepsin (1200–2000 units/mg protein) from Sigma Chemical Company (St. Louis, MO). Bovine serum albumin #7638 was obtained from Sigma Chemical Company. Dialysis tubing was Spectrapore 12,000 dalton MW cutoff from Fisher Scientific.

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Soy protein isolates

Soy protein extract was prepared as by How and Morr (1982) and Brooks and Morr (1982). Defatted soy flakes were sequentially extracted with 10 and 5 volumes of dilute NaOH at pH 8–9. The extracts were clarified by centrifuging at 1,000 × *g*, combined and adjusted to pH 4.5 with 1N HCl. The precipitated protein was recovered by centrifuging at 1,000 × *g*, redispersed in distilled water, and adjusted to pH 8.0 with 1N NaOH or to pH 2.0 with 1N HCl as control soy protein isolate (CSPI).

Phytate and phenolic acids were simultaneously removed from an aliquot of the above extract using the sequential ion exchange process of Brooks and Morr (1982). This method consistently removed ≥95% of the phytate and ≥90% of the phenolic acids from soy protein extracts (Seo and Morr, 1984). The ion exchanged extract was then adjusted to pH 5.0 with 1N HCl and centrifuged at 1,000 × *g* to recover the precipitated protein (Rodriguez et al., 1985). The recovered protein was redispersed in distilled water and adjusted to pH 8.0 or 2.0 as above for phytate-reduced and phenolics-reduced soy protein isolate (PPRSPI).

Free phenolic compounds were removed from a third aliquot of the above soy extract by the activated carbon process of How and Morr (1982). The soy extract was passed through a 200 mL bed of activated carbon. The treated extract was adjusted to pH 4.5 with 1N HCl and the precipitated protein was recovered by centrifuging at 1,000 × g as above. Recovered protein was dispersed in distilled water and made to pH 8.0 or 2.0 as above for phenolics-reduced soy protein isolate (PRSPI).

7S and 11S soy protein fractions were prepared by the method of Brooks and Morr (1984), which was a modification of Thanh and Shibasaki (1976). These proteins were adjusted to pH 8.0 in distilled water with 1N NaOH.

Protein digestibility

In vitro protein digestibility was determined by the pH stat procedure using a REC 80 Servograph, ABU Autoburette, TTT 80 Titrator and PHM 84 Research pH Meter from Radiometer Copenhagen (Copenhagen, DK). Instrumental parameters used were: temperature, 24°C; pH, 8.000; proportional band, 0.2 pH units; autoburette pump speed, 5 on a scale of 5 to 120; and pen speed, 70 min/full scale. The glass and calomel electrodes were calibrated with pH 4.0 and 7.0 buffer according to instructions from the manufacturer. The endpoint was adjusted to pH 8.000 and the autoburette was filled with 0.01N NaOH titrant. Protein dispersions were diluted to contain the required amount of protein by the Biuret assay (Gornall et al., 1949) using bovine serum albumin as reference. Standardized protein dispersions were added to the titration assembly and equilibrated to pH 8.0 with the autoburette. After about 15 min the autoburette was refilled and the prescribed amount of enzyme was added to the protein dispersion in the titration assembly. The protein and enzyme dispersions were preadjusted to 24°C prior to starting the experiment. The volume of titrant was automatically recorded as a function of reaction time. The reaction assembly was not flushed with nitrogen gas prior to starting the experiment (Rothenbuhler and Kinsella, 1985; Hill et al., 1982), since it was found that this treatment did not alter the results of the assay.

The second method for determining protein digestibility was the dialysis equilibrium *in vitro* method of Knuckles et al. (1985) with

slight modification. Soy protein isolates were dispersed and standardized to contain 190 mg/mL protein in 20 mL dilute HCl at pH 2.0 by the Biuret assay (Gornall et al., 1949). The protein dispersions were then transferred into separate dialysis tubing bags containing several glass beads. Two milliliters of enzyme dispersion containing 125 mg/mL pepsin in distilled water were added to provide about 11.5 mg/mL pepsin in the final dispersion. The bags were immediately tied off and inverted several times to provide mixing and placed in separate 250 mL plastic bottles containing 200 mL dilute HCl solution at pH 2.0. Each bottle was capped and mounted on a rotating mixer device operated at about 17–18 rev/min at 20–24°C. Dialysates were sampled from each bottle after various time periods up to 24 hr and analyzed for peptide and amino acid concentrations by the Lowry method (Lowry et al., 1951) using bovine serum albumin as reference.

Kinetic studies

The velocity of the hydrolysis reaction was studied using the pH stat method with a range of substrate concentrations from 6 to 150 μ/mL soy protein in distilled water at pH 8.0. The other experimental parameters for this part of the study were: enzyme concentration, 175 μg/mL pronase; titrant concentration, 0.5 mM for S ≤ 75 μg/mL and 2.0 mM for S ≥ 90 μg/mL; pen speed, 2.8 min/full scale; autoburette speed, 120 on a scale of 5 to 120; proportional band setting, 0.2 pH units; and temperature, 24°C. Initial velocity (V_i) was determined as the initial slope of the autoburette titration curves and expressed as:

$$V_i = \frac{\mu \text{ equiv. peptide bonds hydrolyzed}}{\text{time (min)}}$$

Amino acid analysis

The amino acid composition of soy protein isolates was determined by the method of Moore and Stein (1963) after complete hydrolysis in 6N HCl. Tryptophan was determined by basic hydrolysis (Hugli and Moore, 1972). Cystine and methionine were determined by the performic acid oxidation method of Moore (1963). Free amino acids released by enzyme digestion of soy protein were determined after precipitating residual, intact proteins with 3.6% sulfosalicylic acid and filtering. The concentration of amino acids was determined with a Dionex D-300 Amino Acid Analyzer (Sunnydale, CA) using ninhydrin as indicator.

RESULTS & DISCUSSION

pH stat studies

A variety of experimental conditions were investigated to determine optimum conditions for conducting the pH stat assay in this study. Experiments conducted using redispersed, freeze-dried soy protein isolates produced inconsistent results, presumably due to incomplete protein solubility. Attempts to overcome this problem by preheating the isolate dispersions and by addition of alkali, urea and sodium dodecyl sulfate were unsuccessful. Thus, all further work was done with either freshly prepared soy protein isolate or thawed, frozen protein dispersions that had not been subjected to drying.

Experiments were conducted with a range of enzyme concentrations to determine the minimum enzyme concentration needed to provide maximum protein hydrolysis for soy protein dispersions containing 2.63 mg protein/mL. Limiting pronase and trypsin concentrations were found to be about 350 ± 0.007–0.022 and 530 ± 0.032–0.132 μg/mL, respectively. The higher digestibility value for pronase than for trypsin is predictable since the former is a non-specific protease (Anonymous, 1986), whereas trypsin is restricted to hydrolysis of peptide bonds in which the carbonyl function is donated by either lysine or arginine (Lehninger, 1970). Additional experiments with heated soy protein dispersions demonstrated that these latter differences in enzyme activity were not due to residual trypsin inhibitor.

As indicated in Fig. 1, both PRSPI and PPRSPI gave greater soy protein hydrolysis than for CSPI. These relationships were consistently demonstrated throughout the study. In addition, the slightly greater hydrolysis of PRSPI compared to PPRSPI was also consistently observed in all experiments.

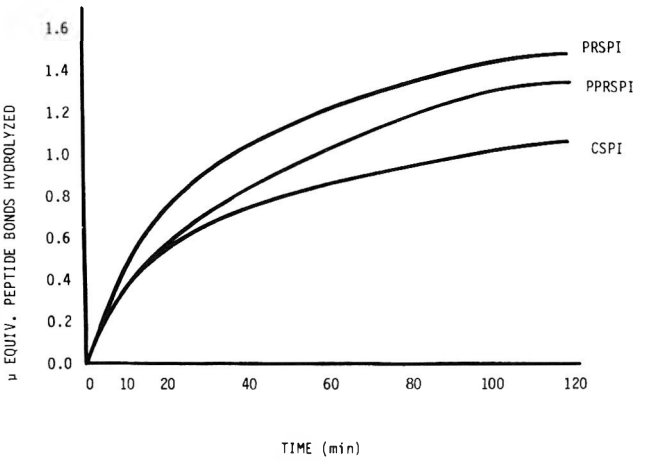

Fig. 1—Typical in vitro *digestibility curves for soy protein isolate by the pH stat method using 350 μg pronase/mL protein dispersion at pH 8.0. PRSPI-phenolics-reduced soy protein isolate; PPRSPI-Phytate-Phenolics-reduced soy protein isolate; and CSPI-Control soy protein isolate.*

Table 1—Percentage of amino acids released by in vitro digestion[a]

Soy protein isolates	Total amino acid content (mg/100 mg protein)	Percent amino acids released
Control soy protein isolate (CSPI)	104.8	25.6
Phenolics-reduced soy protein isolate (PRSPI)	121.5	26.3
Phytate-Phenolics-reduced soy protein isolate (PPRSPI)	120.6	30.0

[a] 530 μg/mL pronase and 70 min reaction period from a single determination

In vitro digestibility values for 7S and 11S soy protein fractions indicated that 11S soy protein was slightly more digestible than 7S soy protein. Although there may be other factors involved in producing these latter differences, it is interesting to note that the greater protein digestibility of the 11S soy protein may also be due in part to its lower phytate content of 0.07% (Brooks and Morr, 1984) compared to a value of 1.41% for 7S soy protein. Rothenbuhler and Kinsella (1985) previously reported that 11S soy protein was more digestible than 7S soy protein when assayed by the pH stat method using pancreatin as the enzyme.

The percentage of total amino acids released during the in vitro digestibility determination of different soy protein isolates was determined as further indication of the availability of their respective peptide bonds to the enzyme pronase (Table 1). The results confirm that PRSPI and PPRSPI are both slightly more digestible than CSPI. However, these results did not support the above indication that PRSPI is more digestible than PPRSPI.

Dialysis equilibrium studies

Results for soy protein hydrolysis by pepsin which is restricted to hydrolysis of peptide bonds in which the amino function is phenylalanine, tryptophan, tyrosine, leucine, aspartic acid and glutamic acid (Lehninger, 1970) at pH 2 over a 24 hr period are given in Fig. 2. These findings were generally similar to those obtained with the pH stat method at pH 8.0 in that PRSPI and PPRSPI were both slightly more digestible than was CSPI. However, the differences in digestibility were smaller than by the pH stat method at pH 8.0.

Kinetic studies

The above results confirm those of others that phytate and phenolic compounds tend to lower the digestibility of proteins (Camus and LaPorte, 1976; Sharma et al., 1978; Lahiry et al., 1977; Hurrel et al., 1982). It has been speculated that this inhibitory action may be due to binding of phytate and phenolic

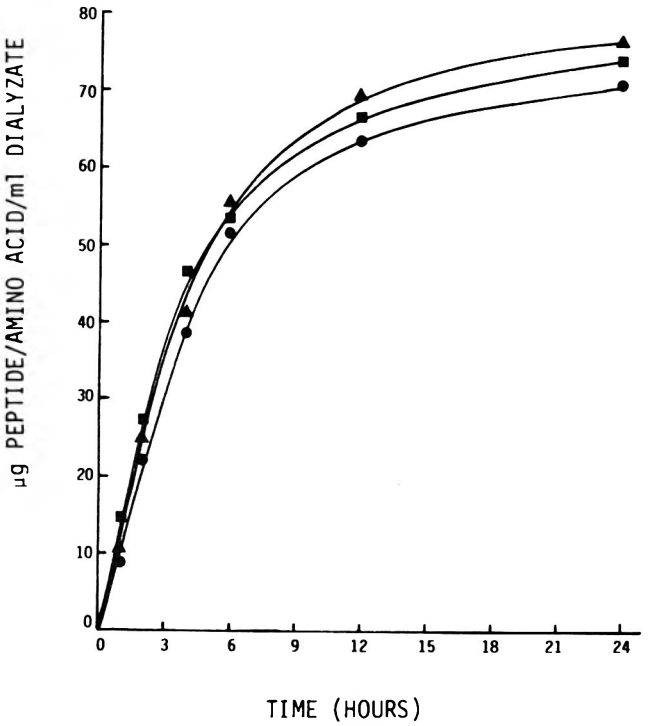

Fig. 2—Dialysis equilibrium in vitro digestibility of soy protein isolates with 11.4 mg pepsin/mL of pH 2.0 protein dispersion: ● Control soy protein isolate; ▲ Phenolics-reduced soy protein isolate; and ■ Phytate-Phenolics-reduced soy protein isolate.

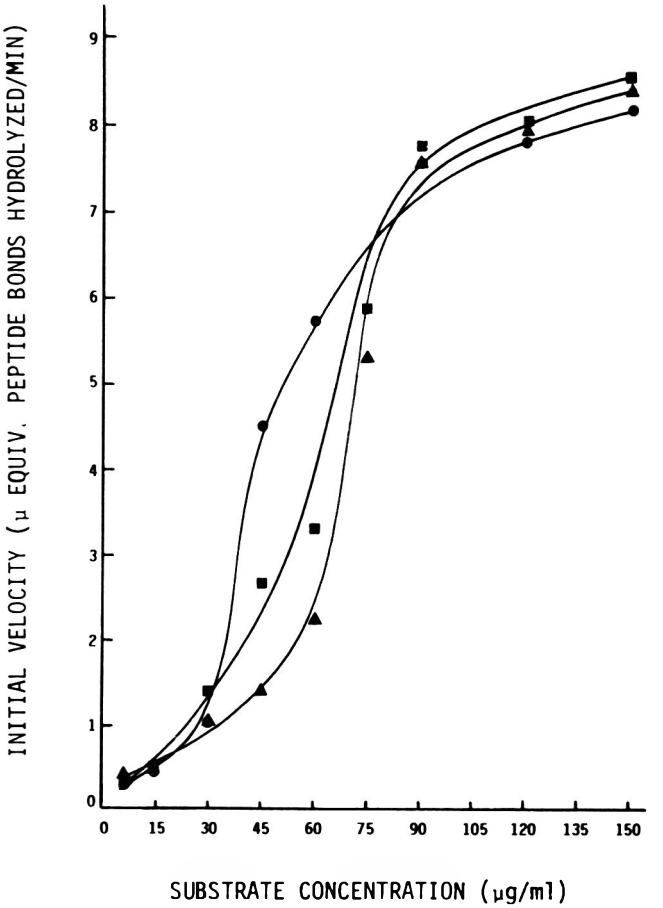

Fig. 3—pH stat in vitro velocity of soy protein hydrolysis by 175 µg pronase/mL of pH 8.0 protein dispersion as a function of substrate concentration: ● Control soy protein isolate; ▲ Phenolics-reduced soy protein isolate; and ■ phytate-phenolics-reduced soy protein isolate.

compounds at or near the substrate-enzyme reaction sites. Such interactions might lead to stearic and charge hindrances capable of lowering the rate and extent of enzyme hydrolysis of the protein. However, results from this study (Fig. 3) indicate the opposite effect, e.g., the presence of phytate and phenolic compounds appeared to activate the enzyme hydrolysis reaction for soy protein isolate. Thus, mechanisms other than stearic hindrance at the active sites are probably responsible for inhibiting soy protein hydrolysis by pronase.

A sigmoidal curve for initial velocity versus substrate concentration in Fig. 3 is in contrast to results of Rothenbuhler and Kinsella (1985) and is indicative of allostery. These latter workers demonstrated that the plot of initial enzyme reaction velocity by pancreatin versus protein concentration for 11S soy protein followed typical Michaelis-Menten kinetics. However, their lowest substrate concentration was approximately 0.25 mg/ml, which was near saturation conditions. As shown in Fig. 3, the use of lower substrate concentrations in these studies provides much more dependable kinetic data.

The allosteric kinetics obtained in this study with pronase at pH 8.0 were somewhat unexpected, since this enzyme is a monomeric enzyme that does not normally exhibit this effect (Palmer, 1981). However, monomeric enzymes may also be subject to allosteric regulation (Monod et al., 1965). Such monomeric enzymes should contain binding sites for substrate, activators and inhibitors. Since phytate and phenolic compounds appear to slightly inhibit proteolysis in the overall reaction, but exert no apparent effect upon the initial phase of the reaction during the first 10–15 min (Fig. 1), it appears that product inhibition may be the controlling factor. Intermediate and final hydrolytic products, especially those that contain phenolic compounds and phytate, may function as competitive inhibitors (Frieden and Walter, 1963). Since the enzyme reaction demonstrates allostery it is likely that noncompetitive product inhibition may be the mechanism for the greater inhibitory effect with CSPI than for PRSPI and PPRSPI. Further kinetic studies are needed to investigate the possible inhibitory effect of hydrolytic end-products upon the soy protein hydrolysis reaction.

It might be expected that the inhibitory effects of end-products upon the soy protein hydrolysis reaction evidenced during *in vitro* studies may be less dramatic when determined by *in vivo* methods. This conclusion is based upon the fact that such end-products would be dissipated from the reaction site by normal transport and absorption processes. In this respect, the dialysis equilibrium *in vitro* digestibility procedure, which allows for dissipation of hydrolytic products from the reaction site, would be expected to provide digestibility results that would compare more closely with those obtained by *in vivo* methods.

Results generally agreed with those of previous workers in that endogenous phytate exerts a small, but consistent inhbiitory effect upon *in vitro* protein digestibility (Abdul-Kadir, 1980; Serraino et al., 1985). Removal of free acidic and neutral phenolic compounds by the activated carbon treatment (PRSPI) was more effective than removal of phenolic acids and phytate by the ion exchange process (PPRSPI) for improving in vitro soy protein digestibility. However, PPRSPI was more completely hydrolyzed than PRSPI on the basis of percent amino acids released by the action of pronase. The pH stat and dialysis equilibrium digestibility methods produced generally similar results for soy protein digestibility, however, the magnitude of these differences was generally smaller by the dialysis equilibrium method than by the pH stat method. Kinetic studies with pronase by the pH stat method indicated allostery and that the binding of phytate and phenolic compounds at the active site is not responsible for the inhibitory effects observed.

—Continued on page 341

c4-Heptenal: An Influential Volatile Compound in Boiled Potato Flavor

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ABSTRACT

Analysis of headspace volatile concentrates from freshly boiled Russet Burbank potatoes revealed a variety of lipid oxidation products including *t*2, *c*6-nonadienal which is further degraded to *c*4-heptenal through a water-mediated retro-aldol condensation reaction. Dilute aqueous solutions of *c*4-heptenal exhibited boiled potato-like aromas, and at relatively high concentrations (greater than 0.7 ppb) added *c*4-heptenal contributed distinct staling-type flavor defects to both fresh, mashed potatoes and reconstituted dehydrated potatoes. When added at levels between 0.1-0.4 ppb, *c*4-heptenal enhanced overall earthy, potato-like flavors in freshly boiled mashed potatoes, but these levels caused stale flavors in reconstituted dehydrated potatoes.

INTRODUCTION

COOKED POTATO FLAVORS vary according to the means of preparation, and in addition to volatile components (Self, 1967; Buttery et al., 1970, 1973; Pareles and Chang, 1974; Sapers, 1975; Coleman and Ho, 1980; Coleman et al., 1981) both amino acids and 5′-ribonucleotides contribute to overall potato flavors (Solms, 1971; Solms and Wyler, 1979). The flavor of baked potatoes contains distinct notes contributed by certain methoxy-alkylpyrazines, methional, and *t*2, *t*4-decadienal which combine along with other aroma compounds to provide an earthy, fresh cooked potato flavor (Buttery et al., 1973; Pareles and Chang, 1974; Coleman and Ho, 1980). On the other hand, boiled potato flavors, which were the subject of several early flavor chemistry investigations, have long been considered to depend on characterizing aroma contributions from methyl mercaptan (Gumbmann and Burr, 1964; Self, 1967) and methional (Burr, 1966) formed through methionine degradations. Methional possesses an aroma quality in modest dilutions which is often described as boiled potato-like. However, during recent handling of *c*4-heptenal in our laboratory, it was noted that low concentrations of this aldehyde also exhibited aroma qualities resembling cooked potatoes, particularly the aroma of cold, boiled potatoes.

The literature does not appear to contain information on the occurrence of *c*4-heptenal in potato flavors. However, we recently reported on the formation of *c*4-heptenal from *t*2, *c*6-nonadienal via a double-bond hydration and retro-aldol condensation mechanism (Josephson and Lindsay, 1987a), and this pathway appears suitable for the production of *c*4-heptenal in potato flavors. Although *t*2, *c*6-nonadienal has not been reported in potato flavor concentrates, it has been found to be the most abundant carbonyl formed through purified potato lipoxygenase-mediated oxidation of linolenic acid (Grosch et al., 1976; Galliard and Phillips, 1971). Since potato lipids contain notable amounts of linolenic acid (Galliard, 1973), formation of *t*2, *c*6-nonadienal in potatoes via lipoxygenase-initiated reactions can be anticipated as well as subsequent formation of *c*4-heptenal through retro-aldol-related mechanisms.

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Therefore, the purpose of this communication is to report the identification of *t*2, *c*6-nonadienal and *c*4-heptenal in boiled potatoes, and to relate observations on the formation and flavor properties of these compounds in potatoes.

MATERIALS & METHODS

Sample preparation

Russet Burbank potatoes were purchased from a local retail market. Samples were prepared by first placing 2 kg washed, whole potatoes with intact skins into 3L boiling water for 30 min. Following boiling, the potatoes were coarsely diced (3–4 cm cubes), and cooled at room temperature to 60°C.

Collection and analysis of boiled potato volatiles

Partially cooled potatoes (1.5 kg) were then added to a 12L flask fitted with a nitrogen purging tube. Just prior to initiating headspace purging, ethyl heptanoate (15 μg in 100 μL ether; internal standard) was added to the inside wall of the flask, and this served to simulate actual concentrations of each volatile in the headspace over the potatoes. Headspace volatiles were collected and concentrated by passing a stream of purified nitrogen (80 mL/min for 15 hr; 21°C) over the samples. The nitrogen stream was first bubbled through water in a separate chamber, and then over the sample to minimize evaporative water losses. Headspace volatiles from the sample were adsorbed onto Tenax GC (60-80 mesh, ENKA N.V., Holland) as described by Olafsdottir et al. (1985). Volatiles were subsequently eluted from the Tenax GC traps with 1 mL redistilled ethyl ether (Fisher Scientific, Fairlawn, NJ), and were concentrated under a slow stream of nitrogen at room temperature (21°C) to approximately 10 μL.

Concentrates of volatile compounds in ethyl ether were then analyzed using a Varian 1740 gas chromatograph equipped with an effluent splitter for simultaneous FID measurement and odor assessment of individual peaks. Separations involving odor evaluations were achieved using a 3 m × 2 mm i.d. glass column packed with 7% Carbowax 20M on Chromosorb W AW/DMCS with a temperature programming rate from 50°C to 220°C at 4°C/min.

Additionally, volatile compounds were analyzed by capillary column gas chromatography in conjunction with mass spectrometry using a Carbowax 20 M (60 m × 0.25 mm i.d.) fused silica capillary column (J & W Scientific, Inc., Rancho Cordova, CA) operated with helium carrier gas. A program rate of 50°C (5 min) to 140°C at 6°C/min followed by a rate of 10°C/min from 140°C to 220°C was employed. Identification of compounds was based on computer matching of mass spectra of unknown compounds with those published in "EPA/NIH Mass Spectral Data Base" (Heller and Milne; 1975, 1980) as well as coincidence of mass spectral patterns from isolated compounds with those of authentic compounds. Coincidence of retention indices (I_E, Van den Dool and Kratz, 1963) with authentic compounds was also employed.

Sensory evaluation of mashed potatoes

A range of concentrations (0.05–2.0 ppb) of *t*2, *c*6-nonadienal and *c*4-heptenal each were added to both fresh mashed potatoes (Russet Burbank) and reconstituted, nitrogen-flushed dehydrated potatoes (Kraft Inc., Glenview, IL) for preliminary assessment of flavor contributions by the authors. Since discernible concentrations of *t*2, *c*6-nonadienal provided an atypical green flavor note to mashed potatoes, only the flavor effects of *c*4-heptenal in mashed potatoes were further tested in formal sensory evaluation trials.

For panel analysis, freshly-boiled, peeled potatoes were prepared along with reconstituted dehydrated potatoes as mashed samples. After

lightly salting each, *c*4-heptenal was added to potatoes from aqueous dilutions to obtain a range of concentrations from 0.01–0.6 ppb in the potato samples. A group of 31 panelists experienced in sensory analysis of foods evaluated the mashed potato samples that were portioned (ca 25g each; 60°C) into individual coded (3-digit random numbers) pleated paper cups. Panelists were seated in individual booths equipped with running water and standard indoor fluorescent lighting (ca 78 ft-c).

Panelists were instructed to first smell samples, then taste, and finally record observations for each attribute on scales typical of descriptive sensory analysis (Stone et al., 1974, 1980). The semi-structured linear scales on ballots were later coded on a 7-point basis. Attributes included overall potato flavor intensity (very weak to very strong), cream-like flavor intensity (absent to very pronounced), freshness (stale, lacks freshness to extremely fresh), and overall preference (dislike extremely to like extremely). Sensory data were subjected to analysis of variance and least significant difference statistical analysis (Steel and Torrie, 1960).

RESULTS & DISCUSSION

A TYPICAL CARBOWAX 20M fused silica capillary column separation of headspace volatiles from freshly boiled Russet Burbank potatoes which exhibited a distinct *c*4-heptenal aroma note during and after boiling is shown in Fig. 1. Identifications and approximate concentration of compounds comprising each numbered peak are recorded in Table 1. *c*4-Heptenal was present at 0.2 ppb which is a concentration substantially above its recognition threshold of 0.04 ppb (McGill et al., 1974). Most of the compounds identified in the boiled potatoes have been observed in other investigations, and these have been summarized by Sapers (1975). 2-Alkenals and alkanals from lipids were the most abundant headspace volatiles identified in freshly boiled potatoes, but evidence for aldehydes formed through Strecker-type degradations was obtained by the identification of phenylacetaldehyde (peak 30). However, neither odor assessment from packed Carbowax 20M column effluent streams nor mass spectral data for methional (I_E region = 8.25-8.30) were obtained in the headspace profiles of the boiled potatoes. Methoxy-alkylpyrazines were not detected by mass spectral analysis, but regions (I_E = 8.0-9.0; Carbowax 20M) in the chromatogram possessing distinct snow pea-pod like, raw potato-like aromas were noted during odor assessments of compounds eluting from the packed Carbowax 20M column which confirmed the presence of these very potent odor compounds in the flavor isolates of boiled potatoes.

Three previously unreported lipid-derived carbonyls were identified in the freshly-boiled potatoes, and these were *c*4-heptenal (peak 9), *t*2, *c*6-nonadienal (peak 27) and 1,5-octadien-3-one (peak A). Although *c*4-heptenal (threshold (T) = 0.04 ppb; McGill et al., 1974), *t*2, *c*6-nonadienal (T = 0.01 ppb; Buttery 1981), and 1,5-octadien-3-one (T = 0.001 ppb; Swoboda and Peers, 1977) all occurred at very low concentrations (<0.3 ppb) in fresh boiled potatoes, the extremely low recognition thresholds (T) which these volatiles exhibit should allow each of them to influence the overall flavor and aroma of boiled potatoes.

*t*2-Nonenal (peak 24) and 1-octen-3-one (peak 13) have been identified earlier in boiled potato isolates by Buttery et al. (1970), and they arise through the oxidation of linoleic acid (C18:2, n-6). Similarly, newly-identified *t*2, *c*6-nonadienal and 1,5-octadien-3-one in potatoes are formed through parallel oxidations of linolenic acid (C18:3, n-3, Grosch et al., 1976; Tressl *et al.*, 1981), and their formation in potatoes would be limited by linolenic acid concentrations in potatoes as well as

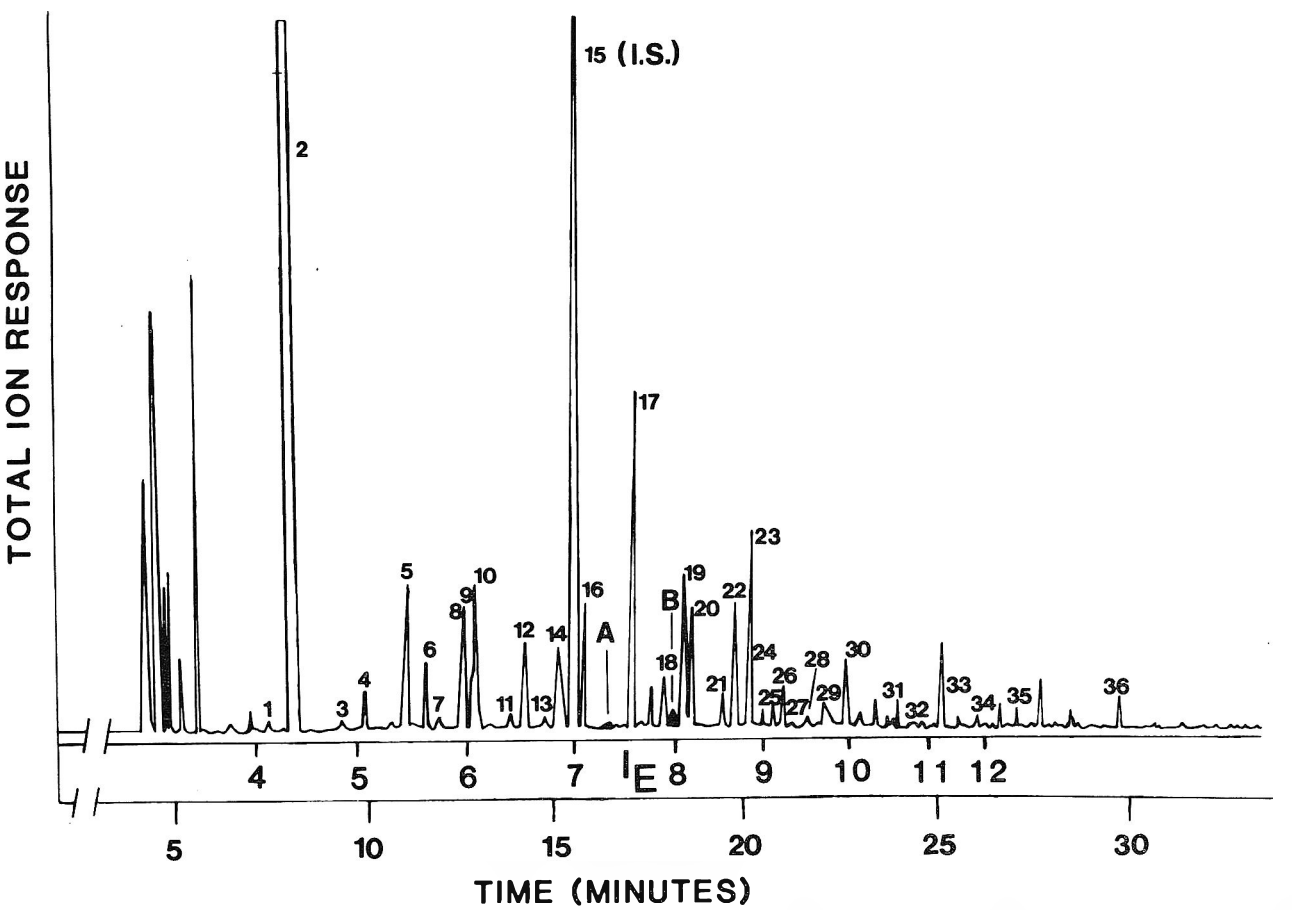

Fig. 1—Fused silica capillary column (Carbowax 20M, 60m × 0.25 mm) separation of headspace volatiles collected from freshly boiled potatoes. The column was programmed from 50°C (hold 5 min) to 140°C at 6°C/min, followed by from 140°C to 220°C at 10°C/min. Chromatographic trace is reproduced from a GC-MS analysis.

Table 1—Volatile aroma compounds identified in Russet Burbank potatoes after boiling for 30 min.

Peak no.[a]	Compound	Estimated concentration (μg/kg, ppb)	Retention indices[b]
1.	2,3-pentandione	0.3	4.10
2.	hexanal	60	4.49
3.	*t*2-pentenal	0.4	4.88
4.	pentyl oxirane (tent.)[c]	1.1	5.06
5.	heptanal	4.1	5.46
6.	3-methyl-l-butanol	2.0	5.58
7.	*t*2-hexenal	0.45	5.81
8.	2-pentyl furan	3.1	5.92
9.	*c*4-heptenal	0.2	6.03
10.	1-pentanol	3.9	6.06
11.	acetoin	0.3	6.43
12.	octanal	2.3	6.52
13.	1-octen-3-one	0.3	6.64
14.	*t*2-heptenal	2.5	6.85
15.	ethyl heptanoate	internal standard	7.00
16.	1-hexanol	4.0	7.18
A.	1,5-octadien-3-one[d]	trace; geranium leaves	7.40
17.	nonanal	8.1	7.56
18.	*t*2-octenal	1.5	7.89
B.	2-methoxy-3-isopropyl-pyrazine[d]	trace; fresh pea pod, raw potato-like	7.99
19.	1-octen-3-ol	3.6	8.12
20.	2-furfuraldehyde	4.0	8.21
21.	*t*2,*t*4-heptadienal	1.0	8.52
22.	decanal	3.4	8.67
23.	benzaldehyde	5.2	8.84
24.	*t*2-nonenal	0.3	9.00
25.	1-octanol	0.3	9.19
26.	*t*3,*t*5-octadien-2-one	0.5	9.30
27.	*t*2,*c*6-nonadienal	0.15	9.46
28.	2-undecanone	0.2	9.63
29.	2-octen-1-ol	0.7	9.72
30.	phenylacetaldehyde	1.9	9.96
31.	*t*2,*t*4-nonadienal	0.3	10.51
32.	ethyl benzaldehyde	0.35	10.58
33.	*t*2,*t*4-decadienal	0.3	11.62
34.	hexanoic acid	0.4	11.91
35.	a hydrocarbon	0.6	---[e]
36.	a hydrocarbon	0.85	---

[a] The peak numbers correspond to the numbers in Fig 1.
[b] Retention Indices (I_E; Van den Dool and Kratz, 1963) on Carbowax 20M.
[c] Tentatively identified based on mass spectral pattern.
[d] Mass spectra was not obtained. Odor eluting from region during packed column analysis.
[e] Not calculated.

by factors such as reaction times provided for lipase-type activity to release esterified fatty acids and lipoxygenase activity to effect hydroperoxide formation. The *c*4-heptenal arises through the alpha/beta double-bond hydration and retro-aldol condensation of *t*2, *c*6-nonadienal as described earlier by Josephson and Lindsay (1987a).

The formation of the hydroperoxide precursor (Galliard and Phillips, 1971) of *t*2, *c*6-nonadienal in boiling potatoes probably occurs in a moving zone of damaged potato tissue that is situated between the cool, undamaged center and the hot, outer layer of the potatoes where enzymes have been inactivated. In the enzymically-active zone, appropriate decompartmentalized lipid substrates and lipoxygenase from damaged cells would react to yield the hydroperoxide which would decompose on further heating to yield *t*2, *c*6-nonadienal. Preliminary trials using microwave cooking of whole, degassed potatoes yielded samples which exhibited suppressed flavors which lacked both baked or boiled potato-like flavor notes. Instead they exhibited strong methoxy-ethylpyrazine-like, earthy flavors which appeared to support a view that the microwave heating inactivated lipoxygenase before it could form *t*2, *c*6-nonadienal.

Although potatoes contain low lipid concentrations (0.1%; Mondy et al., 1963), linolenic acid constitutes 13-24% of these lipids (Galliard, 1973). Thus, there is a significant pool of *t*2, *c*6-nonadienal precursor in the lipids of potatoes. To demonstrate the effects of initial lipoxygenase action in potatoes on the production of flavor compounds, the headspace volatiles of raw potatoes were similarly analyzed after the potatoes were first shredded to disrupt cells and activate lipoxygenase. Following this treatment,about 0.4 ppb of *t*2, *c*6-nonadienal was measured in the raw potatoes compared to about 0.2 ppb in boiled potatoes which supported a view of involvement for potato lipoxygenase in its formation in these systems. However, a portion of the 0.2 ppb of *t*2, *c*6-nonadienal found in boiled potatoes probably arises through purely autoxidative reactions which are accelerated at the elevated temperatures. Only trace amounts of *c*4-heptenal were observed in raw potato volatiles compared to boiled potatoes which contained 0.2 ppb of this compound, and the difference in amounts reflected the rates of conversion of *t*2, *c*6-nonadienal that occurs at the two temperatures (Josephson and Lindsay, 1987a).

Raw, shredded potatoes also contained relatively abundant concentrations of *t*2, *c*4-decadienal (2-3 ppb), *t*2, *t*4-decadienal (5-6 ppb), *t*2-octenal (2-3 ppb) and hexanal (20-25 ppb) which likely also were formed by lipoxygenase-initiated reactions. However, following boiling the potatoes contained only small amounts of *t*2, *t*4-decadienal (0.1-1 ppb), modest amounts of *t*2-octenal (2-5 ppb) and substantial amounts of hexanal (50–60 ppb). These compounds are all related through degradations of 2,4-decadienal which involves either classic Farmer autoxidation or photooxidative mechanisms (Matthews et al., 1971; Michalski and Hammond, 1972; Schieberle and Grosch, 1982) or an alpha/beta double-bond hydration followed by a retro-aldol condensation mechanism (Josephson and Lindsay, 1987b). The latter mechanism first leads to 2-octenal, and it is subsequently converted to hexanal.

Unless protected from oxygen, dehydrated potatoes develop off-flavors derived from lipids to the extent that their use in many market forms of products is severely restricted (Self, 1967; Buttery et al., 1970; Nursten and Sheen, 1974; Sapers, 1975). However, dehydrated potatoes that have been vacuum- or nitrogen-packed generally lack overall potato flavor and still may exhibit stale flavors that have been attributed to browning reaction products (Sapers, 1975). Guadagni et al. (1971) evaluated the enhancement of the flavor of dehydrated mashed potatoes caused by additions of phenylacetaldehyde, 1-octen-3-ol, methional, 2-methoxy-3-isopropylpyrazine, and 2-methoxy-3-ethylpyrazine which were all considered to be influential compounds in raw and boiled potato flavors. However, only 2-methoxy-3-ethylpyrazine was found to effectively enhance the potato flavor quality of dehydrated potatoes.

Adding a relatively high concentration of *c*4-heptenal (0.66 ppb) generally lowered the flavor quality of freshly-mashed potatoes (Table 2). The potatoes used in these tests had an endogenous level of about 0.2 ppb of *c*4-heptenal which combined with higher additions of *c*4-heptenal (0.5-0.8 ppb) to cause staling effects which led to lowering of sensory scores. Additions of 0.1-0.4 ppb of *c*4-heptenal to the fresh potatoes appeared to enhance earthy-like flavors. This was especially

Table 2—Effect of c4-heptenal addition upon the sensory properties of fresh, mashed Russet Burbank potatoes

Samples	Sample attributes: Overall potato flavor intensity[a]	Cream-like flavor intensity[b]	Freshness[c]
	(Mean scores)[d]		
Control	4.13 x	3.68 x	4.0 x
with 0.50 ppb *c*4-heptenal	3.90 x	3.18 y	3.78 x
Control	4.31 x	3.02 x	4.46 x
with 0.66 ppb *c*4-heptenal	3.88 y	2.70 x	4.08 y

[a] Scale: 1 = Very weak; 7 = Very strong.
[b] Scale: 1 = Absent; 7 = Very pronounced.
[c] Scale: 1 = Stale, lacks freshness; 7 = Extremely fresh.
[d] n = 31. Mean scores in same column with same letter are not significantly different at 10% level.

noticeable after butter was added. In the latter case the earthy, potato flavor quality contributed by 2-methoxy-3-ethylpyrazine appeared to blend with the cream-like flavor quality of very dilute concentrations of *c*4-heptenal that were noted earlier by Begemann and Koster (1964).

The addition of *c*4-heptenal to reconstituted dehydrated potatoes (0.1–0.6 ppb) consistently caused stale-potato-type flavors, and resulted in lowered sensory scores. The absence of earthy notes in dehydrated potatoes which are provided by methoxy-alkylpyrazines in fresh potato allowed the aldehydic notes of *c*4-heptenal to shadow the cream-like character that the compound can provide. Addition of low concentrations of *t*2, *c*6-nonadienal (0.01-0.5 ppb) to a number of mashed potato samples did not enhance potato flavors, and detectable concentrations caused green-, cucumber-like flavors which were not characteristic of potatoes.

In summary, *c*4-heptenal was found to be present in freshly boiled potatoes at concentrations about 50× its recognition threshold, and this provided a boiled potato-like characterizing aroma and flavor note to the potatoes. *c*4-Heptenal was formed from a double-bond hydration, retro-aldol degradation of *t*2, *c*6-nonadienal which was also identified in boiled potatoes. Addition of very low levels *c*4-heptenal (0.1-0.4 ppb) to fresh-mashed potatoes provided slight enhancement of earthy, potato-like flavors. However, addition of *c*4-heptenal in high levels to fresh potatoes (>0.5 ppb) and low levels to reconstituted dehydrated potatoes (>0.1 ppb) only caused an enhancement of stale-potato-like flavors.

REFERENCES

Begemann, P.H. and Koster, J.C. 1964. 4-cis-Heptenal: A cream-flavored component of butter. Nature 4932: 552.

Burr, H.K. 1966. Compounds contributing to flavor of potatoes and potato products. In "Proc. Plant Sci. Symp.", p. 83, Campbell Institute, Camden, NJ.

Buttery, R.G., Seifert, R.M., and Ling, L.C. 1970. Characterization of some volatile potato components. J. Agric. Food Chem. 18: 538.

Buttery, R.G., Guadagni, D.G., and Ling, L.C. 1973. Volatile components of baked potatoes. J. Sci. Food Agric. 24: 1125.

Buttery, R.G. 1981. In "Flavor Research-Recent Advances." (Ed.) R. Teranishi, R.A. Flath, H. Sugisawa. p. 199. Marcel Dekker, New York.

Coleman, E.C., Ho, C.-T., and Chang, S.S. 1981. Isolation and identification of volatile compounds from baked potatoes. J. Agric. Food Chem. 29: 42.

Coleman, E.C. and Ho, C.-T. 1980. Chemistry of baked potato flavor. 1. Pyrazines and thiazoles identified in the volatile flavor of baked potato. J. Agric. Food Chem. 28: 66.

Galliard, T. 1973. Lipids of potato tubers. 1. Lipid and fatty acid composition of tubers from different varieties of potato. J. Sci. Food Agric. 24: 617.

Galliard, T. and Phillips, D.R. 1971. Lipoxygenase from potato tubers. Biochem. J. 124: 431.

Grosch, W., Laskawy, G., and Weber, F. 1976. Formation of volatile carbonyl compounds and cooxidation of B-carotene by lipoxygenase from wheat, potato, flax, and beans. J. Agric. Food Chem. 24: 456.

Guadagni, D.G., Buttery, R.G., Seifert, R.M., and Venstrom, D.W. 1971. Flavor enhancement of potato products. J. Food Sci. 36: 363.

Gumbmann, M.R. and Burr, H.K. 1964. Volatile sulfur compounds in potatoes. J. Agric. Food Chem. 12: 404.

Heller, S.R., and Milne, G.W.A. 1975. "EPA/NIH Mass Spectral Data Base." Vol. 1-4. U.S. Government Printing Office, Washington, DC.

Heller, S.R. and Milne, G.W.A. 1980. "EPA/NIH Mass Spectral Data Base." Suppl. 1. U.S. Government Printing Office: Washington, DC.

Josephson, D.B. and Lindsay, R.C. 1987a. Retro-aldol degradations of unsaturated aldehydes: Role in the formation of c-4-heptenal from *t*2, *c*6-nonadienal in fish, oyster and other flavors. J. Am. Oil Chem. Soc. 64: 132.

Josephson, D.B. and Lindsay, R.C. 1987b. Retro-aldol related degradations of 2,4-decadienal in the development of staling flavors in fried foods. J. Food Sci. Submitted for publication.

Matthews, R.F., Scanlan, R.A. and Libbey, L.M. 1971. Autoxidation products of 2,4-decadienal. J. Am. Oil Chem. Soc. 48: 745.

McGill, A.S., Hardy, R., Burt, J.R., and Gunstone, F.D. 1974. Hept-cis-4-enal and its contribution to the off-flavor in cold stored cod. J. Sci. Food Agric. 25: 1477.

Michalski, S.T. and Hammond, E.G. 1972. Use of labeled compounds to study the mechanism of flavor formation in oxidizing fats. J. Am. Oil Chem. Soc. 49: 563.

Mondy, N.I., Mattick, L.R., and Owens, E. 1963. The effect of storage on the total lipids and the fatty acid composition of potatoes. J. Agric. Food Chem. 11: 328.

Nursten, H.E. and Sheen, M.R. 1974. Volatile flavour components of cooked potato. J. Sci. Food Agric. 25: 643.

Olafsdottir, G., Steinke, J.A., and Lindsay, R.C. 1985. Quantitative performance of a simple Tenax-GC adsorption method for use in the analysis of aroma volatiles. J. Food Sci. 50: 1431.

Pareles, S.R. and Chang, S.S. 1974. Identification of compounds responsible for baked potato flavor. J. Agric. Food Chem. 22: 339.

Sapers, G.M. 1975. Flavor stability of dehydrated potato products. J. Agric. Food Chem. 22: 339.

Schieberle, P. and Grosch, W. 1981. Model experiments about the formation of volatile carbonyl compounds. J. Am. Oil Chem. Soc. 58: 602.

Self, R. 1967. Potato flavor. In "Symposium on Foods: The Chemistry and Physiology of Flavors." (Ed.) H.W. Schultz, E.A. Day, and L.M. Libbey, p. 362. AVI Pub. Co., Westport, CT.

Solms, J. 1971. Nonvolatile compounds and the flavor of foods. In "Gustation and Olfaction." (Ed.) G. Ohloff, and A.F. Thomas, p. 92. Academic Press, New York.

Solms, J. and Wyler, R. 1979. Taste components of potatoes. In "Food Taste Chemistry." (Ed.) J.C. Boudreau, p. 175. ACS Symposium Series, American Chemical Society, Washington, DC.

Steel, R.G.D. and Torrie, J.H. 1960. Analysis of variance. I. The one way classification. In "Principles and Procedures of Statistics," p. 99. McGraw-Hill Publ. Co., Inc., New York.

Stone, H., Sidel, J., and Bloomquist, J. 1980. Descriptive sensory analysis. Cereal Foods World 25: 642.

Stone, H., Sidel, J., Oliver, S., Woolsey, A., and Singleton, R.C. 1974. Sensory evaluation by quantitative descriptive analysis. Food Technol. 28: 24.

Swoboda, P.A.T. and Peers, K.E. 1977. Metallic odour caused by vinyl ketones formed in the oxidation of butterfat. The identification of octa-1, cis-5-dien-3-one. J. Sci. Food Agric. 28: 1019.

Tressl, R., Bahri, D., and Engel, K.-H. 1981. Lipid oxidation in fruits and vegetables. In "Quality of Selected Fruits and Vegetables of North America," p. 213. ACS Symposium Series #170, American Chemical Society, Washington, DC.

Van den Dool, H. and Kratz, P.D. 1963. A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. J. Chromatog. 11: 463.

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Determination of Phenolic Compounds of Dry Beans Using Vanillin, Redox and Precipitation Assays

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ABSTRACT

Phenolic compounds of ten genotypes of dry beans (*Phaseolus vulgaris* L.) were investigated using four different methods. Assayable tannins using the 0.5% vanillin assay ranged from 49-306 mg catechin equivalents/100 g beans. Significant differences were observed for tannins measured by the vanillin and the protein precipitable phenol tests. Of the two redox assays, the Prussian blue assay gave significantly higher total phenol values than the Folin-Ciocalteu assay. Both these reagents were more sensitive to tannic acid than to catechin. Although tannin values varied 2–3 fold for a given variety, excellent correlation was observed between the different assays employed for bean tannin analysis.

INTRODUCTION

FLAVONOID COMPOUNDS form the most widely distributed and heterogeneous group of secondary plant products ingested with food. In view of their potentially harmful effects, increasing importance is being attached to the dietary intake of these compounds, particularly of condensed tannins.

The majority of methods for the determination of phenolic compounds in plant products are based on the polarity of the phenolic hydroxyl groups and can be classified as either those which measure all phenols as a group apart from nonphenols or those which assay specific individual substances or classes of phenols. The commonly used methods for legume tannin analysis include the vanillin test (Burns, 1963), the Prussian blue test (Price and Butler, 1977) and the Folin-Denis test (Swain and Hillis, 1959; Burns, 1963). Although the latter two redox assays offer sensitive, versatile methods for the colorimetric determination of total phenols, they do not distinguish nutritionally harmful tannins from other phenolic compounds. The redox assays are also subject to interference by several substances naturally occurring in foods. A measure of actual tannins present in legumes is probably more accurately provided by the vanillin assay when corrected for the background color, although the monomeric proanthocyanidins, which are nutritionally inert, are also detected. The UV spectrophotometric determination of tannins of yellow peas, lentils and white pea beans was reported by Davis (1982). However, such an approach was often affected by the presence of several coloring compounds in dry beans (Deshpande, 1984). Protein precipitation and enzyme inhibition assays correlate most highly with the nutritional quality of foods. The results, however, are often variable and not quite quantitative.

In spite of the availability of such a large number of different approaches for tannin analysis, none has been found completely satisfactory. Bressani et al. (1983) analyzed tannins in 13 samples of red Guatemalan beans by four different chemical assays. Although the results were highly correlated, the degree of variation among the samples differed greatly according to the assay. Recently, Deshpande and Cheryan (1985) suggested modifications to the vanillin test for legume tannins, in addition to those already reported by Price et al. (1978). A number of other methods are also routinely used for tannins in dry beans. Studies regarding the suitability of these methods for bean tannins, however, are lacking. The present investigation was, therefore, undertaken to study the effectiveness of several of these methods and their possible useful relationships in tannin analysis of dry beans.

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MATERIALS & METHODS

THE SOURCE of the dry beans (all *Phaseolus vulgaris* L.) and the preparation of the whole bean flours (820 micron particle size) were reported earlier (Deshpande and Cheryan, 1985). Catechin (+) (3.5 moles of H_2O/mole), tannic acid and bovine serum albumin (fraction V, 96–99% protein) were from Sigma Chemical Co., St. Louis, MO. All other chemicals used were of reagent grade.

Extraction of phenolics

Phenolics from whole bean flours were extracted within 6 hr of grinding using either absolute methanol or acidified-methanol (1% HCl in methanol). In one experiment, to study the relative solubility of bean condensed tannins, the flours were extracted with glass-distilled deionized water and with 1.0M NaCl.

Phenol assays

The 0.5% vanillin assay of Price et al. (1978) was employed for dry bean tannins after determining the optimum conditions for individual varieties (Deshpande and Cheryan, 1985). Total phenols using the Prussian blue assay were determined as described by Price and Butler (1977). Those by the Folin-Ciocalteu assay were measured according to a modification proposed by Swain and Hillis (1959). The Folin-Ciocalteu reagent was substituted for the Folin-Denis reagent. The protein precipitable phenols were measured by the method of Hagerman and Butler (1978).

Statistical analyses

Wherever applicable, the data were analyzed by the analysis of variance procedures of the Statistical Analysis System (SAS, 1979).

RESULTS & DISCUSSION

Vanillin assay

The tannin contents of the ten dry bean varieties using the 0.5% vanillin assay modified as suggested by Deshpande and Cheryan (1985) are summarized in Table 1. Values for the pigmented beans varied widely, ranging from 49–306 mg and 26–291 mg catechin equivalents/100g beans when extracted in methanol and acidified-methanol, respectively. Tannins were not detected by this method in the white seed types (Sanilac, Small White and Great Northern). Pinto and Small Red beans contained more tannins than did Kidney beans. Black Beauty beans were the lowest in tannin of the colored beans. In most cases, extraction in acidified-methanol yielded significantly higher assayable tannin. Only Small Red and Black Beauty beans gave lower tannin values when extracted under acidic conditions. Since tannic acid does not give a positive reaction with the vanillin reagent, it could not be used as a reference standard. The vanillin assay also appears to be less sensitive to catechin than towards condensed tannins (Butler et al., 1982).

Table 1—Assayable tannin of dry beans using the 0.5% vanillin assay[a]

Variety	Extraction medium, 1 hr extraction	
	Absolute MeOH	1% HCl in MeOH
Sanilac	ND[b]	ND
Small White	ND	ND
Great Northern	ND	ND
Cranberry	82	94
Viva Pink	160	181
Pinto	306	291
Light Red Kidney	172	191
Dark Red Kidney	116	143
Small Red	257	239
Black Beauty	49	26
LSD[c] (p = 0.05)	7.14	

[a] Values represent mg catechin equv/100g bean flour (820 micron particle size) on a dry weight basis.
[b] ND = Not detected.
[c] Least significant difference. Differences of two means within/between the varieties exceeding this value are significant.

Table 2—Total phenolics of dry beans as measured by the Folin-Ciocalteu assay[a]

Variety	Methanol extractions		Methanol-HCl extractions	
	Tannic acid equv	Catechin equv	Tannic acid equv	Catechin equv
Sanilac	9	17	24	25
Small White	27	32	36	37
Great Northern	25	31	42	43
Cranberry	66	68	72	73
Viva Pink	64	66	78	79
Pinto	112	110	125	126
Light Red Kidney	67	68	84	85
Dark Red Kidney	55	59	68	69
Small Red	95	94	103	104
Black Beauty	56	59	93	94
LSD[b] (p = 0.05)	5.14		6.13	

[a] Values represent mg total phenols/100g bean flour on a dry weight basis.
[b] Least significant difference. Differences of two means within/between the varieties exceeding this value are significant.

Redox assays

Both the Prussian blue and Folin-Ciocalteu assays were more sensitive to tannic acid than to catechin. These assays were also more sensitive when methanol was used as the extraction medium. Redox assays are known to be affected by the varying hydroxylation pattern and the degree of polymerization of polyphenolics (Price and Butler, 1977). Similarly, phenolic compounds differ in their ability to reduce different reagents. For example, based on the slopes of standard curves from the Prussian blue assay (not shown), tannic acid had approximately 25% more ability to reduce ferric iron than did catechin. Thus, since tannic acid, on a per mole basis, has more phenolic hydroxyls than does catechin, it was better suited as a reference standard for the redox assays.

The total phenol levels of the ten dry bean varieties as estimated by the Folin-Ciocalteu method are summarized in Table 2. The three white varieties had no detectable condensed tannins according to the vanillin assay (Table 1). However, they apparently did contain other phenolic compounds in minor amounts. These three varieties also showed significant differences when the results were expressed as catechin or tannic acid equivalents (absolute methanol extraction). This was presumably because the assay was more sensitive to tannic acid at the lower range of the standard curve. Extraction with methanolic-HCl rather than absolute methanol yielded significantly higher values for total phenols, although no significant differences were observed between the two standards under acidic conditions.

The data on total phenolics of dry beans using the Prussian blue assay are summarized in Table 3. These values were significantly higher than those obtained with the Folin-Ciocalteu assay (LSD = 8.93, p = 0.05). These results of these two assays clearly indicated differences in the ability of phenolic compounds to reduce different solutions, which emphasized the need for caution in interpreting results of redox assays for tannin determination.

Table 3—Total phenolics of dry beans as measured by the Prussian Blue assay[a]

Variety	Total phenols, mg/100g beans	
	Tannic acid equv	Catechin equv
Sanilac	62	78
Small White	66	82
Great Northern	61	87
Cranberry	84	106
Viva Pink	91	114
Pinto	122	153
Light Red Kidney	89	112
Dark Red Kidney	76	96
Small Red	108	136
Black Beauty	90	113
LSD[b] (p = 0.05)	5.81	

[a] Bean flours were extracted in absolute methanol for 1 hr.
[b] Least significant difference. Differences of two means within/between the varieties exceeding this value are significant.

Differences in solubility in aqueous and in salt solutions have been used as a basis for a Prussian blue test which was specific for condensed tannins of sorghum (Price and Butler, 1977). Total phenols of dry beans extracted with water and 1.0M salt solutions are summarized in Table 4. Both the catechin and tannic acid equivalent values for salt extracts were significantly lower than those for water extracts. These values were, in general, significantly lower than those obtained by the vanillin assay (Table 1) which detects both condensed tannins and their monomers. Only Cranberry beans gave similar values by both these methods. The salt extractable polyphenolics apparently varied only within a narrow range. Price and Butler (1977) reported that salt did not reduce the total solubility of all polyphenols, but only the rate at which the polyphenols were dissolved. In the present study, maximum extraction in salt and water was obtained within 1 hr and then declined, presumably due to air oxidation of the extracted material. The reason for lower condensed tannin values obtained by the Prussian blue assay (Table 4) as compared to vanillin assay (Table 1), apart from only tannins being detected, may also be due to the solubility of a relatively high proportion of small molecular weight polymeric tannins (i.e., a lower degree of polymerization) in salt solutions. Also, Price and Butler (1977) suggested that the salt extraction removes only anthocyanidins completely cannot be considered proven. Another major source of error could be due to the extraction in both aqueous and salt solutions of a relatively large number of other substances that may also reduce ferricyanide. Such compounds may include sulfides, aromatic amines, unsaturated aliphatics, glucose, and most important, ascorbic acid (Snell and Snell, 1943).

Protein-precipitable phenols

The values for protein-precipitable phenols (Table 5) were generally much lower than those obtained by the vanillin test (Table 1). Only the two Kidney bean types gave comparable values by both methods. These results were consistent with the observations of Barroga et al. (1985) on polyphenols of mung bean. A major difference, however, was that these authors reported 14–45 times higher values using the 0.5% vanillin assay, compared to only 1.1–6.2 times in the present study. On the other hand, Hagerman and Butler (1978) observed differences in sorghum tannins assayed by these two methods. Different batches of tannic acid used as the reference standard in this test by different researchers may influence the results. For example, in the present study, tannic acid as standard precipitated only half as much protein as reported by

Table 4—Water and salt extractable phenols of dry beans as measured by the Prussian Blue assay

Variety	Tannic acid equv, mg/100g			Catechin equv, mg/100g		
	Water	Salt	Water-Salt	Water	Salt	Water-Salt
Sanilac	132	129	3	171	166	5
Small White	184	163	21	237	210	27
Great Northern	173	159	14	222	205	17
Cranberry	275	215	60	353	276	77
Viva Pink	218	180	38	282	232	50
Pinto	223	191	32	287	246	71
Light Red Kidney	282	209	73	362	269	93
Dark Red Kidney	254	197	57	326	253	73
Small Red	219	183	36	282	236	76
Black Beauty	326	232	94	419	299	120
LSD[a] (p = 0.05)	5.9			6.2		

[a] Least significant difference. Differences of two means exceeding this value are significant.

Table 5—Protein precipitable phenols of dry beans

Variety	Protein precipitable phenols mg Tannic acid equv/100g
Sanilac	ND[b]
Small White	ND
Great Northern	ND
Cranberry	53
Viva Pink	92
Pinto	218
Light Red Kidney	155
Dark Red Kidney	124
Small Red	187
Black Beauty	8
LSD[c] (p = 0.05)	10.9

[a] Bean flours were extracted in absolute methanol for 1 hr.
[b] ND = Not detected.
[c] Least significant difference. Differences of two means exceeding this value are significant.

Table 6—Simple correlation coefficients between different assays used for dry bean tannins

Assays	Folin-Ciocalteu	Prussian Blue	Protein precipitable phenols
Vanillin	0.92[a]	0.86[b]	0.94[a]
Folin-Ciocalteu		0.95[a]	0.79[b]
Prussian Blue			0.67[c]

[a] Significant at 1% level.
[b] Significant at 5% level.
[c] Significant at 10% level.

Hagerman and Butler (1978). Despite its limitations, the protein precipitable phenol method provides information about the biological activity of tannins which cannot be obtained by the other methods.

Simple correlation coefficients between different assays employed for bean tannins are summarized in Table 6. Similar to the observations of Bressani et al. (1983), the results of the corrected vanillin assay and those expressed as protein precipitable phenols were the most highly correlated. The results of the two redox assays were also highly correlated. In general, our correlation coefficients were higher than those reported by Bressani et al. (1983). Although excellent correlations were obtained, tannin contents determined by the different assays varied 2-3 fold within a variety. The vanillin:Folin-Denis (V/FD) ratios have been used to show the degree of polymerization of flavanols in model systems. The V/FC ratios in the present study were highly correlated with protein precipitable phenol values ($r = 0.92$, $p = 0.01$). Using data from Bressani et al. (1983), a correlation of 0.74 ($p = 0.01$) was observed. Although the V/FD (or V/FC) ratios decrease with increasing degree of polymerization, they are probably of little value using crude bean extracts, where the presence of several other reducing compounds influences the FC values.

The results of the present study emphasize the differences in legume tannins determined by the four commonly used methods. The two major factors preventing meaningful comparisons between laboratories using similar methods appear to be the solvent used for tannin extraction and the choice of the reference standard. Extraction in acidified-methanol consistently yielded higher values, and unless corrected for the sample blanks as suggested by Price et al. (1978), may result in erroneous estimations of bean tannins. This may also be a major contributing factor to the controversy over the possible relationship between seed coat color and tannin content assayed by the uncorrected vanillin assay first described by Burns (1963). This was further supported by the fact that although of the colored beans, Black Beauty was lower in tannin, this variety lowered the apparent bean protein digestibility in rats to a similar extent as did the red-colored high-tannin beans (Elias et al., 1979). Since the redox assays do not only detect all phenols but are also influenced by several interfering compounds, the choice of a standard solvent such as absolute methanol and the use of a corrected vanillin-HCl assay in conjunction with a protein precipitation assay may yield more meaningful data on legume tannins.

REFERENCES

Barroga, C.F., Laurena, A.C., and Mendoza, E.M.T. 1985. Polyphenols in mung bean (*Vigna radiata* L. Wilczek): Determination and removal. J. Agric. Food Chem. 33: 1006.

Bressani, R., Elias, L.G., Wolzak, A., Hagerman, A.E., and Butler, L.G. 1983. Tannin in common beans: Methods of analysis and effects on protein quality. J. Food Sci. 48: 1000.

Burns, R.E. 1963. Methods of tannin analysis for forage crop evaluation. Georgia Agric. Exp. Stn. Bull. N.S. 32, Univ. of Georgia, Athens.

Butler, L.G., Price, M.L., and Brotherton, J.E. 1982. Vanillin assay for proanthocyanidins (condensed tannins): Modification of the solvent for estimation of the degree of polymerization. J. Agric. Food Chem. 30: 1087.

Davis, K.R. 1982. Effect of processing on composition and Tetrahymena relative nutritive value of green and yellow peas, lentils, and white pea beans. Cereal Chem. 58: 454.

Deshpande, S.S. 1984. Unpublished data, Dept. of Food Sci., Univ. of Illinois, Urbana-Champaign.

Deshpande, S.S. and Cheryan, M. 1985. Evaluation of vanillin assay for tannin analysis of dry beans. J. Food Sci. 50: 905.

Elias, L.G., de Fernandez, D.G., and Bressani, R. 1979. Possible effects of seed coat polyphenolics on the nutritional quality of bean protein. J. Food Sci. 44: 524.

Hagerman, A.E. and Butler, L.G. 1978. Protein precipitation method for the quantitative determination of tannins. J. Agric. Food Chem. 26: 809.

Price, M.L. and Butler, L.G. 1977. Rapid visual estimation and spectrophotometric determination of tannin content of sorghum grain. J. Agric. Food Chem. 25: 1268.

Price, M.L., Van Scoyoc, S., and Butler, L.G. 1978. A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. J. Agric. Food Chem. 26: 1214.

SAS. 1979. "User's Guide." Statistical Analysis System Institute, Cary, NC.

Snell, F.D. and Snell, C.T. 1953. "Colorimetric Methods of Analysis", 3rd ed., Vol. III, p. 104, Van Nostrand, Princeton, NJ.

Swain, T. and Hillis, W.E. 1959. Phenolic constituents of *Prunus domestica*. I. Quantitative analysis of phenolic constituents. J. Sci. Food Agric. 10: 63.

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Emulsifying Properties of Pea Globulins as Related to Their Adsorption Behaviors

C. DAGORN-SCAVINER, J. GUEGUEN, and J. LEFEBVRE

ABSTRACT

Emulsifying properties of purified pea globulins and of vicilin-legumin mixtures were evaluated through their emulsifying capacity, emulsifying activity index and stability of the resulting emulsions. The results were discussed by reference to the interface adsorption behaviors of these proteins. The influence of the vicilin/legumin ratio on the efficiency of pea isolates as emulsifying agents was also studied. Vicilin which has been shown to be more surface active at air/water and dodecane/water interfaces, led also, in both cases, alone or mixed, to better emulsifying properties than legumin. The globulin composition of the isolates did not completely explain their emulsifying behaviors.

INTRODUCTION

EMULSIFYING PROPERTIES of proteins depend on two effects: (1) substantial decrease in the interfacial tension due to the adsorption of the protein at the oil/water interface; (2) electrostatic, structural, and mechanical energy barrier caused by interfacial layer that opposes coalescence. Nevertheless, the interplay and the relative importance of the various characteristics of protein adsorption and interfacial layer structure as to the different processes implied in emulsion formation and stability remain still largely open to discussion.

The relationship between physicochemical characteristics, interfacial behaviors and emulsifying properties of proteins was investigated by Graham (1976), Graham and Phillips (1976) and Phillips (1981) on model proteins (bovine serum albumin, β- casein and lysozyme). They showed that proteins with highly ordered native structure, giving strongly cohesive interfacial films, were more efficient in stabilizing emulsions, even though they were less surface-active than less rigid proteins with a looser conformation. They emphasized the importance of the thickness and charge of the protein interfacial layer in preventing coalescence. Nakai et al. (1980) observed a positive correlation between surface hydrophobicity, surface tension and emulsifying activity index. These authors did not take into account the kinetics of protein adsorption in the interpretation of their results on protein stabilized emulsions.

In previous work (Dagorn-Scaviner, 1986; Dagorn-Scaviner et al., 1986), the adsorption behaviors of purified pea globulins (legumin and vicilin) and of bovine serum albumin (BSA) were compared at the air/water and dodecane/water interfaces. Surface pressure isotherms showed that, except at very low protein concentrations, pea globulins were more surface active than BSA, and, at the dodecane/water interface, vicilin somewhat more than legumin. However, the comparison of interfacial concentration isotherms indicated that, at all concentrations, BSA coated the dodecane/water interface most effectively; in this respect legumin performed better than vicilin at high concentrations. On the other hand, adsorption of BSA was found to be globally quicker than that of pea globulins. This followed essentially from the fact that the rate constant of the first kinetic step of interfacial tension decrease (mainly diffusion dependent) was appreciably higher in the case of BSA at low concentrations and exceeded those of pea globulins by an order of magnitude at higher concentrations; this process was more rapid for vicilin than for legumin. The rate constants of the subsequent steps were not so much different, although the energy barrier relative to the second step (penetration phase) was found to be noticeably higher for legumin than for vicilin and BSA.

On the whole, vicilin appeared to be a more efficient surface active agent than legumin. This property was retained when vicilin was mixed in solution with legumin, the surface pressure increasing in this case with the relative concentration of vicilin. On the other hand legumin adsorption was disturbed by vicilin, even only by a small amount of this protein in the mixture.

The results, discussed in the light of previous extensive studies (Graham, 1976; Graham and Phillips, 1976), were related to the conformation of pea globulins being more bulky and having less organized secondary and tertiary structures than BSA, with vicilin having a lesser size and a higher flexibility than legumin.

In this paper, the emulsifying properties of pea legumin and vicilin, as well as vicilin and legumin mixtures, were examined and compared to those of BSA. An attempt was made at evaluating the stabilizing effect of proteins towards the different processes of emulsion breaking; for this purpose, the emulsifying capacities, emulsifying activity indices and stabilizing efficiency against coalescence of the proteins were determined using some of the procedures described in the literature, and a test was developed to evaluate the stabilization efficiency against creaming and flocculation. The results were tentatively explained on the basis of the various aspects of adsorption behaviors and interfacial layer structures of the proteins, as brought out in our previous studies.

MATERIALS & METHODS

PEA (variety (AMINO) globulins were separated by chromatography on DEAE Sepharose, purified by gel filtration on ACA 34 ultrogel, and the purity of the resulting legumin and vicilin fractions was checked, as described earlier (Gueguen et al., 1984). Fatty acid free bovine serum albumin (BSA) was purchased from Sigma Chemical Co., St Louis, Mo. Casein (total) was prepared from skimmed milk by acid precipitation at pH 4.6 with 1N HCl.

Isolates were prepared from dehulled pea flour by extraction of the proteins in alkaline medium (pH 9), followed by their precipitation at pH 4.5 (Gueguen et al., 1980). The resulting protein pellet separated by centrifugation was washed, neutralized and then spray dried to give the pea isolates. Four different cultivars were selected: Amino, Colmo, Finale and Sitelle, because of their different vicilin — legumin ratios (Table 1). The composition of the isolates is given in Table 1; crude lipid was determined by hexane extraction after acid hydrolysis of the isolates (Drapron, 1975) and the nitrogen, after mineralization of the sample, using a Technicon automatic analyzer; the nitrogen to protein conversion factor used (5.6) was calculated from the amino acid composition of pea globulins. When expressed conventionaly protein (N

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Table 1—Biochemical composition of isolates

Cultivar	V/L ratio[a] (Flour)	Protein · %(N × 5.6)	Total lipids (g/100 g)	Phospholipid (g/100 g)	Phosphatidyl ethanolamine (g/100 g)	Phosphatidyl choline (g/100g)
Amino	1.65	80.4	8.06	6.01	2.49	3.04
Colmo	1.40	78.1	8.74	n.d	n.d	n.d
Finale	1.50	78.4	8.23	6.44	2.39	3.30
Sitelle	4.90	76.0	12.18	8.11	2.24	4.50

[a] Vicilin to legumin ratio; n.d = not determined; results expressed per 100g of isolates on dry basis.

× 6.25) was comprised between 85 and 90%, and lipid (direct hexane Soxhlet extraction) was around 0.5%.

For the emulsifying tests (emulsifying capacity, stability, activity), purified proteins were dissolved in sodium phosphate buffer ($NaH_2PO_4 \cdot 2H_2O$ — 0.1M, pH 8). Protein isolates were not completely soluble in this buffer; for this reason the emulsifying properties were determined both on the soluble fraction (PBIE) of each isolate and on the total isolate. The concentration of the purified protein solutions was obtained from the absorbance at 278 nm. The specific absorptivity $A^{1cm}_{1\%}$ was determined to be 5.6 and 3.7 for legumin and vicilin, respectively and taken as equal to 6.7 for BSA (Kirschenbaum, 1970).

Emulsifying capacity

The emulsifying capacity (EC) was defined as the maximum amount of oil which could be dispersed in the aqueous phase for a given experimental procedure (Webb et al., 1970); it is usually expressed as EC = (g of oil added to reach inversion point per mg of protein) × 100.

EC was measured by the method of Crenwelge et al. (1974) slightly modified. The inversion point was detected by conductimetry. A commercial soybean oil was continuously added at the rate of 1 g/sec to 150 ml protein dispersion (1 mg/mL) under stirring with a Waring Blendor at 17000 rpm, at room temperature, until the electrical conductivity fell sharply.

Emulsifying activity index

The emulsifying activity index (EAI) was estimated according to the turbidimetric technique of Pearce and Kinsella (1978) with some modifications. Ten milliliters soybean oil were dispersed into 30 ml protein solution (1.3 mg/mL) with a Polytron PT 10 stirrer operated at 20.000 rpm for 30 sec. Aliquots (1 mL) of the emulsions were immediately diluted 250 fold with a solution of sodium chloride (0.1M, pH 7) — 0.1% sodium dodecyl sulfate. The turbidity T (T = 2.303 A/l: A, absorptivity of the emulsion; l, pathlength of the cuvette) of the dilutions was immediately measured at 500 nm. Pearce and Kinsella (1978) defined the EAI as:

$$EAI = 2T/\Phi C \qquad (1)$$

where Φ is the volume fraction of the oil phase (here $\Phi = 0.25$) and C the protein concentration in the aqueous phase; the EAI, expressed in m^2/g, is related to the interfacial area stabilized per unit weight of protein.

Emulsifying stability

The aging stability of emulsions prepared as above for EAI determination was studied. Three different processes can be concomitantly involved in the rupture of the emulsion: creaming, flocculation and coalescence. With our experimental conditions, no separation of an oil phase was observed during the aging under the acceleration of gravity. For this reason the stabilizing effect of proteins against coalescence was estimated by a centrifugation technique.

Creaming and flocculation. Just after preparation, the emulsion was poured into 10 ml graduated cylinders (time zero). The volume of the separated aqueous phase V (mL) was measured at time intervals chosen according to the phase separation rate; they increased from 15 sec to 60 sec during the first steps of the process, finally up to about 1 h.

The progress of creaming plus flocculation was followed by plotting V versus emulsion age (t min); the value V_e of V after 24 hr was taken as the equilibrium limit of the process. An example of such creaming and flocculation curve is given in Fig. 1a.

When coalescence was sufficiently slow, (no oiling off), it could be considered, as in the case of foams, that the separation of the aqueous phase from the emulsion was mainly controlled by a drainage type of process. Several authors (Mita et al., 1977; Waniska and Kinsella, 1979) found experimentally that foam drainage followed first order kinetics. The volume of the separated aqueous phase (V) increased with aging time (t) following the equation $V = V_o \exp(-Kt)$, K being the rate constant of drainage. In consequence, by analogy with the drainage process in foams, the separation kinetics of the aqueous phase in emulsions was taken as a first order reaction, described by the equation:

$$\frac{V_t - V_\infty}{V_o - V_\infty} = \exp \quad (-Kt) \qquad (2)$$

with V_t, volume of separated aqueous phase at t; K, rate constant of the process.

With the volume of the aqueous phase separated at time zero (V_o) being equal to zero, and V_∞ being the equilibrium volume of the separated aqueous phase (V_e), Eq. (2) becomes:

$$(V_e - V_t)/V_e = \exp \quad (-Kt) \qquad (3)$$

or

$$\text{Ln } V_e/(V_e - V_t) = Kt \qquad (4)$$

Analyzing the data by plotting $\ln V_e/(V_e - V_t)$ versus t, it was always observed that the global emulsion breaking can be adjusted with a succession of first order processes, as seen in Fig. (1b) which corresponds to the kinetic interpretation of the raw data in the Fig. (1a). Two first order phases, numbered 1 and 2 were distinguished. This analysis becomes blurred when V_t reaches values not far from V_e, because of the inaccuracy in volume determination. It is safer to discard data corresponding to V_t values higher than 0.85 to 0.90 V_e (part 3 of the plot on Fig. 1b).

Each kinetic phase is then characterized by its rate constant K and its duration Δt. The equilibrium state ($t \to \infty$) of the phase separation is defined by V_e or the limit ϕ_e of the volume fraction of the oil in the concentrated emulsion forming the upper creamed phase. Assuming that when $t \to \infty$, all the oil contained in the initial emulsion is in the upper phase,

$$\phi_e = \frac{2.5}{(10 - V_e) + 2.5} \qquad (5)$$

Stability towards coalescence. Graham's (1976) technique was used after slight modifications. Twelve milliliters emulsion (prepared as above) were poured immediately into a centrifuge tube and centrifuged 100 min at 1000 × *g*. The volume (V_s) of the separated oil phase was determined for different concentrations of proteins in the aqueous phase in the range 10^{-3} to 10 mg/mL. The quantity $100\ V_s/V_i (V_i = 3$ mL, initial volume of the oil in the emulsion), taken as a measure of coalescence, was plotted versus protein concentration.

RESULTS

Emulsifying properties of purified proteins

Emulsifying capacity (EC). The results of EC determinations for legumin, vicilin, bovine serum albumin and casein are shown in Table 2. Pea globulins had lower EC values than the standard proteins taken as reference, about 60g against about 100g oil per 100 mg protein. In the same table are given the corresponding volume fractions ϕ_o of the oil at the point of inversion: ϕ_o was about 0.38 for pea globulins against about 0.55 for BSA.

According to Ostwald's simple geometric theory of emulsion inversion which assumes a monodisperse suspension of

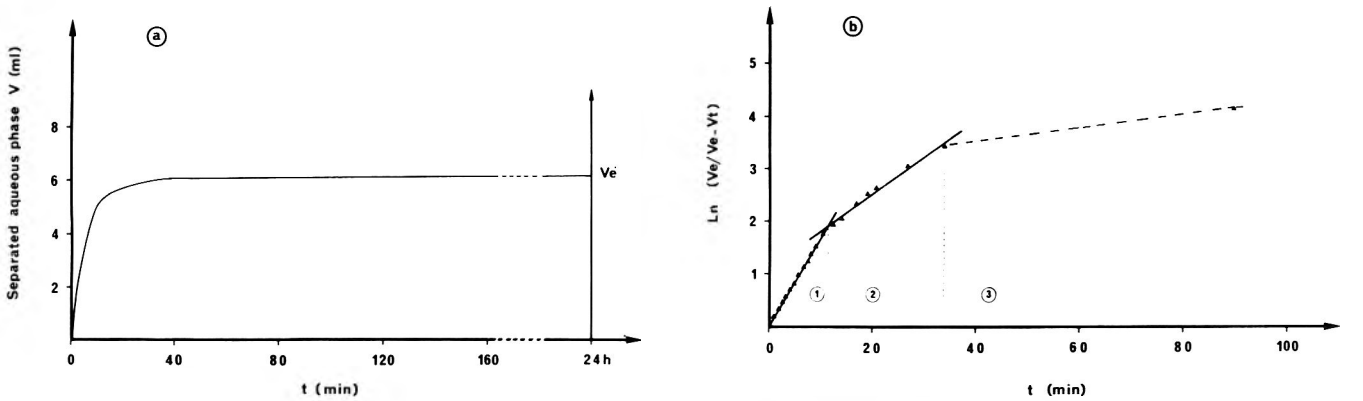

Fig. 1—Flocculation and creaming curve. 1a—Experimental curve; 1b—Kinetic interpretation.

Table 2—Emulsifying properties of purified proteins

Protein	EC[a] (g of oil/100 mg protein)	ϕ_o^b	EAI[c] (m/g)
Legumin	58	0.38	60
Vicilin	61	0.37	111
BSA	112	0.55	133
Casein	90	0.50	110

[a] Emulsifying capacity; measured at 1 mg/mL protein concentration
[b] Volume fraction of the oil at the inversion point of the emulsion
[c] Emulsifying activity index; measured at 1.3 mg/mL protein concentration

spherical undeformable droplets, the volume fraction of the dispersed phase at the inversion point should be $\phi_d = 0.74$. However, because of the vigorous stirring implied by the method used for EC determination, an ordered close packing of oil droplets in the emulsion at the inversion point was not probable, and ϕ_o should be compared to the mean volume fraction of a randomly packed bed of uniform spheres, which has been calculated to be 0.625 (Thomas, 1965). In fact, the pertinent volume fraction to be considered is not the oil phase, but that of the oil droplets surrounded by the interfacial layer; supposing that the dispersed spheres have a uniform radius r = a + h (a, radius of the oil droplet; h, thickness of the interfacial layer), the volume fraction of the dispersed phase at the point of inversion is

$$\phi_d = \phi_o \cdot (a + h)^3/a^3$$

simplifying to:

$$\phi_d = \phi_o [1 + 3 (h/a)] \quad (6)$$

if $h << a$. To explain the discrepancy between $\phi_o = 0.55$ and the theoretical value of 0.63, the ratio h/a has to be around 0.05. This value cannot be reached according to the average size of oil droplets in our emulsions (1 to 10 μm) and to the thickness h (100 A°) obtained by Graham and Phillips (1976) for proteins such as BSA and casein at 1 mg/mL bulk concentration. Thus, the simple geometric argument cannot explain the range of the values of ϕ_o and their differences between proteins. It is clear that the process of inversion as it is classically described (Becher, 1977), must be modulated by the structure and the rheological properties of the adsorbed protein layer; these characteristics of the layer, besides its thickness, could affect the value of ϕ_o. There is nevertheless an argument against a sensible incidence of the rheology of the interfacial layer on the values of EC or ϕ_o in our experimental conditions; at all interfacial concentrations the viscoelastic properties of adsorbed casein films are very low in contrast with those of BSA (Graham and Phillips, 1976), whereas these two proteins show similar EC and ϕ_o values (Table 2). In the last analysis, the primary causes of the discrepancies of ϕ_o values and the differences in EC values between proteins appear to be related to the experimental procedure of the determination of the inversion point. It involves a continuous creation of interfacial area at such a rate that the system is never at equilibrium. The volume of oil at the inversion point, for a given set of conditions, will depend on the ability of the protein to form rapidly enough an interfacial layer sufficient to prevent the coalescence of the newly formed droplets, i.e., chiefly on the adsorption rate and on the amount of the protein in the aqueous phase which is available to coat the excess interfacial area at every moment. Bovine serum albumin adsorbs much faster than pea globulins, and vicilin somewhat quicker than legumin at solution/air interfaces (Dagorn-Scaviner et al., 1986). On the other hand, the comparison of the interfacial concentration and interfacial pressure isotherms showed that BSA is much more efficient in covering the dodecane interface, at all bulk concentrations in the aqueous phase, than pea globulins; in this respect also, vicilin has a slight advantage over legumin (Dagorn-Scaviner, 1986).

Thus the adsorption kinetics and the isotherms of interfacial protein concentration seem to account qualitatively for the differences between the EC values of the proteins considered. The better interface coating behavior of BSA might also explain a better stabilization of the newly formed oil droplets leading to a higher ϕ_o. A real analysis of the intervention of the protein in the process of emulsion inversion would require a thorough characterization of the emulsions at and about the inversion point.

Emulsifying activity index — (EAI). Table 2 gives the results of EAI determination for the four proteins. As in the case of EC, BSA shows the higher value and legumin the lower, but in this case, vicilin does as well as casein.

Replacing in Eq. (1) the turbidity T by the relationship $T = K \pi a^2 N$ where a is the particle radius, N the concentration of particles and K the total scattering coefficient defined as $K = Ko (a/\lambda)^m$ (Reddy and Fogler, 1981) leads to:

$$EAI = 2K \pi a^2 \quad N/\phi C \quad (7)$$

As $\phi = N \times \frac{4}{3} \pi a^2/V_t$, it becomes

$$EAI = \frac{3}{2} \quad K_o \left(\frac{a^{m-1} V_t}{\lambda^m C}\right) \quad (8)$$

where V is the total volume of emulsion, λ the wavelength, K_o a size independent component of the scattering coefficient, and m an exponent which is smaller than 1. Equation (8) shows that in the case of monodisperse emulsion, EAI being a function of the particle size in the newly formed emulsion, is a measure of the ability of the protein to aid the dispersion of the oil phase and to provide very quickly a sufficient coating of the interfacial area to avoid immediate coalescence.

EAI is thus likely to depend first on the kinetics of the early

stagers of protein adsorption. Previously (Dagorn-Scaviner, 1986) the rate constants of the diffusion and penetration steps of the adsorption were shown to be sensibly higher for BSA and vicilin than for legumin; this was consistent with the EAI ranking of these proteins.

The values of EAI reported here for BSA and casein (133 and 110 m^2/g respectively) are somewhat lower than those obtained by Pearce and Kinsella (1978) who found 197 and 166, respectively, with similar experimental conditions except for the type of blender. Due to the fact that the emulsification procedure affects the radius of the droplets (Tornberg, 1978), the EAI, as stated by Pearce and Kinsella (1978), depends on the type of equipment used to produce the emulsion. It is however comforting to point out that the ratio of our EAI values to theirs is the same for BSA as for casein.

Emulsion stability

The different kinetic steps of emulsion breaking due to creaming and flocculation processes under aging, were characterized, as previously explained, by their duration (Δt) and their rate constants (K) for protein-stabilized emulsions at three protein concentrations (0.3, 0.5 and 1 mg/mL in the aqueous phase) (Table 3). It must be stressed that this treatment of the kinetics as a succession of first order steps was purely phenomenal and was intended to allow quantitative comparisons to be made conveniently but not to give an insight on the physical mechanisms underlying the global process. As already stated, the same approach has been applied to the kinetics of foam drainage by several authors (Mita et al., 1977; Waniska and Kinsella, 1979). Besides, it has been shown that emulsion coalescence followed first order kinetics (Becher, 1977).

For the two higher concentrations, two stages could be observed in the case of legumin- and vicilin-stabilized emulsions. The corresponding rate constants were lower for vicilin than for legumin and the Δt values accordingly higher. Phase separation was very much slower in the case of BSA; at 1 mg/mL BSA concentration, the emulsion reached such a high stability that no phase separation could be detected before 35 min. On the other hand, at the lower concentration, the migration of the oil droplets to the surface was so fast in the case of legumin and vicilin that only one step could be observed.

The differences observed in the efficiency of the three proteins in retarding emulsion breaking could reflect directly differences in the flocculation rates, attributable to repulsion forces between oil droplets stronger when they are BSA-coated than when they are vicilin- and especially legumin-coated due to the charge and the thickness of the adsorption layers. An alternative or concurrent explanation could, however, account for the facts. Although proteins are very efficient in preventing coalescence, some fusion of the primary droplets is inevitable during the experiment, at least at short times when the protein interfacial layer is far from fully constituted (protein adsorption is a slow process). The degree of this limited coalescence will control the creaming rate of the emulsion through the size of the effective droplets. The stabilizing effect of the proteins, as evaluated with the method, appeared linked to some degree with the kinetics of the early stages of their adsorption, the rates of which rank in the order BSA> vicilin> legumin (Dagorn-Scaviner, 1986).

At the equilibrium state of the flocculation-creaming process, the closeness of the droplets packing is measured by parameter ϕ_e, volume fraction of the oil in the creamed emulsion given by Eq. (5). The values of ϕ_e reported in Table 3 show that the final packing is denser for vicilin than for legumin and especially BSA. If one assumes that in all cases the limiting "equilibrium" state of the creamed emulsions corresponds to a close packed ordered arrangement of the disperse phase particles (oil droplets coated with interfacial layer), this means (see equation 6) that the effective thickness (including hydration and charge effects) of the interfacial layer is larger for BSA than for pea globulins. This probably contributes also to the higher stabilizing efficiency of the former protein (Graham and Phillips, 1976). The effective thickness increases somewhat with vicilin and legumin concentration.

Figure 2 gives the results of modified Graham's test. Protein concentrations in the aqueous phase required to prevent complete oiling-off were 0.42, 7 and 9 mg/mL for BSA, vicilin and legumin, respectively; they became 0.03, 0.22 and 0.54 mg/mL, respectively, when the 50% oiling-off level was considered. Graham and Phillips (1976) concluded that "for optimum prevention of coalescence the interfacial protein layer had to be as thick as possible and heavily hydrated and charged". The higher resistance to coalescence of BSA films, as compared to those of pea globulins, was in accordance with their higher effective thickness which was inferred from the above results on ϕ_e. However, legumin, which appeared to give thicker films (lower ϕ_e values) than vicilin (Table 3), proves less efficient in preventing coalescence. A tentative conclusion would be that the effective thickness of the interfacial layer was not the sole factor but that differences in the adsorption rates could probably affect the results of the coalescence test in a similar way as explained in the discussion of the results of the creaming-flocculation test.

Emulsifying properties of mixed pea globulins

The emulsifying properties of mixed pea globulins were studied for 4 vicilin/legumin ratios (V/L = 0.33, 0.5, 1.0, 3.0) (Table 4). The emulsifying capacity (EC) was little affected by this ratio and was close to 50g oil per 100 mg of protein except for a vicilin/legumin ratio of 0.33. In this case

Table 3—Comparison of the flocculation and creaming parameters for emulsions obtained with various concentrations of bovine serum albumin, legumin and vicilin

		Flocculation - creaming				Equilibrium state
		step 1		step 2		
Protein	Conc[a] (mg/mL)	Δt_1^b (min)	K_1^b ($10^{-4}s^{-1}$)	Δt_2^b (min)	K_2^b ($10^{-4}s^{-1}$)	ϕ_e^c
Legumin						
	0.33[d]	2.0	178			0.71
	0.50	3.0	96	6.0	29	0.65
	1.06	3.5	71	6.5	24	0.62
Vicilin						
	0.32	9.0	68			0.83
	0.51	6.0	60	8.0	27	0.78
	1.09	11.0	27	23.0	11	0.65
BSA						
	0.33	30.0	3.0	57.0	5	0.59
	0.55	21.0	3.4	63.0	4	0.58

[a] Concentration of the protein in the aqueous phase

[b] Δt and k = duration and rate constant, respectively, of the i^{th} first order kinetic step as defined in Fig 1b.

[c] ϕ_e = oil volume fraction in the creamed emulsion (Eq. 5)

[d] One phase observable only.

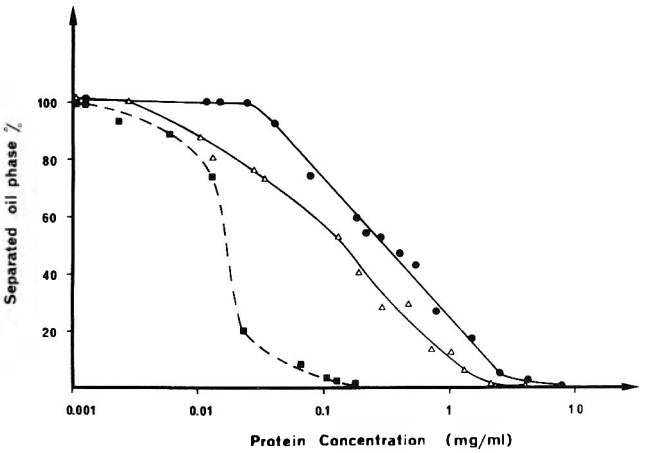

Fig. 2—Coalescence of emulsions expressed as the separated oil volume percent of the total oil phase at different protein concentration for bovine serum albumin (■, legumin (●), and vicilin (△).

it was 45g oil per 100 mg of protein, leading to a slightly lower oil phase volume fraction (ϕ_o) in the emulsion at the inversion point; at V/L = 0.33, ϕ_o was 0.37 instead of 0.40.

The EC values found for each globulin alone were in every case higher than those obtained when both proteins were mixed. According to the definition of emulsifying capacity and as the interfacial area developed by the Waring Blendor was quite constant, the results indicated that in the case of globulin mixtures, a larger amount of protein was needed at the interface to stabilize the emulsions. This could be related to a less efficient organization of the interfacial layers when the two proteins were mixed than when they acted separately.

The comparison of the emulsifying activity index, obtained for the mixed protein solutions, showed that it slightly decreased when the legumin proportion increased, as one could suppose from the EAI values determined for each globulin alone. Besides, when either vicilin or legumin were in high proportion in the mixture (V/L = 3 or 0.33), the EAI reached higher values than those obtained, respectively, with each globulin indicating that in these conditions the minor protein contributed to the emulsification. These EAI results did not agree with the EC data, which showed a lesser efficiency of the mixtures and nearly no influence of the V/L ratio. The EC evaluations used a dynamic method in which the interfacial layers and the droplets were submitted to a continuous drastic shear stress; on the other hand in the EAI method the emulsion was blocked in its initial state by SDS addition and tested at rest. In consequence, in the case of EC measurements, the structure and mechanical properties of the interfacial layers probably played some role in addition to the kinetics of adsorption. The EAI data were in better agreement with the previous results on adsorption kinetics and isotherms of pea globulins (Dagorn-Scaviner et al., 1986). The authors showed indeed that the equilibrium surface pressure (π_e) regularly increased with increasing vicilin/legumin ratio; and π_e rapidly decreased when legumin became the major protein. As the vicilin proportion in the mixture increased, the emulsions stability became higher, but remained, even at V/L = 3, lower than for vicilin alone. It can also be observed that the volume of separated aqueous phase was lower for legumin alone than for V/L = 0.33 mixture. This is more precisely described by the rate constant values (Table 4).

The lesser stability of the emulsions prepared with the protein mixtures was also shown by the higher oil volumes separated during the coalescence test. The separated oil volume was comprised between 22% and 53% of the total oil phase, depending on the V/L ratio, instead of 10% for vicilin and 22% for legumin at the same concentration (1 mg/mL) (Table 4). The oil separated volumes relative to the mixtures were compared to those obtained for each globulin taken alone at the same concentration as in the mixed solution. For example, if V/L = 0.33 for a bulk concentration of 1 mg/mL, the contribution of each protein should be equivalent to that of legumin at 0.75 mg/mL and vicilin at 0.25 mg/ml, if these contributions were additive. This comparison (Fig. 4) shows that the oil separated volumes of the mixtures were very close to those obtained for vicilin alone, especially when vicilin was the major protein. This indicates that legumin contributed only slightly to the emulsion stability but did not inhibit vicilin adsorption; on the other hand, when legumin was the main protein (V/L = 0.33), the coalescence was more pronounced than for legumin alone.

In conclusion, whatever the emulsifying parameters considered, the emulsifying efficiency of mixed pea globulins was lower compared to that of the protein alone. However, even under these conditions, vicilin proved more efficient than legumin alone. In fact, in previous studies on the interfacial behaviors of vicilin and legumin (Dagorn-Scaviner et al., 1986), it was shown that in the case of the protein mixtures the minor protein seems to disturb the molecular rearrangement processes of the major one in the interfacial layer. Moreover, it was observed that a small quantity of legumin added to vicilin did not modify appreciably its surface activity; on the contrary a small amount of vicilin in legumin sample considerably affected its efficiency. It was concluded that few vicilin molecules in the legumin layer suffice to inhibit some interfacial denaturation processes whereas vicilin was less disturbed by legumin.

Thus the lower emulsifying efficiency of the mixed globulins observed in the present paper seemed eventually to be related to the molecular heterogeneity of the interfacial layer,

Table 4—Influence of vicilin/legumin (V/L) ratio on the emulsifying properties of pea globulin mixed solutions

	EC[a] g/oil/100 mg	ϕ_o[b]	EAI[c] (m^2/g)	Stability[d] Flocculation-creaming constant rate K_1 ($\times 10^{-4} sec^{-1}$)	K_2 ($\times 10^{-4} sec^{-1}$)	Coalescence[e] sep. oil vol
Legumin	58	0.42	60	71	24	22
Vicilin	58	0.45	111	27	11	10
Mixtures (vicilin-legumin) V/L ratio						
3	48	0.39	140	46	20	22
1	50	0.40	110	50	16	35
0.50	51	0.41	113	n.d	n.d	n.d
0.33	45	0.37	90	109	42	53

[a] Emulsifying capacity (EC) measured at 1.25 mg/mL protein concentration.
[b] Volume fraction of the oil at the inversion point of the emulsion.
[c] Emulsifying activity index (EAI) measured at 1.30 mg/mL protein concentration.
[d] Stability measured at 1 mg/mL.
[e] Coalescence expressed as the separated oil volume percent of the total oil phase in the emulsion (% v/v); n.d = not determined.

Table 5—Emulsifying properties of various pea isolates

Cultivar	Protein sample	V/L[a]	EC[b] g of oil/100 mg	ϕ_c^b	Coalescence[d] separated oil volume
SITELLE	whole isolate	4.9[e]	59	0.45	10
	PBIE *	2.6	83	0.46	5
AMINO	whole isolate	1.6[e]	59	0.44	14
	PBIE *	1.4	77	0.44	3
COLMO	whole isolate	1.4[e]	62	0.46	7
	PBIE *	1.1	81	0.42	5
FINALE	whole isolate	1.5[e]	59	0.44	8
	PBIE *	0.7	83	0.45	3

[a] Vicilin to legumin ratio.
[b] Emulsifying capacity; protein concentration 1.25 mg/mL.
[c] Volume fraction of the oil at the inversion point of the emulsion.
[d] Coalescence expressed as the separated oil volume percent of the total oil phase in the emulsion (% v/v); protein concentration 1 mg/mL.
[e] Determined in the original flour.
* PBIE, phosphate buffer (pH 8—0.1M) isolate extract.

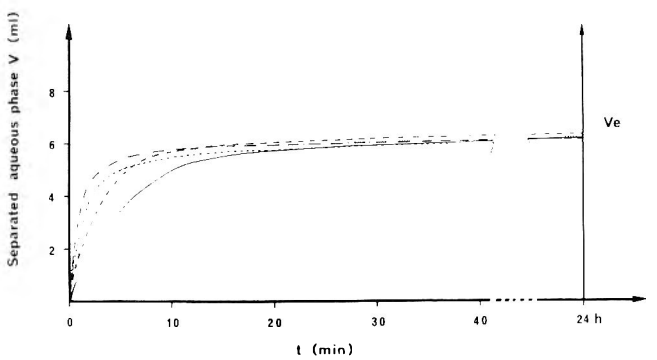

Fig. 3—Flocculation and creaming for emulsions prepared with different V/L protein ratio; (protein concentration, 1.3 mg/mL); vicilin, ———; legumin, ———; V/L = 1/3 —·—; V/L = 1 .....; V/L = 3 — — — —).

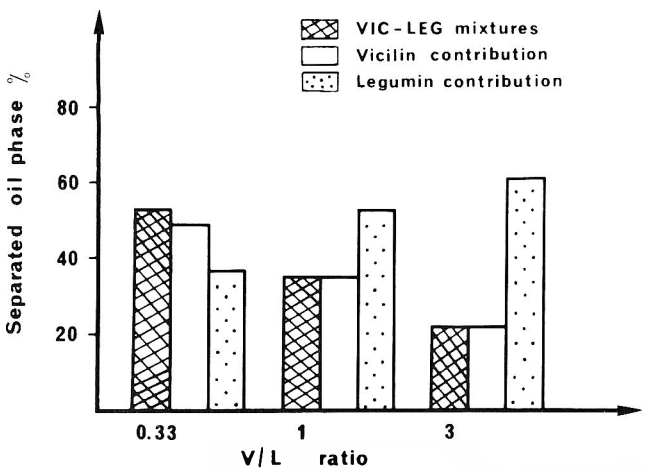

Fig. 4—Coalescence of emulsions prepared at various V/L ratios. Comparison with the data obtained for the globulin as single protein in the mixture.

which might induce higher fragility of the film. Moreover, the effect of vicilin on legumin adsorption, which was observed to be more disturbing than the reverse, may explain the particularly weak stability of the emulsions prepared with legumin as major protein in the mixture (V/L = 0.33).

Emulsifying properties of pea isolates

As a practical consequence of the present results on the emulsifying properties of vicilin/legumin mixtures, the extent of the relation of the emulsifying properties of the isolates to their globulin composition were examined. Because the isolates were only partly soluble in sodium phosphate buffer 0.1M — pH 8 (60-70% solubility), this study was carried out both on the whole isolate and on its soluble fraction. The vicilin/legumin ratios which were easily determined by the rocket immunoelectrophoresis technique of Laurell (Casey et al., 1982) in the case of the soluble fractions could not be directly evaluated on the whole isolates because of their partial insolubility and were taken to be the same as in the corresponding flour. It was indeed shown by Olsen (1977) that vicilin and legumin were quantitatively precipitated in the isolates at pH 4.5.

The emulsifying capacities of the four isolates (Table 5) were very close to each other, around 60 g oil per 100 mg of protein for the whole isolates and 80g for the soluble fractions.

The EC values of the soluble fraction (PBIE), obtained at protein concentration around 1 mg/mL, were higher than those of the globulins, alone or mixed, at the same concentration. This could be related to the presence of polar lipids associated with the proteins in the isolates and especially of phosphatidylcholine (Table 1) which is known to favor the formation of oil-in-water emulsions. The stability towards coalescence of the emulsions prepared with the isolates was studied for 1 mg/mL protein concentration in the aqueous phase. The volume of the coalesced oil phase did not represent more than 7 to 14% of the total oil phase under these conditions. It reached 10% and 22%, respectively, for vicilin and legumin and was between 22% and 53%, according to the V/L ratio for the mixed globulins at the same total concentration.

No evident influence of the vicilin/legumin ratio was observed, whereas in the case of the purified mixed globulins, the emulsion stability increased with the vicilin proportion. The effect of the globulins ratio was perhaps masked by the presence of phospholipids which acted as efficient surface active components.

As indicated by the rather low degree of solubility of the isolates, pea globulins underwent partial denaturation during the technological process of isolate preparation. This also modified their emulsifying behaviors and explained, besides the presence of lipids, that the isolates had different emulsifying properties compared to the native globulins mixtures.

CONCLUSION

VICILIN appeared to be a more efficient emulsifying agent than legumin, but was less effective than BSA. In the case of vicilin-legumin mixtures, the better emulsifying results were obtained when vicilin was the major protein; but, whichever the major protein was, the stability of the emulsion was affected by the minor one.

Besides the effectiveness of proteins in increasing the equilibrium interfacial pressure, as well as the characteristics of the adsorbed layers, the kinetic aspects of protein adsorption,

which are often overlooked, were also important in explaining the emulsifying properties of proteins.

A more direct assessment of the role of the different parameters of protein adsorption would require more elaborate methods to study the emulsifying properties; in those currently used, the various processes implied in emulsion stabilization or breaking occurred in a too intricate way. On the other hand, the creaming-flocculation kinetic interpretation here developed and Graham's coalescence test, seem to be more suited to study the different mechanisms of emulsion breaking.

REFERENCES

Becher, P. 1977. "Emulsions: Theory and Practice." Krieger R.E. Publishing Company Huntington, New York.

Casey, R., Sharmann, J.E., Wright, D.J., Bacon, R.J., and Guldager, 1982. Quantitative variability in Pisum seed globulins: its assessment and significance. Qual. Plant. Plant Foods Hum. Nutr. 31: 333.

Crenwelge, D.D., Dill, C.W., Tybor, P.T., and Landmann, W.A. 1974. A comparison of the emulsification capacities of some protein concentrates. J. Food Sci. 39: 175.

Dagorn-Scaviner, C. 1986. Etude des propriétés tensio-actives des globulins du pois. Application aux isolats protéiques utilisés comme agents d'émulsification ou de moussage. Thesis in Food Science, Univ. of Nantes, (France).

Dagorn-Scaviner, C., Gueguen, J., and Lefebvre, J. 1986. A comparison of interfacial behaviours of pea (Pisum sativum L.) legumin and vicilin at air/water interface. Die Nahrung 30: 337.

Drapron, R. 1975. Méthode rapide de détermination quantitative des lipides totaux dans les produits céréaliers. Ann. Techn. Agric. 24: 117.

Graham, D.E. 1976. Structure of adsorbed protein films and stability of foams and emulsions. PhD. thesis CNAA, London.

Graham, D.E. and Phillips, M.C. 1976. The conformation of proteins at interfaces and their role in stabilizing emulsions. In "Theory and Practice of Emulsion Technology." Smith A.L. (Ed), p. 75. Academic Press, London.

Gueguen, J., Quemener, B., and Valdebouze, P. 1980. Elimination des facteurs antinutritionels de la Féverole Vicia faba L., et du pois Pisum sativum L. au cours de la préparation des isolats protéiques. Lebensm. Wiss. U. Technol. 14: 72.

Gueguen, J., Vu, A.T., and Schaeffer, F. 1984. Large-scale purification and characterization of pea globulins. J. Sci. Food Agric. 35: 1024.

Kirschenbaum, D.M. 1971. Molar absorptivity and $A_{1cm}^{1\%}$ values for proteins at selected wave lengths of the ultraviolet and visible region. II. Int. J. Protein Res. 3: 157.

Mita, T., Nikai, K., Hiraoka, T., Matsuo, S., and Matsumoto, M. 1977. Physico-chemical studies on wheat protein foams. J. Coll. Interface Sci. 59: 172.

Nakai, S., Ho, L., Helbig, N., Kato, A., and Tung, M.A. 190. Relationship between hydrophobicity and emulsifying properties of some plant proteins. Can. Inst. Food Sci. Technol. J. 13: 23.

Olsen, H.S. 1977. Faba bean protein for human consumption. In "Biochemical Aspects of New Protein Food." J. Adler-Nissen, B.O. Eggum, L. Munck, and H.S. Olsen (Ed), p. 31. Pergamon Press.

Pearce, K.N. and Kinsella, J.E. 1978. Emulsifying properties of proteins: Evaluation of a turbidimetric technique. J. Agric. Food Chem. 26: 716.

Phillips, M.C. 1981. Protein conformation at liquid interfaces and its role in stabilizing emulsions and foams. Food Technol. 35: 50.

Reddy, S.R., and Folger, H.S. 1981. Emulsion Stability: determination from turbidity. J. Coll. Interface Sci. 79: 101.

Thomas, D.G. 1965. Transport characteristics of suspension: VIII. A note on the viscosity of Newtonian suspension of uniform spherical particles. J. Colloid Sci. 20: 267.

Tornberg, E. 1978. Functional characterization of protein stabilised emulsions: emulsifying behaviour of proteins in valve homogeniser. J. Sci. Food Agric. 29: 867.

Waniska, R.D. and Kinsella, J.E. 1979. Foaming properties of proteins: evaluation of a column aeration apparatus using ovalbumin. J. Food Sci. 44: 1398.

Webb, N.B., Ivey, F.J., Craig, H.B., Jones, V.A., and Monroe, R.J. 1970. The measurement of emulsifying capacity by electrical resistance. J. Food Sci. 35: 501.

Ms received 6/4/86; revised 11/13/86; accepted 11/13/86.

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REFERENCES

Abdul-Kadir, R.B. 1980. The effect of phytate content on the nutritional quality of soy and wheat bran proteins. M.S. thesis, Univ. of Nebraska, Lincoln.

Anonymous. 1986. Sigma Chemical Catalog, p. 1025. St. Louis, MO.

Brooks, J.R. and Morr, C.V. 1982. Phytate removal from soy protein isolates using ion exchange processing treatments. J. Food Sci. 47: 1280.

Brooks, J.R. and Morr, C.V. 1984. Phosphorus and phytate content of soybean protein components. J. Agric. Food Chem. 32: 672.

Camus, M.C. and LaPorte, J.C. 1976. Inhibition de la proteolyse pepsique in vitro par le ble. Role de l'acide phytique des issues. Ann. Biol. Anim. Biochem. Biophys. 16: 719.

Frieden, E. and Walter, C. 1963. Prevalence and significance of the product inhibition of enzymes. Nature 198: 834.

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

Hill, B.S., Snyder, H.E., and Weise, K.L. 1982. Use of the pH stat to evaluate trypsin inhibitor and tryptic proteolysis of soy flours. J. Food Sci. 47: 2018.

Horigome, T. and Kondatsu, M. 1968. Biological value of proteins allowed to react with phenolic compounds in presence of orthodiphenol oxidase. Agric. Biol. Chem. 32: 1093.

How, J.S.L. and Morr, C.V. 1982, Removal of phenolic compounds from soy protein extracts using activated carbon. J. Food Sci. 47: 933.

Hsu, H.W., Vavak, D.L., Satterlee, L.D., and Miller, G.A. 1977. A multienzyme technique for estimating protein digestibility. J. Food Sci. 42: 1269.

Hugli, I.E. and Moore, S. 1972. Determination of the tryptophan content of proteins by ion exchange chromatography of alkaline hydrolyzates. J. Biol. Chem. 247: 2828.

Hurrel, R.F., Furot, P.A., and Cuq, J.L. 1982. Protein-polyphenol reactions. Brit. J. Nutr. 47: 191.

Knuckles, B.E., Kuzmicky, D.D., and Betschart, A.A. 1985. Effect of phytate and partially hydrolyzed phytate on in vitro protein digestibility. J. Food Sci. 50: 1080.

Kratzer, F.H. 1965. Soybean protein-mineral interrelationships. Proc. Fed. Am. Soc. Exp. Biol. 24: 1498.

Lahiry, N.L., Satterlee, L.D., Hsu, H.W., and Wallace, G.W. 1977. Characterization of the chlorogenic acid binding fraction in leaf protein concentrate. J. Food Sci. 42: 83.

Lehninger, A.L. 1970. In "Biochemistry" p. 97. Worth Publishers, New York.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265.

Monod, J., Wyman, J., and Changeux, J.A. 1965. On the nature of allosteric transitions: a plausible model. J. Mol. Biol. 12: 88.

Moore, S. 1963. On the determination of cystine as cysteic acid. J. Biol. Chem. 238: 235.

Moore, S. and Stein, W.H. 1963. In "Methods in Enzymology," vol. 6, p. 819. Academic Press, N.Y.

Palmer, T. 1981. "Understanding Enzymes," p. 88. Ellis Horwood Ltd., Chichester, England.

Pedersen, B. and Eggum, B.O. 1983. Prediction of protein digestibility by an in vitro enzymatic pH stat procedure. Z. Tierphysiol. Tierernahrg. u. Futtermittelkde. 49: 265.

Ritter, M.A. 1985. Effect of phytate and phenolics upon the in vitro digestibility of soy protein isolates. M.S. thesis, Clemson Univ., Clemson, SC.

Rodriguez, C.J., Morr, C.V., and Kunkel, M.E. 1985. Effect of partial phytate removal and heat upon iron bioavailability from soy protein-based diets. J. Food Sci. 50: 1072.

Rothenbuhler, E. and Kinsella, J.E. 1985. The pH stat method for assessing protein digestibility: An evaluation. J. Agric. Food Chem. 33: 433.

Sekul, A.A. and Ory, R.L. 1977. Rapid enzymatic method for partial hydrolysis of oilseed proteins for food uses. J. Am. Oil Chem. Soc. 54: 32.

Seo, A. and Morr, C.V. 1984. Improved high-performance liquid chromatographic analysis of phenolic acids and isoflavonoids from soybean protein products. J. Agric. Food Chem. 32: 530.

Serraino, M.R., Thompson, L.U., Savoie, L., and Parent, G. 1985. Effect of phytic acid on the in vitro rate of digestibility of rapeseed protein and amino acids. J. Food Sci. 50: 1689.

Sharma, C.B., Goel, M., and Irshad, M. 1978. Myoinositol hexaphosphate as a potential inhibitor of α-amylases. Phytochem. 17: 201.

Singh, M. and Krikorian, A.D. 1982. Inhibition of trypsin activity in vitro by phytate. J. Agric. Food Chem. 30: 799.

Stinson, C.T. and Snyder, H.E. 1980. Evaluation of heated soy flours by measurement of tryptic hydrolysis using a pH stat. J. Food Sci. 45: 936.

Thanh, V.H. and Shibasaki, K. 1976. Major proteins of soybean seeds: A straight-forward fractionation and their characterization. J. Agric. Food Chem. 24: 1117.

Wolf, W.J. and Cowan, J.C. 1975. "Soybeans As a Food Source," Rev. ed. CRC Press, Boca Raton, FL.

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Air Drying Characteristics of Apricots

EL HALOUAT ABDELHAQ and T. P. LABUZA

ABSTRACT

The optimization of the drying of apricots was studied using four treatments: (1) blanching and drying; (2) sulfiting-blanching and drying; (3) blanching-sulfiting and drying; and (4) sulfiting-drying to 50% moisture-blanching and finish drying. Levels of sulfiting were from 0–2000 ppm SO_2 and drying was done at 50° to 80°C. The quality of dried apricots was judged by extent of browning development and hardness determination. A surface response statistical design was applied to evaluate the optimum drying conditions. Sulfiting-drying, using 800–1000 ppm SO_2 at any temperature in the range 50–80°C, was found to be the best treatment. Thus, sulfite was the major factor in controlling dry apricot quality and would be hard to reduce. Drying time was reduced by 50% when apricots were dried at 80°C compared to 50°C, and blanching reduced the time by 10 to 20%. Loss of SO_2 was greater than 50% for all treatments.

INTRODUCTION

BECAUSE of the short harvest season and the sensitivity to storage even at refrigerated conditions, most fresh apricots are preserved in some form. Drying is among the methods which are commonly used (Bolin et al., 1981). Sun drying permits one to produce a product with a rich orange color, a translucent appearance and a desirable gummy texture; however, it has many disadvantages. Notable among these are the slowness of the process, the exposure to environmental contamination, the dependency on weather, and the hand labor requirement (Noyes, 1969; Bolin et al., 1981).

In recent years, air dehydration has been applied to avoid these disadvantages; however, there is a question concerning the pretreatment and drying conditions. Fresh fruit sulfiting (includes use of soluble and gassing methods) is usually used before drying, but the correct amount of SO_2 necessary to prevent browning and ensure stability during storage of dried apricots is not exactly known. Some authors have suggested less than 1000 ppm SO_2 in the fresh tissue (Noyes, 1969; Torrey, 1974), while others have mentioned higher concentrations (Mrak et al., 1943; Dahlenburg, 1976). Blanching is also used sometimes to improve dried fruit quality and to reduce drying time (Mrak et al., 1943). Another unanswered point is the effect of air drying temperature in the range 50–80°C, especially on browning (Bolin et al., 1981; Resnick and Chirife, 1979).

The objective of this study was to determine the effect of pretreatment method and drying temperature on the quality of dried apricots (as determined by color and texture) and to choose the treatment which least affects the color and the texture of the end product.

MATERIALS & METHODS

Materials

Fresh apricots from California were purchased from a Minnesota produce supplier (July through September) and held at 4°C until used (within 2 wk). The Patterson apricot variety (100 kg) was used for drying and sorption isotherm determinations, while the Perfection variety (27 kg) was used to study kinetics of browning and to repeat the best drying treatment. Because of the short production season and limited equipment availability, it was impossible to perform all of the experiments on one variety.

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Experimental design

A Response surface design was used to determine the effect of two independent variables (temperature and SO_2 content) on the dependent variables (browning and hardness). The boundary conditions for this study were the choice of four different pretreatments, different drying temperatures in the range 50–80°C, and sulfiting between 0–2000 ppm. A surface response design was used to determine the optimal conditions. The study was done in five dryers using temperatures in the range 50–80°C, with sulfiting from 0 to 2000 ppm and four different treatments, namely: (1) sulfiting-drying (S/D), (2) sulfiting-blanching-drying (S/B/D), (3) blanching-sulfiting-drying (B/S/D), and (4) sulfiting-drying to 50% moisture-blanching-final drying ($S/D_1/B/D_2$). It has been suggested that partial drying before blanching improves the color and the textural quality of the dried fruit (Nury et al., 1960; Torrey, 1974). For each treatment, nine trials were carried out, supplemented with a duplicate of a center point to estimate experimental error (Fig. 1). A total of 40 runs were randomly conducted and 120 data points were collected and statistically analyzed using the CADE software package based on least squares regression (International Qualtech, Plymouth, MN). The regression coefficients (A_i) for the combined effects of the independent variables were estimated according to a second degree polynomial model.

$$Y = A_o + A_1X_1 + A_2X_2 + A_{12}X_1X_2 + A_1X_1^2 + A_2X_2^2$$

where Y = estimated value for browning or hardness, and A_i = regression coefficients for each parameter X_1 and X_2 in coded units (X_1 represents temperature, X_2 represents sulfite).

In order to visualize the combined effects of the temperature and SO_2 content on the two dependent variables, response surface contour plots were generated using the contour plotting program.

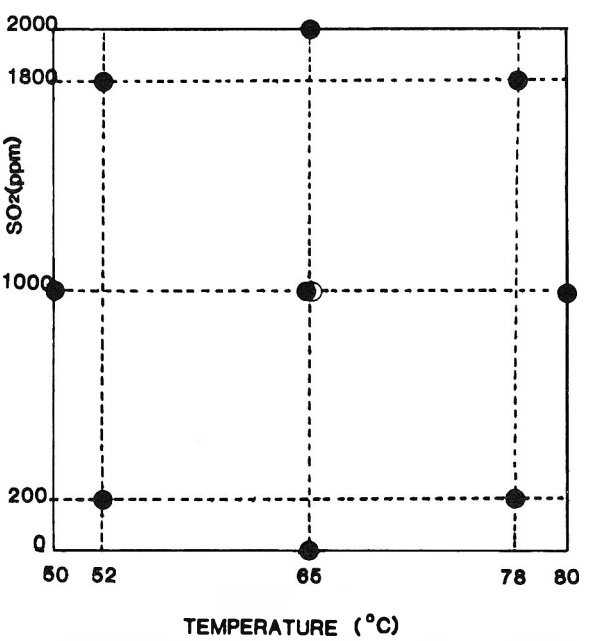

Fig. 1—Response surface Star diagram showing design for experimental optimization.

Methods

Moisture was determined by the AOAC (1984) vacuum oven method at 70°C for 24 hr for the wet slices and six hours for dried fruit. Sulfite content was determined by the AOAC (1984) procedure 201.04.

Isotherms were prepared using the desiccator technique at 25°C (Labuza, 1984) with storage for up to 21 days for adsorption and 45 days for desorption. Freeze dried apricot samples were used for the adsorption studies. To prevent mold growth, a small dish containing 1.5 ml toluene was placed in the glass desiccators used for desorption study at water activities above 0.7.

In order to measure the effect of a temperature shift on the moisture sorption isotherms of apricots, a DT Kaymont Hygroscope (Kaymont Instrument, Huntington Station, NY) was used. Each sample used in the desiccator method was put into the Kaymont chamber and held at 25°C for 2 hr to reach equilibrium with the head space before the reading was taken. The %RH was also read after a temperature shift to 35°C and 45°C (as in the method of Labuza et al., 1985). The Kaymont apparatus was calibrated at 25°, 35° and 45°C by using saturated salt solutions of known a_w.

Fresh apricots were removed from the storage room (4°C) just before drying; they were washed, cut to create two halves and to remove the pit, then pretreated immediately to minimize undesirable enzymatic activity.

Sulfite addition was performed by dipping fresh or blanched apricot halves into Na-bisulfite solutions to achieve levels of 0, 200, 1000, 1800 and 2000 ppm of SO_2 in fresh tissues. Sulfited halves were then placed in the cup-side up position on the dryer trays to prevent bleeding and sticking.

Blanching was done in an autoclave by exposing the trays containing apricot halves, in the cup-up position, to steam at 100°C for 2 min. There was no attempt to optimize blanching time and temperature in this study. Experimental runs were performed in five identical tray dryers which were built specially for this study (Pioneer Products, Minneapolis, MN). The apricot halves were placed on four nylon mesh trays of size 36 cm × 36 cm. The loading was about 2.5 kg/m^2. The selected temperatures of drying were 50°, 52°, 65°, 78° and 80°C for each of the four treatments. Every hour, to get uniform drying, all trays were moved one step up or down depending on their position and also rotated one half turn. The end of drying was fixed at 20% moisture (wb).

Browning was determined by putting a sample of mixed ground dried apricot containing 8 g solids in a 250 mL Erlenmeyer flask containing 100 mL 50% (v/v) ethanol solution. The flask was then covered with parafilm and allowed to remain at room temperature (23°C) for 24 hr with occasional shaking. After the solution was filtered through Whatman #2 filter paper, the O.D. of the eluent was read in a Coleman Junior II Spectrophotometer at 440 mm using a 0.5 cm diameter tube. The results were recorded in absorbance units using a 50% ethanol solution for zero adjustment.

To study browning kinetics, halved and pitted Perfection apricots, pretreated at the optimized conditions (described later), were dried at 50°, 65° and 80°C. About ten samples (containing 10g solids each) were taken over time of drying for browning determination using the procedure described above.

Texture was estimated by a hardness determination on the Instron Universal Testing Machine (Model No. 1122). From each drying run, five dried apricot halves put in the cup side down position were punched five times in different places with a cylindrical 0.1 inch diameter flat-ended punch. The mean of the maximum force for 25 punches was recorded as the hardness of the sample in gram force then converted to Newtons/m^2.

RESULTS & DISCUSSION

Moisture sorption isotherms

The moisture sorption isotherms of apricots, determined at 25°C by the desiccator method (Fig. 2) follow the shape typical of most fruits (Iglesias and Chirife, 1982). Also, because of the high sugar content in apricots (80% of total solids), hysteresis was important. The effect of temperature on the isotherm using the Kaymont shift method is shown in Fig. 3. At higher a_w the a_w is lower for the same moisture content in comparison to the 25°C data. This might be due in part to the fact that equilibrium in the sample head space may not have been reached in the chamber in the two hours, since an increase is expected based on the Clausius Clapeyron equation (Labuza et al., 1985). The crossover of the isotherm curves at high a_w is also typical of high sugar products and may be due to solubility changes (Loncin et al., 1968). The isosteric excess heat of sorption, as determined by the Clausius Clapeyron equation, ranged from 300 cal/mole at a moisture of 6.9g H_2O/100g solids to a value of 600 cal/mole at moisture of 13 g H_2O/100 g. These are in the range of those reported for banana, pineapple, and grapefruit (Iglesias and Chirife, 1976). These data can be used to generate the isotherms at the drying temperatures necessary for prediction of the equilibrium moisture value required for use in determining the diffusion coefficient.

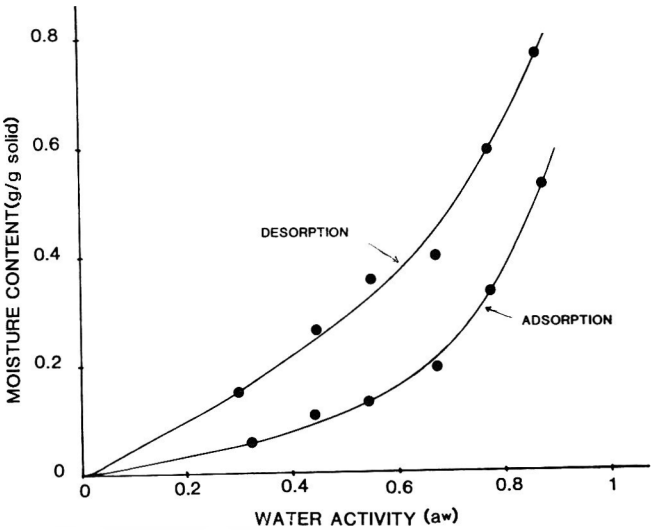

Fig. 2—Adsorption and desorption isotherms at 25°C for dried apricots.

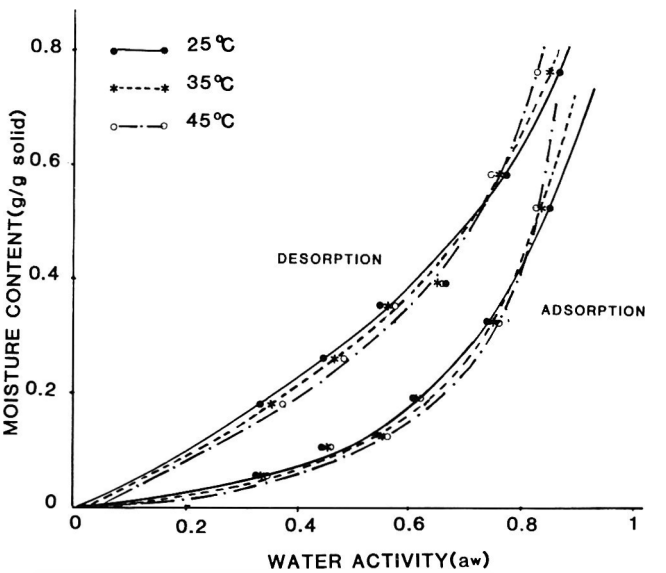

Fig. 3—Effect of temperature shifts at constant moisture on the water activity of dried apricots.

According to these sorption isotherms it should be more efficient to stop drying at 28% (0.4g H_2O/g solid) rather than drying to 20% moisture content (wb) followed by remoisturizing to 25–28% as is typical in the industry (Noyes, 1969; McBean and Wallace, 1967; Torrey, 1974). In the first case the product follows the desorption isotherm curve, thus at 28% moisture (wb), the a_w is around 0.5 which should keep the dried apricot stable towards microbes during storage. However, if the drying goes too far (20% moisture), the dried apricot must then be remoistened to reach 28%. In this case it

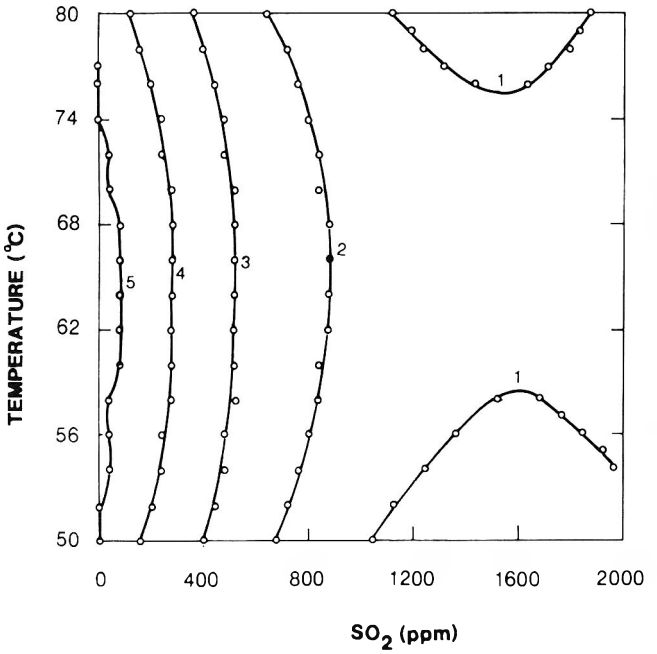

Fig. 4—Surface response contour plot for effect of temperature and SO_2 level on browning (O.D. units) after drying. Contour lines: 1 = 0.05; 2 = 0.10; 3 = 0.15; 4 = 0.20; 5 = 0.25.

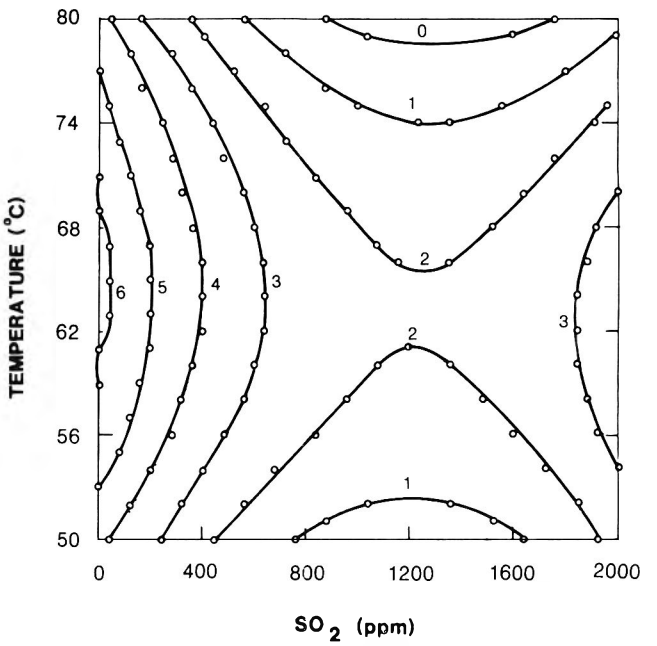

Fig. 5—Surface response contour plot for effect of drying temperature and sulfite level on punch value (hardness in N/m²) for unblanched apricots dried to 28% moisture. Contour lines: 0 = 1.6 × 10⁸ N/m²; 1 ; = 2.0 × 10⁸; 2 = 2.4 × 10⁸; 3 = 2.8 × 10⁸; 4v = 3.2 × 10⁸; 5 = 3.6 × 10⁸; 6 = 10⁸. Sulfite addition only.

will follow the adsorption isotherm curve (Labuza, 1984) where the final a_w is around 0.8, which makes it susceptible to microbiological spoilage, a common problem in the industry (Stadman et al., 1966). Of course since most drying is done on the farm, it is safer to overdry and easier to handle the product at 20% moisture.

Optimization of apricot drying

Although standards for dried apricot quality have not yet been established, the best quality of apricots could be considered to be those which are translucent and gummy. Thus, in this study it was considered that the best treatment would be one with a low degree of browning and a low hardness force. It was assumed that low hardness was also related to the desirable gumminess.

Response surface analysis permits one to visualize the combination effects which are significant at the 5% level (Fig. 4 to 7). This analysis shows that using 800–1000 ppm SO_2, at any temperature in the range 50°–80°C, was the best treatment. Thus blanching could be excluded which also eliminates a cooked flavor.

It appears that temperature in the range studied had only a minimum effect on browning and the hardness of dried apricot while sulfiting was the controlling factor. Most workers in fruit drying confirm the important role played by SO_2 in preventing browning (Joslyn and Braverman, 1954; Burton et al., 1963; McBean et al., 1964; Bolin and Staford, 1974). The extent of browning was negligible (O.D. < 0.1) for the Perfection variety using the 800 ppm SO_2 treatment without blanching and drying at 50°, 65° and 85°C. The results best fit zero order kinetics with an activation energy of about 10 Kcal/mole. This low value is unexpected since most workers show activation energies of 25–40 Kcal/mole for browning (Labuza et al., 1977). However, as seen by the response surface analysis, temperature had no significant effect and thus the rate was controlled more by water diffusion which has a low activation energy. More interesting is the combined effect of temperature and sulfite on hardness. Why hardness depends on sulfite level is unknown.

Residual sulfite in dried fruits is important because of the recent health concerns for asthmatics. For all runs more than

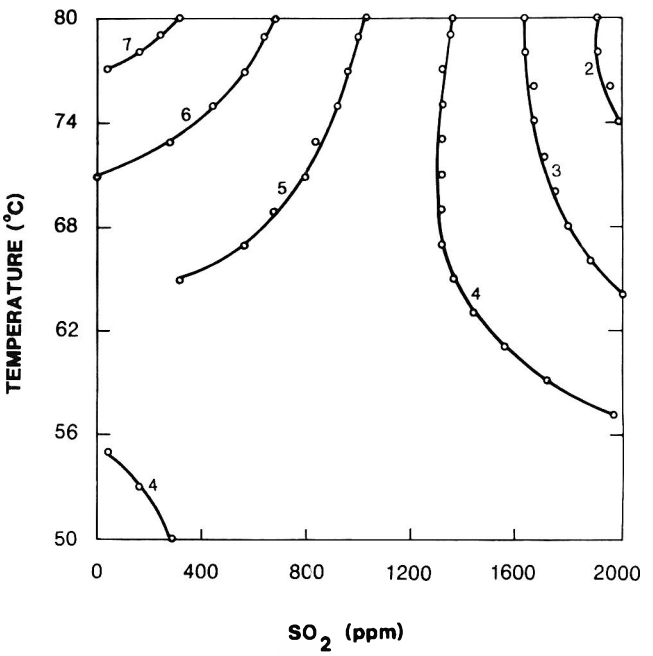

Fig. 6—Surface response contour plot for effect of drying temperature and sulfite level on punch value (hardness in N/m²) for blanched apricots dried to 28% moisture. Contour lines: 2 = 1.6 × 10⁸ N/m²; 3 = 2 × 10⁸; 4 = 2.4 × 10⁸; 5 = 2.8 × 10⁸; 6 = 3.2 × 10⁸; 7 = 3.6 × 10⁸. Apricots blanched before drying (after sulfiting).

50% of the sulfite had been lost from the fruit after drying (Table 1). The losses were a slight function of the temperature with more loss at the lower temperature. This might be due to the conversion of sulfite to sulfate through an oxidative reaction (McWeeny, 1981) which reduces, as high temperature, the SO_2 volatility. As noted, some sulfite also is lost in the blancher (20–60%).

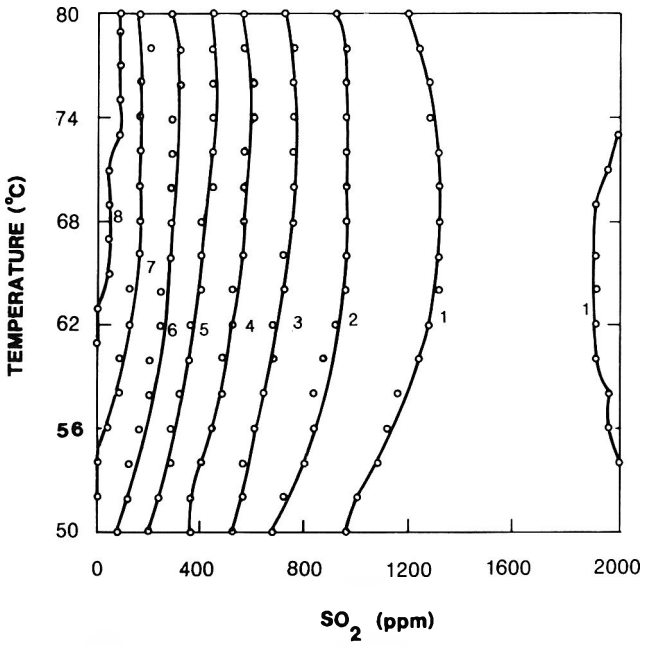

Fig. 7—Surface response contour plot for effect of drying temperature and sulfite level on apricots treated by sulfiting, partial drying, blanching and finish drying. Contour lines: 1 = 1.2 × 10^8 N/m²; 2 = 1.6 × 10^8; 3 = 2 × 10^8; 4 = 2.4 × 10^8; 5 = 2.8 × 10^8; 6 = 3.2 × 10^8; 7 = 3.6 × 10^8; 8 = 4 × 10^8. Apricots blanched before drying (after sulfiting). Values are hardness levels.

Table 1—Losses of sulfite during apricot drying (%)[a]

Temp (°C)	Initial sulfite (ppm)	S/D	S/B/D		B/S/D	S/D1/B/D2
			After blanching	After drying		
50	1000	94	50	90	95	91
52	200	81	65	95	65	64
	1800	61	36	63	57	80
65	0	—	—	—	—	—
	1000	69	45	71	69	74
	1000	70	35	72	62	78
	2000	79	21	57	83	79
78	200	62	35	58	64	57
	1800	78	15	75	70	79
80	1000	60	45	72	58	59

[a] S/D = Sulfiting-Drying
S/B/D = Sulfiting-Blanching-Drying
B/S/D = Blanching-Sulfiting-Drying
$S/D_1/B/D_2$ = Sulfiting-Drying(1)-Blanching-Drying(2)

Time of drying

The drying time of apricots needed to reach 28% moisture content (wb), a level at which texture (gummy fruit) and stability (a_w = 0.5) are both acceptable (Noyes, 1969; McBean and Wallace, 1967; Torrey, 1974), is shown in Table 2. The drying time was reduced by about 50% when the temperature was increased from 50° to 80°C. Also, in most cases, blanching either before or after sulfiting reduced the drying time by 10 to 20% and that the shortest times were when using the B/S/D treatment. Mrak and Perry (1948) mentioned that drying time for peaches, when blanched, was reduced by 30%. Thus, although blanching has no effect on quality, it has an important effect on economics as related to reduced drying time.

Effective water vapor diffusion coefficient (D_{eff})

The Sherwood equation can be used to determine the diffusion coefficient for drying where:

$$\ell n\Gamma = \ell n\left(\frac{m - m_e}{m_c - m_e}\right) = \ell n\left(\frac{8}{\pi^2}\right) - \left(\frac{D_{eff}\,\pi^2\theta}{4\,L_o^2}\right)$$

Table 2—Time of apricot drying (hours) to 28% moisture content[a]

Temp (°C)	SO_2 Content (ppm)	S/D	S/B/D	B/S/D	$S/D_1/B/D_2$
50	1000	20.0	21.0	19.0	26.5
52	200	24.2	21.0	17.3	23.2
65	0	19.2	15.0	15.0	20.3
	1000	15.9	14.7	14.2	19.3
	1000	15.9	14.4	14.4	20.4
	2000	18.0	15.0	14.4	17.0
78	200	12.3	12.0	10.0	13.0
	1800	12.3	10.5	10.2	14.9
80	1000	12.2	10.8	9.8	12.8

[a] S/D = Sulfiting-Drying
S/B/D/ = Sulfiting-Blanching-Drying
B/S/D = Blanching-Sulfiting-Drying
$S/D_1/B/D_2$ = Sulfiting-Drying(1)-Blanching-Drying(2)

m = moisture at any time θ; m_e = final equilibrium moisture determined from the projected isotherm at the air drying temperature and dryer humidity; m_c = moisture at the beginning of falling rate period; L_o = half thickness. A plot of $\ell n\Gamma$ vs time will allow the determination of the diffusivity. The values for all runs ranged from 1 to 3 × 10^{-11} m²/sec depending on the temperature and the treatment. These values are comparable to the 10^{-9} to 10^{-12} m²/sec mentioned for drying by Karel (1975) and reported values of 4.1 × 10^{-10} m²/sec for raisins under vacuum (Chen, 1973), drying of apples 1.1 × 10^{-9} m²/sec (Labuza and Simon, 1970). Vaccarezza et al. (1974) reported a value of 5 × 10^{-10} m²/sec for sugar beet drying at 50°C. As expected, the values are higher than the 4.2 × 10^{-13} m²/sec found by Lomauro et al. (1985) for desorption of moisture from raisins in still air at 25°C.

REFERENCES

AOAC. 1984. "Official Methods of Analysis." 14th ed. S. Williams (Ed.). Association of Official Analytical Chemists. Arlington, VA.

Bolin, H.R. and Staford, A.E. 1974. Effect of processing and storage on provitamin A and vitamin C in apricots. J. Food Sci. 39: 1034.

Bolin, H.R., Huxsoll, C.C., and Salunkhe, D.K. 1981. Fruit drying by solar energy. Utilization Res. 1: 19.

Burton, H.S., McWeeney, D.J., and Biltcliffe, D.O. 1963. Nonenzymatic browning: The role of unsaturated carbonyl compounds as intermediates and of SO_2 as an inhibitor of browning. J. Food Sci. Agr. 14: 911.

Chen, C.S. 1973. Simultaneous heat and mass transfer in convective drying of biological materials. Ann. Technol. Agric. 22(3): 305.

Dahlenburg, A.P. 1976. Sulfuring of tree fruits for drying. Food Technol. (Australia) 27(5): 168.

Iglesias, H.A. and Chirife, J. 1976. Isoteric heats of water vapor sorption on dehydrated foods. Part I. Analysis of the differential heat curves. Lebensm. Wiss. Technol. 9: 116.

Iglesias, H.A. and Chirife, J. 1982. "Handbook of Food Isotherms: Water Sorption Parameters for Foods and Food Components." Academic Press, New York.

Joslyn, M.A. and Braverman, J.B.S. 1954. The chemistry and technology of the pretreatment and preservation of fruit and vegetable products with sulfur dioxide and sulfites. Adv. Food Res. 5: 97.

Karel, M. 1975. Dehydration of foods. In "Principles of Food Science, Physical Principles of Food Preservation." O. Fennema (Ed.). Marcel Dekker, Inc., New York.

Labuza, T.P. and Simon, I.B. 1970. Surface tension effects during dehydration. Air drying of apple slices. Food Technol. 24: 712.

Labuza, T.P., Warren, R., and Warmbier, H. 1977. The physical aspects with respect to water and nonenzymatic browning. Adv. Expt. Med. Biol. 86B: 379.

Labuza, T.P. 1984. "Practical Aspects of Moisture Sorption Isotherm Measurement and Use." American Association of Cereal Chemists, St. Paul, MN.

Labuza, T.P., Kaanane, A., and Chen, J. 1985. Effect of temperature on the moisture sorption isotherms and water activity shift of two dehydrated foods. J. Food Sci. 50: 385.

Lomauro, C.J., Bakshi, A.S., and Labuza, T.P. 1985. Moisture transfer properties of dry and semimoist foods. J. Food Sci. 50(2): 397.

Loncin, M., Biment, T.J., and Lenger, J. 1968. Influence of water activity on the spoilage of foodstuff. J. Food Technol. 3: 131.

McBean, D. McG., Johnson, A.A., and Pitt, J.I. 1964. The adsorption of sulfur dioxide by fruit tissue. J. Food Sci. 29: 257.

McBean, D. McG. and Wallace, J.J. 1967. Stability of moist pack apricots in storage. CSIRO Food Preserv. Q. 27(N2): 19.

McWeeny, D.J. 1981. Sulfur dioxide and the Maillard reaction in food. Prog. Food Nutr. Sci. 5: 395.

Mrak, E.M., Phaff, H.J., Fisher, C.D., and Max Kinney, G. 1943. Dehydration of fruits offers important wartime advantages. Food Ind. 15(4): 59.

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Effect of pH, Certain Chemicals and Holding Time-Temperature on the Color of Lowbush Blueberry Puree

C.S.T. YANG and P.P.A. YANG

ABSTRACT

Main effects of pH, several chemicals, and holding time at 50°C and −20°C on the color of lowbush blueberry puree were evaluated. As pH shifted from low to high, the puree became darker and more bluish-purple. Chemicals such as EDTA and $AlCl_3$ tended to produce a redder and yellower puree whereas $SnCl_2$ and $SnCl_4$ would make the color bluish-purple. The combining effect of pH and chemicals showed a stabilizing effect on puree color which was independent of holding time of 24 hr at 50°C or 4 wk at −20°C. Hue values were recommended to be used rather than absorbance ratios A_{510}/A_{410} to judge color of blueberry puree.

INTRODUCTION

THE LOWBUSH BLUEBERRY of northeastern North America, *Vaccinium angustifolium* Ait., grows in acid soils on barren lands over extensive areas. The berry has a brief harvest season and fresh berries may not be kept for more than 6 wk after harvesting. Until now, most of the crop was frozen with only a small portion canned. Frozen puree prepared from blueberry is one of the promising ingredients for use as baby foods, fruit butter, topping, pie filling, and fruit roll-ups. The stability of color in blueberry is an important aspect of controlling quality during processing and storage. The dominant red and reddish brown anthocyanin pigment of lowbush blueberries has been analyzed (Francis et al., 1966; Francis, 1985), but little is known about the nature of the change in the anthocyanin constituent of blueberry puree during storage. Because of the complex nature of anthocyanin pigments and the different types of reactions that take place in blueberry products after either freezing or other processing methods not much progress has been made in improving the stability of color in blueberry products. It has been shown that the decolorization of anthocyanin pigments was accelerated by ascorbic acid, dehydroascorbic acid and sugar (Beattie et al., 1943; Jurd, 1963; Meschter, 1953; Pratt et al., 1954; Sondheimer and Kertesz, 1953; Tinsley and Bockian, 1960). The reaction rate increased with pH and was directly proportional to the pigment existing as the pseudo base (Jurd, 1963; Lukton et al., 1956; Meschter, 1953). Sistrunk and Cash (1968, 1970) found that color of strawberries could be stabilized with certain chemicals such as $SnCl_4$ and pH level was one of the strongest factors affecting color stability, followed by holding time (at 50°C) and formation of color complexes of metal salts.

This study determines the effect of pH, several chemicals, and holding time-temperature on the color of lowbush blueberry puree over a period of storage.

MATERIALS & METHODS

Raw material

The lowbush blueberries used in this study were obtained from Jasper Wyman and Son Co. (Milbridge, ME). Berries were harvested in 1984, processed by the individually quick freezing (IQF) method (Abdallah, 1966) and packed in 30-lb boxes.

Puree preparation

Frozen berries were thawed overnight at 2°C and blended in a Waring Blendor at medium speed for 2 min. The puree was then treated according to the method reported by Sistrunk and Cash (1970). The entire lot was mixed thoroughly before dividing the puree into three batches which were adjusted to pH 3.0, 3.4 (natural pH), and 3.8 by adding either 50% citric acid or 50% sodium citrate. In each pH batch, the solutions of $AlCl_3$, $SnCl_2$ and $SnCl_4$ were added to make the final concentration to 0.2% of the salt. The EDTA (ethylenediamine-tetraacetate, sodium salt) was added at the rate of 100 ppm. After mixing the individual samples with a Polytron mixer, they were transferred into heat sealable bags with 20g from each lot.

Color measurement

The color of original samples of each lot was read on a Hunter LabScan II Spectrocolorimeter with an 1-3/4 inch aperture and the result was recorded as 0 time. Color data were recorded as Hunter *L*, *a*, *b* and hue values [hue is the angle with tangent *b/a* (Francis and Clydesdale, 1975)]. The remainer of the bags were divided into two portions; one portion was placed in the water bath at 50°C and the other was frozen at −20°C. Samples in the water bath were removed at 3, 6, 9, 12, 15, and 24 hr. After cooling in tap water, color was measured with the Hunter meter. Optical density of each sample was determined according to the method of Sistrunk and Cash (1970) on 5-g samples to which were added 4g NaCl and 25 mL 80% ethanol which was diluted from 100% ethanol with citrate buffers at pH levels of 3.0, 3.4, and 3.8. After stirring and heating 15 min at 60°C the samples were made to 40 ml and filtered on Whatman No. 1 filter paper. Samples were diluted 1:20 with HCl-acetate buffer at pH 1.0. Then optical density ws recordec on a Baush and Lomb Spectronic-20 at 510 mμ (A_{510}). Following extraction with the ethanolic mixture, the residue was extracted twice with 40 mL 5% NaOH. After stirring, the residue and the extractant were centrifuged 10 min at 3,000 rpm and the supernatant transferred into 100 ml volumetric flasks and made to volume with 5% NaOH solution. Optical density was recorded at 410 mμ (A_{410}). Absorbance ratics (AR) were obtained at A_{510}/A_{410}. Color, as determined with the Hunter meter, was also determined on those frozen samples once a week during a 7-wk period.

Statistical analyses

The data were analyzed as a 3 × 5 × 7 factorial experiment and processed by Statistical Analysis System (SAS, 1982) with a Waller-Duncan Test at k ratio of 100 (approximately 5% level of probability).

RESULTS & DISCUSSION

Fresh Samples

The main effects of pH, chemicals, and holding time were sufficient to demonstrate the main changes that occurred during 24 hr holding at 50°C (Table 1).

Effect of pH

Lower pH (3.0) was found to stabilize the color of anthocyanin in the blueberry puree [i.e. higher Hunter *a* value, A_{510}, and AR which denoted a strong red pigment (Meschter, 1953)]. This is because the equilibrium between the colored flavylium and the colorless pseudobase is shifted toward the flavylium, which is much more stable than the pseudobase (Jurd, 1972).

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Table 1—Main effects of pH, chemicals, and holding time at 50°C on color of freshly prepared blueberry puree

Treatment	Hunter *L*	Hunter *a*	Hunter *b*	Hue value	A_{510}	A_{410}	Absorbance Ratio
pH Effect							
3.0	6.78[a]	6.82[a]	0.10[a]	0.84[a]	0.55[a]	0.22[b]	2.56[a]
3.4	6.67[b]	6.16[b]	−0.07[b]	−0.63[b]	0.46[b]	0.28[a]	1.65[c]
3.8	6.63[c]	5.68[c]	−0.12[c]	−1.19[c]	0.42[c]	0.21[c]	2.05[b]
Chemical effect							
Control	7.22[b]	7.06[c]	0.98[c]	7.94[b]	0.48[b]	0.22[c]	2.18[b]
EDTA	7.40[a]	8.03[a]	1.26[a]	8.89[a]	0.48[b]	0.24[b]	1.97[b]
$AlCl_3$	6.30[d]	7.45[b]	1.03[b]	7.89[b]	0.55[a]	0.10[d]	5.66[a]
$SnCl_2$	6.11[e]	4.09[e]	−2.06[e]	−26.76[d]	0.41[c]	0.30[a]	1.36[d]
$SnCl_4$	6.45[c]	4.46[d]	−1.35[d]	−16.89[c]	0.47[b]	0.30[a]	1.55[c]
Holding time: (hr)							
0	6.59[f]	5.94[f]	−0.05[c]	−0.46[c]	0.55[a]	0.21[c]	2.60[a]
3	6.62[e]	6.33[b]	−0.02[a]	−0.14[a]			
6	6.88[a]	6.07[e]	−0.01[a]	−0.11[a]			
9	6.64[d]	6.36[a]	−0.04[c]	−0.38[c]			
12	6.65[d]	6.33[b]	−0.02[a]	−0.17[ab]	0.47[b]	0.25[a]	1.88[b]
15	6.70[c]	6.24[d]	−0.03[b]	−0.23[b]			
24	6.77[b]	6.26[c]	−0.04[c]	−0.39[c]	0.42[c]	0.24[b]	1.73[b]

[a–e] Data of Hunter and hue values are means of four measurements; data of absorbance are means of three measurements. Means within columns having a common superscript letter are not significantly different (P≥0.05).

The strong red pigment and higher Hunter *b* and hue value which corresponded to a more yellow hue (Skrede, 1985) might contribute to a lighter color (high *L*). At higher pH (3.8) a reduction of Hunter *a*, *b*, hue value, A_{510} and A_{410} was found which made the puree darker and bluish. At pH 3.8, a reduction of A_{410} was found, causing a higher AR value which was in conflict with the Hunter values and previous reports (Meschter, 1953; Markakis, 1974; Francis, 1985). The result suggests the hue values may be more suited for color evaluation of blueberry puree than the AR values. A similar conclusion was reached by Skrede et al. (1983) and Skrede (1985) on color evaluation of blackcurrent syrup.

Effect of chemical treatment

Puree treated with EDTA was lighter, redder, and yellower than the purees with other treatments, including control sample. This could have resulted from the chelation effect of EDTA in coordinating with metal to form metal complexes and thus inhibiting browning reaction (Furia, 1964). Puree treated with $AlCl_3$ had a redder, yellower, yet darker, color than that of the control sample, and the A_{510} in the ethanolic extract of the puree with $AlCl_3$ was higher than those with any of the other chemicals, which is in agreement with the report by Sistrunk and Cash (1970). This high A_{510} resulted in a high AR value. Both $SnCl_2$ and $SnCl_4$ caused a significant decrease in Hunter *L*, *a*, *b* readings, hue values, and AR values, indicating a shift of color spectrum from reddish-purple to a bluish-purple. This is different from the effect of these two chemicals on stabilizing color of strawberries as reported by Sistrunk and Cash (1968). Since there is neither a reported standard color of lowbush blueberry puree nor a consumers' preference record, treatments which can maintain the color closer to that of the fresh blueberry puree are thereby considered the appropriate stabilizing methods. Both EDTA and $AlCl_3$ tended to intensify the red and yellow color whereas $SnCl_2$ and $SnCl_4$ tended to reduce the red color and shift the yellow spectrum toward the blue zone. EDTA and $SnCl_2$ treatments were the two extremes that resulted in two completely different colored puree products.

Effect of holding time

A lower Hunter *b* and hue value was found as the temperature of the puree reached 50°C, meaning a less yellow color. A reduction of A_{510}, AR, and an increase of A_{410} were found after 12 hr holding, indicating a decoloration effect of rising temperature on anthocyanin as reported by Markakis (1974). However, since blueberries have a relatively high pigment content (Francis, 1985),a change in yellow hue of some anthocyanin pigments does not seem to induce a drastic change in the overall color of puree (i.e. consistent *L* and *a* values in Table 1). There was no apparent trend of color change during the 24 hr holding period, suggesting that the interaction of both pH and chemicals had a prolonged stabilizing effect on puree color after an initial color change due to the elevated temperature.

Frozen samples

The main effects of pH, chemicals, and holding time on the color of blueberry puree held at −20°C are presented in Table 2. The effects of pH and chemicals were similar, suggesting that both were independent of the temperature of storage. The puree became darker at the end of 4 wk and a stronger yellow hue started to develop after 6 wk. The ascorbic acid in puree and hydrogen peroxide produced by the oxidation reaction between ascorbic acid and air incorporated through puree preparation might gradually decolor the anthocyanin (Beattie et al., 1943; Pederson et al., 1947; Markakis, 1974) and result in a darker color.

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Table 2—Main effects of pH, chemicals, and holding time at −20°C on color of frozen blueberry puree

Treatment	Hunter *L*	Hunter *a*	Hunter *b*	Hue value
pH Effect				
3.0	6.03[a]	6.59[a]	0.12[a]	1.04[a]
3.4	5.90[b]	5.83[b]	−0.04[b]	−0.39[b]
3.8	5.87[c]	5.55[c]	−0.08[c]	−0.83[c]
Chemical effect				
Control	6.20[b]	6.57[b]	1.08[b]	9.34[b]
EDTA	6.42[a]	7.28[a]	1.24[a]	9.67[a]
$AlCl_3$	5.63[d]	7.29[a]	1.08[b]	8.43[c]
$SnCl_2$	5.42[e]	3.96[d]	−2.08[d]	−27.71[e]
$SnCl_4$	5.99[c]	4.82[c]	−1.31[c]	−15.21[d]
Holding time: (wk)				
0	6.59[e]	5.94[cd]	−0.05[d]	−0.48[af]
1	5.99[c]	6.13[a]	0.03[ab]	0.28[c]
2	6.11[b]	5.87[e]	−0.04[cd]	−0.39[e]
3	6.09[b]	6.03[abc]	−0.06[d]	−0.57[f]
4	5.64[d]	5.98[bcd]	0.03[ab]	0.29[c]
5	5.70[d]	5.80[e]	0.00[bc]	0.00[d]
6	5.69[d]	6.08[ab]	0.05[a]	0.47[b]
7	5.64[d]	6.09[ab]	0.07[a]	0.66[a]

[a–f] Data are means of four measurements. Means within columns having a common superscript letter are not significantly different (P≥0.05).

Sensory Techniques for Measuring Differences in California Navel Oranges Treated with Doses of Gamma-Radiation Below 0.6 Kgray

M. O'MAHONY and L. R. GOLDSTEIN

ABSTRACT

Navel oranges from California were given low postharvest doses of gamma radiation: 0.32–0.37 and 0.52–0.60 KGy (32–37 and 52–60 Krad); they were compared with nonirradiated controls for visual appearance, flavor by mouth, odor, taste and taste after sweetness suppression by gymnema sylvestre. Practiced judges were used as an analytical tool, with minimum cross-sensory interference, while untrained subjects were used to determine whether changes might be distinguished by 'nonexperts'. Differences were found in appearance, flavor, taste and odor although they were less extreme at the lower dose. Untrained judges could discriminate the juice at the higher irradiation level only.

INTRODUCTION

SINCE BALOCK et al. (1956) first introduced the idea of postharvest irradiation of fruit, its potential for disinfestation and prolongation of shelf life has been reviewed (Anon, 1982: Burditt, 1982; Maxie and Abdel-Kader, 1966; Moy, 1977). With the banning of ethylene dibromide as a fumigant and approval by the F.D.A. of irradiation up to 1 KGy (100 Krad) as a postharvest treatment, an examination of the effects of irradiation on sensory characteristics of food becomes timely.

For oranges in particular, the effects of irradiation on the sensory characteristics have been studied using techniques ranging from visual inspection and hedonic measurement (Ahmed et al., 1966; Barkai-Golan and Kahan, 1966; Belli-Donini and Baraldi, 1977; Braddock et al., 1970; Bramlage and Couey, 1965; Dennison and Ahmed, 1966; Guerrero et al., 1967; Monselise and Kahan, 1968; Riov, 1975; Rouse et al., 1966) to sensory evaluation (Maxie et al., 1964; 1969; Grierson and Dennison, 1965; Guerrero et al., 1967). With some exceptions (Barkai-Golan and Kahan, 1966; Bramlage and Couey, 1965; Monselise and Kahan, 1968) the sensory effects for oranges were reported for irradiation doses above 1 KGy (range 1-3 KGy; 100-300 Krad). Recently, Nagai and Moy (1985) performed sensory analyses on Valencia oranges irradiated at doses ranging 0.3-1.0 KGy (30-100 Krad) and O'Mahony et al. (1985) examined Navel oranges irradiated at 0.60-0.85 KGy (60-85 Krad).

The purpose of this study was to demonstrate a novel methodological approach to measuring the sensory effects of irradiation on oranges, and to determine whether dose levels as low as 0.3 KGy (30 Krad) which would sterilize insect pests, had less of a sensory impact than higher doses designed to kill.

MATERIALS & METHODS

Specification of fruit

California navel oranges (size 72) were examined; they were harvested in March, 1986 from Lindsay, California.

Transport, irradiation and storage of fruit

On the day of harvesting, the fruit were washed, waxed and packed in flat cardboard boxes (20 oranges per box). They were stored at Sunkist Co. (Lindsay, CA) for 2 days after the harvesting day, at ambient temperature: pulp temperature 15.5°C, relative humidity 88%.

In the afternoon of the next day, the oranges were transported by road at ambient temperature to the irradiation facility, International Nutronics Inc. (Irvine, CA): transport time 24 hrs, pulp temperature 14-17°C, relative humidity 70–100%. The long transport time included a stop at a separate facility to irradiate a separate batch of fruit; the present batch of oranges were not disturbed or removed from the van during this period. It is worth noting that there were heavy rain storms and flooding during the days of harvest and collection of the fruit; some of the boxes became damp though not wet.

At the irradiation facility, all the fruit was unloaded. A quarter of the fruit was irradiated at ambient temperature at 0.32–0.37 KGy (32–37 Krad), a quarter at 0.52-0.60 KGy (52–60 Krad), while the remaining half of the batch of fruit was taken into the facility but not actually irradiated: time at facility was 6 hrs, pulp temperature 17–18°C, relative humidity 68–70%. The radiation source was cobalt-60. The fruit to be irradiated at the lower dose was placed in front of the source for 4.6 min; the boxes were then turned over and irradiated for a further 4.6 min. For the higher dose, the irradiation time was doubled. Dose delivered was measured by optichromic (70–83) dosimeters (Far West Technology, Inc., Goleta, CA).

Immediately after irradiation, the oranges were transported by road to Davis, CA: transport time 12 hrs, pulp temperature 16–18°C, relative humidity 68–70%. At Davis the fruit was stored prior to testing: ambient temperature 7.2°C, pulp temperature 7.2°C, relative humidity 60%. Thus, storage at Davis began 5 days after harvest. Oranges developing blue-green mold during storage were discarded. Sensory testing took place during the 4th and 5th week after harvest, for 9 days.

Judges

Practiced judges were used as analytical instruments for testing samples of fruit; more than one judge was used as a failsafe rather than to constitute a statistical sample. Their purpose was to use their senses as analytical tools to provide information about the physical changes in the fruit. For each test, judges were selected after three orientation and practice sessions to learn the appropriate behavioral procedures and sensory cues, detailed later; this provided a pool of 8 practiced judges (5F, 3M, 20–28 yr). Only those judges who showed success in making discriminations over their first ten replicate testings were used to perform further tests; the purpose was to use only the most sensitive judges to test large samples of the fruit.

A sample of untrained judges, consumers of oranges, were also tested. They were used to make inferences about the population. Sixty-two students and staff at U.C.D. (28M, 34F, age 19–43 yr) were selected at random and merely required to understand the task at hand before commencing the simple behavioral procedures, detailed later.

Titratable acidity and soluble solids

Five weeks after harvesting, juice was made from batches of 20 randomly sampled oranges from each treatment. The titratable acidity and soluble solids (TS Meter Refractometer; American Optical, Buffalo, NY) were determined for each batch of juice, to determine the mean soluble solids/titratable acid ratio.

Evaluation of surface appearance

The oranges were assessed for the occurrence of brown pitting on the surface of the peel. Two practised judges compared a sample of 186 oranges irradiated at 0.52–0.60 KGy with a sample 191 nonirradiated control oranges and a sample of 174 oranges irradiated at 0.32–0.37 KGy with 199 controls. This represented a sampling of

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approx. 60% of each orange treatment in storage. Each judge evaluated the same oranges to give a measure of interjudge consistency. Judges worked separately and categorized the oranges as: 'definitely blemished', 'perhaps blemished', 'definitely unblemished', 'perhaps unblemished'. The categorization was accomplished by judges sorting batches of oranges into four appropriately labeled trays. Judges were given blemished and unblemished standards prior to testing. From these data, R-index measures of the comparative degree of blemishing can be computed (O'Mahony, 1979a, 1983, 1988) for each orange treatment and each judge.

Over the 9 days of testing, approx. one ninth of the total sample of oranges was evaluated on each day, by each judge. Session lengths each day ranged 5-10 mins. The oranges were viewed under standard lighting conditions described previously (O'Mahony et al., 1985).

Evaluation of flavor by mouth

Practised judges determined, at their own speed, the difference between the juice from the irradiated and nonirradiated control fruit, using dual standard tests (Peryam, 1958, Peryam and Swartz, 1950). The tests were performed under red light to mask color differences and juice was used to eliminate texture cues.

Juice was prepared immediately before testing, from random samples of oranges using a Waring juicer Model JC110-8 (Waring Products Division, Dynamics Corp. of America, New Hartford, CT). Approximately 25 mL samples were presented at constant room temperature (22–24°C) in 1 oz paper portion cups (Lily Portion cups, Type 100: Lily-Tulip Inc., Toledo, OH).

Judges first went through a 'warm-up' procedure (O'Mahony and Odbert, 1985) by alternate ad-lib tasting of the two standards, until they felt confident to proceed; this procedure increased sensitivity to the differences. Judges then performed ten successive dual standard tests (O'Mahony et al., 1986). They were allowed as much retasting as desired, further portion cups of juice being provided as necessary. Judges did not always find it necessary to taste both standards for each test. This is a modification of the strict dual standard procedure, making it arguably like a paired comparison test in which judges had learned the dimension along which the samples differed. Responses were given verbally. Judges rinsed with deionized water between tastings to clear the mouth of residual stimuli and lessen adaptation effects (O'Mahony, 1979b). Prior experimentation had indicated that performance was maintained better when judges chose their own interstimulus rinsing regimes, to strike the compromise between enough rinsing to clean the mouth and too much rinsing time causing memory loss of the prior taste. Judges were taught a technique of rapid successive sipping of two juice samples, so that each was delivered to different sides of the mouth. In this case, no rinsing was required between the two juice samples to be compared, so that the comparison of sensations was made close in time; this alleviated memory loss. Prior experimentation indicated that a quarter of the judges exhibited better discrimination with this technique; the remainder did not feel comfortable with the technique and used successive whole mouth tasting. Judges using one technique did not appear superior to those using the other.

Judges' performance was inspected over the first ten dual standard tests. Those able to make discriminations were recalled for further sessions of 10 dual standard tests, so that large samples of the juice could be evaluated by the most sensitive judges.

Three judges (3F, age 20–24 yr) performed three sessions each (30 dual standard tests); one (M, 28 yr) performed two sessions (20 dual standard tests). Session lengths ranged 20–45 min.

To ensure that the color of the juice and its 'body' did not provide inadvertant nongustatory cues during the assessment of flavor and taste, control experiments were performed in which judges were required to discriminate between the control and the irradiated juices by color and by 'body'.

The color controls were performed under the same red lighting conditions as the experiment. Judges viewed the juice in their portion cups; they did not smell or taste the juice, so avoiding nonvisual cues. Each judge performed ten dual standard tests and all performed no better than chance (binomial $p > 0.172$); this corresponded with subjective reports.

For the assessment of 'body', the judges were first taught the concept by being given a juice with more body (pure juice) and one with less body (juice diluted by 25% with purified water). All judges found these stimuli easily distinguishable. They then performed ten dual standard tests in the same manner as the judgements for flavor by mouth, except that noseclips were worn to eliminate olfactory cues. No judges performed better than chance ($p > 0.172$). In this case, gustatory cues could not be eliminated from the judgements, but it is worth noting that all judges who could discriminate by taste were unable to discriminate by body.

These control experiments were performed at the end of all the testing so as not to alert judges inadvertently to these cues.

Evaluation of odor

The procedure was essentially the same as for evaluation of flavor by mouth, except that judges smelled orange halves instead of sipping juice. The orange half was squeezed during smelling to expose the juice and thereby release the volatiles. Instead of using one standard from each treatment, two standards were given for each treatment; during 'warm-up' the four standard orange halves were used to allow the formation of a broader concept of the odor differences (Miller and Johnson-Laird, 1976; O'Mahony and Ishii, 1987). These standards were then used during testing. The standards and the samples presented to any one judge all came from separate oranges. The judges did not place the orange in the mouth so avoiding taste and oral texture cues; they were blindfolded to avoid visual cues. It was possible, however, that potential differences in resistance to squeezing could interfere with this judgement. If pitted oranges were used, a half without pits was used, to avoid tactile cues.

Three judges (2F, 1M, age 21–28 yr) performed two sessions each; session lengths ranged 15–35 min. Responses were given verbally.

Evaluation of taste

The procedure was the same as for testing flavor by mouth, except that judges wore a noseclip which eliminated odor cues. Three judges (2M, 1F, age 21–28 yr) performed two sessions each and one (F, age 22 yr) performed three sessions. Sessions lengths ranged 25–45 min.

Evaluation of taste without sweetness

The procedure was exactly the same as for the evaluation of taste, except that judges were given a prior treatment with *Gymnema sylvestre* to block the sweet taste receptors; the remaining taste quality was predominantly sourness. A strong tea was made from *Gymnema sylvestre* leaves (O'Mahony et al., 1983). Judges held approx. 50 mL in the mouth for 1.5 min with agitation. On expectoration, judges rinsed the mouth *ad lib* with deionized water, paused and rinsed again until the flavor of the *Gymnema sylvestre* had subsided. They then tasted a sugar cube to ensure that the sweet taste receptors were blocked. Judges then rinsed again before commencing testing. Three judges (3F, age 21–22 yr) performed two sessions each; session lengths ranged 30–45 min.

Evaluation by untrained judges of gross sensory differences in the juice

Sixty-two untrained judges were each required to perform a triangle test to determine the difference between the control juice and each irradiated treatment. Juice samples (60 mL) were presented in 3 oz waxed paper cups (Lily-Tulip Inc., Toledo, OH). Judges responded verbally and gave reasons for their selection. Judges were instructed to rinse *ad lib* between tasting. Both irradiation treatments were tested in the same session; for each triangle, the irradiated fruit was the odd sample. No practice was given although it was ensured that the simple behavioral task was understood at the beginning of the session. Session lengths ranged 7–15 min.

RESULTS

Mold

By the end of the 5th week of storage, the percentages of the untreated control oranges (N = 640) and of the irradiated oranges (N = 290 per treatment) that were discarded because they developed blue-green mold were: 1.6% for untreated controls, 1.4% for the 0.32–0.37 KGy dose, 1.7% for the 0.52–0.60 KGy dose. Irradiated oranges did not show a significantly reduced tendency to mold (binomial comparison proportions $p = 0.94$) Despite the damper conditions of harvesting, the occurrence of mold was less than in a previous study (O'Mahony

et al., 1985). The lack of mold indicates the efficiency of fungicides added routinely during waxing and washing.

Titratable acidity and soluble solids

The soluble solids, titratable acidity and soluble solids/titratable acidity ratios, 5 weeks after harvesting, are given in Table 1. The irradiated oranges had a greater solids/acid ratio and lower solids and titratable acidities than the controls.

A higher solids/acid ratio was found by O'Mahony et al. (1985) for navel oranges at 0.60–0.85 KGy. The present study indicates that the effect persisted at even lower doses. O'Mahoney et al. (1985) also found lower titratable acidity but did not note any consistent change for soluble solids. Other studies with various doses and orange varieties give no consistent pattern for titratable acidity or soluble solids (Braddock et al., 1970; Guerrero et al., 1967; Monselise and Kahan, 1968; Nagai and Moy, 1985).

Evaluation of surface appearance

From the sorting data for each judge, R-index values (O'Mahony, 1979, 1983, 1988) can be computed giving measures of the degree of discrimination between orange treatments, in terms of brown blemishing. Here, an R-index is the probability of picking an irradiated orange by its blemishing, when it is presented in paired comparison with a control orange (50% = chance discrimination; 100% = perfect discrimination). In signal detection terms (Green and Swets, 1966), the R-index is a measure of discriminability of a blemish signal in the irradiated oranges, from the noise level blemishing in the controls. Rank sums tests (O'Mahony, 1986a) were performed to determine the statistical significance of each R-index value.

The R-index values giving the degree of discrimination between the irradiated oranges and the controls, are given in Table 2. Also given are R-index values indicating the degree of discrimination between oranges at the two levels of irradiation. It is clear that oranges from both irradiation treatments could be discriminated fairly easily from the controls; there was even a low, yet significant, discrimination between oranges given different irradiation doses.

A consistency check between the two judges was performed by using the data for a given orange treatment and comparing the sorting data between judges. Between judges R-indices were calculated giving a measure of the comparative response bias (Green and Swets, 1966) of the judges. Values ranged 50.2–51.7 and were not significant ($p > 0.23$), indicating a high degree of agreement between the judges.

Both these measures reflect the fact that the pitting was generally pronounced and extreme. It was far more extreme than in a comparable prior study (O'Mahony et al., 1985) and was probably due to the dampness on the day of irradiation. Moisture on the surface tends to elicit pitting after irradiation; the extreme pitting encountered here would suggest that irradiation is only a suitable treatment for oranges under dry conditions. Development of some degree of pitting and blemishing under dry conditions, during storage after an irradiation treatment, has also been reported in studies at comparable (0.50–0.52 KGy) dose levels by casual observation (Barkai-Golan and Kahan, 1966; Bramlage and Couey, 1965; Monselise and Kahan, 1968) and by sensory evaluation (Nagai and Moy, 1985; O'Mahony et al, 1985). Further work at doses above 1 KGy also indicates blemishing for oranges (Barkai-Golan and Kahan, 1966; Belli-Donini and Baraldi, 1977; Braddock et al., 1970; Bramlage and Couey, 1965; Dennison et al., 1966; Grierson and Dennison, 1965; Mahmood, 1972; Maxie et al., 1969; Monselise and Kahan, 1968; Riov, 1975).

Table 1—Mean values for titratable acidity, soluble solids and the soluble solids/total acid ratio for irradiated and control oranges 5 weeks after harvest[a]

Irradiation dose KGy	Soluble solids °Brix	Titratable acidity g acid/100mL juice	Soluble solids/ titratable acid ratio
0	11.8	1.11	10.6
0.32-0.37	11.5	1.05	11.0
0.52-0.60	11.5	1.05	11.0

[a] Means represent two determinations for soluble solids and four for titratable acidity. Standard deviations range: 0.1-zero for soluble solids 0.02-0.04 for titratable acidity.

Table 2—R-index percentage values[a] with significance levels[b] for each judge, indicating their degree of differentiation between irradiated and control oranges, in terms of brown blemishing

Orange treatments		Judge	
Noise	Signal	A	B
Control	0.32-0.37 KGy	84.3 (<0.0002)	83.6 (<0.0002)
Control	0.52-0.60 KGy	88.7 (<0.0003)	90.0 (<0.0003)
0.32-0.37 KGy	0.52-0.60 KGy	55.5 (0.012)	56.9 (0.003)

[a] The higher the R-index value above 50, the greater the degree of blemishing in the 'signal' treatment, compared with the 'noise' treatment.

[b] Significance levels in parentheses.

Evaluation of flavor by mouth

The proportions of correct dual standard tests for judges who were recalled for more than one session are given in Table 3. The significance levels were determined by binomial statistics (Roessler et al., 1978). Generally, both treatments could be discriminated from the control. For three of the judges discrimination was better with the high dosed oranges; a binomial comparison of proportions (O'Mahony, 1986a) indicated that for judges #2 and #3 this difference was significant ($p < 0.035$).

O'Mahony et al. (1985) found differences in flavor by mouth for navel oranges irradiated at 0.60–0.85 KGy. The present study indicates that flavor differences occur for even lower doses of irradiation. However, the effect appears less at the lower dose. A similar dose effect was found by Macfarlane and Roberts (1968). Nagai and Moy (1985) did not find flavor differences for Valencia oranges irradiated at doses below 0.5 KGy; however, they used less sensitive scaling procedures rather than difference tests. Other studies have reported flavor changes for doses above 1 KGy (Braddock et al., 1970; Bramlage and Couey, 1965; Grierson and Dennison, 1965; Maxie et al., 1964).

Evaluation of odor

The proportions of correct dual standard tests for judges who were recalled for more than one session are given in Table 4. Significance levels were determined by binomial statistics (Roessler et al., 1978). The 0.52–0.60 KGy treatment could be distinguished from the control but the low dose treatment could not. More dual standard tests were performed correctly for the higher dose treatment (binomial comparison of proportions, judges #2 and #3 $p = 0.03$; judge #1, $p = 0.057$).

The differences at the higher dose level confirm O'Mahony et al.'s (1985) results for doses of 0.60–0.85 KGy. Nagai and Moy (1985) did not find differences at 0.5 KGy for Valencia oranges using scaling. Reports of odor differences have also been given for doses above 1 KGy (Braddock et al., 1970; Maxie et al., 1964).

Evaluation of taste

The proportions of correct dual standard tests for judges who were recalled for more than one session are given in Table 5. Significance levels were determined by binomial statistics (Roessler et al., 1978). As with odor, the irradiated juice could only be discriminated from the control at the higher dose; although judge #4 failed to discriminate

Table 3—Proportion of correct dual standard tests, with significance levels[a] for each judge, indicating the degree of discrimination between irradiated and control oranges, in terms of flavor by mouth of the juice

	Irradiation dose (KGy)	
Judge	0.32-0.37	0.52-0.60
1	25/30 (<0.001)	22/30 (0.008)
2	21/30 (0.021)	28/30 (<0.001)
3	18/30 (0.181)	30/30 (<0.001)
4	15/20 (0.021)	19/20 (<0.001)

[a] Significance levels given in parentheses.

Table 4—Proportion of correct dual standard tests, with significance levels[a] for each judge, indicating the degree of discrimination between irradiated and control oranges, in terms of odor

	Irradiation dose (KGy)	
Judge	0.32-0.37	0.52-0.60
1	13/20 (0.13)	18/20 (<0.001)
2	12/20 (0.25)	18/20 (<0.001)
3	11/20 (0.41)	17/20 (0.001)

[a] Significance levels given in parentheses.

Table 5—Proportion of correct dual standard tests, with significance levels[a] for each judge, indicating the degree of discrimination between irradiated and control oranges by taste of the juice

Judge	Irradiation dose (KGy) 0.32-0.37	0.52-0.60
1	15/30 (0.57)	26/30 (<0.001)
2	15/20 (0.021)	17/20 (0.001)
3	14/20 (0.058)	17/20 (0.001)
4	12/20 (0.25)	11/20 (0.41)

[a] Significance levels given in parentheses.

Table 6—Proportion of correct dual standard tests, with significance levels[a] for each judge, indicating the degree of disrimination between irradiated and control oranges by taste without sweetness of the juice

Judge	Irradiation dose (KGy) 0.32-0.37	0.52-0.60
1	15/20 (0.02)	15/20 (0.02)
2	11/20 (0.41)	12/20 (0.25)
3	9/20 (0.75)	14/20 (0.058)

[a] Significance levels given in parentheses.

at either dose level. For judge #1, the proportion of correct tests was significantly higher at the higher dose level (binomial comparison of proportions, $p = 0.002$), while for judges #2 and #3, the effect was not significant ($p > 0.26$). O'Mahony et al. (1985) found a weak tendency for taste discrimination for 0.60–0.85 KGy doses.

The solids/acid ratio was higher for the irradiated oranges, indicating a sweeter orange. However, whereas the ratio was the same for both dose levels, taste discrimination was better at the higher dose level. Either the taste test was very sensitive to the small difference in acid/sugar ratio or it was detecting further tastes.

Evaluation of taste without sweetness

The proportions of correct dual standard tests for judges, who were recalled for more than one session, are given in Table 6. Significance levels were determined by binomial statistics (Roessler et al., 1978). Only judge #1 was able to determine differences; there was no difference in discriminability with dose level. This result follows the pattern for titratable acidity. It is interesting to note that a further judge, who was discounted because she could tell differences in body of the juice, and who could not tell difference by taste, could tell differences in this test for only the higher dose treatment.

Evaluation by untrained judges of gross sensory differences in the juice

The proportions of untrained judges who performed their triangle test correctly were: 25/62 (0.32–0.37 KGy; binomial $p = 0.15$) and 36/62 (0.52–0.60) KGy; binomial $p < 0.00005$).

The significant discrimination by untrained judges, between the control and irradiated juices at the higher dose level, parallels the finding for flavor by mouth for expert judges. Differentiation by color or body is also possible. Subjective reports, although unreliable, did not indicate use of these latter cues; judges generally alluded to differences in 'sweetness', 'sourness' or various flavor aspects like 'orangeness', 'freshness' etc.

Whereas O'Mahony et al. (1985) had indicated that untrained judges could not discriminate between irradiated and control whole oranges, the present study would indicate differentiation between juices, at comparable dose levels. At the lower dose level, however, discrimination was not significantly better than chance.

DISCUSSION

THE AIM of this study was to demonstrate a novel strategy for sensory testing and how the goals of the test affect the test design chosen. In what could be called Sensory Evaluation I, judges were used as an analytical tool. Practiced judges under controlled conditions, with minimal cross-sensory interference (O'Mahony et al., 1983; 1985) were used as instruments to assess samples of food. The practice of maintaining a fixed panel was abandoned in favor of a strategy whereby the most sensitive judges were singled out to perform more tests than is usual. This economy of testing allowed the most sensitive instruments to maximize their sampling of the oranges, while the insensitive instruments were discarded. It is interesting to note that sensitivity in one test was not a good predictor of sensitivity in another test. In what could be called Sensory Evaluation II, untrained judges who were also consumers of the food, were used under ordinary tasting conditions when the goal was to assess whether differences would be noticed in the marketplace. In this study, the primary concern was flavor rather than appearance and accordingly, juice was tasted; the pitting effects were of secondary concern here, not being so pronounced when irradiation was performed under dry conditions (O'Mahony et al., 1985).

The flavor by mouth of the juice of oranges irradiated at 0.32–0.37 and 0.52–0.60 KGy could be discriminated from the juice of the nonirradiated controls; the juice from the oranges with the lower dose was less discriminable, however. The same pattern was found for taste; with odor only the higher dose juice could be discriminated. In this case, it would seem as if taste were a greater contributor to flavor discrimination although odor would seem to be important for differences found between the two dose levels. Slight discrimination was also found for taste with the sweet taste receptors blocked; this paralleled titratable acidity. Untrained judges could also distinguish between the irradiated and nonirradiated juices at the higher dose level but could not at the lower dose. Unlike an earlier study (O'Mahony et al., 1985) which used whole oranges, discrimination at the higher dose levels seemed possible. A lower dose at which insect pests are sterilized, would seem to give less chance of detecting differences. It is important to stress, however, that the present study is reporting differences not preferences. Even if irradiated oranges can be discriminated from controls, it does not mean that they will not be liked. It was also noted that irradiation of damp oranges results in extreme pitting of the surface which had an undesirable cosmetic effect. Irradiation treatments should be given with caution in conditions of high humidity or where the orange packages are exposed even briefly to rain.

REFERENCES

Ahmed, E.M., Knapp, F.W., and Dennison, R.A. 1966. Changes in peel color during storage of irradiated oranges. Proc. Fla. St. Hort. Soc. 79: 296.

Anon. 1982. Is gamma radiation a substitute for EDB? Citrograph 67: 180.

Balock, J.W., Christenson, L.D., and Burr, G.O. 1956. Effect of gamma rays from cobalt 60 on immature stages of the oriental fruit fly (Dacus doralis Hendel) and possible application to commodity treatment problems. Proc. 31st Ann. Meet. Hawaii Acad. Sci., p. 18.

Barkai-Golan, R. and Kahn, R.S. 1966. Effect of gamma irradiation on extending the storage life of oranges. Plant Dis Rept. 50: 874.

Belli-Donini, M.L. and Baraldi, D. 1977. Relationship between peel damage and the accumulation of limonene in four varieties of irradiated oranges. Env. Exp. Bot. 17: 161.

Braddock, R.J., Wolford, R.W., Dennison, R.A., and Ahmed, E.M. 1970. Irradiation-induced changes in volatile constituents of 'Valencia' oranges. J. Amer. Soc. Hort. Sci. 95: 437.

Bramlage, W.J. and Couey, H.M. 1965. Gamma irradiation of fruits to extend market life. U.S. Dept. Agric. Mktg. Res. Rept. No. 717.

Burditt, A.K. 1982. Food irradiation as a quarantine treatment of fruits. Food Technol. 36: 51.

Dennison, R.A. and Ahmed, E.M. 1966. Review of the status of irradiation effects on citrus fruits. In "Food Irradiation," p. 619. Proc. Int. Symp. Food Irrad., Karlsruhe.

Dennison, R.A., Grierson, W., and Ahmed, E.M. 1966. Irradiation of Duncan grapefruit, pineapple and Valencia oranges and Temples. Proc. Fla. St. Hort. Soc. 79: 285.

Green, D.M. and Swets, J.A. 1966. "Signal Detection and Psychophysics." Wiley, New York.

Grierson, W. and Dennison, R.A. 1965. Irradiation treatment of 'Valencia' oranges and 'Marsh' grapefruit. Proc. Fla. St. Hort. Soc. 7: 233.

Guerrero, F.P., Maxie, E.C., Johnson, C.F., Eaks, I., and Sommer, N.F. 1967. Effects of postharvest gamma irradiation on orange fruits. Proc. Amer. Soc. Hort. Sci. 90: 515.

Macfarlane, J.J. and Roberts, E.A. 1968. Some effects of gamma radiation on Washington Navel and Valencia oranges. Australian J. of Exp. Agr. and Animal Husband. 8: 625.

Maxie, E.C. and Abdel-Kader, A. 1966. Food irradiation-physiology of fruits as related to feasiblity of the technology. Adv. Food Res. 15: 105.

Maxie, E.C., Johnson, C.F., and Boyd, C. 1964. Oranges. In: Radiation technology in conjunction with postharvest procedures as a means of extending the shelf life of fruits and vegetables. U.S. Atomic Energy Comm. Rept. No. UCD-34P80-2, p. 109.

Maxie, E.C., Sommer, N.F., and Eaks, I.L. 1969. Effect of gamma irradiation on citrus fruits. Proc. First Int. Citrus Symp., Riverside, Vol. 3, p. 1375.
Miller, G.A. and Johnson-Laird, P.N. 1976. "Language and Perception." Cambridge University Press, Cambridge.
Monselise, S.P. and Kahan, R.S. 1968. Effect of gamma radiation on appearance, composition and enzymatic activities of citrus fruits. In "Preservation of Fruit and Vegetables by Radiation," Int. Atomic Energy Agency, Vienna, p. 39.
Moy, J.H. 1977. Potential of gamma irradiation of fruits: A Review. J. Food Technol. 12: 449.
Nagai, N.Y., and Moy, J.H. 1985. Quality of gamma irradiated California Valencia oranges. J. Food Sci. 50: 215.
O'Mahony, M. 1979a. Short-cut signal detection measurements for sensory analysis. J. Food Sci. 44: 302.
O'Mahony, M. 1979b. Salt taste adaptation: the psychophysical effects of adapting solutions and residual stimuli from prior tastings on the taste of sodium chloride. Perception 8: 441.
O'Mahony, M. 1983. Adapting short cut signal detection measures to the problem of multiple difference testing: The R-Index. In "Sensory Quality in Foods and Beverages, Definition, Measurement and Control." (Ed.) A.A. Williams and R.K. Atkins, p. 69. Ellis Horwood, Chihester.
O'Mahony, M. 1986a. "Sensory Evaluation of Food. Statistical Methods and Procedures." Marcel Dekker, New York.
O'Mahony, M. 1988. Sensory difference and preference testing: The use of signal detection measures. In H. Moskowitz (Ed.) "Applied Sensory Analysis of Foods." CRC Press, Boca Raton, FL.
O'Mahony, M., Buteau, L., Klapman-Baker, K., Stavros, I., Alford, J., Leonard, S.J., Heil, J.R., and Wolcott, T.K. 1983. Sensory evaluation of high vacuum flame sterilized clingstone peaches, using ranking and signal detection measures with minimal cross-sensory interference. J. Food Sci. 48: 1626.
O'Mahony, M. and Ishii, R. 1987. 'The Umami taste concept: Implications for the dogma of four basic-tastes.' Y. Kawamura, M. Kare (Ed.) In: "Umami; A Basic Taste." Marcel-Dekker, N.Y.
O'Mahony, M. and Odbert, N. 1985. A comparison of sensory difference testing procedures: Sequential sensitivity analysis and aspects of taste adaptation. J. Food Sci. 50: 1055.
O'Mahony, M., Wong, S.-Y., and Odbert, N. 1985. Sensory evaluation of navel oranges treated with low doses of gamma-radiation. J. Food Sci. 50: 639.
O'Mahony, M., Wong, S.-Y., and Odbert, N. 1986. Sensory difference tests: Some rethinking concerning the general rule that more sensitive tests use fewer stimuli. Lebensm.-Wiss. u-Tecnol. 19: 93.
Peryam, D.R. 1958. Sensory difference tests. Food Technol. 12: 231.
Peryam, D.R. and Swartz, V.W. 1950. Measurements of sensory differences. Food Technol. 4: 390.
Riov, J. 1975. Histochemical evidence for the relationship between peel damage and the accumulation of phenolic compounds in gamma-irradiated citrus fruit. Radiat. Bot. 15: 257.
Roessler, E.B., Pangborn, R.M., Sidel, J.L., and Stone, H. 1978. Expanded statistical tables for estimating significance in paired-preference, paired-difference, duo-trio and triangle tests. J. Food Sci. 43: 940.
Rouse, A.H., Dennison, R.A., and Atkins, C.D. 1966. Irradiation effects on juices extracted from treated 'Valencia' oranges and 'Duncan' grapefruit. Proc. Fla. St. Hort. Soc. 79: 292.
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BLUEBERRY PUREE COLOR. . .From page 347

CONCLUSIONS

A LOW pH and EDTA concentration should be used if a lighter, redder, and yellower blueberry puree is preferred. The addition of $AlCl_3$ improved the red color but also resulted in a darker product. A higher pH and $SnCl_2$ and $SnCl_4$ concentrations produce a darker and bluish color. A stable puree color can be obtained by the joint action of pH and these chemicals with a minimum effect from holding time and temperature combination.

REFERENCES

Abdallah, D.A. 1966. The IQF Industry. In "Proceeding of the North American Blueberry Worker's Conference, April 6-7, 1966." p. 132–133. Maine Agri. Exp. Sta., UMO, Maine Misc. Report 118.
Beattie, H.G., Wheeler, K.A., and Pederson, C.S. 1943. Changes occurring in fruit juices during storage. Food Res. 8: 395.
Francis, F.J. 1985. Blueberries as a colorant ingredient in food products. J. Food Sci. 50: 754.
Francis, F.J. and Clydesdale, F.M. 1975. "Food Colorimetry: Theory and Applications." p. 208. AVI Publishing Co., Westport, CT.
Francis, F.J., Harborne, J.B., and Barker, W.G. 1966. Anthocyanins in the lowbush blueberry, *Vaccinium angustifolium*. J. Food Sci. 31: 583.
Furia, T.E. 1964. EDTA in foods. Food Technol. 18: 1874.
Jurd, L. 1963. Anthocyanins and related compounds. I. Structural transformations of flavylium salts in acidic solutions. Org. Chem. 28: 987.
Jurd, L. 1972. Anthocyanin-type plant pigments. In "The Chemistry of Plant Pigments," C.O. Chichester (Ed). Academic Press, New York.
Lukton, A., Chichester, C.O., and Mackinney, G. 1956. The breakdown of strawberry anthocyanin pigment. Food Technol. 10: 427.
Markakis, P. 1974. Anthocyanins and their stability in food. CRC Critical Rev. Food Technol. 4: 437.
Meschter, E.E. 1953. Effects of carbohydrates and other factors on strawberry products. J. Agri. Food Chem. 1: 574.
Pederson, C.S., Beattie, H.G., and Stolz, E.H. 1947. Deterioration of processed fruit juices. N.Y. Agric. Exp. Sta. Bull. 728.
Pratt, D.E., Balkcom, C.M., Powers, J.J., and Mills, L.W. 1954. Interaction of ascorbic acid, riboflavin and anthocyanin pigments. J. Agri. Food Chem. 2: 367.
SAS. 1982. "SAS User's Guide." Statistical Analysis System, p. 151. SAS Institute, Inc., Cary, NC.
Sistrunk, W.A. and Cash, J. 1968. Stabilizing the color of frozen strawberries. Ark. Farm. Res. Vol. XVII (3):2.
Sistrunk, W.A. and Cash, J. 1970. The effect of certain chemicals on the color and polysaccharides of strawberry puree. Food Technol. 24: 473.
Skrede, G., Naes, T., and Martens, M. 1983. Visual color deterioration in blackcurrant syrup predicted by different instrumental variables. J. Food Sci. 48: 1745.
Skrede, G. 1985. Color quality of blackcurrant syrups during storage evaluated by Hunter L', a', b' values. J. Food Sci. 50: 514.
Sondheimer, E. and Kertesz, Z.I. 1953. Participation of ascorbic acid in the destruction of anthocyanins in strawberry juice and model systems. Food Res. 18: 475.
Tinsley, Ian J. and Bockian, A.H. 1960. Some effects of sugars on the breakdown of pelargonidin-3-glucoside in model systems at 90°C. Food Res. 25: 161.
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Volatile Flavor Components in the Headspace of the Australian or "Bowen" Mango

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ABSTRACT

The volatile constituents of the "Bowen" mango (*Mangifera indica*, Kensington Pride) have been isolated by concentration of the headspace vapors on Tenax-GC. The major components in the ripe fruit are ethyl butanoate and terpinolene, although a number of other esters and terpenes are present in smaller amounts. The concentration of esters was markedly decreased when the volatile constituents were isolated by simultaneous distillation-extraction and the method is, therefore, considered to be less useful than headspace concentration. As the fruit is allowed to ripen on storage the concentration of ethyl butanoate decreases accompanied by an increase in the concentration of higher esters.

INTRODUCTION

THE ASSESSMENT of the flavor of fruits and vegetables is an important aspect in both the development of new species and in the control of quality during production of canned and pulped products.

Mango (*Mangifera indica*) is one of the most appealing of tropical fruits and several hundreds of different cultivars are grown in various parts of the world. These are known to vary markedly in their flavor characteristics (Nagy and Shaw, 1980). It is, therefore, not surprising that several workers have investigated the volatile organic components by gc/ms and reported considerable variation in the chemical composition, particularly with respect to terpenes (MacLeod and Pieris, 1984; Gholap and Bandypadhyay, 1975) and esters (Engel and Tressel, 1983).

The varieties which have been most extensively studies are Venezuelan (MacLeod and Troconis, 1982) and Alphonso (Idstein and Schreier, 1985; Hunter et al., 1974). However, no reports have appeared on the volatile components of the "Bowen" Mango, *Mangifera indica* (Kensington Pride), which is one of the most important tropical fruits grown in the Northern parts of Queensland, Australia. Furthermore, with the exception of the work by Ackerman and Torline (1984), all the studies on other varieties have used classical procedures of flavor isolation such as steam distillation or solvent extraction. Such procedures are known to cause both qualitative and quantitative changes in the volatile components (Schamp and Dirinck, 1982).

Since the pioneering work of Jennings et al. (1972) and Bertsch et al. (1974) the technique of headspace concentration on Tenax-GC (Dirinck et al., 1977) has gained wide acceptance and has been extensively applied to the analysis of volatiles from food products (Ioff and Vitenberg, 1984; Charalambous, 1978). In fact, the technique has been reported to give more meaningful results than total volatile analysis using distillation or extraction procedures (Galt and MacLeod, 1984; Adam, 1984; Pankow and Ligocki, 1985).

The purpose of this study was to identify the chemical components responsible for the unique flavor of the "Bowen" mango and to compare its flavor profile with those of other varieties reported in the literature. We have also examined the way in which the composition of the volatile material changes as the fruit ripens on storage.

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MATERIALS & METHODS

FRESH RIPE MANGOS were collected from a normal harvest in the Bowen district of Northern Queensland.

Triangle testing was used to establish that the chosen fruit was characteristic of the variety under study. A panel of eight trained panelists familiar with the sensory characteristics of the variety was used. One of the samples in each set was from fruit of authenticated plants and panelists were asked to select the different sample. All the samples of fruit chosen were found to match with authentic samples of the "Bowen" mango.

The chosen fruit was divided into three groups of eight mangos in each group. The first group was processed immediately and was designated as "Ripe Fruit." The second group was allowed to ripen for eight days at 25°C and designated as "Very Ripe Fruit." The third group was allowed to ripen for a further eight days at 25°C and was designated as "Over Ripe Fruit." The skin of these latter mangos had darkened to a very deep brown and the fruit was very soft to touch.

Before analysis the kernels and skins were removed and the pulp from each group blended. Two 250 g samples of pulp were then taken from each group and the volatile components obtained. Two different extraction methods were used [Headspace Concentration and Simultaneous Distillation Extraction (SDE)], although the SDE method was applied only to the "Ripe Fruit."

Headspace concentration

The freshly macerated pulp was placed in a two necked flask. Two glass lined stainless steel tubes (1.5 mm × 115 mm) packed with Tenax-GC (2-3 mg, 60-80 mesh) were connected in series between the flask and a rotary vacuum pump. A nitrogen bleed was installed in the other neck of the flask and purified nitrogen drawn over the surface of the fruit pulp at 15 cm^3/min. for two hours (1.8 dm^3 N_2).

Each tube was then desorbed separately in an SGE Unijector at 250°C for 2 min. (flow rate 5 cm^3/min.) and trapped on a 50 m fused silica BP-1 column at 5°C using liquid CO_2 as coolant. The column was then temperature programmed to 150° at 2°/min. The output from the gas chromatograph was fed directly to the source of a Kratos MS-25 mass spectrometer scanning continuously at 1 sec./decade and chromatograms were obtained as plots of total ion current (TIC) versus time.

The tubes were usually run on the same day that the samples were collected. However, there were no observable changes after storage of the tubes in closed glass vessels for a period of two weeks.

Simultaneous distillation-extraction (Likens-Nickerson)

A second sample of pulp was placed in a flask connected to one arm of the Likens-Nickerson apparatus (Likens and Nickerson, 1964) and enough water added to just cover the fruit pulp. Approximately 100 cm^3 of a 1:1 mixture of redistilled pentane and redistilled diethyl ether was added to a flask connected to the second arm of the apparatus. Extraction was continued for a period of 3 hours, the extract dried (sodium sulphate), and the volume reduced to about 5 cm^3 by careful distillation using a Vigreux column. Further concentration to a volume of 100 µl was achieved by gas entrainment (−20°, 0.5 mm Hg).

Five microliter samples were then subjected to GC/MS analysis under the same conditions as described above but using the split mode of injection.

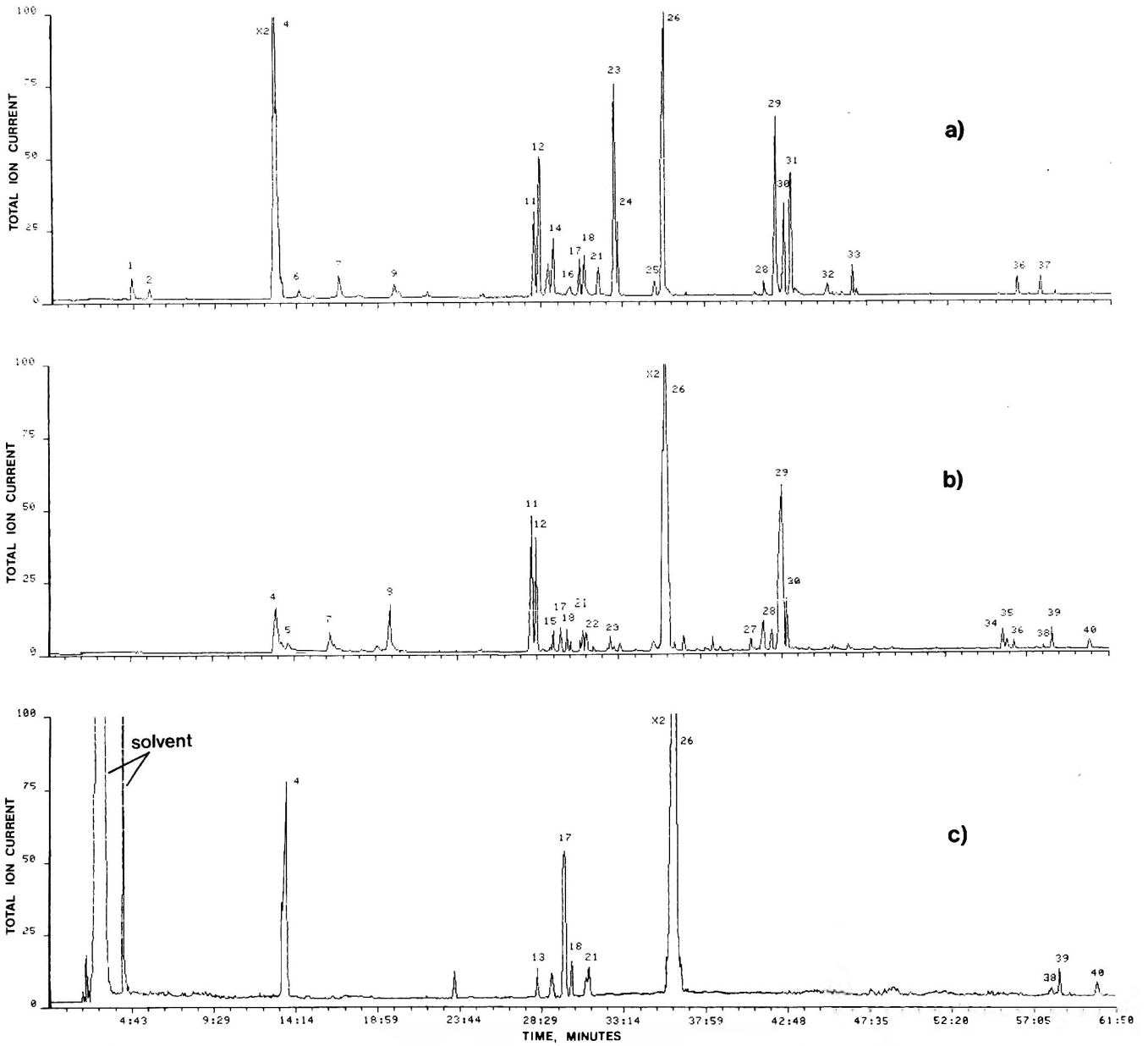

Fig. 1—Gas Chromatograms of "Bowen" Mango Constituents. (a) Fresh, Ripe Fruit (Headspace); (b) Over Ripe Fruit (Headspace); (c) Fresh, Ripe Fruit (SDE).

RESULTS & DISCUSSION

TYPICAL CHROMATOGRAMS of the flavor components observed by the headspace method are shown in Fig. 1a and 1b for both "ripe" and "over-ripe" specimens. For comparison, a chromatogram of the components obtained by simultaneous distillation-extraction is given in Fig. 1c for the "ripe" fruit only. The components were identified by comparison of their mass-spectra and Kovats Indeces with those of authentic samples and with published data (Ryhage and von Sydow, 1963; Jennings and Shibamoto, 1980). A list of the compounds identified is given in Table 1.

The flavor profile of the "Bowen" mango differs markedly from that of the other varieties reported in the literature (Engel and Tressel, 1983; MacLeod and Pieris, 1984; Sakho et al., 1985; Idstein and Schreier, 1985). Forty components were observed and the chromatograms were dominated by two major components (ethyl butanoate and terpinolene) which together accounted for 72% of the total volatile organic material in the fresh ripe fruit.

The large proportion of ethyl butanoate (45%) and other aliphatic esters is unusual. Most other species contain only small quantities of esters and only Baladi (Engel and Tressel, 1983) is reported to contain large amounts of ethyl butanoate. Most of the monoterpenes present in other species were also detected in the present case, but this region of the chromatogram was dominated, in the present case, by terpinolene (27%). Only the Willard and Parrot varieties are reported to contain large amounts of terpinolene (MacLeod and Pieris, 1984) and these contain only small amounts of ethyl butanoate. Furthermore, the varieties Alphonso, Baladi, and Jaffna are reported to contain large amounts of myrcene, ocimene and α-pinene. Only myrcene was dectected in the present case. Again, a number of varieties have been shown to contain large amounts of 3-carene (MacLeod and Troconis, 1982; MacLeod, 1985) but, in the "Bowen" mango, only moderate amounts of this compound were detected.

Although, previous studies have revealed the presence of substantial amounts of aliphatic alcohols (Engel and Tressel, 1983; Idstein and Schreier, 1985), furfural (MacLeod and Tro-

Table 1—Volatile constituents of Bowen mango

Peak no.	Compound	K.I.	Amount (%) Headspace Ripe	Very ripe	Over ripe	SDE Ripe
1	Ethyl acetate	655	0.30	-	-	-
2	3-Methyl-2-butanone	670	0.10	-	-	-
3	1-Butanol	700	-	0.1	0.05	-
4	Ethyl butanoate	793	44.60	20.50	5.33	21.51
5	Butyl acetate	810	-	-	0.20	-
6	1-Methylethyl 2-propenoate	805	0.17	-	-	-
7	Ethyl 2-butenoate	835	0.87	1.61	3.33	-
8	1-Hexanol	860	-	3.40	9.86	-
9	3-Methylbutyl ethanoate	865	0.41	0.19	-	-
10	2-Methylpropyl butanoate	935	-	-	0.20	-
11	Butyl butanonate (or 1-methylpropyl)	980	2.61	9.80	30.38	-
12	Ethyl hexanoate	985	2.87	2.49	6.09	-
13	Myrcene	985	2.26	1.51	-	1.23
14	3-Hexenyl ethanoate	990	0.31	-	-	-
15	Hexyl acetate	995	-	0.19	0.91	-
16	Thujene	1000	0.23	0.21	-	1.62
17	3-Carene	1005	1.01	1.10	0.39	10.38
18	α-Terpinene	1008	1.35	0.91	0.84	1.78
19	p-Cymene	1010	-	-	0.14	-
20	β-Phellandrene	1018	-	-	0.03	-
21	Limonene	1020	1.28	0.90	0.52	2.52
22	Butyl 2-butenoate	1023	-	-	0.15	-
23	Pentyl butanoate	1040	6.68	4.32	0.29	-
24	2-Methylbutyl 2-methylpropanoate	1045	0.97	-	-	-
25	1-Methyl-4-(1-methylethenyl)benzene	1060	0.28	0.18	0.41	-
26	Terpinolene	1076	26.91	46.43	64.41	54.99
27	4-Methylacetophenone	1145	-	-	0.06	-
28	2-Methyl-1-propenylbenzene	1155	0.09	0.07	0.4	-
29	3-Hexenyl butanoate	1165	3.59	-	0.31	-
30	Hexyl butanoate	1174	1.40	2.82	9.89	-
31	Ethyl octanoate	1180	2.43	2.78	0.38	-
32	3-Hexenyl pentanoate	1210	0.07	0.12	-	-
33	3-Methylbutyl octanoate	1230	0.29	0.08	-	-
34	Hexyl hexanoate	1365	-	-	0.14	-
35	Octyl butanoate	1370	-	-	0.04	-
36	Ethyl decanoate	1375	0.18	0.10	0.03	-
37	n-Tetradecane	1400	0.13	-	-	-
38	α-Gurjunene	1404	-	-	0.01	0.56
39	β-Caryophylene	1411	-	-	0.15	0.12
40	α-Humulene	1439	-	-	0.03	0.80

conis, 1982), lactones (Idstein and Schreier, 1985) and aliphatic acids (Sakho et al., 1985) we could detect none of these compounds in the fresh ripe fruit, even by the use of single ion monitoring techniques. It is interesting to note that in all cases where these compounds have been detected, distillation or solvent extraction techniques have been used. Furthermore, Sakho et al. (1985), who used headspace methods as well as steam distillation found such compounds only in the steam distillate. It is also significant that Hunter et al. (1974) observed high concentrations of furfural, furanones, and lactones in the volatile components of *cooked* canned mangos. These findings imply that such compounds may not be part of the true flavor profile but that they may be artifacts of the extraction process. Thus, although the chromatograms from the Headspace Concentration technique are less complex than those observed by some workers for other mango varieties, they may more closely reflect the true flavor profile of the fruit.

The results from the Likens-Nickerson simultaneous distillation extraction (SDE) technique showed little correlation with the headspace results (Table 1 and Fig. 1c). In particular, the concentration of esters detected was considerably reduced compared with the headspace method. Only ethyl butanoate was present in detectable quantity. This observation may be a consequence of the volatility of some of the esters but it is likely that some hydrolysis has also occurred as a result of the conditions used in this technique. It is worth noting, however, that the terpenic region more closely resembled that observed for other varieties by some previous workers. For example, up to 10% of 3-carene was detected together with small amounts of α-pinene. These observations, once again raise doubts about the validity of extraction methods for determining flavor profiles. The sesquiterpenes (caryophylene, gurjunene, and humulene) were detected by this method but, although they were also observed in over-ripe fruit examined by the headspace method, the importance of these less volatile materials to the flavor of the fruit is questionable.

In fact, the reproducibility of the SDE method was poor and a considerable variation in both the total amount of extractable material and in the composition of the extractables was observed depending on the severity of the boiling process and the duration of the extraction. This method was, therefore, applied only to the fresh ripe fruit in order to provide some means of comparison with previous work on other species.

Effects of storage

It is well known that the unique flavor of the "Bowen" mango is seriously affected if the fruit is allowed to become over-ripe. It is, therefore, not surprising that as the fruit was allowed to ripen by storage at 25° subtle changes in the flavor profile were observed. The effect can be seen in the data reported in Table 1. The composition of the flavor profile after eight days of ripening represented an intermediate stage between the fresh ripe fruit and that which had been ripened for sixteen days. Thus, only the results for the "over-ripe" fruit (16 days) are discussed in detail. Fig. 1a and 1b show respectively, the chromatograms for the "ripe", and "over-ripe" fruit.

The most significant change was the dramatic decrease in the concentration of ethyl butanoate and a corresponding increase in the higher esters (butyl butanoate and hexyl butanoate) and in aliphatic alcohols (butanol and hexanol). These changes are apparently due to trans-esterification processes, presumably the result of enzymic activity. This would dramatically alter the flavor of the fruit since ethyl butanoate is a very volatile substance and is undoubtedly a major contributor to the unique flavor of the fresh fruit of this particular variety.

Ripening was also accompanied by a large increase in the terpinolene content but since this component is already present in significant amounts in the ripe fruit the subjective effect on the flavor may not be as obvious as the decrease in the ethyl butanoate level. An increase in sesquiterpene content was also observed but, as mentioned above, these compounds probably do not markedly influence the flavor of the fruit.

Thus the "Bowen" mango contains a unique combination of volatile components and this undoubtedly accounts for the unique flavor reported from sensory trials of the species. However, the volatility of one of the major components, accompanied by chemical changes, results in rapid deterioration of the flavor on storage.

REFERENCES

Ackerman, I.G.J. and Torline, P.A. 1984. Volatile Components in the Headspace of Eight Mango Cultivars. Lebensm. -Wissens und Technol. 17(6): 339.

Adams, S. 1984. Analysis of volatile aroma components in fruit and vegetable juices. In "Chromatography and mass spectrometry in nutrition science and food safety," A. Frigerio and H. Milon (Ed.), p. 67. Elsevier, Amsterdam.

Bertsch, W., Chang, R.C., and Zlatkis, A. 1984. The determination of organic volatiles in air pollution studies. J. Chromat. Sci. 12: 175.

Charalambous, G. (Ed.). 1977. "Analysis of Foods and Beverages; Headspace Techniques." New York: Academic Press, 1978.

Dirinck, P., Schreyen, L., and Schamp, N. 1977. Aroma quality evaluation of tomatoes, apples and strawberries. J. Agric. Food Chem. 25: 759.

Engel, K.H. and Tressl, R.J. 1983. Studies on the volatile constituents of two mango varieties. Food Agric. Chem. 31: 796.

Galt, M.G., and MacLeod, G. 1984. Headspace sampling of cooked beef aroma using Tenax-GC. J. Agric. Food Chem. 32: 59.

Gholap, A.S. and Banbypadhyay, C. 1975. Comparative assessment of aromatic principles of ripe Alphonso and Langra mango. J. Food Sci. Technol. 12: 262.

Hunter, G.L.K., Bucek, W.A., and Radford, T. 1974. Volatile components of canned Alphonso mango. J. Food Sci. 39: 900.

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Measurement of Papaya Maturity by Delayed Light Emission

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ABSTRACT

Papaya at five stages of maturity were evaluated for differences in intensity of delayed light emission (DLE), chlorophyll, β-carotene and Hunter color values. Results showed there were high correlations between DLE intensity and chemical and physical properties that relate to papaya maturity. DLE has a high potential as a rapid screening technique for identifying papayas which are one-half ripe and riper.

INTRODUCTION

THE 1984 PRODUCTION of papayas in Hawaii was 80 million pounds with an estimated value of $9 million. About 70% of this production was shipped to the United States mainland and Canada, 15% was exported to Japan, and only 15% was consumed in Hawaii. The export market is very important to the Hawaiian papaya industry.

Hawaiian papayas (*Carica papaya* L.) are infested by the oriental fruit fly, (*Dacus dorsalis* Hendel), the melon fly (*Dacus cucurbitae* Coquillett), and the Mediterranean fruit fly (*Ceratitis capatata* [Wiedeman]). All species can be spread as larvae or eggs in fruits shipped from infested areas. Prior to 1983, quarantine restrictions required that papayas be treated with a hot water dip and then fumigated with ethylene dibromide (EDB) prior to shipment to the mainland (Anonymous, 1978; Havens et al., 1979; Seo et al., 1979). However, in September, 1984, EDB was banned as a fumigant for papayas in interstate commerce. Discontinuance of EDB treatments threatened the continuing success of the important export market. A new quarantine procedure was developed as an alternative to fumigation with EDB (Couey and Hayes, 1986). The new procedure is based on the nonsusceptibility to fruit-fly infestation of papayas which are less than one-half ripe and on the lethality of a two stage heat treatment to eggs of the fruit flies. Seo et al. (1982) and Couey et al. (1984) found that papayas which are riper than half-ripe are the most susceptible to infestation by the fruit flies. Presently, papayas that are ripe enough to support fly infestation are identified by measuring the amount of yellow color present on the surface of the fruit with a Hunter colorimeter. Fruit having an amount of yellow color that is typically present on half-ripe fruit are excluded from export shipments to minimize the risk of the spread of the fruit flies.

Colorimeters currently used for estimating papaya maturity were not designed for high speed sorting; therefore, there is a need for equipment that will nondestructively and rapidly sort papayas into categories of maturity. Birth et al. (1984) demonstrated that immature and mature-green fruit, which were indistinguishable by visual examination, could be separated by body transmittance spectroscopy into nonripening and ripening groups. The equipment used in this work also was not designed for high speed sorting.

Papayas undergo large changes in chlorophyll and related compounds during maturation; therefore, it seemed possible to sort them into categories of maturity by delayed light emission (DLE). It has been demonstrated that if chlorophyll containing plant materials are illuminated with a light source and then placed in darkness, they will emit a very low intensity light for several sec or min (Strehler and Arnold, 1951). The duration and intensity of the DLE has been shown to be positively correlated with the concentration of chlorophyll and related compounds in the material (Tollin et al., 1958; Jacob et al., 1965). Previous research has included investigations to determine the feasibility for applying DLE to determine the maturity of tomatoes (Chuma and Nakaji, 1976; Forbus et al., 1985b), Satusma oranges (Chuma et al., 1977), fresh tea leaves (Nakaji et al., 1978) and bananas (Chuma et al., 1980).

The objectives of this study were to evaluate the effects of changes in the physical and chemical properties of papaya during maturation on the intensity of the DLE they produce and to determine the feasibility for sorting papayas into categories of maturity by DLE.

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MATERIALS & METHODS

TWENTY-EIGHT PAPAYAS at five different stages of maturity were harvested in Hawaii on August 8, 1985. On the same date they were shipped by air and were received in Athens, GA on August 12. Each fruit was marked to indicate its stage of maturity at harvest based on visual examination of its external surface color by experienced personnel. A mark was placed on the abaxial side of the fruit which was exposed to direct sunlight while it was attached to the tree. Stages of maturity used were similar to those defined by Akamine and Goo (1971) and were as follows: (1) immature green - entire external surface dark green and fruit will never ripen; (2) mature green - entire surface dark green but fruit will ripen normally; (3) color turning - barely visible yellow streaks emanating from blossom end; (4) eighth ripe - ⅛ of external surface area has turned yellow; (5) quarter ripe - ¼ yellow; (6) half ripe - ½ yellow; and (7) three quarter ripe - ¾ yellow. Only fruit of the maturity stages through quarter ripe were harvested because it was anticipated that some ripening would occur during shipment. Immediately after arrival, each fruit was evaluated subjectively by visual examination to determine its stage of maturity. Samples were held in darkness for 1 hr prior to making DLE measurements to eliminate the effects of previous light excitation. DLE measurements were made on the blossom end of each fruit and at four positions, 90° apart around the circumference, midway between the stem and blossom end. The first measurement around the circumference was made on the abaxial side of the fruit. Looking at the blossom end, the fruit was rotated 90° at a time in a clockwise direction to obtain readings on the remaining sides, which will hereafter be referred to as the abaxial + 90°, the adaxial, and the adaxial + 90°.

The experimental DLE meter developed by Forbus et al. (1985a) was modified and used for making the DLE measurements. A schematic drawing of the meter is shown in Fig. 1. The meter is a totally dark enclosure having an upper and lower compartment. To make a DLE measurement, the papaya (P) was placed over the 5 cm diameter round opening in the bottom of the upper compartment. The papaya was oriented so that the area of the fruit surface on which the measurement was to be made covered the opening and faced in the direction of the lower compartment. The light source (L), an 80-watt 30 volt tungsten halogen projector lamp (Sylvania EKP-ENA), was turned on for 3 sec. The 3 sec illumination time was used because it conveniently resulted in detector responses to DLE in the range 0–1 volt for the full range of papaya maturity. Light from the source was reflected by the tilting mirror (TM) through the round opening and

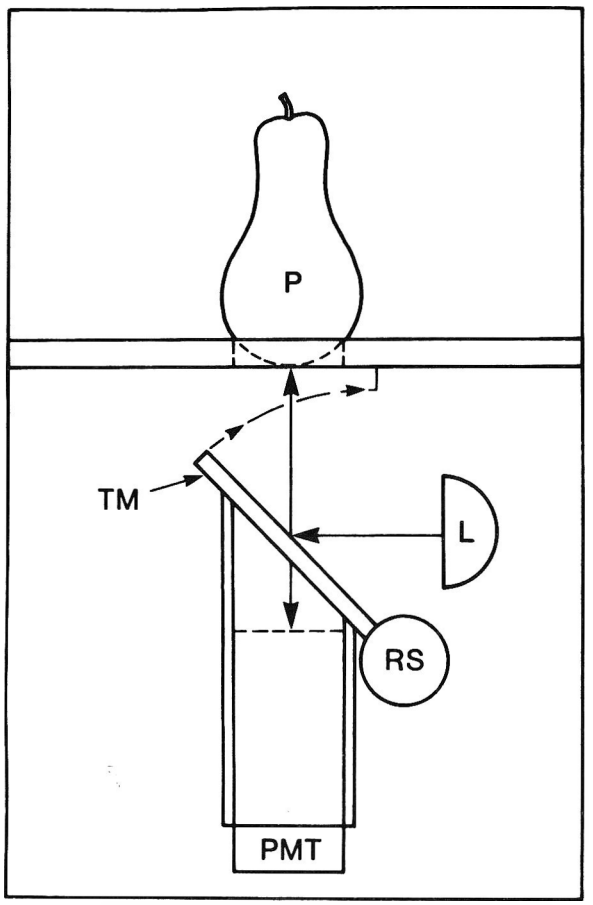

Fig. 1—Schematic diagram of experimental equipment for measuring delayed light emission (DLE): P = papaya; L = lamp; TM = timing mirror; RS = rotary solenoid; PMT = photomultiplier tube.

onto a 78.5 cm^2 area of the sample surface. The lamp was turned off, and the tilting mirror was rotated 45° upward to the vertical position by the action of the Ledex model 5SR rotary solenoid (RS). With the tilting mirror in the vertical position, the end window of the RCA 8645 photomultiplier tube (PMT) was exposed and the DLE from the sample surface caused a low intensity current to flow from the anode of the photomultiplier. The current was amplified, converted to a d-c voltage and recorded with a strip chart recorder (Heath Model SR-204) during a 15 sec interim for each measurement. In this study, DLE intensity is expressed as volts of detector response. Papayas were held in darkness for 10 min between measurements at each of the 5 positions previously described.

Hunter color values were determined with a Hunterlab D25-2 colorimeter using a 5.08 cm viewing port on the optical head. L (lightness), a (green to red) and b (yellow to blue) values were measured on the external surface of each fruit at the same 5 positions DLE measurements were made. L, a and b values were also determined for internal flesh by excising a section between the internal seed cavity and the blossom end from each papaya. The Hunter a and b values obtained were used to compute values for hue angle ($\theta = \tan^{-1} b/a$), a parameter that has been shown to be useful for predicting the visual color appearance of food products (Little, 1975).

Chlorophyll a and b and β-carotene of each papaya were determined by spectrophotometric procedures similar to those of Brown et al. (1970) and Forbus et al. (1985a). Five samples of epidermal tissue, averaging ca 1.5g ea, were excised from each papaya at the same positions on the fruit where the DLE measurements were made. Sections were taken parallel to the skin to a depth not exceeding 5 mm. The samples were then blended for 5 min at medium speed with a Polytron homogenizer (Brinkman Corp.) with 50 mg $CaCo_3$ and 50 mL acetone. The solvent and extracted chlorophylls were removed with a Buchner funnel, filter aid and vacuum after which the pad and pulp were returned for an additional 5 min of blending with 50 mL of a mixture of acetone-ethyl ether (4:1 v/v). This procedure was repeated again with 50 mL of a mixture of acetone-ethyl ether (2:3 v/v) for complete extraction of the carotenoids. The combined extracts were placed in a separatory funnel where the acetone was removed with repeated washings with water saturated with NaCl. The ether suspension of pigments was brought to 100 mL volume and dried with anhydrous Na_2SO_4. Absorbances were measured at 660 and 642 nm (Goodwin, 1976) with a Perkin-Elmer, Lambda Model 2b, spectrophotometer for determination of chlorophyll a and b, respectively. Absorbances were converted to μg quantities with the spectrophotometer from determined concentration factors ("K" factors) of standard solutions of authentic compounds (Sigma Chemical Co., St. Louis, MO). For the determination of β-carotene, the previous aliquots were recombined with the original extracts, 10 mL methanol saturated with KOH were added and the mixtures were allowed to stand for 30 min after vigorous shaking. The methanol soluble mixtures of saponified materials were then drawn off and the residual contaminants were removed from the ether-carotenoid mixtures by repeated washings with water saturated with NaCl. The solutions were brought to 100 mL volume with ethyl ether, dried with anhydrous Na_2SO_4, and the absorbances were measured at 453 nm (Goodwin, 1976) with the spectrophotometer and results were expressed as μg of β-carotene/g of fresh wt. material. Soluble solids, measured with an Abbe' refractometer and expressed as % soluble solids, were determined on samples removed from the designated areas with a #10 cork borer. After maceration followed by centrifugation at 10,000 × g, the clarified juice was representative of cross sectional areas from the epidermal tissue to the seed cavity.

Analysis of variance was performed on the entire data set to determine whether there were significant differences between measurements taken at different positions on each fruit. Position means for all variables were compared by Duncan's Multiple Range Test (Duncan, 1955). The 28 papayas were ranked in order of increasing maturity based on decreasing internal θ values and regressions of DLE on papaya by position were determined. All possible correlations between variables were determined.

RESULTS & DISCUSSION

A TYPICAL RECORDER response to DLE for papayas is shown in Fig. 2. Point A on the baseline is the instant the photomultiplier was exposed to the papaya. Point B indicates the magnitude of detector response to DLE 0.25 sec after the light source was turned off. The response curve shows that the maximum DLE intensity occurred immediately after the illumination was removed and then rapidly decayed. Point C is the instant the recorder was turned off. The DLE values reported in this study are the maximum values obtained at Point B.

Analysis of variance F values for position effects and position means for the variables measured on 28 papayas at different stages of maturity are given in Table 1. There were differences ($P < 0.01$ or $P < 0.05$) in measurements by po-

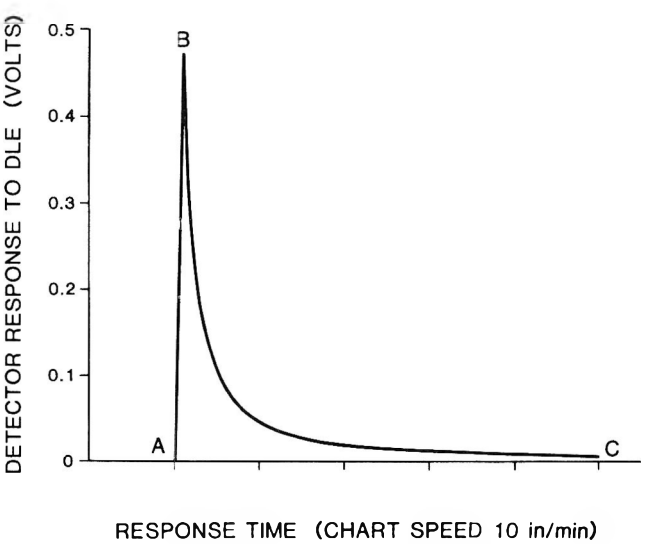

Fig. 2—Typical detector response to DLE for papayas.

Table 1—Analysis of variance F values for position effects and position means for variables measured on papaya fruit

Variable	F value	Position means[a]				
		Blossom end	Abaxial side	Abaxial side + 90°C	Adaxial side	Adaxial side + 90°C
DLE, volts	6.94**	0.72^{cd}	0.67^{d}	0.70^{cd}	0.80^{b}	0.73^{c}
Total chlorophyll, μg/g	5.96**	418.96^{c}	638.54^{b}	656.04^{b}	400.82^{c}	489.75^{c}
β-Carotene, μg/g	37.68**	163.43^{cd}	253.50^{b}	265.68^{b}	150.18^{b}	185.71^{c}
Soluble solids, %	1.40	9.75	11.49	9.57	9.55	9.90
External θ, degrees	2.60*	111.50^{c}	116.29^{bc}	118.77^{b}	118.79^{b}	118.91^{b}

[a] Means for DLE are based on 4 measurements at each position for the 28 papaya and means for all other variables are based one one measurement at each position for each papaya.

[b-d] Means in same row with a common superscript are not significantly different ($P < 0.05$).

* Significant at 95% level.

** Significant at 99% level.

sition for all variables except soluble solids. Chlorophyll and β-carotene measurements taken on the abaxial side of the fruit were greater ($P < 0.05$) than measurements taken on the adaxial side. Soluble solids measurements were slightly higher for the abaxial side than for the adaxial side but the differences were not statistically significant at the 5% level. External θ values for the blossom end were lower ($P < 0.05$) than for all positions except the abaxial side. The lower values of external θ indicated less green and more yellow color on the fruit surface.

It was interesting to note that DLE measurements taken on the abaxial side of the papayas were lower ($P < 0.05$) than those taken on the adaxial side even though chlorophyll was higher for the abaxial side. This is contrary to previous studies (Jacob et al., 1965; Forbus et al., 1985a) where DLE intensity has been shown to be positively correlated with chlorophyll. The lower DLE intensities associated with higher chlorophyll on the abaxial side of the fruit may be explained by the possibility that photosynthesis was taking place at a faster rate there because of the longer exposure to direct sunlight. When the abaxial side surface of the fruit was illuminated, for taking a DLE measurement, a large amount of the incident light energy may have been absorbed and utilized to continue photosynthesis. This would have reduced the amount of light energy available for contributing to DLE and may explain the lower level of DLE intensities obtained on the abaxial side of the fruit. Since there were significant differences in DLE by fruit position, it was necessary to determine the position that would be most effective for predicting papaya maturity. This was achieved by ranking the 28 papayas in order of increasing maturity based on decreasing values of the internal θ measurements made 4 days after harvest. We believed that these color measurements taken on the internal flesh would be the best estimate of relative maturity. Regression analyses by position of DLE on the ranked papayas showed that the DLE measurements taken on the external surface at the blossom end were the most effective for predicting papaya maturity. The R^2 values obtained were 0.60 for the blossom end, 0.19 for the abaxial side, 0.04 for the abaxial side + 90°, 0.25 for the adaxial side and 0.33 for the adaxial side + 90°. Previous work (Forbus et al., 1985b) has also shown that blossom end DLE measurements were most effective for predicting tomato maturity.

Subjective estimates of maturity at harvest and 4 days after harvest and values for the chemical and physical properties measured on the blossom ends of the 28 papayas evaluated are given in Table 2. In the table the papayas were ranked in order of increasing maturity based on decreasing values of internal θ obtained 4 days after harvest. The decrease in the values of internal θ indicated the gradual change in color of the internal flesh from greenish white to yellow which occurred during normal ripening. Differences between the estimates of maturity at harvest and 4 days after harvest demonstrated the difficulty of subjectively sorting papayas into categories of maturity by evaluation of external color. Although it was recognized that changes in maturity occurred during the 4 days between harvest and testing, it was obvious that some of the papayas were misclassified at harvest. For example, if papayas 7, 8 and 11 were properly classified at harvest, they would have decreased in maturity during the 4 days between harvest and testing. It also appeared that papayas 23 and 26 were judged to be less mature than they actually were. These results demonstrated the need for technology to accurately and nondestructively estimate papaya maturity.

Values of the chemical and physical properties for these 28 papayas illustrated the changes in these properties that occurred during the ripening process. The gradual decrease in external and internal θ values reflected the change in color of the external fruit surface from dark green to yellow and the internal flesh from white to yellow, respectively. Total chlorophyll of the papayas decreased during maturation while soluble solids increased.

The DLE values for those papayas classified as immature green (IG) in Table 2 were variable, but after the immature green stage, DLE values decreased at a fairly uniform rate. The relationship between DLE intensity and papaya maturity for the 28 papayas evaluated is shown in Fig. 3. The regression equation for the curve is:

$$DLE = -0.001P^2 + 0.26P + 0.650 \quad (R^2 = 0.60)$$

where DLE = detector response to DLE in volts and P = papaya rank in order of increasing maturity based on values of internal θ. The means of the observed values of DLE were plotted around the regression curve and papayas were identified by rank. The variability of the observed DLE values for papayas (1-8) indicated that some factor associated with immature green papayas interfered with their ability to produce DLE and suggested that DLE sorting might only be applicable for papayas which were riper than the immature green stage. Table 3 shows that the correlations between DLE and all variables, except β-carotene, were much higher for papayas 9-28 than for papayas 1-28. The relationship between DLE intensity and papaya maturity for papayas 9-28 is shown in Fig. 4. The regression equation for the line is:

$$DLE = -0.018P + 1.054 \quad (R^2 = 0.84)$$

where DLE = detector response to DLE in volts and P = rank in order of increasing maturity. These results showed that when only papayas 9-28 were considered, rather than papayas 1-28, the R^2 value for the regression of DLE on papaya rank was improved from 0.60 to 0.84.

To determine the feasbility for DLE sorting of papayas for maturity, the means of the observed values of DLE were plotted around the regression line for papayas 9-28 (Fig. 3) with points being identified by rank. The correlation between DLE intensity and papaya maturity was high ($r = -0.92$) for the 20 papayas; however it appeared that it might be difficult to effectively sort papayas into all categories of maturity. The data indicated that it may be feasible to separate fruit that was less than ½ ripe from fruit that was ½ ripe or riper by DLE. Table 2 shows that papayas 9-20 were judged to be less than ½ ripe 4 days after harvest and papayas 21-28 were classified as ½ ripe or riper. Fig. 3 shows that a DLE sorting system using a 0.66 V detector response, indicated by the broken line,

Table 2—Stages of maturity[a] at harvest and maturity and values of physical and chemical properties determined after harvest for 28 papayas ranked in order of increasing maturity based on decreasing values of internal θ

Papaya rank	Maturity at harvest[b]	Maturity 4 days after harvest	Values[d] for blossom end: External θ (degrees)	Internal θ (degrees)	Total chlorophyll (μg/g)	β-carotene (μg/g)	Soluble solids (%)	DLE (volts)
1	IG	IG	123.1	107.9	637	149	4.4	0.81
2	IG	IG	123.7	106.3	866	211	4.8	0.74
3	IG	IG	123.6	105.8	726	171	5.0	0.60
4	IG	IG	123.0	105.4	786	157	4.7	0.69
5	IG	IG	123.7	104.3	882	200	5.2	0.57
6	IG	IG	125.2	104.3	783	169	5.4	0.79
7	MG	IG	125.3	104.3	570	133	4.8	0.73
8	MG	IG	125.8	102.2	780	205	5.5	0.81
9	MG	MG	120.5	83.1	582	145	9.7	0.86
10	MG	MG	118.4	79.2	361	106	9.5	0.80
11	CT	MG	118.4	78.2	402	147	11.1	0.86
12	MG	MG	120.5	78.1	561	184	9.4	0.87
13	MG	CT	118.0	76.3	484	158	12.1	0.81
14	CT	CT	116.6	75.4	412	158	12.6	0.84
15	CT	CT	115.8	75.0	298	139	12.7	0.81
16	CT	1/8	114.2	73.2	254	147	11.7	0.79
17	CT	1/4	103.5	69.8	176	159	12.9	0.77
18	1/8	1/4	109.5	67.4	287	172	13.3	0.78
19	1/8	1/4	105.5	66.6	344	181	11.6	0.67
20	1/8	1/4	106.5	66.1	175	113	11.9	0.74
21	1/8	3/4	97.3	65.5	224	197	14.3	0.65
22	1/4	3/4	92.6	64.9	149	140	14.6	0.65
23	CT	3/4	93.9	63.7	79	195	12.7	0.60
24	1/4	1/2	97.6	63.5	191	158	12.5	0.64
25	1/4	3/4	91.8	63.1	101	133	13.7	0.54
26	1/8	1/2	102.2	62.5	268	209	9.9	0.65
27	1/4	1/2	89.0	60.9	173	191	12.8	0.47
28	1/4	3/4	96.2	60.6	180	149	14.0	0.60

[a] Stages of papaya maturity: IG-immature green; MG-mature green; CT-color turning; 1/8-1/8 ripe; 1/4-1/4 ripe; 1/2-1/2 ripe.
[b] Based on subjective evaluation of external color.
[c] Based on subjective evaluation of internal color.
[d] Values for all variables except soluble solids and DLE are based on one measurement per fruit. DLE values are the means of 4 measurements and soluble solids values are the means of 4 measurements per fruit made 90° apart around the circumference midway between the stem and blossom end.

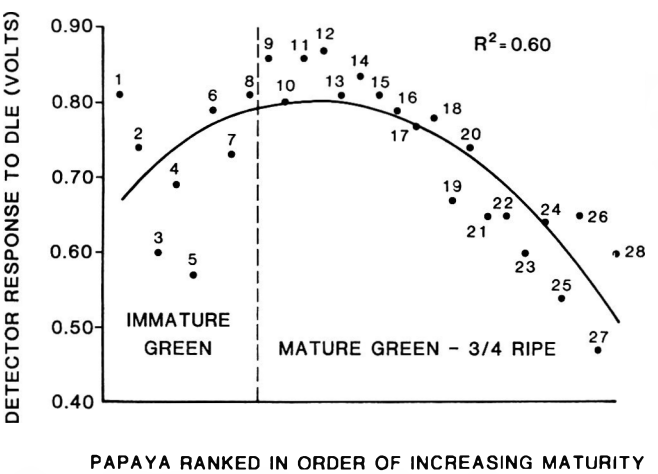

Fig.3—Regression of DLE on 28 papayas ranked in order of increasing maturity.

Table 3—Simple correlations[a] between DLE and variables evaluated for 28 papayas

Variable	Correlation coefficients: Papaya 1–28	Papaya 9–28
Total chlorophyll	0.29	0.77
β-Carotene	−0.27	−0.32
Soluble solids	−0.29	−0.71
External θ	0.64	0.95
Internal θ	0.26	0.89
Maturity ranking[b]	−0.77	−0.92

[a] $P < 0.05$ for $r > 0.53$ and $P < 0.01$ for $r > 0.63$.
[b] Ranked in order of increasing maturity based on decreasing values of internal θ.

would have properly separated all 20 papayas into two broad maturity classes.

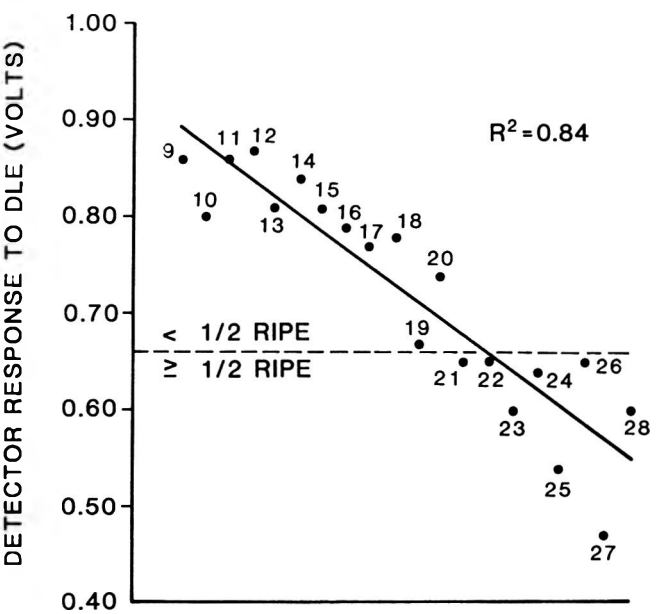

Fig. 4—Regression of DLE on 20 papayas ranked in order of increasing maturity.

These results indicate that DLE sorting may not be feasible for sorting papayas into all stages of maturity. However, it appeared that DLE sorting could provide the papaya industry with a rapid and nondestructive technique for identifying papayas which are ½ ripe or riper. Papayas of this maturity have

been shown to be the most susceptible to infestation by fruit flies (Seo et al., 1982; Couey and Hayes, 1986). Identification and removal of this fruit from export shipments would reduce the risk of spreading fruit flies in uninfested areas. Additional work is needed to confirm that these results are applicable to sorting freshly harvested Hawaiian papayas under commerical operating conditions.

REFERENCES

Akamine, E.K. and Goo, T. 1971. Relationship between surface color development and total soluble solids in papaya. HortScience 6: 567.

Anonymous, 1978. Combination hot water dip and fumigation, (Rev.) T102(5) (ai), p. 9. In "Plant Protection and Quarantine Treatment Manual." USDA, Animal and Plant Health Inspection Service, Plant Protection and Quarantine Services. Section VI.

Birth, G.S., Dull, G.G., Magee, J.B., Chan, H.T., and Cavaletto, C.G. 1984. An optical method for estimating papaya maturity. J. Am. Soc. Hort. Sci. 109(1): 62.

Brown, H.E., Meredith, F.I., Saldano, G., and Stevens, T.S. 1970. Freeze peeling improves quality of tomatoes. J. Food Sci. 35: 485.

Chuma, Y. and Nakaji, K. 1976. Optical properties of fruits and vegetables to serve the automatic selection within the packing house line (4) - Delayed light emission as a means of automatic selection of tomatoes. J. Soc. Agr. Mach. (Japan) 38(2): 217.

Chuma, Y., Nakaji, K., and Okura, M. 1980. Maturity evaluation of bananas by delayed light emission. ASAE Trans. 23(4): 1043.

Chuma, Y., Sein, K., Kawano, S., and Nakaji, K. 1977. Delayed light emission as a means of automatic selection of Satsuma oranges. ASAE Trans. 20(5): 996.

Couey, H.M. and Hayes, C.F. 1986. Quarantine procedure for Hawaiian papaya using fruit selection and a two stage hot water immersion. J. Econ. Entomol. (In press)

Couey, H.M., Linse, E.S., and Nakamura, A.N. 1984. Quarantine procedure for Hawaiian papayas using heat and cold treatments. J. Econ. Entomol. 77: 984.

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

Forbus, W.R. Jr., Hardigree, G.A., and Adams, J.H. 1985a. Experimental delayed light emission meter for horticultural crops. ARS-41 USDA.

Forbus, W.R. Jr., Senter, S.D., and Wilson, Ruel L. 1985b. Measurement of tomato maturity by delayed light emission. J. Food Sci. 50: 750.

Goodwin, T.W. 1976. "Chemistry and Biochemistry of Plant Pigments," 2nd ed. Academic Press, New York.

Havens, C., Hu, B.K.S., Farias, G.J., Seo, S.T., and Harris, E.J. 1979. Dacus dorsalis: fumigation of papayas with ethylene dibromide. J. Econ. Entomol. 72: 4.

Jacob, F.C., Romani, R.J., and Sprock, C.M. 1965. Fruit sorting by delayed light emissions. Trans. ASAE 8(1): 18.

Little, A.C. 1975. Off on a tangent. J. Food Sci. 40: 410.

Nakaji, K., Sagiura, Y., and Chuma, Y. 1978. Delayed light emission of green tea as a means of quality evaluation (1) - Delayed light emission of fresh tea leaves. J. Soc. Agric. Mach. (Japan) 39: 483.

Seo, S.T., Chambers, D.L., Akamine, E.K., Harris, E.J., Lee, C.Y.L., and Komura, M. 1979. Oriental fruit fly: factors affecting ethylene dibromide fumigation of infested papayas. J. Econ. Entomol. 72: 350.

Seo, S.T., Farias, G.J., and Harris, E.J. 1982. Oriental fruit fly: ripening of fruit and its effect on the index of infestation of Hawaiian papayas. J. Econ. Entomol. 75: 173.

Strehler, B.L. and Arnold, W.A. 1951. Light production by green plants. J. Gen. Physiol. 34: 809.

Tollin, G., Fumimori, E., and Calvin, M. 1958. Action and emission spectra for the luminescence of green plant materials. Nature 181: 1266.

Ms received 7/14/86; revised 10/4/86; accepted 10/23/86.

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AIR DRYING APRICOTS. . .From page 345

Mrak, E.M. and Perry, R.L. 1948. Dehydrating freestone peaches. California Agricultural Experiment Station circular No. 381.

Noyes, R. 1969. Dehydration processes for convenience food. p. 164. Noyes Development Corporation, London.

Nury, F.S., Taylor, D.H., and Brekke, J.E. 1960. Apricots: Research for Better Quality in Dried Fruits. Report of Agricultural Research Service, U.S. Department of Agriculture, Albany 10, CA.

Resnik, S. and Chirife, J. 1979. Effect of moisture content and temperature on some aspects of nonenzymatic browning in dehydrated apple. J. Food Sci. 44(2): 601.

Stadman, E.R., Barkh, H.A., Haas, V., and Mrak, E.M. 1966. Storage of dried fruit influence of temperature on deterioration of apricots. Ind. Eng. Chem. 38: 541.

Torrey, M. 1974. Dehydration of Fruits and Vegetables. Food Technol Review No. 13. Noyes Data Corporation, New Jersey.

Vaccarezza, L.M., Lombardi, J.L., and Chirife, J. 1974. Heat transfer effects on drying rate of food dehydration. Can. J. Chem. Eng. 52: 576.

Ms received 6/8/86; revised 11/3/86; accepted 11/5/86.

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VOLATILE COMPONENTS OF BOWEN MANGO. . .From page 355

Idstein, H. and Schreier, P. 1985. Volatile constituents of Alphonso mango. Phytochemistry 24: 2313.

Ioff, B.V. and Vitenberg, A.G. 1984. "Headspace Analysis and Related Methods in Gas Chromatography." Wiley, New York.

Jennings, W.G., Wohleb, R., and Lewis, M.J. 1972. Gas chromatographic analysis of headspace volatiles in alcoholic beverages. J. Food Sci. 37: 69.

Jennings, W. and Shibamoto, T. 1980. "Qualitative Analysis of Flavor and Fragrance Volatiles by Glass Capillary Gas Chromatography." Academic Press, New York.

Likens, S.T. and Nickerson, G.B. 1964. Detection of certain Hop oil constituents in brewing products. Proc. Am. Soc. Brew. Chem. 5.

MacLeod, A.J. 1985. Volatile compounds of two cultivars of mango from Florida. J. Agric. Food Chem. 33: 380.

MacLeod, A.J. and de Troconis, N.G. 1982. Volatile flavor components of mango fruit. Phytochemistry 21: 2523.

MacLeod, A.J. and Pieris, N.M. 1984. Comparison of the volatile components of some mango cultivars. Phytochemistry 23: 361.

Nagy, S. and Shaw, P.E. (Ed.) 1980. "Tropical and Subtropical Fruits." AVI Publ. Co., Westport.

Pankow, J.F. and Ligocki, M.P. 1985. Assessment of adsorption/solvent extraction with polyurethane foam and adsorption/thermal desorption with Tenax-GC for the collection and analysis of ambient organic vapors. Anal. Chem. 57: 1138.

Ryhage, R. and von Sydow, E. 1963. Mass spectrometry of terpenes. Acta Chem. Scand. 17: 2025.

Sakho, M., Crouzet, J., and Seck, S. 1985. Volatile components of African mango. J. Food Sci. 50: 548.

Schamp, N. and Dirinck, P. 1982. The use of headspace concentration on Tenax for objective evaluation of fresh fruits. In "Chemistry of Foods and Beverages," G. Charalambous (Ed.), p. 25. Academic Press, New York.

Ms received 7/2/86; revised 9/23/86; accepted 10/21/86.

Quality of Fresh-Market Peaches Within the Postharvest Handling System

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ABSTRACT

Peaches (cv 'Redglobe') were sampled at six points in the postharvest handling system to determine the effects of physiological maturity at harvest and identify the step(s) where alternate handling techniques would lead to improved quality at point of purchase. Samples were evaluated for maturity, color, firmness, soluble solids, and total acidity. Harvest maturity and temperature management were identified as the most critical factors in the postharvest system. Firmness was the most limiting quality attribute. Improvement of peach quality can be achieved within the existing handling system by strict control of harvest maturity and allowing peaches to ripen at ambient temperatures (18–24°C) at the retail outlet or in the home just prior to consumption.

INTRODUCTION

THE FRESH-PEACH INDUSTRY is concerned with the recent decline in peach consumption despite a national trend toward increased purchase and consumption of fresh fruits and vegetables (Bunch, 1984). One explanation for this decline is the practice of harvesting fruit at too early a stage of physiological maturity which results in the inadequate quality development during postharvest ripening (Baumgardner, 1985).

Peach quality has been characterized for fresh (Rood, 1957; Salunkhe et al., 1968; Kader et al., 1982), canned (Culpepper et al., 1955; Bedford and Robinson, 1955; Boggess and Heaton, 1973; Fuleki and Cook, 1975; Kader et al., 1982) and frozen (Culpepper et al., 1955; Bedford and Robertson, 1955) products. The peach, a climacteric fruit, continues to ripen off the tree as evidenced by changes in quality characteristics. Ground color (green-yellow portion of the fruit) changes from green to full yellow as chlorophyll disappears and carotenoids are synthesized (Salunkhe et al., 1968). Blush color (bright red areas) is not usually appreciably affected during postharvest storage as anthocyanin synthesis is primarily catalyzed by sunlight in the orchard (Bedford and Robertson, 1955). Culpepper et al. (1955) noted, however, an increase in anthocyanin synthesis at higher temperature storage (30°C). Softening during postharvest ripening is attributed to solubilization of pectin in cell walls (Postlmayr et al., 1956; Shewfelt, 1965) as accomplished by polygalacturonase activity (Pressey et al., 1971; Pressey and Avants, 1973). Flavor development upon ripening is characterized by an increase in sugars (Salunkhe et al., 1968; Sandu et al., 1983), a decline in acids (Salunkhe et al., 1968) and development of flavor volatiles (Lim and Romani, 1964; Do et al., 1969).

Peach quality is affected by numerous factors both prior to, during and after harvest. Preharvest effects on quality that have been identified include cultivar selection (Kader et al., 1982; Sandu et al., 1983), fertilization (Cummings and Reeves, 1971), pruning (Marini, 1985), application of growth regulators (Baumgardner et al., 1972; Morris et al., 1978; Dekazos, 1985) and tree position (Marini and Trout, 1984). Physiological maturity at harvest (Culpepper et al., 1955; Rood, 1957; Deshpande and Salunkhe, 1964; Salunkhe et al., 1968; Fuleki and Cook, 1975; Baumgardner, 1985; Shewfelt et al., 1986a) and mechanical damage (Ginn, 1962; O'Brien et al., 1963, 1978) influence subsequent quality of the fruit. Storage temperature (Culpepper et al., 1955; Dekazos, 1985) and relative humidity (Sommer and Mitchell, 1959) are major factors in determining postharvest quality. Chilling injury as evidenced by wooliness has been described after storage at 0°C (Fisher et al., 1943; Ben-Arie et al., 1970; Anderson and Penney, 1975; Buescher and Furmanski, 1978).

Most studies of peach quality during postharvest handling and storage have focused on a particular step in the handling system. Little effort has been made to follow changes in quality through realistic handling regimes. The systems approach has been employed to study quality changes of southern peas (Shewfelt et al., 1985), tomatoes (Shewfelt et al., 1986b) and snap beans (Shewfelt et al., 1986c). The systems approach allows for the assessment of the effect of each handling step on final product quality and provides the basis for establishing coordination and control of quality within the operation (Thompson, 1984; Shewfelt, 1986). Systems approaches have been used to assess damage and handling of peaches in an orchard (Horsfield et al., 1971; Sims et al., 1973) and during transportation to a processing plant (O'Brien et al., 1978), but no previous studies have used a systems approach to evaluate quality changes of peaches during postharvest handling.

The objectives of this study were (1) to document a typical fresh-market peach handling system, (2) to determine the effects of physiological maturity at harvest on quality changes during postharvest handling, and (3) to identify the factor(s) in the system that limit quality at the point of purchase.

MATERIALS & METHODS

A TYPICAL postharvest handling system for fresh-market peaches was documented through interviews with peach growers and packers, wholesale produce distributors, retail produce managers, extensions specialists and colleagues in peach research. From information obtained, an idealized, defined system was established as outlined in Fig. 1. Samples were collected at six steps in the handling system as follows: (A) upon arrival at a peach packinghouse in Fort Valley, GA; (B) after hydrocooling, grading, sorting and packing in the packinghouse (peaches were packed on the day of arrival but could be held up to 6 hr at 5°C prior to packing; only fruit graded as U. S. No. 1 for shipping was used from this point on.); (C) after transport 40 km to the Georgia Experiment Station (exposure to non-refrigerated conditions for up to 3 hr); (D) departure from 3 days simulated warehouse storage conditions at 5°C; (E) departure from 2 days simulated retail conditions at 5°C, and (F) after 2 days simulated consumer storage at 21°C.

Initial evaluation at steps A and B (prior to leaving the peach packinghouse) was conducted in a mobile laboratory (Prussia and Tollner, 1984) located at the packinghouse facility. All samples from steps A and B were evaluated for maturity at the greenest part of the peach using color chips (Delewiche and Baumgardner, 1985). The color of

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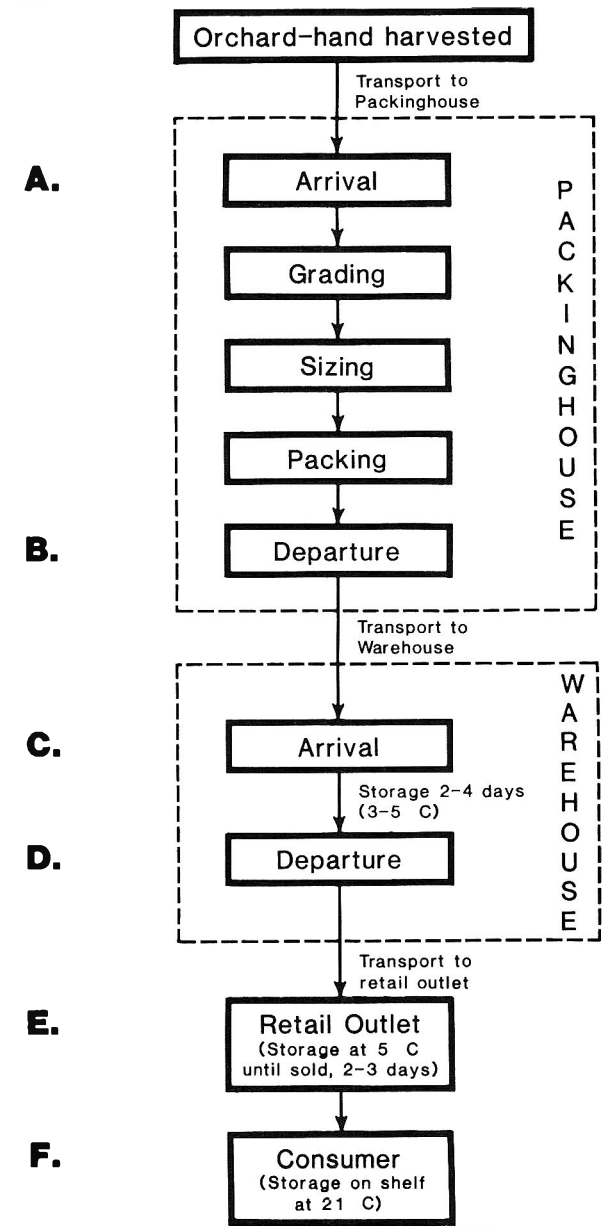

Fig. 1—Postharvest handling system for fresh-market peaches.

each peach was then determined using a Gardner XL-845 colorimeter recording blush color (reddest point on the equator of the peach) and the ground color (least red point on equator).

Unless the peaches were being evaluated in the mobile laboratory (~ 22°C), they were stored in the packinghouse refrigerated storage area (~ 5°C). Twenty peaches from step A and 40 peaches from step B were selected by a predetermined sampling plan for destructive measurements (described below). The remaining samples from step B (160 fruit) were labelled and handled under conditions simulating the postharvest system described above. Forty fruits were selected based on a predetermined numbering scheme for color and destructive measures at each sampling point (steps C-F).

Firmness measurements were determined at 2 points on the equator of the peach 90° apart by puncture. A Universal Fruit Tester with a foot pedal attachment was used to allow for more even application of pressure. Fruit were peeled prior to puncture. Due to the large volume of samples, peaches that had been punctured were halved and the unpunctured half was frozen for later analysis (moisture, soluble solids, pH and total acidity). Moisture concentration was determined by drying to a constant weight at 110°C. Percentage soluble solids was measured on juice squeezed from individual fruit with a Bausch & Lomb Abbe refractometer equipped with a flowing water chamber to maintain constant temperature (20°C). Total acidity was determined on a blended sample (1:5 dilution with distilled H_2O) by titration to pH 8.1 using 0.1N NaOH and reported as malic acid. Subsequent analyses (steps C - F) were performed at the experiment station laboratories following similar regimes.

Standard analyses of variance techniques for unbalanced designs were performed to determine the effects of handling step and maturity on quality attributes (SAS, 1982). Due to inadequate sample sizes of the more mature fruit, the 6-point maturity scale of Delewiche and Baumgardner (1985) was reduced to 4 points (1, 2, 3 and 4 - where physiological maturity increases with the number designation).

RESULTS & DISCUSSION

BLUSH, ground and average color changes during handling are shown in Table 1. Average color changes observed during handling were characterized by a gradual lightening (higher L values) and a decrease in hue angle (lower $\tan^{-1}$ b/a). Changes in ground color during handling were most consistent and appeared to have the greatest influence on average color values. A decrease in hue angle for ground color corresponds to less green (− to + a) and more yellow (higher + b) character. These changes are consistent with an increase in carotenoids accompanying a decline in chlorophyll (Salunkhe et al., 1968). Little change in hue was observed in ground color until the retail storage part of the study (step E) with most pronounced changes occurring under higher temperatures (21°C, step F). Differences in blush color readings were not as consistent and were difficult to interpret. Since all samples were screened from light, except during sample measurement, little or no anythocyanin synthesis would be expected. Culpepper et al. (1955) observed biosynthesis of anythocyanins in peaches stored at 30°C and above, but it is not clear whether those peaches were exposed to light during storage.

No significant interaction effects were observed on lightness or hue for maturity at harvest by handling step. A significant main effect was observed for maturity for each of the hue angle values (Table 2). No separation was observed for any of the L measurements. Blush hue angle provided the best means of separation of immature color classes (1 from 2), but ground hue angle provided better separation of samples of recommended maturity (3 and 4) from the immature peaches (1 and 2). In both cases the 'a' value (green-red) had greater influence on hue than the 'b' value (yellow-blue). Less negative 'a' values for ground color correspond to lower chlorophyll concentrations. More positive 'a' values for blush color correspond to higher anthocyanin concentrations.

An interaction effect of maturity and handling step on peach firmness was observed. Mean separations by Duncan's multiple range analysis are shown in Table 3. All peaches were firm at the packinghouse (steps A & B) and softening was rapid in fruit from all levels when the temperature was raised to 21°C (step F). Softening became apparent at different points in the handling system based on initial maturity level. Peaches harvested at maturity level 1 remained firm through step E while those at maturity levels 2 and 3 showed evidence of softening at step E, and level 4 at step D. The rate of fruit softening after harvest was greatest for fruits that were most mature at harvest.

Inadequate sample sizes for chemical data forced a consolidation of fruit at maturity levels 3 and 4 [recommended as optimal harvest maturity by Baumgardner (1985)]. Significant interaction effects of maturity at harvest by handling step were observed for °Brix, total acidity and the Brix-to-acid ratio (BAR). Changes in BAR during handling as affected by maturity are shown in Fig. 2. The significant decrease in Brix-to-acid ratio (BAR) in the 3/4 maturity level between steps A and B may reflect the sorting out of fruit at the packinghouse judged by the graders as over-ripe. Peaches at the 3/4 maturity level appeared to ripen gradually from steps B to E while levels 1 and 2 did not ripen until removal to room temperature (step F). The ultimate BAR was lower in maturity level 1 than from the other maturity levels. Note that no significant changes in sol-

Table 1—Effect of handling step on instrumental color readings

Handling Step	n[x]	Blush L	Blush a	Blush b	Blush tan⁻¹ b/a	Ground L	Ground a	Ground b	Ground tan⁻¹ b/a
A. Packinghouse arrival	100	38.0 ab	8.5 a	15.9	61.5 a	50.4 a	−2.3 a	22.8 a	95.2 a
B. Packinghouse departure	200	37.0 a	12.7 b	16.0	51.8 b	51.6 ab	−2.1 a	24.1 bc	94.8 a
C. Warehouse arrival	40	36.7 a	12.9 b	15.1	49.7 b	53.3 bc	−2.4 a	24.8 c	95.1 a
D. Warehouse departure (2 days, 5°C)	40	40.9 bc	12.0 b	15.7	52.3 b	54.9 c	−2.2 a	25.0 c	94.9 a
E. Retail departure (3 days, 5°C)	40	41.1 bc	11.7 b	15.8	53.3 b	54.4 c	−0.2 b	23.3 ab	90.0 b
F. Consumer (2 days, 21°C)	40	42.7 c	17.8 c	16.6	42.8 c	57.4 d	6.6 c	26.4 d	76.0 c

[a-d] Means in a column with the same letter are not significantly different by the Duncan's multiple range test ($P \leq 0.05$).
[x] n = number of fruit evaluated per step.

Table 2—Effect of maturity on instrumental color readings

Color chip	n[x]	Blush L	Blush a	Blush b	Blush tan⁻¹ b/a	Ground L	Ground a	Ground b	Ground tan⁻¹ b/a
1	76	39.0	9.0 a	16.6	61.2 a	52.8	−3.6 a	24.2	98.4 a
2	112	38.2	11.2 b	15.8	54.8 b	53.0	−2.9 a	24.2	96.8 a
3	95	37.4	12.4 bc	15.7	52.2 b	51.6	−1.5 b	23.8	93.6 b
4	67	37.2	13.3 c	15.3	49.7 b	51.3	−0.1 c	23.6	90.1 c

[a-c] Means in a column with the same letter are not significantly different by the Duncan's multiple range test ($P \leq 0.05$).
[x] n = number of fruit evaluated for each maturity level.

Table 3—Effect of maturity at harvest and handling step on fruit firmness (N) as measured by puncture

Handling step	n[x]	Color chip 1	2	3	4	Avg.
A. Packinghouse arrival	15	-[y]	84 ab	75 a	79 a	79 a
B. Packinghouse departure	38	89 a	89 a	72 a	81 a	83 a
C. Warehouse arrival	39	87 a	85 a	88 a	79 a	85 a
D. Warehouse departure (2 days, 5°C)	34	93 a	91 a	78 a	56 ab	83 a
E. Retail departure (3 days, 5°C)	37	91 a	67 b	54 b	49 b	65 b
F. Consumer (2 days, 21°C)	36	0 b	0 c	0 c	0 c	0 c
	Avg.	72	73	54	46	65
	n	45	64	52	38	199

[a-c] Means in a column followed by the same letter are not significantly different as evaluated by the Duncan's multiple range test ($P \leq 0.05$).
[x] n = number of fruit evaluated for each step or maturity level.
[y] No observations.

Table 4—Effect of maturity at harvest and handling step on soluble solids (°Brix) and total acidity (%)

Handling Step	n[x]	Soluble solids 1	2	3/4	Avg.	Total acidity 1	2	3/4	Avg.
A. Packinghouse arrival	8	-[y]	9.7 a	10.1 a	9.9 a	-[y]	0.70 ab	0.55 a	0.63 a
B. Packinghouse departure	19	11.4 a	9.9 ab	10.7 ab	10.6 ab	0.77 ab	0.69 ab	0.69 a	0.71 b
C. Warehouse arrival	20	11.2 a	10.4 ab	10.2 a	10.7 b	0.81 b	0.77 b	0.68 a	0.77 b
D. Warehouse departure (2 days, 5°C)	14	11.2 a	11.0 bc	11.9 bc	11.3 b	0.79 ab	0.71 ab	0.69 a	0.73 b
E. Retail departure (3 days, 5°C)	19	10.7 a	11.6 c	11.4 abc	11.3 b	0.70 ab	0.73 ab	0.68 a	0.71 b
F. Consumer (2 days, 21;°C)	18	11.9 a	13.7 d	12.7 c	12.6 c	0.65 a	0.63 a	0.59 a	0.62 a
Avg.		11.3	10.9	11.4	11.2	0.75	0.72	0.65	0.70
n		34	28	36	98	34	28	36	98

[a-d] Means in a column with the same letter are not significantly different by the Duncan's multiple range test ($P \leq 0.05$).
[x] n = number of samples evaluated at each step or maturity level.
[y] No observations.

uble solids were observed at maturity level 1 and that no significant changes in acidity were observed in maturity level 3/4 (Table 4). A gradual increase was observed in soluble solids accumulation through the postharvest system. Total acidity reached a maximum at step C followed by a decline.

Implications for the postharvest handling system

In the orchard, fruit harvested at maturity levels 2, 3 and 4 [based on the color chips developed by Delewiche and Baumgardner (1985)] appeared to be acceptable for maintenance of firm 'Redglobe' peaches during handling while achieving high Brix-to-acid ratios. Optimum harvest maturity levels may vary for other cultivars.

Softening reduces quality of fruit at all maturity levels. A lowering of postharvest temperatures might be effective in maintaining firmness, particularly for fruit harvested at high levels of maturity (Shewfelt et al., 1986a). From a practical standpoint, however, changing warehouse temperatures in the fresh produce industry for a single commodity is unlikely. It has been our observation that most wholesale and retail distributors use 5°C as a compromise temperature that provides minimal damage to most chilling-sensitive commodities while maintaining acceptable quality of most chilling-resistant commodities.

Rapid changes in color and firmness, soluble solids and acidity occurred as peaches were transformed from 5°C to 21°C to simulate shelf-top storage in the home. Thus, loose fruit expected to be sold and consumed that day should be allowed to ripen at ambient temperature (18–24°C), while peaches sold in larger-quantity containers should be maintained at low temperatures (< 5°C) to allow the consumer to ripen the fruit as needed. Ironically, most retail peaches are marketed loose in supermarkets at low temperature and in bulk at roadside stands at ambient temperature. The consumer is advised to use refrigeration to hold peaches and to ripen them for 12–24 hr at ambient temperatures before consuming them when fruit give to slight finger pressure.

The next step in the analysis of the postharvest handling system of peaches is to more fully understand the costs and

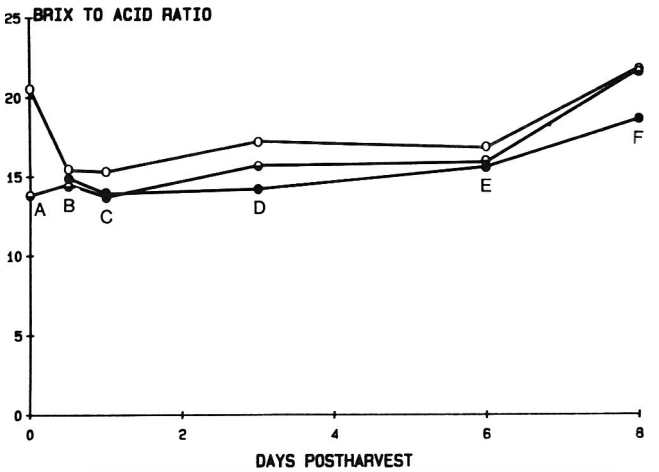

Fig. 2—Effect of maturity and handling step on Brix-to-acid ratio of fresh-market peaches. Maturity levels correspond to color chips 1 (●), 2 (◒) and 3 & 4 (○) with 1 representing the least mature stage. Handling steps sampled included A, packinghouse arrival; B, packinghouse departure; C, warehouse arrival; D, warehouse departure (after storage 2 days, 5°C); E, retail departure (3 days, 5°C); and F, consumer (2 days, 21°C).

benefits of changes in the system. The costs of changes in handling techniques are usually known. What characteristics of highest value that most affect the price of peaches can help determine the economic feasibility of alternative handling techniques or new technologies.

The emphasis of this article, like others using the systems approach (Shewfelt et al., 1985, 1986b, 1986c), has been on maintenance of quality during realistic handling conditions and not on shelf-life extension. Extensive observation of packing, warehouse and retail sales operations have led us to conclude that perishable produce moves through the system based on rigid inventory turnover schedules and extended shelf life is to no handlers' economic advantage unless it changes the product from perishable to non-perishable. On the other hand, maintenance of optimum quality between harvest and purchase or consumption is important in consumer satisfaction and could influence repeat purchase decisions.

SUMMARY & CONCLUSIONS

THE POSTHARVEST handling system of southeastern U.S., fresh-market peaches was documented and analyzed. Harvest maturity and temperature control were identified as the most critical factors in ultimate market quality. To maintain quality and markets for fresh peaches, growers have the responsibility for harvesting fruit at optimal maturity (levels 2–4 for 'Redglobe') and packers have the responsibility of enforcing these standards. In addition, education of retail personnel and consumers on temperature management of peach ripening just prior to consumption could lead to greater consumer satisfaction.

REFERENCES

Anderson, R.E. and Penney, R.W. 1975. Intermittent warming of peaches and nectarines stored in a controlled atmosphere or air. J. Am. Soc. Hort. Sci. 100: 151.

Baumgardner, R.A. 1985. Harvesting quality peaches. Proc. Natl. Peach Council, p. 17.

Baumgardner, R.A., Stembridge, G.E., Van Blaricom, L.O., and Gambrell, C.E. 1972. Effects of succinic acid-2, 2-dimethylhydrazide on the color, firmness, and uniformity of processing peaches. J. Am. Soc. Hort. Sci. 97: 485.

Bedford, C.L. and Robertson, W.F. 1955. Harvest maturity and ripening of peaches in relation to quality of the canned and frozen products. Mich. State Agric. Exp. Sta. Tech. Bull. 245.

Ben-Arie, R., Lavee, S., and Guelfat-Reich, S. 1970. Control of woolly breakdown of 'Elberta' peaches in cold storage, by intermittant exposure to room temperature. J. Am. Soc. Hort. Sci. 95: 801.

Boggess, T.S. and Heaton, E.K. 1973. Canning southeastern freestone peaches. Univ. Ga. Agric. Exp. Sta. Res. Rept. 159.

Buescher, R.W. and Furmanski, R. J. 1978. Role of pectinesterase and polygalacturonase in the formation of woolliness in peaches. J. Food Sci. 43: 264.

Bunch, K. 1984. Food consumption, prices, and expenditures. U.S. Dept. Agric. Econ. Res. Serv. Stat. Bull. 713.

Culpepper, C.W., Haller, M.H., Demaree, K.D., and Koch, E.J. 1955. Effect of picking maturity and ripening temperature on the quality of canned and frozen Eastern-grown peaches. USDA Tech. Bull. No. 1114.

Cummings, G.A. and Reeves, J. 1971. Factors influencing chemical characteristics of peaches. J. Am. Soc. Hort. Sci. 96: 320.

Dekazos, E.D. 1985. Effects of postharvest treatments on ripening and quality of 'Baby gold 7' peaches. HortScience 20: 240.

Delewiche, M.J. and Baumgardner, R.A. 1985. Ground color as a peach maturity index. J. Am. Soc. Hort. Sci. 110: 53.

Deshpande, P.B. and Salunkhe, D.K. 1964. Effects of maturity and storage on certain biochemical changes in apricots and peaches. Food Technol. 18: 1195.

Do, J.Y., Salunkhe, D.S., and Olson, L.E. 1969. Isolation, identification and comparison of volatiles of peach fruit as related to harvest maturity and artificial ripening. J. Food Sci. 34: 618.

Fisher, D.V., Britton, T.E., and O'Reilly, H. J. 1943. Peach harvesting and storage investigations. Sci. Agric. 24: 1.

Fuleki, T. and Cook, F.I. 1975. Relationship of maturity as indicated by flesh color to quality of canned clingstone peaches. J. Inst. Can. Sci. Technol. Aliment. 9: 43.

Ginn, J.L. 1962. Evaluation of selected consumer packages and shipping containers for peaches. U.S. Dept. Agric. AMS Mark. Res. Rept. 533.

Horsfield, B.C., Fridley, R.B., and Claypool, L.L. 1971. Systems analysis of postharvest handling of mechanically harvested peaches. Trans. ASAE 14: 1040.

Kader, A.A., Heintz, C.M., and Chordas, A. 1982. Postharvest quality of fresh and canned clingstone peaches as influenced by genotypes and maturity at harvest. J. Am. Soc. Hort. Sci. 107: 947.

Lim, L. and Romani, R.J. 1964. Volatiles and the harvest maturity of peaches and nectarines. J. Food Sci. 29: 246.

Marini, R.P. 1985. Vegetative growth, yield, and fruit quality of peach as influenced by dormant pruning, summer pruning, and summer topping. J. Am. Soc. Hort. Sci. 110: 133.

Marini, R.P. and Trout, J.R. 1984. Sampling procedures for minimizing variation in peach fruit quality. J. Am. Soc. Hort. Sci. 109: 361.

Morris, J.R., Ray, L.D., and Cawthon, D.L. 1978. Quality and postharvest behavior of once-over harvested clingstone peaches treated with daminozide. J. Am. Soc. Hort. Sci. 103: 716.

O'Brien, M., Claypool, L.L., and Leonard, S.J. 1963. Effect of mechanical vibrations on fruit damage during transportation with special reference to cling peaches. Food Technol. 17: 1578.

O'Brien, M., Fridley, R.B., and Claypool, L.L. 1978. Food losses in harvest and handling systems for fruits and vegetables. Trans. ASAE 21: 386.

Postlmayr, H.L., Luh, B.S., and Leonard, S.J. 1956. Characterization of pectin changes in freestone and clingstone peaches during ripening and processing. Food Technol. 10: 618.

Pressey, R. and Avants, J.K. 1973. Separation and characterization of endopolygalacturonase and exopolygalacturonase from peaches. Plant Physiol. 52: 252.

Pressey, R., Hinton, D.M., and Avants, J.K. 1971. Development of polygalacturonase activity and solubilization of pectin in peaches during ripening. J. Food Sci. 36: 1070.

Prussia, S.E. and Tollner, E.W. 1984. Human factors for mobile agricultural research laboratory. Trans. ASAE 27: 997.

Rood, P. 1957. Development and evaluation of objective maturity indices for California freestone peaches. Proc. Am. Soc. Hort. Sci. 70: 104.

Salunkhe, D.K., Deshpande, P.B., and Do, J.Y. 1968. Effects of maturity and storage on physical and biochemical changes in peach and apricot fruits. J. Hort. Sci. 43: 235.

Sandu, S.S., Dhillon, B.S., and Randhawa, J.S. 1983. Chromatographic estimation of sugars from the components of developing fruits of early- and late-maturing peach cultivars. J. Hort Sci. 58: 197.

SAS. 1982. "SAS User's Guide: Statistics." SAS Institute, Inc. Cary, NC.

Shewfelt, A.L. 1965. Changes and variations in the pectic constitution of ripening peaches as related to product firmness. J. Food Sci. 30: 573.

Shewfelt, R.L. 1986. Postharvest treatment for extending the shelf life of fruits and vegetables. Food Technol. 40(5): 70.

Shewfelt, R.L., Myers, S.C., and Resurreccion, A.V.A. 1986a. Effect of physiological maturity at harvest on peach quality during low temperature storage. J. Food Qual. In press.

Shewfelt, R.L., Prussia, S.E., Hurst, W.C., and Campbell, D.T. 1986b. Quality changes of vine-ripened tomatoes within the postharvest handling system. J. Food Sci. In press.

Shewfelt, R.L., Prussia, S.E., Jordan, J.L., Hurst, W.C., and Resureccion, A.V.A. 1986c. A systems analysis of postharvest handling of fresh snap beans. HortScience 21: 470.

Shewfelt, R.L., Prussia, S.E., Hurst, W.C., and Jordan, J.L. 1985. A systems approach to the evaluation of changes in quality during postharvest handling of southern peas. J. Food Sci. 50: 769.

Sims, E.T., Webb, B.K., Hood, C.E., and Gambrell, C.E. 1973. Quality of fresh market peaches subjected to a mechanical harvesting and field handling: grading system. J. Am. Soc. Hort. Sci. 98: 253.

Sommer, N.F. and Mitchell, F.G. 1959. Fruit shrivel control in peaches and nectarines. Proc. Am. Soc. Hort. Sci. 74: 199.

Thompson, R.A. 1984. Introduction to systems analysis. In "A Systems Approach to Vegetable Handling and Processing." W.C. Hurst (Ed.), p. 1. Misc. Publ. 191 Univ. GA Coop. Ext. Serv. Athens, GA.

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A Shelflife Evaluation of an Oriented Polyethylene Terephthalate Package for Use with Hot Filled Apple Juice

M.R. McLELLAN, L.R. LIND, and R.W. KIME

ABSTRACT

A year long storage study was undertaken to evaluate an oriented polyethylene terephthalate (OPET) container for use with hot filled juice. Freshly made juice as well as single strength juice reconstituted from commercial apple juice concentrate were evaluated. The results indicated that the oxidation of the juice by way of oxygen permeation through the OPET barrier was slow enough to allow for a satisfactory shelf life as compared to glass-packaged samples over a 6–9 month period. Surface-to-volume effects did not become significant until late in the study. Difference and preference tests were used to establish shelflife comparison between OPET packages and glass-packaged products.

INTRODUCTION

NEW PACKAGING OPTIONS for processors has become quite extensive. Trade journals constantly invite packaging specialists in the fruit juice industry to look at new films, new laminates, and new technolgies (Peters, 1985). In recent years, a technique of forming polyethylene terephthalate (PET) containers under carefully controlled conditions of temperature, stretch and flow has led to an oriented polymer (OPET). This biaxial orientation of the polymer has led to a packaging container with improved impact strength, gas barrier properties, stiffness and clarity (Irwin, 1985). The OPET container is currently in use by the fruit juice industry for the packaging of hot filled cranberry juice (Anon. 1985).

Although the OPET container has significant improvements over its nonoriented counterpart, it still is far from the impervious barrier provided by glass. It is well known that storage conditions can have a dramatic effect on the quality of bottle apple juice and presence of oxygen would have particular impact (Moyer and Aitken, 1980; Pollard and Timberlake, 1971). However, economies motivate the processor to assess the alternatives to glass. Depending upon the properties of the new containers, there may exist additional advantages to the product and process other then those of economies.

The objectives of this study were to evaluate the use of OPET containers for the packaging of hot filled apple juice. Consideration was given to possible alternative processing methods as well as storage longevity as determined by sensory techniques.

MATERIALS & METHODS

OPET CONTAINERS, both 2 quart and 1 quart, were provided by Monsanto Corporation (Monsanto Plastics & Resins Co., Bloomfield, CT.). Table 1 shows some of their physical characteristics. Two quart glass containers were used to provide a control for comparison purposes.

Two sources of apple juice were used in this study: [1] a fresh apple juice made from a blend of six cultivars in our pilot plant and [2] a juice reconstituted from a commerical concentrate according to the producer's instructions.

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The fresh juice was made into a 'natural style' as well as a clarified product. Apples were pressed as a blend in a hydraulic press. The raw juice was then subdivided into clarified and natural style lots. The clarified lot was heated to 48.8°C (120°F) and treated with commercial pectinase (G.B. Fermentation Industries Inc., Charlotte, NC.). At the end of a 2.5 hr period, the juice was cooled through a tube and shell heat exchanger, then stored at 1.1°C (34°F). After 24 hr, the refrigerated juice was flash heated to 93.3°C (200°F), immediately cooled and filtered through a plate and frame diatomaceous earth filter. A precoat of Filter Cell 201 and a body feed of Celite 577 (Manville Products Group, Toledo, OH) was used for filtration. The clarified juice was then pasteurized at 87.7°C (190°F) and bottled.

The natural style product was produced by heat stabilizing the suspended cloud in the juice followed by a partial filtration utilizing cloth filter elements to retain only the larger particle sizes. The fresh cider was flash heated to 200°F and immediately cooled. The juice was then cloth filtered, pasteurized at 190°F and bottled.

The commercial concentrate was an aseptically packaged product shipped in a bag-in-a-box container. Dilution of the concentrate was performed on the same day it was to be processed using fresh tap water in a 4:1 dilution ratio according to the manufacture's instructions. This reconstituted juice was pasteurized at 190°F and bottled.

Four different packaging conditions were included in the study. The codes appended to the OPET labels were: QC — for a fast cooling or quench cooling process which achieved room temperature in 10 min and GC — for the slow cooling process which achieved room temperature in 40 min. The samples were [1] a quench cooled, 2 quart, OPET container [OPET/QC-2Qt]; [2] a slow-cooled, 2 quart, glass container [GLASS]; [3] a slow cooled, 2 quart, OPET container [OPET/GC-2Qt] and [4] a quench cooled, 1 quart, OPET container [OPET/QC-1Qt].

The first three package conditions were evaluated at 0, 1, 3, 6, 9, and 12 months. The OPET/QC-1Qt was evaluated at 0, 3, 6, 9 and 12 months. Analyses of samples included turbidity, lightness, hue and chroma. Samples for turbidity measurements were prepared by a standard shaking of the sample container prior to removal of a 30 ml aliquot for analysis. Turbidity measurements, reported in nephelometric turbidity units [NTU], were made on a Hach Model 18900-10 Ratio Turbidimeter. The Hunter Model D-25 Tristimulas Colorimeter was used to measure L,a, and b values. From these, values representing hue and chroma were calculated (Little, 1975).

Sensory analysis included specific difference and preference tests and quantitative descriptive analysis [QDA] (Stone *et al.*, 1974). Difference tests were based on a triangle test method and paired preference tests with significance based on published expanded tables (Roessler *et al.*, 1978). Sensory judges were experienced and trained panelists, familiar with these test procedures and with processed apple products in general. Sixteen judges were selected for the difference tests and the QDA method based upon their ability to discriminate and reproduce results. Panels were performed under controlled environmental conditions in a sensory laboratory containing partitioned booths. All lighting in the laboratory was standardized northern daylight. For the QDA method, samples were evaluated in random order with triplicate evaluations made for each sample. Groups of three juice samples, were served in wine glasses at room temperature. Difference tests and

Table 1—Some physical characteristics of the OPET container

	OPET Package Characteristics:
Density	= 1.34 - 1.36 g/cc
Flexural Modulus	= 340,000 psi
Oxygen Permeability	= 5-8 cc/mil/100 in²/24hr @ 22.7°C (73°F), 1 atm
Appearance	= Glossy, clean, colorless-translucent white
Maximum Fill Temperature	= 93.3°F (200°F)

Table 2—Difference and preference tests conducted on the 'natural style' fresh apple juice over the period of the shelflife study[a]

Difference [D] & Preference [P] Tests		Months in storage 0	1	3	6	9	12
OPET/QC vs glass	[D]	6/16 ND	8/16*	8/16*	10/16**	15/16**	16/16**
	[P]	NT	6/8NP	5/8NP	6/10NP	11/16NP	13/16GL
OPET/GC vs glass	[D]	8/16*	7/16ND	11/16**	15/16**	15/16**	16/16**
	[P]	5/8NP	NT	6/11NP	10/15NP	12/15GL	11/16NP
OPET 2Qt vs 1Qt.	[D]	4/16ND	--	6/16ND	9/16*	--	8/16*
	[P]	NT	--	NT	5/9NP	--	5/8NP

[a] Juice source: Fresh apples - 'Natural style'
[b] NT = not tested; ND = no significant difference; NP = no significant preference; GL = statistically significant preference for glass at the 95% level.
* Statistically significant differences at the 95% level
** Statistically significant differences at the 99% level

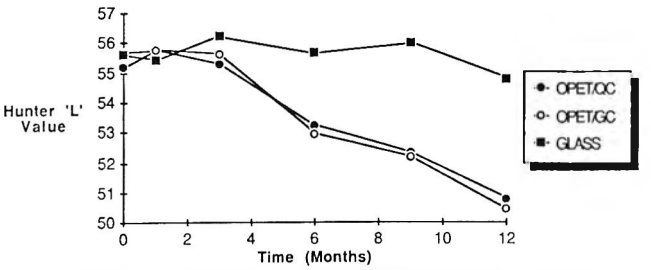

Fig. 1—Hunter 'L' values for clarified fresh apple juice over the period of the study. Tukey's Minimum significant difference = 0.750 'L' value units at the 95% confidence level for all treatment combinations. OPET/QC is a rapidly cooled 2 quart OPET polymer container. OPET/GC is a 2 quart OPET polymer container which has been slowly cooled at the rate which a glass container is normally cooled.

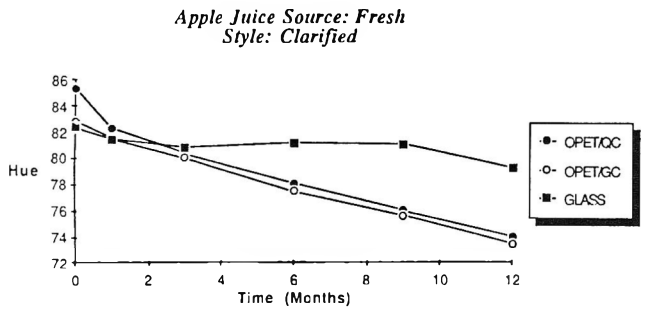

Fig. 2—Hue values for clarified fresh apple juice over the period of the study. Tukey's Minimum significant difference = 0.662 Hue degrees at the 95% confidence level for all treatment combinations. OPET/QC is a rapidly cooled 2 quart OPET polymer container. OPET/GC is a 2 quart OPET polymer container which has been slowly cooled at the rate which a glass container is normally cooled.

Table 3—F-values from the analysis of variance on OPET/QC, OPET/GC and glass packaged juice for sensory characteristics

Sensory attribute	Package [P]	Time [T]	P×T
'Natural Style'			
Lightness	17.612**	4.643**	2.126*
Fruitiness	1.448NS[a]	3.091*	0.845NS
Sweetness	0.004NS	6.488**	0.740NS
Sourness	0.230NS	2.162NS	1.418NS
Syrupiness	0.001NS	1.852NS	0.519NS
Acceptability	2.698NS	2.426*	0.608NS
Enzymatically clarified			
Lightness	22.454**	10.600**	2.086*
Fruitness	0.009NS	3.404**	0.553NS
Sweetness	0.796NS	5.845**	0.121NS
Sourness	0.157NS	3.300**	0.422NS
Syrupiness	0.488NS	5.484**	0.522NS
Acceptability	0.960NS	2.313*	0.652NS

[a] NS = not statistically significant at the 95% level
* 95% significance level
** 99% significance level

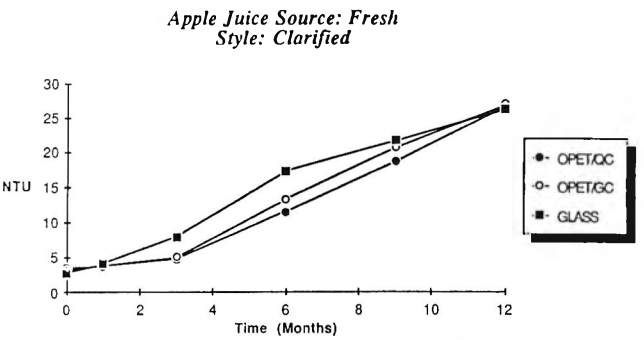

Fig. 3—Nephelometric turbidity units (NTU) of clarified fresh apple juice sample over the period of the study. Tukey's minimum significant difference = 1.243 NTU at the 95% confidence level for all treatment combinations. OPET/QC is a rapidly cooled 2 quart OPET polymer container. OPET/GC is a 2 quart OPET polymer container which has been slowly cooled at the rate which a glass container is normally cooled.

preference tests were performed without replication by individual judges under the same controlled environmental conditions.

Statistical analysis was completed on the difference and preference test based upon tabular values for 95% level of significance (Roessler *et al.*, 1978). Analysis of the QDA data was based on and analysis of variance, ANOVA, performed using a statistical analysis package, SAS, operating on a Prime computer, and documented methods (SAS, 1985).

RESULTS & DISCUSSION

Fresh apple juice—natural style

Results of the difference and preference tests for this juice are shown in Table 2. Although trained panelists were able to detect statistically significant differences early in the study, a statistically significant preference for the glass package was not expressed until 9 and 12 months of storage. These preference tests were run using trained judges. It is understood by the authors that this is not desirable; however, limitations during the study precluded our access to general public assessments. Expressions of confidence in these preference results should therefore be accepted as only indications of trend rather than as actual measures of population preference.

An assumption, prior to these experiments, was that cooling a hot-filled juice product quicker than the typical slow cooling process used for glass containers may impart a beneficial result on the product quality. The first indications of support for this supposition was found in the results shown in Table 2. The glass packaged product was prefered over the OPET/GC-2Qt container at 9 months of storage whereas the glass packaged product was not prefered over the OPET/QC-2Qt container until 12 months storage. Since no other differences between

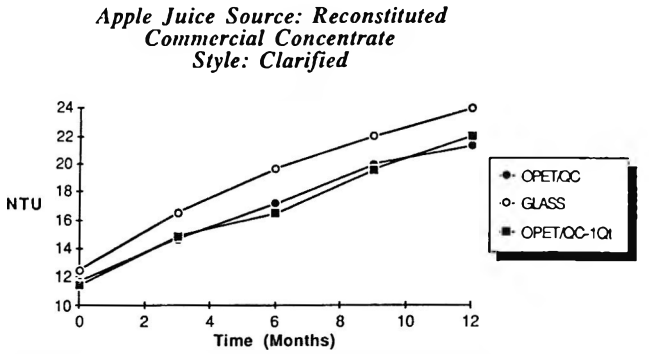

Fig. 4—Nephelometric turbidity units (NTU) of reconstituted commercial apple juice sample over the period of the study. Tukey's Minimum significant difference = 1.444 NTU at the 95% confidence level for all treatment combinations. OPET/QC is a rapidly cooled 2 quart OPET polymer container. OPET/GC is a 2 quart OPET polymer container which has been slowly cooled at the rate which a glass container is normally cooled.

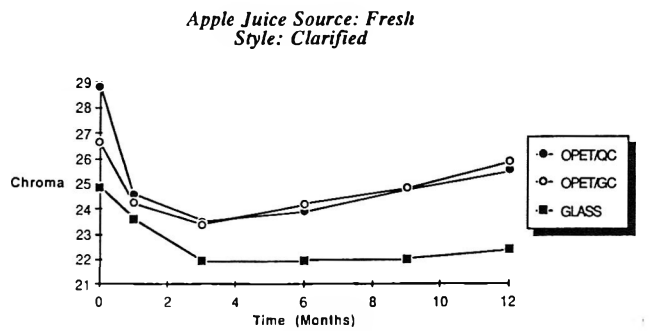

Fig. 5—Chroma values for clarified fresh apple juice over the period of the study. Tukey's Minimum significant difference = 0.394 Chroma units at the 95% confidence level for all treatment combinations. OPET/QC is a 2 quart OPET polymer container which has been slowly cooled at the rate which a glass container is normally cooled.

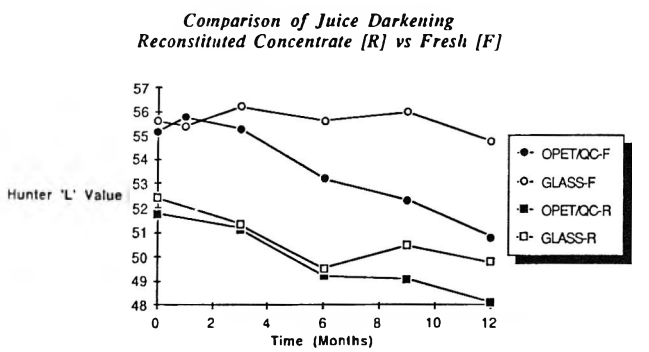

Fig. 6—Comparison of juice darkening in reconstituted concentrate and fresh apple juice. OPET/QC is a rapidly cooled 2 quart OPET polymer container. OPET/GC is a 2 quart OPET polymer container which has been slowly cooled at the rate which a glass container is normally cooled.

the two containers, OPET/GC-2Qt and OPET/QC-2Qt, exist other than the rapid cooling process, one could surmise that the quench cooling proces significantly retained product quality over a storage life of 6–9 months into the study.

Most noticeable of the results of analytical measurements were the changes in lightness and hue. Lightness of the glass packaged product remained stable over the period of the study while the OPET packaged products darkened as oxidation proceeded due to oxygen permeation through the OPET container walls and this trend was similarly noted in the enzyme clarified fresh juice samples (Fig. 1). Similar results were seen with the hue value, a measure of the color of the juice. The GLASS packaged juice remained stable over the period of the study with only slight color shift towards the red-brown hues as compared to the extensive shift in this direction seen with the OPET containers and this trend was also seen in the enzyme clarified fresh juice samples (Fig. 2).

Sensory differences based on the QDA tests (Table 3) revealed that packaging differences accounted for differing perceptions of the lightness of the juice. Other measured characteristics such as, fruitiness, sweetness, sourness, syrupiness, and acceptability were not affected.

Fresh apple juice — Clarified

For the purposes of comparing the various packaging systems, a high quality juice was prepared using a well balanced blend of apple cultivars. Differences were detected over the period of the study with a preference for the glass packaged product over the OPET/QC-2Qt by 12 months (Table 4). In these clarified samples, we also detected significant surface-to-volume effects in the OPET containers. The two quart OPET/QC container was prefered over the one quart size container by 12 months storage.

QDA results (Table 3) again indicated significant packaging effects on the perception of lightness; however, the other assessed attributes were not significantly affected other than by duration of storage.

Visual attributes measured using the turbidity meter and the colorimeter were important indicators of the changes and differences in and among these package types over the period of the study. Figure 3 illustrates the change in turbidity of the clarified juice during storage. As measured in nephelometric turbidity units (NTU), formation of turbidity in the glass package proceeded at a faster rate, early in the study, than in the OPET packaged samples. These findings were observed in the reconstituted commercial apple juice (Fig. 4). There is an apparent reduction in the rate of turbidity formation in the OPET containers versus in the glass containers. This is irregardless of the cooling period after bottling. This could be due to an interaction between the chemistry of the OPET polymer and that of the juice.

The change in lightness, hue and chroma for the clarified fresh apple juice over the period of the study are shown in Fig. 1,2, and 5. There were significant differences between the glass packaged products and the OPET packages. The glass packaged product remained approximately constant over the storage period. The lightness of the OPET packaged juice dropped as the product oxidized and aged during storage. The difference between the glass packaged product and the OPET packaged was possibly due to the gas permeation through the OPET polymer. The quench cooled product, OPET/QC-2Qt, was significantly more yellow (hue) and of a more saturated color (chroma) than all of the other packages at the start of the study, however, that advantage was lost after the first month or two into storage. From that point on, degradation of the OPET containers, both quench cooled [OPET/QC] and slow cooled [OPET/GC] continued at an even rate while the GLASS packaged juice remained relatively stable.

Reconstituted commercial apple juice concentrate

A commercial concentrate was acquired for use in this experiment. The reconstituted juice in the OPET container was preferred over the glass packaged product during the evaluations at 3 and 6 months (Table 5). We believe that the juice concentrate, having lost flavor during processing, actually improved in acceptability as it oxidized. Continued oxidation eventually degraded the product to the point that the panelists did not prefer it over the glass packaged juice (9 months of storage). Figure 6 plots the lightness values for the reconsti-

Table 4—Difference and preference tests conducted on enzymatically clarified fresh apple juice over the period of the shelflife study[a,b]

Difference [D] & Preference [P] Tests		Months in storage 0	1	3	6	9	12
	[D]	11/16**	11/16**	12/16**	16/16**	15/16**	16/16**
OPET/QC vs glass	[P]	6/11NP	6/11NP	11/12OP	10/16NP	11/16NP	13/16GL
	[D]	6/16ND	11/16**	14/16**	16/16**	15/16**	16/16**
OPET/GC vs glass	[P]	NT	6/11NP	7/14NP	9/16NP	12/16NP	11/16NP
OPET	[D]	7/16ND	--	4/16ND	8/16*	--	8/16*
2Qt vs 1Qt.	[P]	NT	--	NT	6/8NP	--	8/82Qt

[a] Juice source; Fresh apples-clarified

[b] NT = not tested; ND = no significant difference; NP = no significant preference; GL = statistically significant preference for GLASS at the 95% level; OP = statistically significant preference for OPET at the 95% level; 2Qt = statistically significant preference for 2Qt at the 95% level.

* statistically significant differenees at the 95% level

** statistically significant differences at the 99% level

Table 5—Difference and preference tests conducted on juice made from reconstituted commercial apple juice concentrate over the period of the shelflife study[a,b]

Difference [D] & Preference [P] Tests		Months in storage 0	3	6	9	12
	[D]	9/16**	9/16**	12/16**	13/16**	13/16**
OPET/QC vs glass	[P]	5/9NP	8/90OP	10/12OP	7/13NP	9/13NP
OPET	[D]	8/16*	7/16ND	7/16ND	8/16*	ND
2Qt vs 1Qt.	[P]	6/8NP	NT	NT	6/8NP	NT

[a] Juice source: Reconstituted apple concentrate

[b] NT = not tested; ND = no significant difference; NP = no significant preference; GL = statistically significant preference for GLASS at the 95% level; OP = statistically significant preference for OPET at the 95% level.

* statistically significant differences at the 95% level

** statistically significant differences at the 99% level

tuted juice as well as for the freshly made juice. In the reconstituted juice, oxidation rates for the glass and OPET containers were very similar. However; the fresh juice showed, as described earlier, discreet differences between the package types. In fact the lightness of the reconstituted juice at the start of the storage study (0 months) was essentially the condition of the fresh juice in OPET containers after completing the entire study (12 months).

CONCLUSIONS

DEPENDING UPON METHODS of processing, an OPET container may provide a six to nine month shelflife for single strength apple juice under normal storage conditions. Evaluating freshly made apple juice which had been packaged in two systems (glass vs OPET), judges were able to distinguish between the two; however, significant preferences were not expressed for either of two types until 12 months into storage when the glass packaged product was preferred. When juice made from commercial concentrate was evaluated at 3 months and at 6 months, the OPET/QC packaged juice was preferred over the glass packaged juice.

REFERENCES

Anonymous. 1985. Ocean Spray: Hot fillable OPET to replace glass. Packaging. 30(12): 12.

Irwin, C. 1985. Blow-molding extrusion. In "Packaging Encyclopedia & Yearbook, 1985." Cahners Publishing Co., Boston, MA.

Little, A.C. 1975. Off on a tangent. J. Food Sci. 40: 410.

Moyer, J.C. and Aitken, H.C. 1980. Apple juice. *In* "Fruit and Vegetable Juice Processing Technology." P.E. Nelson and D.K. Tressler (Ed.), p. 212. AVI Publishing Co., Westport, CT.

Peters, J.W. (Ed.). 1985. "Packaging Encyclopedia & Yearbook, 1985." Cahners Publishing Co., Boston, MA.

Pollard, A. and Timberlake, C.F. 1971. Fruit Juices. *In* "The Biochemistry of Fruits and Their Products." A.C. Hulme (Ed.), p. 573. Academic Press Inc. New York.

Roessler, E.B., Pangborn, R.M., Sidel, J.L., and Stone H. 1978. Expanded statistical tables for estimating significance in paired-preference, paired-difference, duo-trio and triangle tests. J. Food Sci. 43: 940.

SAS Institute Inc. 1985. "SAS® User's Guide: Statistics," Version 5 Ed. p. 113. SAS Institute, Inc., Gary, NC.

Stone, H., Sidel, J., Oliver, S., Woolsey, A., and Singlton, R.C. 1974. Sensory evaluation by quantitative descriptive analysis. Food Technol. 11: 24.

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Fouling and Flux Restoration of Ultrafiltration of Passion Fruit Juice

BEEN HUANG CHIANG and ZER RAN YU

ABSTRACT

Fouling phenomena of passion fruit juice in tubular ultrafiltration (UF) system were studied. The membrane deposit was found to be composed of pectin, sugars, citric acid, hemicellulose, and cellulose. Rinsing behaviour of the fouled membrane indicated that the deposit might be in the form of gel. Scanning electron microscopic examinations showed slime-like deposit on the fouled membrane. Intermittent on-off operation was able to restore the flux effectively. Rebounding of the gel layer at sudden release of pressure may explain the effect of on-off operation on flux restoration.

INTRODUCTION

FLUX DECLINE due to fouling and concentration polarization is one of the major problems of membrane processes. The materials that often deposit on the surface of the membrane for various food fluids have been investigated by a number of workers. Casein, α-lactalbumin, β-lactoglobulin and γ-globulin were found to be the major components accumulated at membrane surface during cheese whey processing (Peri and Dunkly, 1971; Lim et al., 1971; Lee and Merson, 1975; Cheryan and Merin, 1980). For whole milk, casein micelles and calcium salt were the major components in the deposit (Glover and Brooker, 1974; Skudder et al., 1977). Watanabe and coworkers (1979a,b) found that the major fouling components of mandarin orange juice were pectin and cellulose.

Many methods have been developed to restore flux during ultrafiltration (UF) and reverse osmosis (RO). The method of intermittent lateral surface flushing was developed by Watanabe and coworkers (1978) for RO of orange juice. Turbulence promoters were used to improve flux in RO of various food fluids (Lowe and Durkee, 1971; Peri and Dunkly, 1971; Dejmek et al., 1974; De Boer et al., 1980). Kennedy et al. (1974) found that pulsed RO system with pulsing frequencies up to 1 Hz could increase the permeation rate of sucrose solution more than 70%.

In a previous study (Yu et al., 1986), it was found that many major flavor constituents of passion fruit juice could be recovered well by UF membrane. This suggests that UF may be a useful tool for preconcentration of passion fruit juice if a reasonably high flux can be maintained throughout the process. The purposes of this study were to investigate the nature of membrane surface deposits which cause flux decline during UF of passion fruit juice and to study the possibility of using intermittent on-off operation to maintain the flux.

MATERIALS & METHODS

Passion fruit juice preparation

The passion fruit juice was extracted from hybrid variety of *Passiflora edulis Sims* (female) and *Passiflora edulis* var. *flavicarpa* (male). The procedures of passion fruit juice manufacturing, incuding depectin, de-starch and pasteurization, have been described in detail (Yu et al., 1986).

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Fouling and flux restoration studies

The UF processes were operated at constant conditions of 30 L/min feed rate, 20°C, and 12 bar of operation pressure using a tubular membrane system (PCI laboratory UF unit, Paterson Candy International Ltd., Witchurch, England). The membrane module of the sys-

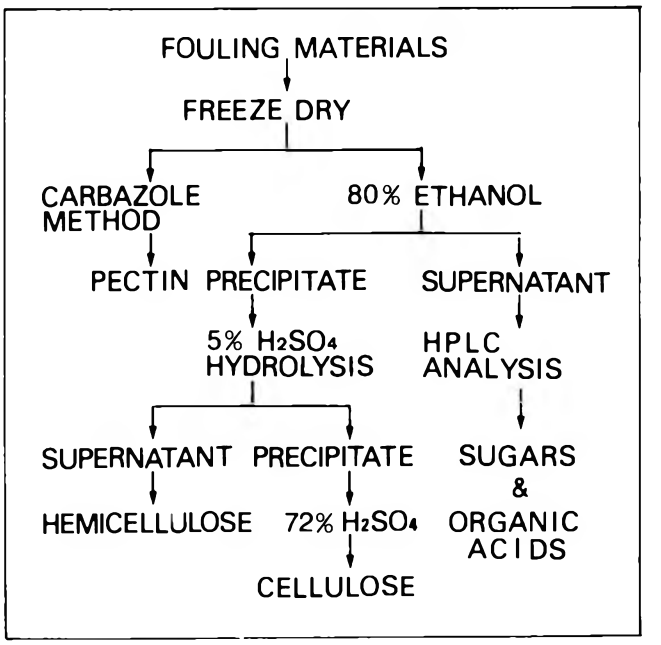

Fig. 1—Analyses of chemical composition of membrane fouling material.

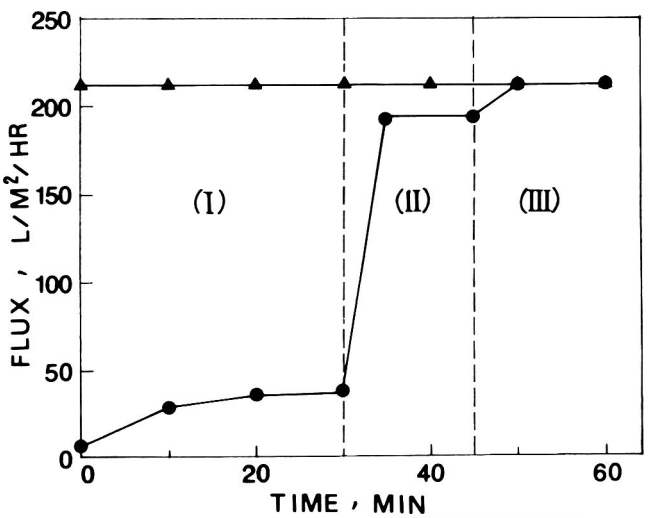

Fig. 2—The effect of rinsing on permeate flux: (I) Rinse with pure water for 30 min (II) Rinse with 0.1% NaOH solution for 5 min followed by rinsing with pure water for 10 min (III) Rinse with 0.1% NaOH for 5 min followed by rinsing with pure water for 10 min. ▲, the water flux when membrane was clean. ●, the flux during rinsing the fouled UF membrane.

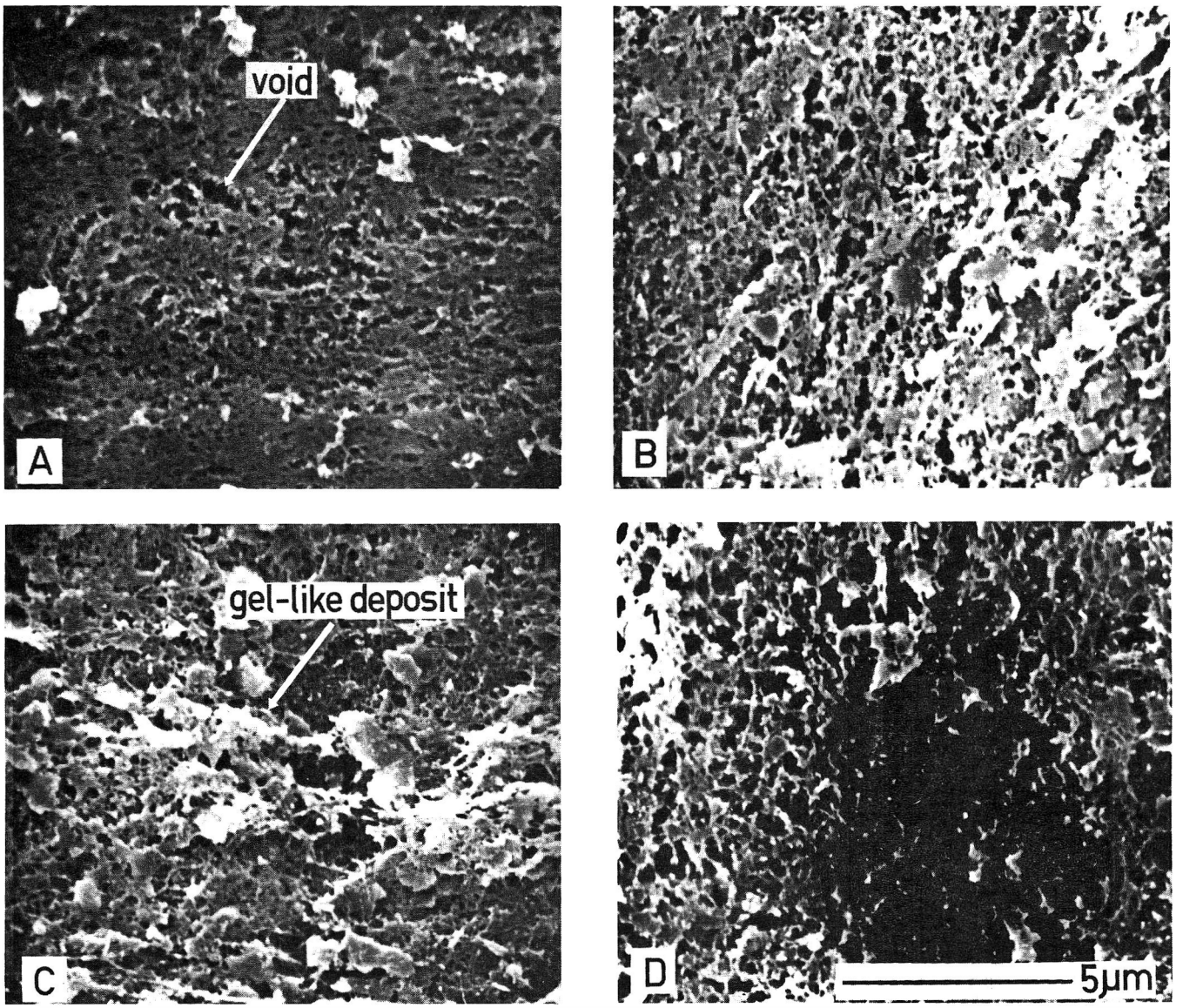

Fig. 3—Scanning electron micrographs of fouling deposits. (A) Cleaned UF membrane (B) Fouled UF membrane (C) Fouled UF membrane after rinsing with pure water for 30 min. (D) Fouled UF membrane after rinsing with 0.1% NaOH solution for 5 min.

tem was 1.2 meter in length and contained 18 tubes of 1.25 cm diameter in series, providing 0.9 m^2 membrane area. The BX6 UF membrane, with a molecular weight cut-off of 25,000 daltons, was chosen for this study based on the previous finding (Yu et al., 1986). The system was equipped with a 1 hp constant speed piston pump to provide fluid flow. A pressure control valve was located at the retentate outlet for controlling the operation pressure.

To study the fouling phenomena of UF at constant feed concentration, both permeate and retentate were recycled to the feed tank and the permeate flux was recorded periodically. For flux restoration study, the pressure of the UF system was first decreased by fully opening the pressure control valve, then the feed pump was shut off for a few seconds and restarted. This intermittent on-off operation was conducted at one hr intervals during the UF process. The permeation rates were measured before and after each on-off operation.

The effect of intermittent on-off operation during concentration process was also studied. The passion fruit juice was concentrated by recycling the retentate to the feed tank while the permeate was collected. The on-off operations were conducted at each hr during the concentration process.

Nature of fouling material studies

The nature of fouling materials on UF membrane was studied by chemical analysis and scanning electron microscopy. After 4 hr of UF operation, the unit was rinsed with water at 8 bar, 20°C and 30 L/min feed rate for 30 min. The membrane was then flushed with 50L 0.1% NaOH solution for 5 min followed by 50L water for 10 min. The washing procedure was repeated one more time. The rinsing fluids, 100L NaOH solution and 100L water, containing the fouling materials, were pooled. The mixture was concentrated by vacuum evaporation at 45°C, 25 mm Hg absolute pressure using a rotatory vacuum evaporator (Model N1, Tokyo Rikakikai Co., Japan), and then freeze-dried (Model RFS 450-5, Refrigeration for Science, Inc., New York). The dried sample was analyzed for carbohydrates, pectin, organic acid, hemicellulose and cellulose.

The chemical analysis procedures are outlined in Fig. 1. The carbohydrates and organic acids were analyzed by HPLC, and details of the analytical procedures are given elsewhere (Yu et al., 1986). The pectin in the sample was estimated by the D-galacturonic acid content, determined by the carbozole method (Bitter and Muir, 1962). The hemicellulose and cellulose were hydrolyzed by sulfuric acid (Anderson and Clydesdale, 1980). The glucose liberated was then analyzed by Somogyi method (AOAC, 1980).

For scanning electron microscope (SEM) studies, the 9th, 10th, 11th and 12th membrane tubes were removed from the module before processing, after fouling, after water flushing and after NaOH washing, respectively. The membranes were cut into 3 × 3 mm pieces and fixed in 2.5% glutaldehyde at 4°C for 3 hr. The pieces were washed three times for 10 min each with 0.1M phosphate buffer at pH 7.2, dehydrated in a 50, 70, 80, 90, 95 and 100% ethanol series for 10 min at each concentration. The sample pieces were then dehydrated twice in 100% ethanol for 10 min each and treated 3 time

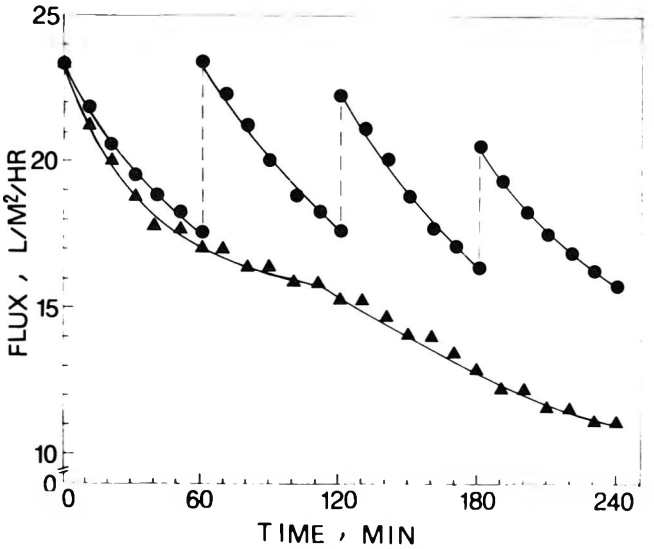

Fig. 4—Changes of flux during UF of single strength passion fruit juice. ▲, fouling curve without intermittent on-off operations. ●, flux restoration curve by intermittent on-off operations.

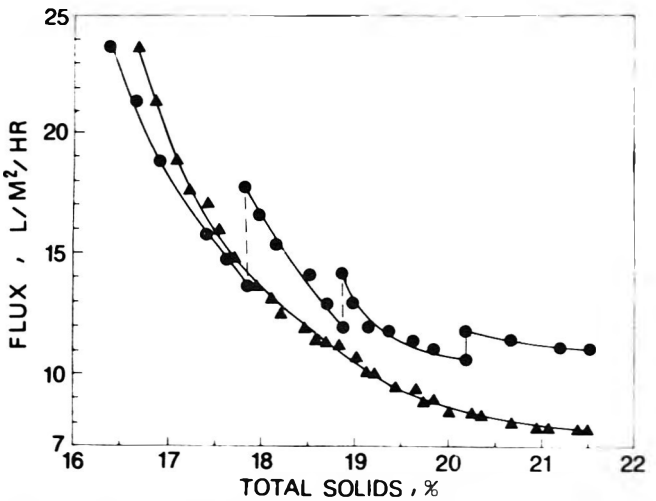

Fig. 5—changes of flux during UF concentration process of passion fruit juice (Symbols same as Fig. 4).

with isoamyl acetate, 10 min each time. Further dehydration was done in a critical point dryer (model HCP-1, Hitachi, Ltd., Japan). The dried pieces were mounted on aluminum stubs with two-face Scotch tape, coated with gold using an ion coater (Model IB-2, Eiko Engineering, Ltd., Japan). The prepared samples were scanned in an Hitachi S-550 SEM (Hitachi Ltd., Japan) at an acceleration valtage of 20 kV.

RESULTS & DISCUSSION

Nature of the membrane deposit

The changes of permeate flux during the washing procedures is shown in Fig. 2. The fact that rinsing the membrane module with pure water could not restore the flux indicated the low water solubility of the deposit. On the other hand, 0.1% NaOH solution was able to reduce the resistance of the deposit and restored the flux readily. Analyzing the composition of the rinsing fluids showed that the major foulants were pectin 34.6%; sucrose 21.6%; glucose 20.4%; fructose 10.3%; cellulose 3.5%; hemicellulose 1.5%, and citric acid 1.2%, on dry basis. Since sugars are water soluble in pure form but are capable of forming gels with pectin and acid under suitable conditions (Hodge and Osman, 1980) it was believed that these componenets formed a low water soluble gel matrix on the membrane. When the membrane was rinsed with NaOH solution, the gel matrix disintegrated. Thus, the deposit was removed and the flux increased.

Fig. 3 shows SEM micrographs of the UF membrane before (Fig. 3A) and after the processes. After UF operation of passion fruit juice a slime-like material was observed on the membrane (Fig. 3B). Rinsing the membrane with pure water for 30 min removed little membrane deposit (Fig. 3C). The NaOH solution, however, appeared to be very effective in washing the slimy fouling material from the membrane surface (Fig. 3D).

The formation of gel-like matrix on the membrane may be attributed to concentration polarization. During UF processing, a concentration boundary layer developed near the membrane surface, where the solutes concentration was much higher than the bulk concentration. The macromolecules, such as pectin, hemicellulose and cellulose, retained by the membrane, therefore, had an opportunity to interact with sugars and citric acid to form a gel matrix of low water solubility, which caused membrane fouling and reduced the rate of permeation.

Fouling and flux restoration by intermittent on-off operations

Fouling of UF of the single strength passion fruit juice and the restoration of flux by intermittent on-off operations are shown in Fig. 4. In a constant process condition, the permeate flux declined continuously as the operation time increased. However, the on-off operation each hour during processing restored the flux effectively. A possible explanation is that when the UF system was shut off, the sudden release of pressure (ca. 8 bar) on the gel layer caused part of the fouling materials to rebound and disassociate from the membrane, an action similar to the sudden release of a compressed spring. Following the re-start-up operation, the flow wave would flush the loosened fouling materials away, thus removed part of the resistance and increasing flux. As the operation time increased, the gel layer accumulated on the membrane, which was under a long term pressure effect, became more compact. Therefore, the effectiveness of on-off operation for loosening the gel from the membrane was reduced. It was suspected that shutting off the UF system at a certain minimum operation pressure was essential for loosening the fouling materials. However, the fact that the minimum pressure of this PCI tubular UF system was at 8 bar when the pressure control valve was fully opened made further testing on the effect of on-off operation at lower pressure impossible. Nevertheless, similar experiments conducted on a Romicon HFXS-5/10 UF unit (Romicon, Inc., Wolburn, MA) at relatively low pressure (~2 bar), showed that the flux was not restored by on-off operation.

The effect of on-off operation on flux restoration during concentration process is shown in Fig. 5. The gel layer formed on the membrane at high concentrations may be more compact than that at low concentrations. Therefore, restoring the flux by on-off operation was not as effective as in the process of single strength juice. It is believed that increasing the frequency of on-off operation would improve its effect on flux restoration during the concentration process.

CONCLUSION

FORMATION of a gel-like deposit appeared to be responsible for fouling of the ultrafiltration membrane by passion fruit juice. Pectin, sugars, citric acid, cellulose and hemicellulose were the major constituents of the foulant. Possibly due to rebounding of the gel-like deposit upon sudden release of pressure, intermittent on-off operations restored the permeate flux significantly. It was suspected that shutting off the UF system at high operation pressure was essential for effective flux restoration. However, further study is needed to determine the role of pressure in on-off operation. Work is also needed to

—Continued on page 380

The Contributing Effect of Apple Pectin on the Freezing Point Depression of Apple Juice Concentrates

A.F. HOO and M.R. McLELLAN

ABSTRACT

Frozen apple juice concentrate was prepared using conventional procedures. Response surface methodology was used to statistically model the results. A regression equation was derived as a function of °Brix and pectin concentration. The results of this investigation indicated that pectin contributed to the freezing point depression of apple juice concentrate, the effect of which was greater with increasing concentration. The untreated apple juice concentrate (with pectin intact) had a lower freezing point compared to the depectinized juice. The freezing point of apple juice concentrate decreased as the apples ripened as would be expected due to an increase in soluble pectin.

INTRODUCTION

Commercially available apple juice concentrate freezes at −8°C, which is a higher temperature than that of most storage freezers (−12°C to −21°C). It is, therefore, desirable to develop a product capable of maintaining its pourability at freezer temperatures, thus eliminating the inconvenience of thawing out the product prior to use. Rich Products Corporation (Kahn and Eapen, 1980) have received a patent for a soft intermediate-moisture frozen beverage concentrate in which the major contributor to the freezing point depression was the addition of sugars. The patent stated that more than 70% of the juice concentrate contained sugar.

Pectin, a polymer composed of polygalacturonic acids, is known as a cryoprotectant in the field of cryobiology. Shuman (1956) stated that hydrocolloids can affect crystal growth in various ways and postulated that hydrocolloids and crystals compete for the blocks which go into making the crystal. Thus, in the crystallization of sugar from solution the hydrocolloid could bind sugar molecules and thereby hinder growth of sugar crystals. A second action of hydrocolloids is their combination with impurities which would otherwise affect crystal growth.

Omran and King (1974), Stocking and King (1976) and Shirai et al (1985) have observed the kinetics of ice crystallization in various solutions. They found that pectin in solutions suppressed the nucleation of ice crystals. The decrease of the nucleation rate constant in polymer solutions was related to the increase of viscosity.

The objectives of this investigation were: (1) to conduct a controlled study of apple ripening in storage as it affects soluble pectin concentration and freezing point; (2) to analyze and quantify the freezing point effects of soluble pectin in apple juice concentrates by response surface methodology (RSM).

MATERIAL & METHODS

Raw material

Apples were harvested at optimal harvest dates as determined over a 10 year average from the NYS Agricultural Experiment Station's orchards (LaBelle, 1973). The cultivars studied were *McIntosh, Empire, R.I. Greening, Twenty Ounce, Cortland, Monroe, Northern Spy, Spigold, Rome* and *Golden Delicious*. The fruits were stored under conditions of high relative humidity (90%) at 0°C and 25°C. Apples stored at 0°C were used for RSM study. Those stored at 25°C were drawn weekly over a 3 week period to study the effects of ripening, the control being the apples stored at 0°C.

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Processing

The apples were processed using a blend of the above cultivars following conventional processsing procedures. Prior to filtration, the juice was divided into two lots, untreated and pectic enzyme treated (Klerzyme 200, GB Fermentation Industries Inc., Charlotte, NC). The juice was then vacuum concentrated to 60°Brix. Each lot was further divided into two lots, one was diluted to 42°Brix (typical of commercially frozen juice concentrates) and the other was left at 60°Brix (soluble solids of commercially available pourable frozen concentrates).

Analytical procedures

Freezing point was measured using the Beckmann Freeze Point Apparatus (Barrow, 1973). The holding tube was placed in an isopropyl alcohol-dry ice bath held below −60°C. A stirrer which was attached to a motor was set to give a constant stirring rate. An Analog Devices digital thermometer AD2070 (Norwood, MA) with a copperconstantan thermocouple was used to measure freezing point. A serial communication link was used between it and a Macintosh 512K computer which collected temperature data in real time and generated freezing curves.

Pectin concentration was determined using a modification (increased reagent volumes by 5) of the method described by Blumenkrantz and Asboe-Hansen (1973) which expressed concentration of pectin as μg/mL of galacturonic acid.

Galacturonic acid in the soluble pectin of blended apple juice was determined (Ahmed and Labavitch, 1977), by extracting pectin from the apple juice with three volumes of 95% ethanol. After sitting for 4 hr, the suspension was filtered through Whatman No. 1 filter paper using a Buchner funnel. The alcohol insoluble solids (AIS) were collected and dried overnight under vacuum at 60°C. The AIS were rehydrated with sulfuric acid and assayed for uronic acids using the modification of the m-hydroxydiphenyl method as described above.

Response surface methodology experiment

Response surface methodology (RSM) is a statistical model used to identify the combination of quantitative levels of two or more factors to generate an optimum response. The use of RSM has become increasingly popular in the field of food science (Heinka, 1982; Giovanni, 1983; McLellan et al., 1984). The experimental design for two factors (°Brix and pectin concentration) were described by Mullen and Ennis (1979). For the two factors, nine level combinations are used. The level (0,0) was repeated five times, thus a total of 13 formulations was used. Table 1 illustrates the range of the two variable levels.

Table 1—Variable levels for a two factor case in a response surface methodology experiment

Level of coded variable	Level of °Brix	Level of pectin conc. (g/L)
−1.414	10.0	1.593
−1.0	17.3	5.155
0	35.0	13.755
1.0	52.7	22.356
1.414	60.0	25.917

Statistical analysis of the data was done using statistical analysis system (SAS, 1985).

Sucrose was added to adjust the solids of the concentrate. Soluble solids, measured as °Brix, was determined using the Abbe refractometer. Apple pectin (Herbstreith NSS-40, Neuenburg, Wurtt, Germany) was added to adjust the pectin concentration for the RSM study. Vigorous stirring for 24 hr was used to ensure that the pectin was completely dissolved.

Determination of the effect of added commercial pectin on the freezing point

A 1% concentration of apple pectin (Herbstreith NSS-40) was added to 12°Brix solution, 42°Brix solution and 60°Brix solution. The solutions were stirred for 24 hr to ensure that the pectin was totally dissolved. Freezing point analysis was done in duplicate for each sample.

Determination of the effect of invertase on the freezing point of the concentrate

Various amounts of invertase (Nutritional Biochemical Corp., Cleveland, OH) were added to 12°Brix, 42°Brix and 60°Brix apple juice concentrates to give a final concentration of 0.001 g/mL, 0.003 g/mL and 0.006 g/mL, respectively. The solutions were stirred at room temperature (25°C) for 24 hr prior to determining in duplicate the freezing points.

Determination of the effect of demethylation on the freezing point of the concentrate

Pectin-esterase (Rohm pectin-esterase 7020, New York, NY) was added to apple juice to give a final concentration of 0.002g/mL. The mixture was stirred for 5 hr at 120°F (49°C) and vacuum concentrated to 60°Brix before analysis of freezing points.

Pourability study on the frozen concentrate

The apple juice was vacuum concentrated to 42°Brix, 45°Brix, 48°Brix, 51°Brix, 54°Brix, 57°Brix and 60°Brix. These concentrates were placed in a −18°C freezer for 3 days. Samples were visually inspected by tilting the sample flask at a 90° angle for differences in pourability.

Sensory evaluation

An experienced panel composed of 16 judges evaluated the visual, aroma, flavor and mouthfeel characteristics of untreated versus depectinized single-strength apple juice. The panelists were also asked to rank the two samples according to preference.

RESULTS AND DISCUSSION

Apple juice concentrate processed from apples stored under different storage conditions: freshly harvested and held at 0°C; 1 wk, 2 wk and 3 wk at 25°C showed differences in the soluble pectin concentration and freezing point. Soluble pectin concentration was confirmed to be directly correlated to apple ripening (Kertesz, 1951; Smock and Neubert, 1950). As the apples were stored in the ripening room at 25°C for a period of 0 to 3 weeks, the overall amount of soluble pectin in the juice increased (Fig. 1). The increase in soluble pectin was greatest over the first week and decreased with storage time.

The effect of soluble pectin concentration on the freezing point is illustrated in Fig. 2. As the °Brix increased, there was an observed decrease in freezing point. As the level of soluble pectin concentration increased keeping the level of °Brix constant, the freezing point was further depressed. The effect of pectin in depressing the freezing point was greater with higher soluble solids.

To confirm the above observation, two lots of apple juice were prepared. One lot was enzyme treated with pectinase to degrade the pectic substances and the other lot was not treated

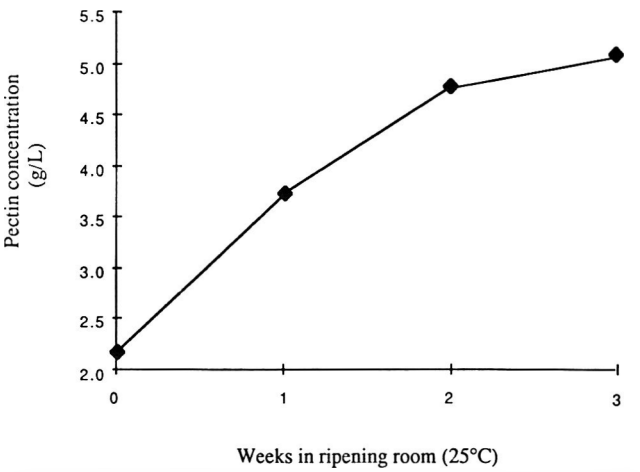

Fig. 1—Changes in pectin concentration of juice prepared from apples stored for various lengths of time in the ripening room at 25°C.

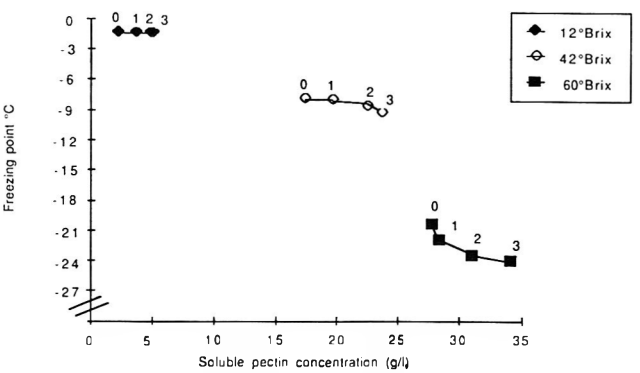

Fig. 2—Effect of pectin concentration of apple juice concentrate (12°, 42°, 60°Brix) prepared from apples stored in ripening room at 25°C on freezing point. The numbers above the symbols represent weeks of storage at 25°C.

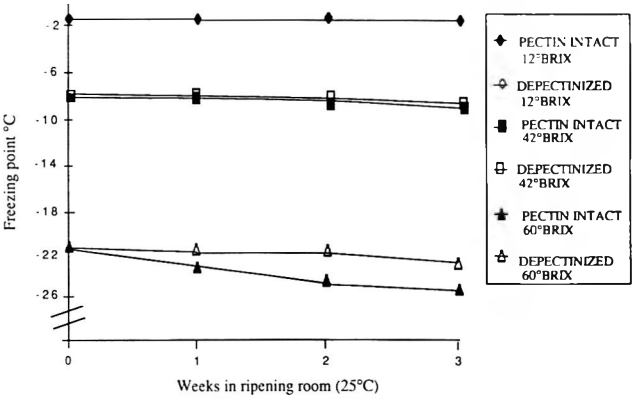

Fig. 3—Changes in freezing point of untreated and pectinase treated juice prepared from apples stored for various lengths of time in the ripening room at 25°C. (Note: first two lines overlay one another)

(pectin intact). A the same °Brix level, the juice with the pectin intact had a lower freezing point than the depectinized juice (Fig. 3). At 12°Brix, the two curves, pectin intact verses depectinized, were nearly indistinguishable, however, the effect was greater as the soluble solids increased. From these results it might be concluded that the hydrocolloid effect of pectin was more effective in depressing the freezing point than the solute effect of polygalacturonic acid. The lower freezing point of the concentrate-with-pectin might be explained by competition between hydrocolloids (pectin) and crystal for the blocks which make up the ice crystal (Shuman, 1959). In addition,

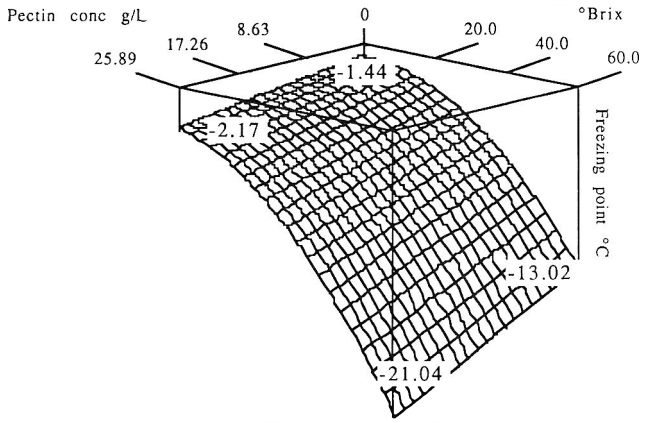

Fig. 4—Three-dimensional plot of the relationship between pectin concentration and °Brix on the freezing point of untreated apple juice.

the mixture of high sugar concentration and polymer solutions had a greater effect on freezing point because of the decrease of the growth and nucleation rates of ice crystals in solution (Omran and King, 1974; Shirai et al., 1985; Stocking and King, 1974).

Response surface methodology was the method used to model the freezing point depression based on a specified range of two factors, °Brix and pectin concentration. Figure 4 illustrates the pectin-°Brix interaction in the pectin intact apple juice. At 0°Brix and 0 g/L pectin, the predicted freezing point of the juice was −1.44°C, due mainly to the presence of other solutes in the juice. At 25.89 g/L pectin and 0°Brix, the predicted freezing point of the juice was −2.17°C; at 60°Brix and 0 g/L pectin, the predicted freezing point was −13.02°C. This clearly demonstrated that soluble solids were more effective in depressing the freezing point than added pectin. However, the model predicted that the combined effect of added pectin and °Brix (60°Brix and 25.89g/L pectin) would depress the freezing point of the apple juice concentrate even further to −21.04°C, possibly due to an interaction between pectin and soluble solids.

A regression equation for the freezing point of pectin intact apple juice was derived as follows:

$$F.P. = -1.44 + 0.1214(°B) - 0.028(P) - 0.00524(°B)^2 - 0.00469(°B)(P)$$

where: F.P = Freezing point (°C), °B = °Brix, and P = Pectin concentration (g/L).

These variables were found to be significant at the α-level of 0.05. The predicted freezing point calculated from the equation is a fairly good estimate (±1.5C°) of the actual freezing point. This slight discrepancy between actual studies and the predicted model could have been due to the following: (1) varietal and growing season differences among apples; (2) the fact that for the predicted model °Brix was adjusted by adding sucrose while for actual studies °Brix was adjusted by vacuum evaporation, possibly resulting in a difference in concentration of other solutes which affect the freezing point.

Galacturonic acid in the pectin of apple juice extracted by ethanol and determined by a modified Blumenkrantz and Asboe-Hansen (1973) method was calculated to be 52.09%, the rest being extraneous, nongalacturonic acid material. Commercial pectin which has undergone several purification steps contains about 70% galacturonic acid. However, it should be noted that the percent of galacturonic acid in pectin varies among cultivars, storage conditions and method of extraction (Boothby, 1980; Joslyn and Deuel, 1963).

The addition of various amounts of commercial apple pectin, approximately 70% galacturonic acid, to pure water resulted in a depression of freezing point. With water as the solvent, the interferences by other solutes was eliminated. The addition of 1% pectin to a 12°Brix, 42°Brix and 60°Brix sucrose solution depressed the freezing point by 0.3°C, 0.6°C and 0.8°C, respectively. As sugar was increased, the combined effect of added solute and added pectin further depressed the freezing point.

Table 2—Effect of added invertase on the freezing point of apple juice concentrate

Initial °Brix	Final °Brix	Freezing point °C
12.3	12.3	−1.20
12.3*	13.0	−1.40
42.3	42.3	−8.40
42.3*	42.9	−9.00
60.6	60.6	−20.1
60.6*	62.8	−23.7

*Added invertase

Invertase was found to depress the freezing point of apple juice concentrate compared to samples without added enzyme (Table 2). This was due to an increase in solute concentration as a result of the hydrolysis of sucrose to fructose and glucose.

A gelling problem was observed with 60°Brix pectin intact concentrate. To circumvent this problem, two possible approaches were tested. One approach was to determine empirically the maximum °Brix that would allow pourability at freezer temperature. At −18°C, the maximum level of sugars that gave a pourable concentrate was found to be within the range of 54° −57°Brix. At lower sugar concentration, the apple concentrate was frozen and at higher sugar concentration, the concentrate was a gel. Another approach was to demethylate the juice prior to concentration with the use of pectinesterase, this would degrade the gel without degrading the pectin (Baron et al 1981; Schachet and Raymond, 1959). This resulted in a pourable frozen concentrate even at 60°Brix.

Sensory evaluation was conducted on the 12°Brix apple juice. This is normal for single-strength juice. The majority (87.5%) of the panelists preferred the pectin intact juice over the depectinized juice. The panelists perceived the pectin intact juice as having more apple aroma and character and a heavier body.

CONCLUSION

SINCE SOLUBLE PECTIN increased with ripening, the apple juice concentrate processed from apples held longer in storage had a lower freezing point.

The addition of invertase to the apple juice concentrate enhanced the freezing point depression. The maximum °Brix that allowed pourability at −18°C, home freezer temperature, was between 54°Brix and 57°Brix. Conversely, the addition of pectin esterase to the apple juice concentrate also improved the pourability at freezer temperature.

Sensory evaluation indicated that apple juice with pectin was preferred by 87.5% of the panelists over depectinized apple juice.

Added pectin had the ability to depress the freezing point of apple juice concentrates. This effect was even greater in the presence of sugars due to an interaction between soluble solids and pectin. A regression equation was derived to model the predicted freezing point based on soluble solids and pectin concentration in apple juice.

REFERENCES

Ahmed, A.E.R. and Labavitch, J.M. 1977. A simplified method for accurate determination of cell wall uronide content. J. Food Biochem. 1: 361.

Baron, A., Prioult, C., and Drilleau, J.F. 1981. Gelation of apple pectin 1. Experimental methods and study of the influence of pectin esterase concentration on pectin gelation. Sciences Des Aliments 1: 81.

Barrow, G.M. 1973. The thermodynamic treatment of multicomponent systems. In "Physical Chemistry," 3rd ed. McGraw-Hill Book Company, New York, NY.

Blumenkrantz, N. and Asboe-Hansen, G. 1973. New method for quantitative determination of uronic acids. Anal. Biochem. 54: 484.

Boothby, D. 1980. The pectic components of plum fruits. Phytochemistry 19: 1949.

Giovanni, M. 1983. Response surface methodology and product optimization. Food Technol. 11: 41.

Henika, R.G. 1982. Use of response-surface methodology in sensory evaluation. Food Technol. 11: 96.

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Clarification of Apple Juice by Hollow Fiber Ultrafiltration: Fluxes and Retention of Odor-Active Volatiles

M. A. RAO, T. E. ACREE, H. J. COOLEY, and R. W. ENNIS

ABSTRACT

Fresh apple juice was clarified in a pilot scale ultrafiltration (UF) unit, with membranes made of polysulfone and polyamide and plate and frame and vacuum drum filters. Flux of apple juice (L/m^2 hr) vs UF transmembrane pressure data showed an optimum at a pressure of about 140 kPa. Retention of odor-active volatiles was highest in plate and frame filtered apple juice and lowest in vacuum drum filtered juice; the retention of odor-active volatiles in UF juice was intermediate to the two traditional filtration methods. Retention of odor-active volatiles in the permeate of a polyamide membrane was higher than that of a polysulfone membrane.

INTRODUCTION

APPLE JUICE is an important item of commerce whose demand in the USA has increased to the extent that concentrated apple juice is being imported from West Germany, Argentina, South Africa, and other countries. Single strength apple juice retailed in the USA is clarified prior to packaging. For clarification of apple juice, either traditional techniques such as plate and frame (PFF) and vacuum drum (VDR) filtration or the relatively new ultrafiltration (UF) can be employed. In the former, because diatomaceous earth (DE) is used to precoat the surface of the filter and as body feed in the juice, they also are called DE filtration methods.

UF, used extensively in the processing of whey (Fenton-May et al., 1971) has also been used for pilot scale recovery of soy proteins (Cheryan, 1977). Porter and Michaels (1971) reviewed potential applications of UF in the food and pharmaceutical industries.

The advantages of UF for the clarification of apple juice include lower operating costs as a result of not requiring filter aids and lower labor requirements due to automation of the operation. Heatherbell et al. (1977) clarified apple juice by UF and obtained a stable clear product. UF also has been employed for the clarification of pear juice (Kirk et al., 1983) who found that the permeate flux reached a maximum value at an average transmembrane (TM) pressure of 157 kPa.

Little information can be found on the fluxes and the retention of odor-active volatiles of UF clarified apple juice. One objective of the present study was to determine on a pilot scale-hollow fiber UF unit the fluxes (L/m^2 hr) of fresh depectinized and undepectinized apple juice as a function of TM pressure and its turbidity. A second objective was to determine the retention of odor-active volatiles of depectinized apple juice clarified by PFF filtration, VDR filtration, and by UF. In the case of UF, polysulfone and polyamide membranes were evaluated.

MATERIALS & METHODS

Apple juice

Apples (Matsu) stored under controlled atmosphere for about 4 months were used. They had an average firmness of 11.0 lbf (48.9 Newtons) determined with an Effegi pressure tester (McCormick's Fruit Tree Co., Yakima, WA). Juice was produced by first crushing the apples in a hammer mill (Fitzpatrick Co., Chicago, IL, 4200 rpm, 0.635 cm screen) followed by pressing in a hydraulic press (Orchard Equipment, Conway, MA). Different lots of depectinized and undepectinized apple juice were employed in studies on fluxes with the UF unit. Only depectinized juice from a single lot was used in studies on changes in odor-active volatiles as a result of DE filtration (PFF and VDR) and UF.

Ultrafiltration unit

A pilot scale UF unit (model HF-LAB-5, Romicon Inc., Woburn, MA) with a single hollow fiber cartridge was used. The unit had a 19L stainless steel storage tank, a sanitary stainless steel pump, pressure gauges at the inlet and outlet of the cartridge, a flow reversing valve, and a back pressure control valve (Fig. 1). The permeate stream was collected and the retentate was returned to the feed tank. TM pressure was controlled by the back pressure control valve. A polysulfone membrane cartridge (PM-50) with a molecular weight cut-off of about 50,000 daltons was used in the studies on fluxes with depectinized and undepectinized apple juice. For studies on changes in odor-active volatiles, in addition to the polysulfone membrane cartridge, a polyamide membrane cartridge (PA-30) with a molecular weight cut-off of about 30,000 daltons was employed. Juice samples from the feed tank, and the permeate and retentate streams were collected for analyses of odor-active volatiles.

The UF cartridge was cleaned with aqueous 1% NaOH, bleach, and distilled water as recommended by the manufacturer. Data for flux of water were determined after an initial run of 15 min. Prior to obtaining data on fluxes of apple juice, the unit was flushed twice with water for 5 min, operated with distilled water for 15 min, and the flux checked at a TM pressure of 137.9 kPa. Flux data with apple juice were recorded after an initial run time of 30 min to ensure that water in the UF cartridge was displaced. Subsequent flux data were recorded as soon as the pressure readings and the flow rates of permeate and retentate had stabilized. Following the tests with apple juice, the unit was rinsed with solutions of 1% NaOH, commercial bleach and finally with water.

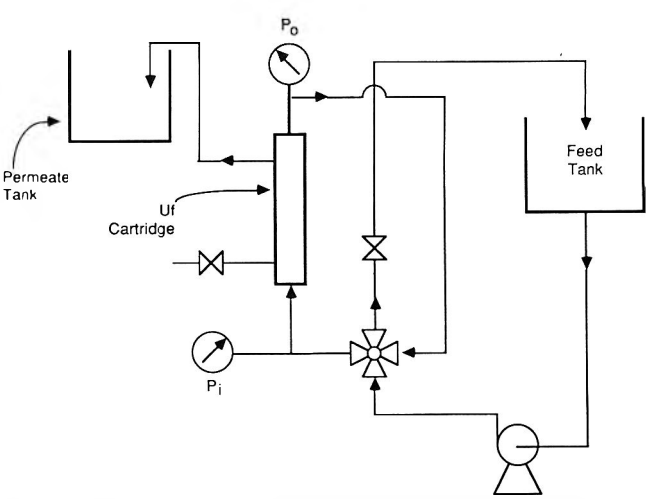

Fig. 1—Schematic diagram of ultrafiltration equipment; Uf stands for ultrafiltration, and P_i and P_o for inlet and outlet pressures.

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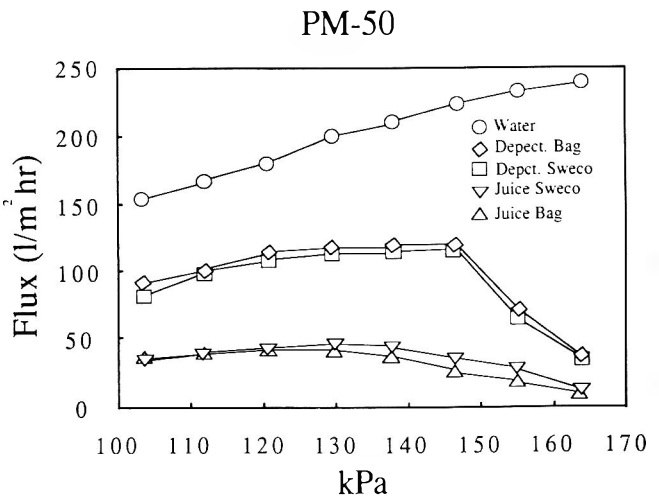

Fig. 2—Transmembrane pressure versus flux of water, and depectinized and undepectinized apple juices with polysulfone UF cartridge.

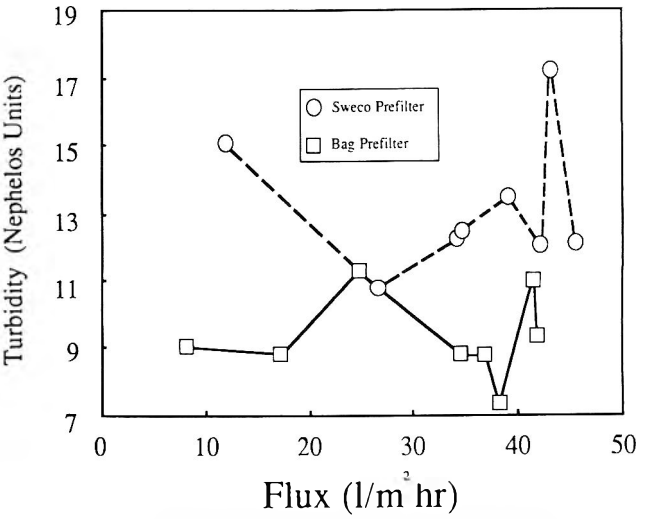

Fig. 3—Turbidity as a function of flux of depectinized apple juice using a bag and a screen for pre-filtration.

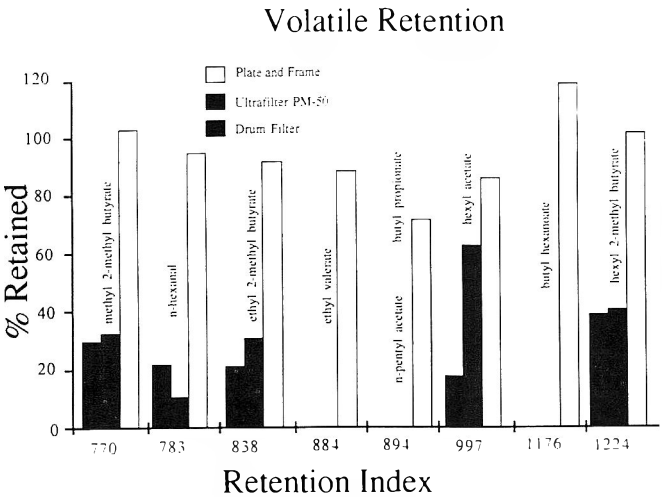

Fig. 4—Retention of odor-active volatiles in depectinized apple juice clarified with a polysulfone UF membrane, a plate and frame filter, and a vacuum drum filter.

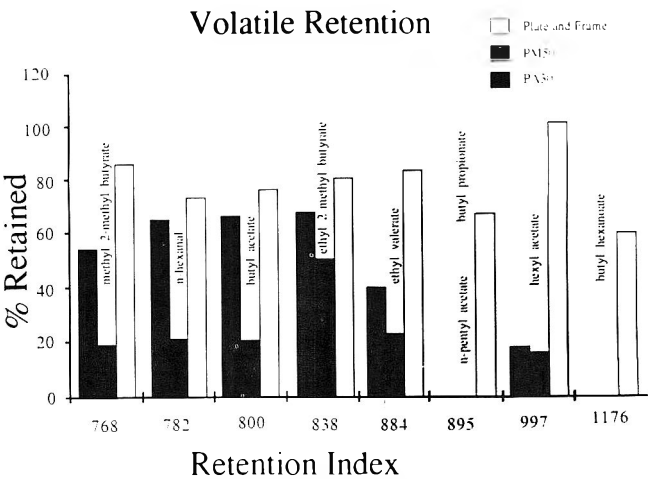

Fig. 5—Retention of odor-active volatiles in depectinized apple juice clarified with a polysulfone UF membrane, a polyamide UF membrane, and a plate and frame filter.

Vacuum drum (VDR) and plate and frame filters (PFF)

Pilot scale PFF (T. S. Shriver and Co., Harrison, NJ, size 7) and VDR (Filtration Engineers, East Moline, IL, size 20 in (50.8 cm) dia × 12 in (30.5 cm) wide) filters were used in the study. The former was operated with 7% precoat and 1.5% body feed of DE filter aid (Celite 503, Manville Corp., Denver, CO); the latter was operated with 1.5% body feed and 20 g/plate precoat of DE filter aid (Celite 503).

Turbidity

Turbidity of the samples was determined with a nephocolorimeter (Coleman Instruments, Maywood, IL) after its calibration using appropriate Nephlos standards.

Odor-active volatiles

Odor-active volatiles in the permeate and the original feed streams were determined with a gas chromatograph (Model 5840, Hewlett Packard, Palo Alto, CA) as described by Cunningham et al. (1986).

RESULTS & DISCUSSION

Fluxes and turbidity

The fluxes of distilled water, depectinized and undepectinized apple juice as a function of the TM pressure are shown in Fig. 2. Two types of pre-filters, a cloth bag (average pore size 200 μm) and a screen (Sweco Separator, Southwestern Engineers Co., Los Angeles, CA) with screen size of 140 μm were used with the apple juice samples.

The slight deviation from linear increase in flux of water (J_w) with TM pressure over the range 100–160 kPa is attributed to compaction of the membrane resulting in deviation from magnitudes of flux predicted by Hagen-Poiseuille relationship (Cheryan, 1977; Tarnawski and Jelen, 1986):

$$J_{wO} = A_{mO} \frac{\Delta P}{\eta} \qquad (1)$$

where J_{wO} is the water permeate flux without compaction effects (m^3/m^2 sec), A_{mO} is the membrane coefficient without compaction effects (m), ΔP is the TM pressure (N/m^2), and η is the viscosity (Pa sec).

The flux of apple juice increased with TM pressure until it reached a maximum value at a TM pressure of about 140-145 kPa and then decreased with further increase in TM pressure. This pressure for optimum flux of apple juice is in good agreement with the 157 kPa reported by Kirk et al. (1983) for pear juice. The lower fluxes for apple juice in comparison with those of water were due in part to the higher viscosity of apple

juice and in part to membrane fouling. Fouling of the UF membrane by depectinized apple juice can be attributed to the suspended solids that were not removed by the coarse filters. Fouling by the undepectinized apple juice was due to both the suspended solids and the pectic substances.

The mechanism of fouling of UF membranes during the clarification of fruit juices appears to have not been well documented. A simple hypothesis is that there was a gradual build up of a layer of suspended solids on the tubes of the UF cartridge in the case of the depectinized apple juice. In the case of undepectinized juice, the layer was made up of suspended solids and pectic substances. The resistance due to the built up layer was responsible for the lower fluxes of apple juice compared to those of water. The resistance of the layer was higher in the case of the undepectinized apple juice due to the presence of both suspended solids and dissolved pectic substances. The observed decrease in flux after reaching a maximum value with increase in TM pressure was probably due to the collapsing of the fouling material that in turn closed membrane pores (Kirk et al., 1983).

Turbidity

The juice treated with the bag prefilter was lower in turbidity (Nephlos units) than that treated with a screen prefilter (Fig. 3).

Retention of odor-active volatiles

Eight odor-active volatiles previously identified (Cunningham et all, 1986) as contributing to the odor of apple juice were detected in the apple juice samples. The odor-active volatiles retained in the PM-50 polysulfone membrane permeate and in the filtrate of PFF and VDR are shown in Fig. 4. The odor-active volatiles retained in the filtrate using PFF were higher than those of the filtrate from the VDR and the permeate from the PM-50 polysulfone membrane. In general, the volatiles retained in the permeate of the UF membrane were higher than those in the filtrate using the VDR. The loss of volatiles in the operation of VDR can be attributed to the use of vacuum that resulted in the evaporation of volatiles from the juice, to absorption by DE, and to oxidation. The losses in the UF operation were probably due to the adsorption of the volatiles on the UF membrane surface as well as vaporization through recirculation of the retentate for 30 min before sampling.

Because of the low retention of volatiles in the VDR filtrate, it was not employed in the other tests on the retention of volatiles. Tests were conducted with a PA-30 polyamide UF membrane, a PM-50 polysulfone UF membrane and the PFF and the volatiles retained after each test are shown in Fig. 5. Retention of volatiles in the permeate of the PA-30 polyamide membrane was higher than that of the PM-50 polysulfone membrane. However, the volatiles retained in the PFF filtrate were higher than those in the permeates of the two UF membranes.

CONCLUSIONS

PERMEATE FLUXES of depectinized and undepectinized apple juice with a polysulfone membrane were the highest at a TM pressure of about 145 kPa so for optimum performance the UF operation should be carried out at 145 kPa. Retention of odor-active volatiles was low in VDR filtrate indicating that it may not be a desirable method for the clarification of fresh apple juice. Retention of volatiles was higher in the case of a polyamide UF membrane than with a polysulfone UF membrane.

REFERENCES

Cunningham, D.G., Acree, T.E., Barnard, J., Butts, R.M., and Braell, P.A. 1986. Charm analysis of apple volatiles. Food Chem. 19: 137.

Cheryan, M. 1977. Mass transfer characteristics of hollow fiber ultrafiltration of soy protein systems. J. Food Proc. Eng. 1: 269.

Fenton-May, R.I., Hill, C.G., and Amundson, C.H. 1971. Use of ultrafiltration/reverse osmosis systems for the concentration and fractionation of whey. J. Food Sci. 36: 14.

Heatherbell, D.A., Short, J.L., and Strubi, P. 1977. Apple juice clarification by ultrafiltration. Confructa 22: 157.

Kirk, D.E., Montgomery, M.W., and Kortekaas, M.G. 1983. Clarification of pear juice by hollow fiber ultrafiltration. J. Food Sci. 48: 1663.

Porter, M.C. and Michaels, A.S. 1971. Membrane ultrafiltration. Chem. Technol. 1: 56.

Tarnawski, V.R. and Jelen, P. 1986. Estimation of compaction and fouling effects during membrane processing of cottage cheese whey. J. Food Eng. 5: 75.

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*EFFECT OF PECTIN ON THE FREEZING POINT. . .*From page 374

Joslyn, M.A. and Deuel, H. 1963. The extraction of pectins from apple marc preparations. J. Food Sci. 28: 65.

Kahn, M.L. and Eapen, K.E. 1980. Soft intermediate-moisture frozen beverage concentrates. U.S. patent 4,235,936, November 25.

Kertesz, Z.I. 1951. Proportion of and changes in pectic components in some plants. Chap. XIII. In "The Pectic Substances," Z.I. Kertesz (ed.), p 281. Interscience Publishers, Inc., New York.

LaBelle, R.L. 1973. Mean data of harvest for apple cultivars as grown at Geneva, NY. Mimeocircular, New York State Agriculatural Experiment Station, Geneva, NY.

McLellan, M.R., Barnard, J., and Queale, D.T. 1984. Sensory analysis of carbonated apple juice using response surface methodology. J. Food Sci. 49: 1595.

Mullen, K. and Ennis, D.M. 1979. Rotable Designs in product development. Food Technol. 7: 74.

Omran, A.M. and King, C.J. 1974. Kinetics of ice crystallization in sugar solutions and fruit juices. AIChE J. 20: 795.

SAS. 1985. "SAS® User's Guide: Statistics", 5th ed. SAS Inst. Inc., Institute Inc., Cary, NC.

Schachat, R.E. and Raymond, L.Z. 1959. Some Aspects of Hydrocolloid Gelation. Adv. Chem. Series 25, American Chemical Society, Washington, D.C.

Shirai, Y., Nakanishi, K., Matsuno, R., and Kamikubo, T. 1985. Effects of polymers on secondary nucleation of ice crystals. J. Food Sci. 50: 401.

Shuman, A.C. 1959. "Theoretical Aspects of Hydrocolloids in Controlling Crystal Structure in Foods. Adv. Chem. Series 25, American Chemical Society, Washington D.C.

Smock, R.M. and Neubert, A.M. 1950. Chemical changes and physiology of apple fruit after harvest. Chap. VI. In "Apples and Apple Products," Z.I. Kertesz (Ed.), p 95. Interscience Publishers, Inc., New York.

Stocking, J.H. and King, C.J. 1974. Secondary nucleation of ice in sugar solutions and fruit juices. AIChE J. 22: 131.

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Effect of Assay Temperature on Activity of Citrus Pectinesterase In Fresh Orange Juice

L. WICKER, R.J. BRADDOCK, and M. VASSALLO

ABSTRACT

The effect of temperature on pectinesterase activity in crude enzyme extract from Valencia pulp was determined. The effect is best described by the regression equation: LN PEU = 14.3446 − 3415 (1/T) with a correlation coefficient of 0.991. The activation energy was determined to be 28,392 joules/mol. This regression equation was used to predict the enzyme activities of different juice samples and verified by assay of Valencia juice at various temperatures. Duncan analysis indicated no significant differences in the predicted pectinesterase activities of the concentration-corrected crude extract and collective means of the Valencia juices at the respective temperature.

INTRODUCTION

CLOUD RETENTION in citrus juices is a major concern of citrus processors. Loss of cloud has been attributed primarily to the deesterification of pectin by pectinesterase (PE). The resulting pectic acid complexes with calcium to form insoluble calcium pectate (Joslyn and Pilnik, 1961). It has been reported the temperatures of 90°C for at least 15 sec are required to inactivate PE in citrus (Rouse and Atkins, 1962; Versteeg et al., 1980). Commercially, enzyme stabilization is accomplished during citrus juice concentration in the evaporator. Heat and vacuum result in loss of the volatile aromas. Technologies such as freeze concentration or reverse osmosis offer nonthermal means to concentrate juice without loss of delicate aromas associated with citrus juice. No suitable method exists to stabilize the juice against pectinesterase except for heat. Residual PE activity following pasteurization or the effect of storage temperature has been related to cloud loss of unstabilized juice (DuBois and Kew, 1951; Rothschild and Karsenty, 1974). This study was undertaken to determine the effects of assay temperature on PE activity and to evaluate the potential effects of pectinesterase activity at freezing or near freezing product temperatures.

MATERIALS & METHODS

Preparation of crude enzyme extract and juice

Mid-season Valencia orange pulp was extracted during the 1985 processing season at the Citrus Research and Education Center and stored at −20°C until needed.

The frozen pulp was homogenized in a 1:5 ratio (w/v) of pulp to 0.25M Tris-Cl, 0.3M NaCl, pH 8.0 at high speed in four, 15 sec bursts using a blender (Astramixer, Model M-100). The mixture was stirred for 1 hr at room temperature and centrifuged at 16,000 X *g* for 25 min at 4°C. The supernatant, constituting the crude enzyme extract, was stored at −20°C in small aliquots until needed. Analyses were conducted in triplicate on the enzyme extract at temperatures between 5°C and 60°C.

Juice was extracted from Valencia oranges at three times during the 1986 processing season at the Citrus Research and Education Center and collected after the juice finisher or from the State Test House Extractor. A FMC 291 B-100 extractor was used to process fruit on March 21, 1986. A 7/16 in long bore orifice tube, 0.040 strainer tube, 3/4 in beam setting, and 3 in orange cups were used. A FMC Model 35 finisher with 0.020 screens and 46 psi pressure was used. The State Test House Extractor was used to process fruit on March 26 and April 14, 1986. The settings for a 3 in cup were 0.025 strainer tube, 4 in window ring, 3 in barbed split ring, 1/8 in beam, and 45 psi pressure. Analyses of the three replicates were conducted on juice between 5°C and 40°C in triplicate without further modification.

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Statistical analysis

Data were examined with analysis of variance and Duncan's multiple range test (Allen, 1982). The means of the three juice samples and concentration-corrected crude extract were analyzed separately at each temperature. The results are summarized in Table 1. The means of the three juice samples were also analyzed collectively at each temperature and compared to the concentration-corrected extract.

Pectinesterase assay

The method of Rouse and Atkins (1955) was used to determine the activity of PE in the crude enzyme extract or juice at pH 7.0 and pectinesterase units (PEU) are expressed as the microequivalents of ester hydrolyzed per min per ml juice or extract. The temperature of the assay was controlled with a circulating water bath maintained at the report temperature. The stock pectin, NaOH, and assay mixture were maintained at the reported temperature for at least 10 min before addition of enzyme.

RESULTS & DISCUSSION

THE EFFECT of assay temperature on crude pectinesterase (PE) activity is shown in Fig. 1. In a linear plot of this data, it was observed that the PE activity increased as the assay temperature increased. Korner et al. (1980) observed similar temperature profiles with purified Shamouti and Valencia orange PE and reported a rapid decrease in PE activity above 60°C, presumably due to enzyme denaturation. If our PE extract had been assayed above 60°C, the same effect would be expected since it was observed that the PE activity decreased during the time of the assay even at 55°C.

A step wise multiple regression analysis (Ryan et al., 1982) was performed to determine the relationship of assay temperature and PE activity. The effect is best described by the regression equation:

$$\text{LN PEU} = 14.3446 - 3415\ (1/T)$$

The terms LN PEU and 1/T refer to the natural logarithm of the PEU and the reciprocal of the absolute temperature in °Kelvin, respectively. The correlation coefficient adjusted for the degrees of freedom is 0.991. Using an Arrhenius model, the activation energy (E_a) of pectin deesterification was calculated from the slope of the regression line to be 28,392 joules/mol. Korner et al. (1980) purified two PE isozymes from Shamouti orange pulp and reported activation energies of 23,442 and 24,028 joules/mol, respectively.

A similar linear relationship in Valencia orange juice is de-

Table 1—Comparison of predicted and measured activity[a] of pectinesterase of Valencia juices

Temp (°C)	Model[b] predicted	Extract[bc] measured	OJ-1[cd] measured	OJ-2[ce] measured	OJ-3[cf] measured	OJ[fg] mean
5	0.68	0.70[ab] ± 0.16	0.88[b] ± 0.12	1.44 ± 0.07	0.46[a] ± 0.04	0.93 ± 0.49
10	0.97	1.09[b] ± 0.19	0.85[ab] ± 0.11	1.71 ± 0.05	0.62[a] ± 0.11	1.06 ± 0.57
15	1.20	1.39[b] ± 0.14	1.20[ab] ± 0.17	1.90 ± 0.32	0.89[a] ± 0.14	1.33 ± 0.52
30	2.15	2.29[b] ± 0.23	2.06[b] ± 0.15	3.15 ± 0.20	1.45[a] ± 0.08	2.22 ± 0.86
40	3.08	3.13[b] ± 0.17	3.35[b] ± 0.28	5.18 ± 0.33	2.50[a] ± 0.50	3.68 ± 1.37

[a] Activity expressed as microequivalents of ester hydrolyzed per min per ml of juice or extract.
[b] PEU predicted from the regression equation and the measured activity of the crude extract have been divided by ten.
[c] Duncan's analysis conducted on measured PE activity of concentration-corrected crude extract, OJ-1, OJ-2, and OJ-3. Means within each row with the same letter are not significantly different at $P < 0.05$.
[d] Valencia orange juice extracted on March 21, 1986.
[e] Valencia orange juice extracted on March 26, 1986.
[f] Valencia orange juice extracted on April 14, 1986.
[g] Mean PEU of three juice samples. No significant difference ($P < 0.05$) between mean of three juice samples to concentration-corrected crude extract.

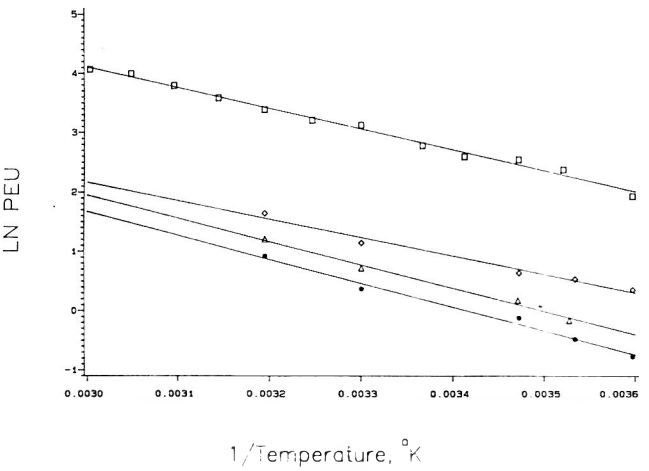

Fig. 1—Arrhenius plots of temperature effect on pectinesterase activity (PEU): □ crude enzyme extract, Ea = 28,392 joules/mol; △ Valencia orange juice, Replication 1, Ea = 28,536 joules/mol; ◇ Valencia orange juice, Replication 2, Ea = 25,640 joules/mol; ○ Valencia orange juice, Replication 3, Ea = 33,316 joules/mol.

picted in Fig. 1. The activation energies determine from the three samples were 28,536, 25,640, and 33,316 joules/mol, respectively. The activation energy for Replicate 3 was higher than the activation energies for the first two juice replicates. Although the Arrhenius plot of the three juice samples indicates similar reaction kinetics, enzyme activity at 30°C was variable. Variation in PE activity has been related to several factors such as fruit variety and maturity, pulp content of juice, processing conditions, etc. (Rouse and Atkins, 1955). The observed activity in the three juice samples did not directly correlate with variables which have been related to increased PE activity. For example, juice extracted on March 26 had 10% pulp and 3.15 PEU at 30°C, but juice extracted on April 14 had 12% pulp and 1.45 PEU at 30°C. Further, juice prepared with the FMC extractor and finisher was intermediate in PE activity between the two juice samples prepared in the State Test House Extractor, which were not finished. Seasonal effects were expected to be minimal since Rouse et al. (1962) reported that PE activity from Valencia was relatively constant during the time period of this study.

The regression equation derived for the crude extract data was used to predict enzyme activities at low temperatures in juices and verified by enzyme assay in juice. Rouse and Atkins (1955) conducted a survey of PE activity assayed at 30°C in commercial samples of frozen concentrated citrus juice from 23 concentrate plants. A mean and standard deviation of 1.3316 ± 0.7198 was calculated from their data and corrected for single-strength juice. The mean enzyme activity for the three juice replicates in this study was 2.22 ± 0.86 PEU at 30°C. Since the Rouse and Atkins (1955) data was collected from concentrate produced from low temperature evaporators, the enzyme was not inactivated (Rouse, 1952). Therefore, the PE activity reported is an estimate of the enzyme activity observed in fresh, unconcentrated juice. The concentration of pectinesterase in the crude extract was adjusted higher than expected in juice to maximize detection of pectinesterase at low temperatures. If the concentration is corrected for this 10-fold difference, then the regression equation derived form the crude extract provides a good estimate of the enzyme activity at low temperatures as seen in Table 1. Duncan analysis (Allen, 1982) indicated no significant differences ($P > 0.05$) in the pectinesterase activities of the concentration-corrected crude extract and the three Valencia juices analyzed collectively at the respective temperatures. If analyzed independently at 30°C, OJ-2 was significantly different ($P < 0.05$) from the crude extract, OJ-1, and OJ-3. The crude extract and OJ-1 were not significantly different but both differed significantly from OJ-3 ($P < 0.05$) (see Table 1). The OJ-2 and OJ-3 juices represent the high and low measured PEU values, respectively, whereas OJ-1 represents an intermediate PE activity. Since the average PEU value is within the standard deviation calculated from the Rouse and Atkins (1955) data, we believe the regression equation represents a direct estimate of PE activity at temperatures where the linear relationship holds.

Previous studies of the effect of PE on cloud loss were indirectly conducted by storage of juice at different temperatures and measurement of cloud density. DuBois and Kew (1951) reported the effects of storage temperature on citrus concentrate produced by low temperature vacuum concentration. At temperatures less than −17.8°C, the cloud was stable for 10 months in orange, grapefruit, and tangerine concentrate. As the storage temperature was increased to −6°C, the cloud was stable only for a few days in the three cultivars. The PE load was not reported in these samples. Rothschild and Karsenty (1974) related low PE activity and cloud loss during storage of different varieties of citrus juice. They reported that at 4°C, the cloud was stable from 0–24 months depending on the variety, PE concentration, juice concentration, and acidity. At 4°C, Valencia orange juice cloud ws stable for 24 months at 0.5×10^4 PEU/g single strength juice stored as single strength juice. At room temperature storage, cloud was lost at 0.25×10^4 PEU/g single strength juice within 15 months when stored as single strength juice.

Chandler and Robertson (1983) observed a continual decline in cloud density of pasteurized juice with storage time at 30°C and postulated that cloud loss was not due to residual enzyme activity but rather due to the result of PE activity before pasteurization and the physico-chemical reactions so initiated continued after pasteurization. Krop (1974) reported that a calcium pectate gel could be formed by high methoxyl apple pectin of 75% DE when the DE had only decreased by 5% when enzymatically deesterified.

The significance of these data is in the application of technology such as freeze or reverse osmosis concentration of unheated citrus juice and residual effects of PE at lower

temperatures during processing or frozen storage. Factors such as thermal processing and juice handling prior to and after concentration have been shown to be more important to final quality than the freeze concentration process itself (Braddock, 1986). Enzyme activities in single strength juices reported in Table 1 would be 3- to 4-fold higher in a 45°Brix concentrate prepared by freeze concentration of the fresh juice.

In summary, the effect of assay temperature on a crude extract of pectinesterase was measured directly. A regression equation was developed to predict PE activity in juice. A good correlation between the predicted activity of the crude PE extract and Valencia juices was found. In combination with previous storage studies, the regression equation can be used to predict PE activity at any temperature where the linear relationship holds. Processors using nonthermal concentration techniques can use known time/temperature profiles to predict enzyme activity at each point in the concentration process. This should prove to be a more accurate tool with which to predict cloud loss in orange juice.

REFERENCES

Allen, A. (Ed.). 1982. "SAS User's Guide: Basics, 1982 edition," SAS Institute, Cary, NC.
Braddock, R.J. 1986. Freeze concentration of grapefruit juice. XIV International Fruit Juice Producers Symposium. The Hague, The Netherlands (In press).
Chandler, B.V. and Robertson, G.L. 1983. Effect of pectic enzymes on cloud stability and soluble limonin concentration in stored orange juice. J. Sci. Food Agric. 234: 599.
DuBois, C.W. and Kew, T.J. 1951. Storage temperature effects on frozen citrus concentrates. Presented at 38th Spring Meeting of Amer. Soc. of Refrigerating Engineers, Detroit, May 28–30.
Joslyn, M.A. and Pilnik, W. 1961. Enzymes and enzyme activity. In "The Orange: Its Biochemistry and Physiology." W.B. Sinclair (Ed.)., p. 395. Univ. of Calif. Press, Davis.
Korner, B., Zimmermann, G., and Berk, Z. 1980. Orange pectinesterase: Purification, properties, and effect on cloud stability. J. Food Sci. 45: 1204.
Krop, J.J.P. 1974. The mechanism of cloud loss phenomena in orange juice. Ph.D. dissertation, Center for Agricultural Publishing and Documentation, Wageningen, Netherlands.
Rothschild, G. and Karsenty, A. 1974. Cloud loss during storage of pasteurized citrus juices and concentrates. J. Food Sci. 39: 1037.
Rouse, A.H. 1952. Pectinesterase retention in citrus juices stored at various temperatures. Proc. Fla. State Hortic. Soc. 65: 220.
Rouse, A.H. and Atkins, C.D. 1952. Heat inactivation of pectinesterase in citrus juices. Food Technol. 6: 291.
Rouse, A.H. and Atkins, C.D. 1955. Pectinesterase and pectin in commercial citrus juices as determined by methods used at the Citrus Experiment Station. Univ. of Fla. IFAS Bull. 570, Gainesville, FL.
Rouse, A.H., Atkins, C.D., and Moore, E.L. 1962. Seasonal changes occurring in the pectinesterase activity and pectic constituents of the component parts of citrus fruits. I. Valencia oranges. J. Food Sci. 27: 419.
Ryan, T.A., Jr., Joiner, B.L., and Ryan, B.R. 1982. Minitab Reference Manual. Duxbury Press. Boston, MA.
Versteeg, C., Rombouts, F.M., Spaansen, C.H., and Pilnik, W. 1980. Thermostability and orange juice cloud destabilizing properties of multiple pectinesterases from orange. J. Food Sci. 45: 969.
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investigate the effectiveness of this flux restoration method in large scale equipment in commercial processing. In particular, its effect on membrane life needs to be evaluated.

REFERENCES

Anderson, N.E. and Clydesdale, F.M. 1980. An analysis of the dietary fiber content of a standard wheat bran. J. Food Sci. 45: 336.
AOAC, 1980. "Official Method of Analysis," 13th ed. Association of Official Analytical Chemists, Washington, DC.
Bitter, T. and Muir, H.M. 1962. A modified uronic acid carbazole reaction. Anal. Biochem. 4: 330.
Cheryan, M. and Merin, U. 1980. A study of the fouling phenomenon during ultrafiltration of cottage cheese whey. In "Ultrafiltration membranes and Application,' (Ed.) A.R. Cooper, p. 619. Plenum Pub., Co., New York.
De Boer, R., Zomerman, J.J., Hiddink, J., Aufderheyde, J., Van Swaay, W.P.M., and Smolders, C.A. 1980. Fluidized beds as turbulence promoters in the concentration of food liquids by reverse osmosis. J. Food Sci. 45: 1522.
Dejmek, P., Funeteg, B., Hallstrom, B., and Winge, L. 1974. Turbulence promoters in ultrafiltration of whey protein concentrate. J. Food Sci. 39: 14.
Glover, F.A. and Brooker, B.E. 1974. The structure of the deposit formed on the membranes during the concentration of milk by reverse osmosis. J. Dairy Res. 41: 89.
Hodge, J.E. and Osman, E.M. 1976. Carbohydrates. Ch. 3. In "Principles of Food Science, Part I. Food Chemistry," (ed.) O.R. Fennema, 41. Marcel Dekker, Inc., New York.
Kennedy, T.J., Merson, R.L., and McCoy, B.J. 1974. Improving permeation flux by pulsed reverse osmosis. Chem. Eng. Sci. 29: 1927.
Lee, D.N. and Merson, R.L. 1975. Examination of cottage cheese whey proteins by scanning electron microscopy: Relationship to membrane fouling during ultrafiltration. J. Dairy Sci. 58: 1423.
Lim, T.H. Dunkely, W.L., and Merson, R.L. 1971. Role of protein in the reverse osmosis of cottage cheese whey. J. Dairy Sci. 58: 658.
Lowe, E. and Durkee, E.L. 1971. Dynamic turbulence promotion in reverse osmosis processing of liquid foods. J. Food Sci. 36: 31.
Peri, C. and Dunkely, W.L. 1971. Reverse osmosis of cottage cheese whey. 1. influence of composition of the feed. J. Food Sci. 36: 25.
Skudder, P.J., Glover, F.A., and Green, M.L. 1977. An examination of factors affecting the reverse osmosis of milk with special reference to deposit formation. J. Dairy Res. 44: 293.
Watanabe, A., Kimura, S., and Kimura, S. 1978. Flux restoration of reverse osmosis membranes by intermittent lateral surface flushing for orange juice processing. J. Food Sci. 43: 985.
Watanabe, A., Kimura, S., Ohta, Y., Randall, J.M., and Kimura, S. 1979a. Nature of the deposit on reverse osmosis membranes during concentration of pectin/cellulose solutions. J. Food Sci. 44: 1505.
Watanabe, A., Ohta, Y., Kimura, S., Umeda, K., and Kimura, S. 1979b. Fouling materials on the reverse osmosis membrane during concentration of mandarin orange juice. J. Japan Soc. Food Sci. & Tech. 26: 260.
Yu, Z.R., Chiang, B.H., and Hwang, L.S. 1986. Retention of passion fruit juice compounds by ultrafiltration. J. Food Sci. 51: 841.
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Preparation and Storage of 72° Brix Orange Juice Concentrate

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ABSTRACT

Each year, thousands of tons of citrus concentrate are stored and transported throughout the world at 62° Brix. Increasing this concentration to 72° Brix can result in substantial savings. Fresh orange juice, treated mechanically or with pectic enzymes to reduce its viscosity, was concentrated to 72° Brix in a commercial processing plant, then samples were stored in barrels in a tank farm at −7 or at 4°C. At monthly intervals, samples were blended to make 41.8° Brix Frozen Concentrated Orange Juice. The enzyme-treated samples had lower viscosity than those mechanically treated. Vitamin C retention levels were > 94% after 6 months storage at 4°C. Taste evaluations rated the product 'very good' and furfural levels were well below that which indicates off flavors. Browning tended to increase with storage time and temperature.

INTRODUCTION

CITRUS IS ONE of the largest fruit crops of the United States with 6.7×10^6 metric tons (t) grown during the 1984-85 season. Florida is the major producer in the United States with more than 70% of the orange crop, about 95% of which goes for processing (USDA, 1985). With these large volumes of citrus concentrate being produced each year, citrus processors are constantly looking for new ways to reduce storage and transportation costs.

Since the early 1960's, freshly extracted orange juice has been concentrated to about 62° Brix (% soluble solids) in high temperature, falling film evaporators (Cook, 1963). At first, this concentrate was stored in 200 L drums, but more recently in bulk storage tanks ranging in size from 5.7×10^5 L to 1.8×10^7 L (Hendrix and Ghegan, 1980) and held at storage temperatures from 0 to −11°C (Crandall et al., 1981). Currently, there is sufficient interest in increasing the concentration of orange juice above 62° Brix so that specially designed evaporators have been constructed to concentrate the juice to 72° Brix (Gherardi, 1980; Fischer et al., 1983). Brazil is also a major processor of citrus, processing an estimated 8.3×10^6 t of oranges in 1985 (USDA, 1985). Much of Brazil's orange concentrate is also stored in bulk tanks prior to export, then shipped in bulk tanker ships holding about 1×10^4 t. This concentrate is shipped to plants located near major orange juice consumption areas for reprocessing into single-strength juice. These plants dilute the concentrate and package the single-strength juice into retail containers. The energy and transportation savings of 72° Brix have been studied (Stegelin and Crandall, 1981; Crandall and Beilock, 1985).

High viscosity is one problem encountered in concentrating, storing and transporting 72° Brix orange concentrate (Crandall et al., 1981). Tests in the pilot plant had shown that the TASTE evaporator was capable of producing 72° Brix concentrate when the pulp levels were less than 9% by volume (Crandall et al., 1986). There are commercial methods of reducing juice viscosity by: mechanically separating the juice streams at the finisher, reducing the extractor and finisher pressure or screen sizes, centrifuging to remove the fine pulp or using pectic enzymes. The mechanical separation method has been used in the citrus industry to produce a less bitter tasting juice, but it has not been adopted to reduce the viscosity. Mechanical separation has several advantages over using a centrifuge: the capital costs would be less than 1% of that for centrifuges, the free flow juice from the finisher has had lower extraction pressure and the remaining juice from the finisher can be blended with normally finished juice so there would be a minimal yield loss. There is a loss of entrained juice solids when the pulp is removed by centrifugation. The mechanical separation of the juice streams for viscosity reducing does not violate the Standards of Identity for orange juice.

Previous research has shown pectic enzymes are effective in reducing the viscosity of citrus concentrates (Crandall et al., 1981; Braddock, 1981). However, to date, Federal Standard of Identity Regulations have not permitted the use of Generally Recognized as Safe (GRAS) pectic enzymes to be used as processing aids in citrus juice. The use of these enzymes is widespread in other fruit juices (Rombouts and Pilnik, 1978) and in pulp wash made from water-leached citrus pulp (Braddock and Kesterson, 1979). The advantage of using pectic enzymes over the other three methods is that the processor could use standard extraction and finishing techniques and then treat all of the juice with enzymes.

The current study is a continuation of our work on energy savings by storing a high Brix orange concentrate at refrigerated temperatures. The first pilot plant study showed no appreciable quality change in 72° Brix samples stored at less than 4.4°C for at least 6 months (Crandall et al., 1981; Crandall and Graumlich, 1982). The second pilot plant study (Crandall et al., 1986) demonstrated that adding 70 ppm pectic enzyme to orange juice prior to concentrating to 72° Brix reduced the initial apparent viscosity by about 25%. Vitamin C levels were above 32 mg/100 mL after 6 months at −7°C and there was no significant taste difference between control and 70 ppm enzyme treated samples.

The objectives of this study were: (1) to examine alternative mechanical and enzymatic treatment methods for reducing the viscosity by using existing commercial evaporators to concentrate orange juice to 72° Brix; (2) to measure the quality of the Frozen Concentrated Orange Juice (FCOJ) produced from these treated concentrates; and (3) to determine the storage effects of these treatments.

MATERIALS & METHODS

TWO EXPERIMENTAL TESTS were carried out in cooperation with a Florida commercial citrus processor. One test was conducted at the peak processing time for the early-midseason oranges and the other at the peak of the Valencia season. In each test, about 20 t of fruit were processed by each of the following methods. The first involved standard commercial extracting and finishing conditions using Brown equipment (Brown International, Covina, CA). After finishing, the juice was pumped into a 7600 L evaporator feed tank where 1200 ppm pectic enzyme (Bio Con 100) was added. This enzyme has 100 units polygalacturonase activity per gram as measured by the dinitrosalicylic acid method (Bio Con, Lexington, KY). The enzyme was diluted with juice, added near the tank's paddle agitator while the tank

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was being filled, and allowed to react for 50 min at 22°C. The juice was then fed into a 9,000 kg water removal/hr TASTE evaporator to inactivate the pectic enzyme, destroy the microorganisms and concentrate the juice. After the output from the evaporator reached a stable Brix concentration, a sample of 625 L of 72° Brix concentrate was blended into a uniform batch. Samples from this batch will be referred to as enzyme (enz)-treated juice.

The second juice processing method involved placing a vertical, movable, separating pan in the juice trough of the Brown finisher (Fig. 1). This pan split the juice from the finisher into two separate streams: juice which flowed freely from the pulp near the entrance of the finisher, called "free run" juice, and juice which was removed with higher finisher pressure near the pulp discharge end of the finisher. The "free run" juice will be referred to as light end (lt end) juice in this experiment. It was stored in a separate evaporator feed tank without the addition of pectic enzymes and was evaporated to 72° Brix, then a sample taken as in the first method.

Each batch was uniformly blended with 0.01% v/v cold-pressed orange oil and divided into two separate subsamples, each transferred to commercial concentrate storage drums. These steel drums have two non-sterile polyethylene liners which were twisted and tied shut without modification of the headspace gas. One subsample was stored in a commercial tank farm storage room at −7°C which simulated bulk storage temperatures. The other subsample was stored in a refrigerated storage room at 4°C. Each month (mo), samples were taken of the concentrates at each of the two storage temperatures and blended to 41.8° Brix, equivalent to FCOJ, in a commercial citrus laboratory. Treated water and cold-pressed orange oil were added according to standard industrial practice. Duplicate quality analyses were performed on each sample initially, at monthly intervals up to 6 mo and again after 12 mo storage. Soluble solids and vitamin C were determined according to Praschan (1976). Serum viscosity and percent light transmission were determined according to established procedures (FMC, 1983). Nonenzymatic browning was determined by measuring the absorbance at 420 nm on an alcohol extract of the juice (Meydav et al., 1977), furfural was measured spectrophotometrically at 515 nm (Dinsmore and Nagy, 1974).

Sensory evaluations were made by the Citrus Research and Education Center's (CREC) 12-member experienced taste panel using a nine-point hedonic scale where 9.0 is "like extremely" and 1.0 is "dislike extremely." They compared initial samples of enz-treated and lt end for each cultivar and samples stored at −7° and 4°C. These samples were evaluated at 11.8° Brix after they had been concentrated to 72° Brix then made into 41.8° Brix FCOJ by adding cold-pressed oil and water. Samples were presented under red light, to eliminate color biases, at room temperature, and presented in a random order. In addition, the samples were graded by three commercial plant laboratory personnel who are experienced in grading citrus juice using the USDA grade standards for FCOJ (USDA, 1983).

Apparent viscosity was measured using a Haake rotational viscometer, RV-12, M-500 measuring head, and a SVII rotor using a Hewlett Packard HP 86 computer and plotter (Haake Buchler, Saddle Brook, NJ) and a Brookfield Viscometer Model LVT, Spindle No. 2, 12 rpm at 30°C (Brookfield Eng. Lab., Stoughton, MA).

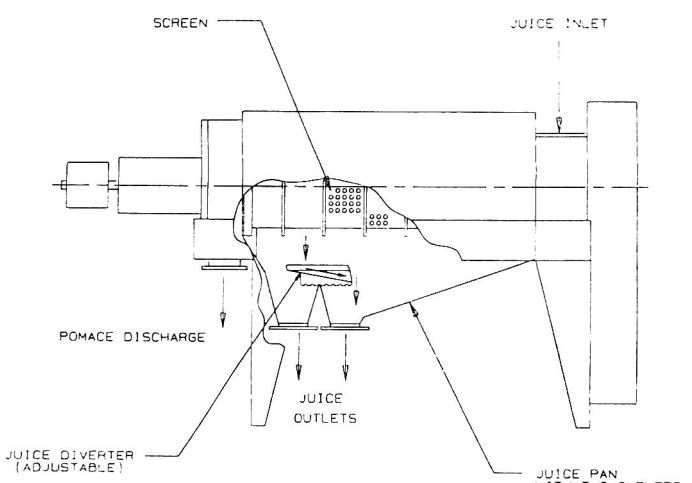

Fig. 1—Brown Model 2507 Finisher showing vertical separating pan (courtesy Brown International Corp., Covina, CA).

Each treatment was analyzed separately by an analysis of variance (ANOVA) using a split plot design and the analytical factors were checked for correlation using the statistical program MINITAB for linear regression. SAS (1985) version 4.10's ANOVA was used to analyze the hedonic taste data. A significance level of $P < 0.01$ was used throughout.

RESULTS & DISCUSSION

THE MOVABLE PAN in the finisher (Fig. 1) separated the juice which flowed freely from the juice removed by higher finisher pressures. The finisher pressure averaged 415 kPa and ranged from 310 to 515 kPa near the pulp discharge end. Rouse et al. (1960) found that as the finisher pressure increased from a range of 34–69 kPa to 103–138 kPa there was a change in the pectic fractions of the juice. The concentration of the water-soluble, ammonium oxalate-soluble and sodium hydroxide-soluble pectins all increased. Water-insoluble solids and pulp also increased with higher finisher pressure and in general, serum viscosity increased with greater amounts of water-soluble pectin.

Apparent viscosity

The effect of treatment and cultivar on the apparent viscosity of 72° Brix concentrate after 12 months storage is shown in Fig 2. This τ/D sol curve is the down portion of the flow curve and shows how these concentrates behave after being sheared (pumped). The viscosity is determined by dividing the τ value by the D value. For both cultivars, the enz-treated samples had a lower viscosity than the lt end. There was a larger than expected viscosity reduction for the enz-treated Valencia sample, due in part to differences between the cultivars. The highest viscosity at $D = 121\ sec^{-1}$ is 11.4 Pa·s for the early-mid lt end concentrate, which is in agreement with previously reported work that showed concentrate from early-mid cultivars were higher than Valencia (Crandall et al., 1982). The viscosity of the enz-treated early mid-sample was 5.83 Pa·s. The early-mid enz-treated sample had a lower Brix of 69.4° but a higher amount of pulp 8.5%. The lt end pulp was 7.0% with 73.4° Brix.

For the Valencia sample, the viscosity of the lt end sample was higher at 6.7 Pa·s for $D = 122\ sec^{-1}$. The enzyme treatment reduced the viscosity of the Valencia sample to 2.8 Pa·s. The enz-treated sample was 68.8° Brix and had 8.0% pulp. The lt end sample had 9.5% pulp and 70.4° Brix.

The ANOVA on the 72° Brix concentrate showed that for both cultivars there were significant effects on the viscosity of the enz-treated samples due to storage temperature and time (Table 1). For the lt end juice, in addition to the temperature and time contributing significantly to the differences in the viscosity readings, there was a significant temperature × time interaction which means the effect of temperature on viscosity was dependent on the length of time it was stored. This interaction makes it difficult to determine the effect of either major contributor.

In each of the four cases (enz-treated, 4°C, −7°C and lt end, 4°C, −7°C), there was an increase in viscosity from the initial to the one mo reading. This indicates a rebuilding of the internal structure which continued to increase the viscosity during the 6-mo storage time. In each case, the mean viscosities for 6 mo were greater than those for 0 mo and the viscosity increased with time in each case.

Table 2 shows the range of values for commercial FCOJ and bulk orange juice concentrate. These values are from Brookfield measurements at 30°C. The FCOJ samples were prepared to 41.8° Brix. For the bulk concentrated orange juice (BCOJ), the average Brix was 64.1, 63.0 and 62.9° for the 1981 to 1979 seasons, respectively. The values for both the enz-treated and lt end FCOJ were well within the range now handled for ths product.

Table 1—Summary of main effect means affected by storage temperature and time[a]

Cultivars	Early midseason								Valencia							
	Enzyme				Lt end				Enzyme				Lt end			
Treatment	Time, mo		Temp, °C		Time, mo		Temp, °C		Time, mo		Temp, °C		Time, mo		Temp, °C	
Response	0	6	−7	4	0	6	−7	4	0	6	−7	4	0	6	−7	4
Vitamin C mg/100 mL	45.0	42.2	43.5	42.8	50.4	48.7	49.6	47.8	39.9	39.2	39.9	38.9	40.4	39.1	39.4	39.3
	0.22[b]		0.13		0.19		0.11		0.34		0.20		0.37		0.21	
Taste[c] 9 point	6.1	4.8	6.0	5.6	5.8	4.7	5.8	5.5	6.3	5.0	6.0	5.8	5.6	6.3	6.0	5.8
hedonic scale	1.23		0.71		1.23		0.71		0.82		0.48		0.82		0.48	
Browing (Absrob.	0.119	0.127	0.122	0.123	0.126	0.121	0.118	0.121	0.195	0.222	0.198	0.206	0.177	0.201	0.182	0.189
at 420 nm)	0		0		0.002		0.001		0.003		0.002		0.030		0.017	
Apparent viscosity,	0.561	0.411	0.371	0.394	0.175	0.283	0.231	0.248	0.229	0.249	0.252	0.250	0.332	0.396	0.365	0.371
41.8°Brix (Pa·s)	0.978		0.566		0.435		0.251		0.254		0.146		0.758		0.437	
Serum viscosity	1.44	1.63	1.54	1.56	1.44	1.47	1.50	1.52	1.15	1.51	1.36	1.41	1.46	.165	1.55	1.61
mPa·s)	0.30		0.17		0.14		0.08		0.75		0.45		0.45		0.26	
Apparent viscosity,	2.57	3.49	3.25	2.82	3.51	3.76	3.69	3.37	.475	.670	.650	.604	2.31	3.15	3.23	2.74
72°Brix (Pa·s)	7.9		4.6		1.1		0.6		0.3		0.2		1.3		0.8	

[a] Values are the means of duplicate readings.
[b] Confidence limit, $P < 0.01$.
[c] Means of 12 taste evaluations.

Table 2—Viscosity data for commercial frozen concentrated orange juice (FCOJ) and bulk concentrated orange juice (BCOJ)[a]

		FCOJ				BCOJ			
Season	Measurement	Number samples	Min	Max	Mean	Number samples	Min	Max	Mean
1980-81	Viscosity (Pa·s)	106	0.300[b]	1.55	0.710	33	2.15	10.2	6.35
	Centrifuged pulp (%)	106	7.0	15.0	10.1	33	8.0	14.0	11.2
1979-80	Viscosity (Pa·s)	137	0.438	1.76	0.881	47	1.01	21.8	5.64
	Centrifuged pulp (%)	137	9.0	13.0	11.3	47	6.0	12.0	10.0
1978-79	Viscosity (Pa·s)	135	0.363	1.95	0.976	36	3.0	27.5	9.04
	Centrifuged pulp (%)	135	9.0	13.0	11.0	36	8.0	12.0	10.8

[a] From Sica (1982); Steverson (1980).
[b] Viscosity measured with Brookfield LVT, Spindle No. 2 at 30°C.

The ANOVA on the apparent viscosity of reconstituted 41.8° Brix early-midseason samples showed both the enz-treated and lt end treatments had a significant contribution to their differences by temperature, time and temperature × time interaction (Table 1). For the Valencia samples, both the enz-treated and lt end treatments had significant time, temperature × time interaction.

The results showed no significant correlations $R^2 < 0.5$ between the apparent viscosity of the 72° Brix bulk concentrate and the apparent viscosity of the 41.8° Brix samples reconstituted from them. This indicates high viscosity bulk concentrate can be stored or transported then reprocessed into FCOJ with 'normal' to low viscosity. The 41.8° Brix samples were handled according to commercial practice where they were diluted with purified water and cold pressed oil added (Matthews, 1984).

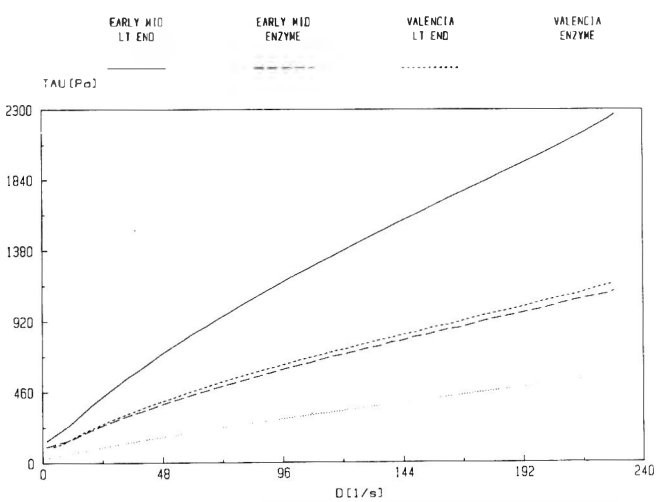

Fig. 2—Flow curve, shear stress (τ) vs shear rate (D), for early-mid season and Valencia orange juice concentrates.

Serum viscosity

The ANOVA showed time was the only factor that significantly affected all four cases and serum viscosity tended to increase with storage time (Table 1). Marcy et al. (1984) found no significant effect of storage time on the serum viscosity of a lower Brix concentrate.

It is standard industrial practice to run serum viscosity on the clear serum from the reconstituted 11.8° Brix product. This requires an approximate six-fold dilution of the serum from the 72° Brix original sample. This limits the usefulness of these viscosity measurements in explaining differences among the treatments because of the dilution effect of water on the interaction of small insoluble particles, soluble pectin and ions.

Vitamin C

Initial vitamin C values for the early-mid cultivars were higher than for the Valencia (Table 3). This difference due to cultivars was also seen by Nagy and Smoot (1977). For both cultivars, the lt end treatment gave slightly higher vitamin C values than did the enzyme treatment. There were statistically significant decreases in vitamin C after 6 mo storage at −7°C and 4°C. However, after 6 mo storage at 4°C, there was an excellent retention of 96% of the vitamin C for the early-mid lt end, 95.1% for early-mid enz-treatment, 94.6% for Valencia

Table 3—Vitamin C levels in commercially prepared bulk orange concentrate, initially and after 6 months storage

Treatment	Storage temp °C	Early-Midseason Vitamin C (mg/100 mL) Initial	6 months	Valencia Vitamin C (mg/100 mL) Initial	6 months
Pectic enzyme	−7	45.1	41.6**	40.0	39.0**
	4	45.0	42.8**	39.8	39.3**
Light end	−7	50.5	49.1**	40.4	40.0**
Light finisher	4	50.4	48.4**	40.4	38.2**
Pressure					

** Significant at $P \leq 0.01$.

lt end and 98.7% for Valencia enz-treated. These results show a small but statistically significant decrease in vitamin C during storage. Kanner et al. (1982) reported more than 90% retention of vitamin C after 6 mo storage at 12°C. The ANOVA showed that in each of the cases there were significant temperature by time interactions which indicates a complex relationship between time and temperature in the degradation of vitamin C (Table 1). Nagy and Smoot (1977) reported vitamin C levels on fresh commercially processed single-strength orange juice from several plants over a season. This juice was just pasteurized, not concentrated. The average value for early-mid orange juice ranged from 50.2 to 57.3 mg/100 ml and for Valencia 38.9 to 46.5. Most (three quarters) of the initial vitamin C values for the 72° Brix samples are within this range. This indicates that concentrating orange juice to 72° Brix under commercial conditions produced a juice with a vitamin C content equal to the milder pasteurization treatment.

Taste

The hedonic taste evaluation of the reconstituted juice by the CREC taste panel showed no significant difference among the initial, 0 time, enz-treated and lt end treatment for either cultivar. The average for the initial taste evaluations (0 time) were 6.6 ± 1.3 for the early-mid enz-treated, 6.7 ± 0.8 for early-mid lt end, 5.5 ± 2.7 for Valencia enz-treated and 5.1 ± 2.1 for Valencia lt end. This corresponds to the "like moderately" to "neither like nor dislike" categories, respectively. Comparing the two temperatures and two treatments over time, showed the minimum hedonic scores occurred at the 12 mo evaluation for both storage temperature and treatments. The exception was Valencia lt end treatment. The mean scores are shown in Table 4.

The ANOVA for the early-mid showed temperature, time and their interaction all contributed significantly to the observed differences (Table 1). For the Valencia, the ANOVA showed significant variation due to time, temperature and time interaction and treatment and time interaction. For both cultivars, the main effect storage time changed in a complex way with temperature.

A summary of the commercial taste panel evaluations on the reconstituted FCOJ samples is given in Table 5. The flavor scores in the range from 36 to 40 are in the "very good" range and could meet this criteria for USDA Grade A product. A very good flavor is described as fine, distinct, typical of orange juice from fresh, sweet oranges and is free from off-flavors of any kind (USDA, 1983). During the 6 mo storage period, all of the samples were judged to have a very good flavor. This indicates that bulk concentrate produced from either mechanical or enzyme tratment is similar in taste quality to commercial concentrate. Storage at tank farm or refrigerated temperatures maintained this quality for at least 6 mo.

Marcy et al. (1984) used a multiple comparison difference test to compare samples of 66° Brix orange concentrate stored at 4.4°C to a reference stored at −17.7°C. Experienced taste panelists were unable to detect a significant difference until after 5 mo storage at 4.4°C. However, these juice samples did not have the addition of a cold-pressed flavoring oil which is standard commercial practice in making FCOJ from bulk concentrate. Kanner et al. (1982) tested the flavor of aseptically canned 58° Brix orange concentrate in a triangle test against a reference sample stored at −18°C. They found no significant flavor change in the reconstituted sample after storage at 5°C for 17 mo. Again, this comparison was made without the addition of cutback flavoring oils.

Cloud

Retention of orange juice cloud is one of the principal reasons for pasteurizing citrus juice. Consumers generally believe that high quality citrus-containing beverages should be cloudy whereas drinks should be clear. Cloud readings are expressed as percent light transmittance and values ranging from 9 to 18% are considered acceptable (Sica, 1982). The cloud values for the early-mid samples were 10% for enz-treated and 12% for lt end. For Valencia, they were 11% for the enz-treated and 12% for the lt end. Baker and Bruemmer (1972) showed careful enzyme treatment increased cloud values over unheated controls. However, Chandler and Robertson (1983) found that both added enzyme and naturally occurring pectinesterase decreased cloud stability. Some of the differences among these results are due to the use of different methods and procedures used to measure cloud and its stability.

Browning

The absorbance values at 420 nm give an estimation of the extent of formation of brown pigments in orange juice due to heat processing and to the effects of temperature and time in storage (Meydev et al., 1977). Absorbance values are presented in Table 6 for the samples initially and then after 6 mo storage. The ANOVA showed time and temperature significantly affected all four cases but the mean square error term was very small. Browning did tend to increase with time and temperature but not uniformly.

Furfural

The accumulation of significant levels of furfural has been correlated with the formation of off-flavors in overheating and storage abuse (Dinsmore and Nagy, 1974; Nagy and Randall, 1973). Absorbance values are presented in Table 6 for the samples initially and then after 6 mo storage. All of the furfural values are well below the 30 ppb level for taste significance found by Nagy and Randall (1973). These values indicate that commercial evaporation to 72° Brix and storing 6 mo at refrigerated temperatures can be used to maintain orange juice quality.

CONCLUSIONS

MECHANICAL TREATMENT or the addition of pectic enzymes can be used to reduce the viscosity and enable commercial TASTE evaporators to produce 72° Brix concentrate.

Table 4—Twelve member experienced taste panel comparison of storage temperature and time for enzymatically and mechanically treated early-mid and Valencia orange juice[a]

Storage time (mo)	Early-Midseason				Storage time (mo)	Valencia			
	−7°C		4°C			−7°C		4°C	
	Enz[b]	Lt end	Enz	Lt end		Enz	Lt end	Enz	Lt end
2	6.17[c]	6.17	6.00	5.50	3	5.83	5.58	6.83	5.58
4	5.83	6.08	6.00	6.33	4	6.92	6.08	6.33	5.53
5	6.75	5.33	5.92	5.92	7	5.75	5.58	5.58	5.58
7	6.67	6.58	5.08	5.17	12	5.67	6.67	4.33	6.00
12	4.58	4.75	4.92	4.67					

[a] Samples diluted to 11.8° Brix for analysis.
[b] Pectic enzyme and light finisher pressure treatments.
[c] Mean of nine point hedonic scale (9 is like extremely and 1 is dislike extremely), confidence limit calculated from ANOVA $P < 0.01$ is 0.71 for early-mid and 0.47 for Valencia.

Table 5—Three member experienced commercial taste panel evaluation of reconstituted FCOJ

Storage time	Early-Midseason Enzyme[a] Light end Storage temperature				Valencia Enzyme Light end Storage temperature			
	20	40	20	40	20	40	20	40
Initial	36[b]	36	36	36	38	38	38	38
1 month	37	37	36	36	--	--	--	--
2	--	--	--	--	--	--	--	--
3	37	--	36	--	36	36	36	36
4	37	37	36	36	36	36	36	36
5	37	37	36	36	36	36	36	36
6	36	36	36	36	36	36	36	36
12					36	35	36	36

[a] Pectic enzyme and light finisher pressure treatments.

[b] Values are the mean of three taste evaluations made according to the USDA (1983) system where the flavor must be very good to score between 36–40 points.

Table 6—Browning and furfural levels in commercially prepared bulk orange juice concentrate, initially and after 6 months storage

Treatment	Storage temp °C	Early-midseason		Valencia	
		Initial	6 months	Initial	6 months
			-------Browning[a]-------		
Enzyme	−7	0.119	0.130	0.195	0.221
	4	0.118	0.124	0.195	0.224
Light End	−7	0.128	0.117	0.177	0.193
	4	0.125	0.126	0.177	0.209
			-------Furfural[b]-------		
Enzyme	−7	2[c]	5	2	4
	4	2	3	2	4
Light End	−7	2	4	4	4
	4	2	5	4	4

[a] Absorbance at 420 nm.

[b] All values are means of duplicate readings.

[c] ppb.

Mechanical treatment may currently be used, but the addition of pectic enzymes to citrus juice has not yet been approved. Results of quality evaluations of these 72° Brix concentrates showed good flavor, vitamin C retention. low furfural. and low browning during 6 mo storage at frozen and refrigerated temperatures. These results were obtained in a commercial plant but should be evaluated by individual companies before adoption. The storage and transportation of higher Brix concentrates at refrigerated temperatures offers substantial savings.

REFERENCES

Baker, R.A. and Bruemmer, J.H. 1972. Pectinase stabilization of orange juice cloud. J Agric. Food Chem. 20(6): 1169.

Braddock, R.J. 1981. Pectinase treatment of raw orange juice and subsequent quality changes in 60° Brix concentrate. Proc. Fla. State Hort. Soc. 94: 270.

Braddock, R.J. and Kesterson, J.W. 1979. Use of enzymes in citrus processing. Food Technol. 33(11): 78.

Cook, R.W. 1963. High temperature short time evaporation. Trans. 1963 Citrus Eng. Conf. 9: 1.

Chandler, B.V. and Robertson, G.L. 1983. Effect of pectic enzymes on cloud stability and soluble limonin concentration in stored orange juice. J. Sci. Food Agric. 34: 599.

Crandall, P.G. and Beilock, R. 1985. The costs & benefits of transporting 72° Brix orange concentrate. Proc. Fla. State Hort. Soc. 98: 200.

Crandall, P.G., Chen, C. S., and Carter, R.D. 1982. Models for predicting viscosity or orange juice concentrate. Food Technol. 36(5): 245.

Crandall, P.G., Chen, C.S., and Graumlich, T.R. 1981. Energy savings from storing a high degree Brix orange juice concentrate at elevated temperatures in an inert atmosphere. Proc. Int. Soc. Citriculture 2: 855.

Crandall, P.G., Chen, C.S., Marcy, J.E., and Martin, F. 1986. Quality of enzymatically treated 72° Brix orange juice concentrate stored at refrigerated temperatures. J. Food Sci. 51(4): 1017.

Crandall, P.G. and Graumlich, T.R. 1982. Storage stability and quality of high Brix orange concentrate. Proc. Fla. State Hort. Soc. 95: 198.

Dinsmore, H.L. and Nagy, S.J. 1974. Improved colorimetric determination for furfural in citrus juices J. AOAC 57(2): 332.

Fischer, M., Jacobsen, J.F., and Robe, K. 1983. Evaporator concentrates juices to 70° Brix in single pass vs. 2 to 3 passes before. Food Processing 44(1): 92.

FMC. 1983. Procedures for analysis of citrus products. Rev. 6. FMC Corporation, Lakeland, FL.

Gherardi, S. 1980. Production of "super-concentrated" pulpous citrus juices and their chilled storage. Industria Conserve 55: 185.

Hendrix, D.L. and Ghegan, R.C. 1980. Quality changes in bulk stored citrus concentrate made from freeze-damaged fruit. J. Food Sci. 45(6): 1570.

Kanner, J., Harel, S., Fishbein, Y., and Shalom, P. 1982. Furfural accumulation in stored orange juice concentrates. J. Agric. Food Chem. 29: 948.

Marcy, J.E., Graumlich, T.R., Crandall, P.G., and Marshall, M.R. 1984. Factors affecting storage of orange concentrate. J. Food Sci. 49:1628.

Matthews, R.F. 1984. Frozen concentrated orange juice from Florida oranges. Food Science Fact Sheet FS 8, University of Florida, Gainesville, FL.

Meydav, S., Saguy, I., and Kopelman, I.J. 1977. Browning determination in citrus products. J. Agric. Food Chem. 25(3): 602.

Nagy, S. and Randall, V. 1973. Use of furfural content as an index of storage temperature abuse in commercially processed orange juice. J. Agric. Food Chem. 21: 272.

Nagy, S. and Smoot, J.M. 1977. Temperature and storage effects on percent retention and percent U.S. recommended dietary allowance of vitamin C in canned single-strength orange juice. J. Agric. Food Chem. 25(1): 135.

Praschan, V.C. 1976. Quality control manual for citrus processing plants. Intercit, Inc., Safety Harbor, FL.

Rombouts, F.M. and Pilnik, W. 1978. Enzymes in fruit and vegetable juice technology. Proc. Biochem. 13(8): 9.

Rouse, A.H., Atkins, C.D., and Moore, E.J. 1960. Effect of pectinesterase on the stability of frozen concentrated orange juice. Proc. Fla. State Hort. Soc. 94: 270.

SAS Institute, Inc. 1985. SAS. Cary, NC.

Sica, V.A. 1982. Quality summary citrus juice products from FMC citrus juice extractors—1981-82 Florida processing season. FMC Corp., Lakeland, FL.

Stegelin, F.E. and Crandall, P.G. 1981. Technologic and economic considerations of citrus concentrate storage. Proc. Fla. State Hort. Soc. 94: 273.

Steverson, E.M. 1980. Quality summary citrus juice products from FMC citrus juice extractors—1981-82 Florida processing season. FMC Corp., Lakeland, FL.

USDA. 1983. United States standards for grades of orange juice. Jan: 55. Washington, DC.

USDA. 1985. Horticultural products. Foreign Agric. Circ. July: 18. Washington, DC.

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Use of Sulfur Dioxide in Winemaking

C. S. OUGH and E. A. CROWELL

ABSTRACT

Ten juices of common wine cultivars were studied to determine if pretreatment of the juice with air, blanketing the juice with nitrogen or pretreatment with sulfur dioxide (SO_2) was the most satisfactory method to make the better wine. Further treatment with SO_2 was made later at intervals. The pretreatment with aeration lowered the final color in the wine compared to nitrogen blanketing. SO_2 pretreatment gave the lowest colored juice for the white wines. All juices treated with SO_2 later had much lower color. Wines without SO_2 were generally less liked. Pretreatment of juice with SO_2 helped preserve good color and sensory attributes of the wines but later treatments with SO_2 were more essential in improving and maintaining quality. Oxidation was probably the most important control exercised by SO_2 additions.

INTRODUCTION

RECENTLY, much ado has been made concerning the use of SO_2 in wines. For example, Yang and Purchase (1985) condemn wine in a blanket condemnation of the use of sulfites. With all the negative reactions, the careful work of the committee to reevaluate the GRAS status of sulfiting agents (Select Committee on GRAS, 1985) seems to have been more or less ignored. SO_2 has been used as a preservative in wines and other food for centuries (Ough, 1983). It serves several purposes in wine (Amerine et al., 1980): (a) it has a bleaching effect, eliminating brownish colors; (b) it works as an antioxidant in that it reacts with components such as hydrogen peroxide, oxidized phenols and aldehydes and reduces or reacts with them to form less harmful components; and (c) has an antimicrobial activity on wild yeasts and bacteria. The major action on these problems comes from the SO_2 in the nonionized form. It is a weak acid that can exist in several forms depending on the pH.

$$SO_2(H_2O) \xrightleftharpoons{pKa_1 = 1.8} HSO_3^- + H^+ \xrightleftharpoons{pKa_2 = 7.2} SO_3^{2-} + 2H^+$$

Since wine has pH values ranging from 2.8 to 4.2 with most falling in the range 3.2 to 3.6, the highest percentage of the SO_2 is in the HSO_3^- form. The amount in the nonionized form can be estimated (Ough, 1986). Reactions of HSO_3^- with aldehydes to form the aldehyde bisulfite complexes (Amerine et al., 1980) remove these sometimes unpleasant compounds from contributing to the aroma.

The SO_2 can also inhibit the polyphenol oxidase enzymes which are normally present in grape juice. As shown by White and Ough (1975), as little as 35 mg/L are sufficient to inhibit this enzyme completely if the enzyme is tyrosinase. If it comes from a mold source, than SO_2 is not a good inhibitor of that polyphenol oxidase (laccase) as shown by Ribereau-Gayon et al. (1976).

It has been observed by winemakers over the years that wine made without the use of SO_2, even though it may be well protected from oxygen, heat and microbial activity is less appreciated for quality than those made with reasonable amounts of SO_2. There is little in the literature to properly support this. Therefore, a series of experiments were set up to determine if this assumption was valid and to document some of the more obvious chagnes which could occur.

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Table 1—Location and must analysis of the cultivars tested

Cultivar	Location	Must analysis °Brix	Must analysis Total acidity as tartaric acid g/100 mL	Must analysis pH
Chardonnay I	Oakville	23.0	0.77	3.10
Sauvignon blanc I	Davis	21.4	0.79	3.21
Chardonnay II	Oakville	23.4	1.07	3.19
Sylvaner	Oakville	20.4	0.92	2.99
White Riesling	Oakville	20.4	0.73	3.10
Chenin blanc	Davis	20.8	0.97	3.20
Thompson Seedless	Davis	21.2	0.68	3.47
French Colombard	Davis	23.0	0.80	3.28
Sauvignon blanc II	Oakville	21.1	0.73	3.10
Cabernet Sauvignon[a]	Oakville	22.1	0.54	3.23

[a] As a "blush" wine.

METHODS & MATERIALS

GRAPES, clean with essentially no rot or mold, were harvested at a good maturity level from both of the University vineyards (Davis and Oakville). The juice analysis given in Table 1 indicates the juice composition which is in commonly accepted ranges. The cultivars are for the most part those used for the white wines sold on the market. One light rose or "blush" wine was included. The grapes were crushed and pressed in the usual manner. The juice obtained would be of similar composition to that obtained from industrial drainers or the equivalent of "free-run" juice similar to that used for quality wines.

The individual juices were each divided into three lots. One was aerated vigorously with air for 15 min using a fritted glass sparger to assure either saturation or at worst far more oxygen being absorbed than with any standard commercial operation. Saturation was achieved as indicated by oxygen electrode measurements. The second lot was sparged with oxygen-free nitrogen for 15 min with a similar sparger and nitrogen blanketed carefully. Measurement with the O_2 electrode indicated no oxygen was present. Without SO_2 this would be expected as seen from the work of White and Ough (1975). In several instances, delays in the start of the fermentation were noted with the nitrogen treatment. Yeast cultures are more quickly started if some oxygen is present. In the others there was no apparent delay in growth of the yeast or in fermentation. The third lot was treated in the usual manner; that is no sparging of any kind and the juice was treated with about 50 mg/L of SO_2 in the form of 5% water solution within 1 hr after crushing and pressing. All the grapes were at 23–28°C at time of crushing and pressing. The juices settled overnight at 15°C and the sediment was racked off. The nitrogen sparged sample was racked into a nitrogen filled container and reblanketed with nitrogen. No special precautions to prevent oxidation were made other than the usual fermentation traps that were used on all the 15°C fermentations. About 1×10^6 cells/mL of starter culture, *Saccharomyces cerevisiae* strain Montrachet (Red Star) were added to each. The dry yeast culture was reconstituted in 104°F (40°C) water for 30 min prior to addition to the juices.

After fermentation was complete, each wine was tested for residual reducing sugar which was found to be less than 0.30 g/100 mL using the Rebelein method (Amerine and Ough, 1980). The wines were cared for in a normal manner. Wines were protected from further direct oxidation by blanketing with nitrogen as much as possible, keeping them in full glass containers and, in general, making a real effort to minimize air contact. The wines were all stored at 12°C from finish of fermentation except during stabilization. The wines were cold

Table 2—Changes in SO_2 and color in wines with various must and wine treatments

Variety	Must treatment	SO_2 added later	At completion of fermentation 1985				January 1986[a]				May 1986[b]			
			SO_2 (mg/L)		Color[c]		SO_2 (mg/L)		Color[c]		SO_2 (mg/L)		Color[c]	
			Free	Total	420	520	Free	Total	420	520	Free	Total	420	520
Chardonnay	Aeration	(−)	—	2.9	0.089	—	—	—	—	—	tr	3.1	0.073	—
	N_2	(−)	—	3.1	0.117	—	—	—	—	—	tr	8.4	0.114	—
	SO_2	(−)	tr	34.3	0.103	—	—	—	—	—	tr	28.3	0.107	—
	Aeration	(+)	—	—	—	—	8.2	41.2	0.080	—	15.2	49.6	0.076	—
	N_2	(+)	—	—	—	—	8.6	41.7	0.091	—	15.2	51.2	0.079	—
	SO_2	(+)	—	—	—	—	8.6	74.3	0.091	—	18.3	82.4	0.077	—
Sauvignon blanc	Aeration	(−)	—	1.7	0.132	—	—	—	—	—	tr	3.0	0.123	—
	N_2	(−)	—	4.8	0.107	—	—	—	—	—	tr	3.3	0.147	—
	SO_2	(−)	tr	36.5	0.067	—	—	—	—	—	tr	23.1	0.108	—
	Aeration	(+)	—	—	—	—	8.2	41.0	0.152	—	21.9	63.0	0.121	—
	N_2	(+)	—	—	—	—	8.6	43.0	0.156	—	12.4	38.9	0.127	—
	SO_2	(+)	—	—	—	—	8.4	69.2	0.072	—	18.3	77.3	0.080	—
Chardonnay	Aeration	(−)	—	12.4	0.073	—	—	—	—	—	tr	1.6	0.101	—
	N_2	(−)	—	5.0	0.092	—	—	—	—	—	tr	3.0	0.106	—
	SO_2	(−)	tr	43.5	0.084	—	—	—	—	—	tr	33.8	0.102	—
	Aeration	(+)	—	—	—	—	9.9	46.1	0.090	—	16.5	46.5	0.087	—
	N_2	(+)	—	—	—	—	8.7	51.0	0.087	—	6.6	51.1	0.084	—
	SO_2	(+)	—	—	—	—	11.9	79.4	0.085	—	17.3	87.3	0.078	—
Sylvaner	Aeration	(−)	—	tr	0.081	—	—	—	—	—	tr	tr	0.067	—
	N_2	(−)	—	tr	0.095	—	—	—	—	—	tr	tr	0.100	—
	SO_2	(−)	—	35.4	0.079	—	—	—	—	—	tr	28.0	0.090	—
	Aeration	(+)	—	—	—	—	6.7	41.4	0.060	—	21.4	48.1	0.063	—
	N_2	(+)	—	—	—	—	5.8	39.9	0.081	—	11.5	48.0	0.088	—
	SO_2	(+)	—	—	—	—	7.1	77.6	0.070	—	9.9	88.3	0.068	—
White Riesling	Aeration	(−)	—	1.6	0.084	—	—	—	—	—	tr	tr	0.080	—
	N_2	(−)	—	tr	0.099	—	—	—	—	—	tr	tr	0.111	—
	SO_2	(−)	tr	39.0	0.085	—	—	—	—	—	tr	25.0	0.083	—
	Aeration	(+)	—	—	—	—	6.7	44.8	0.060	—	4.6	49.8	0.066	—
	N_2	(+)	—	—	—	—	5.8	44.7	0.081	—	4.9	48.1	0.094	—
	SO_2	(+)	—	—	—	—	7.1	70.9	0.070	—	6.8	84.6	0.067	—
Chenin blanc	Aeration	(−)	—	6.4	0.050	—	—	—	—	—	tr	6.4	0.082	—
	N_2	(−)	—	6.4	0.053	—	—	—	—	—	tr	1.8	0.055	—
	SO_2	(−)	tr	35.7	0.046	—	—	—	—	—	tr	6.3	0.072	—
	Aeration	(+)	—	—	—	—	6.6	49.4	0.069	—	8.4	52.7	0.058	—
	N_2	(+)	—	—	—	—	8.2	49.6	0.093	—	14.0	54.3	0.066	—
	SO_2	(+)	—	—	—	—	8.6	77.6	0.075	—	11.5	52.6	0.058	—
Thompson Seedless	Aeration	(−)	—	12.4	0.049	—	—	—	—	—	tr	4.8	0.088	—
	N_2	(−)	—	6.2	0.053	—	—	—	—	—	tr	3.0	0.094	—
	SO_2	(−)	tr	32.9	0.052	—	—	—	—	—	tr	24.7	0.021	—
	Aeration	(+)	—	—	—	—	11.4	53.6	0.066	—	10.0	59.7	0.079	—
	N_2	(+)	—	—	—	—	10.7	41.2	0.069	—	5.6	46.1	0.086	—
	SO_2	(+)	—	—	—	—	13.5	72.7	0.062	—	8.2	68.6	0.070	—
French Colombard	Aeration	(−)	—	9.3	0.083	—	—	—	—	—	tr	9.6	0.123	—
	N_2	(−)	—	8.5	0.066	—	—	—	—	—	tr	5.1	0.114	—
	SO_2	(−)	tr	39.0	0.059	—	—	—	—	—	tr	34.6	0.095	—
	Aeration	(+)	—	—	—	—	11.7	49.8	0.112	—	10.7	49.4	0.100	—
	N_2	(+)	—	—	—	—	11.9	48.0	0.100	—	9.9	49.4	0.084	—
	SO_2	(+)	—	—	—	—	16.3	76.6	0.084	—	11.7	77.1	0.072	—
Sauvignon blanc	Aeration	(−)	—	2.3	0.060	—	—	—	—	—	tr	tr	0.066	—
	N_2	(−)	—	tr	0.118	—	—	—	—	—	tr	8.7	0.087	—
	SO_2	(−)	tr	31.2	0.069	—	—	—	—	—	3.0	23.1	0.077	—
	Aeration	(+)	—	—	—	—	6.6	44.5	0.061	—	4.9	50.3	0.062	—
	N_2	(+)	—	—	—	—	8.2	45.3	0.081	—	6.6	54.4	0.073	—
	SO_2	(+)	—	—	—	—	6.8	71.9	0.069	—	7.6	55.7	0.065	—
Cabernet Sauvignon	Aeration	(−)	tr	tr	0.251	0.198	—	—	—	—	tr	tr	0.211	0.156
	N_2	(−)	tr	tr	0.280	0.258	—	—	—	—	tr	5.4	0.283	0.226
	SO_2	(−)	tr	35.7	0.252	0.272	—	—	—	—	tr	19.8	0.323	0.346
	Aeration	(+)	—	—	—	—	6.8	45.3	0.232	0.157	4.6	44.5	0.235	0.157
	N_2	(+)	—	—	—	—	8.4	45.8	0.249	0.185	4.9	47.3	0.250	0.181
	SO_2	(+)	—	—	—	—	8.4	77.3	0.241	0.215	6.6	84.7	0.243	0.216

[a] Measured 1 wk after addition of about 40 to 45 mg/L of SO_2 to the (+) samples.
[b] Measured 4 days after addition of about 25 mg/L of SO_2 to the (+) samples.
[c] Measured as absorbance at indicated wavelength (nm).

stabilized at −2°C for more than 2 wk then filtering them through medium grade cellulose pads at that temperature to remove excess potassium bitartrate. Each was then divided into two lots. One was untreated, and the other was treated with about 40–45 mg/L of sulfur dioxide about 3 or 4 months after the finish of fermentation. In another 4 months, another 20–30 mg/L were added. Then about a week or two later, all the wines were tasted by the experienced panel of judges (average experience about 20 years) for quality and, in addition, they were asked if the wines were oxidized or not.

Sensory evaluations were made using the system suggested by Amerine and Roessler (1983). A 20 point score card was used. The wines were presented in individually coded glasses. Analysis of var-

Table 3—Sensory mean scores and significant differences between the total treatments and for individual experiments

	Juice treatment					
	Aeration	N_2 blanket	SO_2	Aeration	N_2 blanket	SO_2
	SO_2 additions to wine					
Cultivar	(−)	(−)	(−)	(+)	(+)	(+)
Chardonnay I	11.18[d]	11.73[c]	12.13[b]	12.03[bc]	12.48[a]	12.33[ba]
Sauvignon blanc I	10.62[d]	10.43[d]	11.78[b]	11.13[c]	10.78[dc]	12.25[a]
Chardonnay II	10.18[d]	10.95[c]	11.48[b]	11.98[a]	11.92[a]	12.00[a]
Sylvaner	10.93[cd]	10.73[d]	11.50[b]	12.00[a]	11.37[cb]	11.23[cb]
White Riesling	9.87[c]	10.83[b]	10.93[b]	11.45[a]	11.37[a]	11.53[a]
Chenin blanc	11.81[b]	11.53[b]	11.58[b]	12.47[a]	12.22[a]	12.38[a]
Thompson Seedless	10.67[d]	10.98[d]	11.53[c]	12.00[ab]	11.72[bc]	12.15[a]
French Colombard	11.55[b]	11.53[b]	12.07[a]	12.15[a]	12.18[a]	12.13[a]
Sauvignon blanc II	9.78[c]	9.78[c]	10.80[b]	10.98[b]	10.98[b]	11.37[a]
Cabernet Sauvignon	10.72[d]	10.77[d]	12.10[a]	11.13[c]	11.57[b]	11.60[b]
Total	10.372[e]	10.926[d]	11.590[c]	11.732[b]	11.659[bc]	11.897[a]

[a-e] Quality scoring scale is 1 to 20 points. Wines having the same superscript are not significantly different at the 5% level of probability.

Table 4—Number of decisions describing wine as either oxidized or not oxidized

Wine treatment	Must treatment					
	Aeration		Nitrogen		SO_2	
	Oxidation decision					
SO_2 added	yes	no	yes	no	yes	no
No	394	206	425	175	346	254
Yes	202	398	226	374	157	443

All paired comparisons are significantly different at the 0.1% level using chi^2 analysis. aeration + No SO_2 = 58.28, nitrogen + No SO_2 = 103.34, SO_2 + No SO_2 = 13.80, aeration + SO_2 = 63.38, nitrogen + SO_2 = 36.02 and SO_2 + SO_2 = 135.38

iance of the replciated scores were made and the significant difference calculated using Duncan's Multiple Range Test. Chi square analysis was used on data resulting from yes or no responses to questions asked: "If the wine was oxidized or not?"

Chemical analyses were made using the methods of Amerine and Ough (1980). The sulfur dioxide was measured using the aeration oxidation method which has proved excellent for accurate white wine analysis. Color was determined as absorbance at 420 nm for whites and 420 and 520 nm for reds using a flow through cell spectrophotometer.

RESULTS & DISCUSSION

THE CHANGES with time for the total and free SO_2 and the color for the 10 wines and the various treatments are given on Table 2. The total SO_2 generated by the yeast when no SO_2 was added ranged from a trace (less than 1 mg/L) to 9.6 mg/L at the end of the experiment. The accuracy at these very low levels of total SO_2 was not more than ± 3 to 5 mg/L. When the determination only took one drop of base for the color change in the indicator (<1 mg/L of SO_2), it was judged as a trace amount.

A certain amount of SO_2 is oxidized to sulfate. Therefore, the total SO_2 declines. The free SO_2 (that in the unbound form) also declines as equilibrium reactions occur with aldehydes, ketones and other reactive substances. These rates of disappearance are temperature dependent (Ough, 1985). The schedule for adding SO_2 was not unlike that used commercially. Those treated in the "normal" manner of SO_2 addition at the start ended up with about 50 to 80 mg/L total SO_2. Variations can occur from cellar addition errors or by variation in the rate of changes occurring in the wines due to composition effects on the reactions.

The wines without SO_2 added until filtration (3–4 months later) ended up with 40 to 50 mg/L total SO_2. In general, the free SO_2 was not that much different from the one with SO_2 added before fermentation. The variations noted in these final free SO_2 values were within experimental error for these wines. What is interesting is that about the same free SO_2 could be maintained in these wines with a much lower total SO_2 in the aerated and N_2 samples compared to the SO_2 treated juice.

The color values showed that SO_2 additions caused a significant decline in the color of the wines. In comparing the color of the nitrogen protected wines versus the wines which came from aerated juices, the latter had generally lower color. Also, with a couple of exceptions, the aerated juice samples had about the same end color when treated later with SO_2 as did the SO_2 treated juice samples which also had additional SO_2 added. The "blush" Cabernet wine showed a lower 420/520 color ratio for the SO_2 juice-treated samples in both cases. This indicates a control of browning by the SO_2 additions. The early lack of SO_2 allowed the brown oxidized colors to build up. While later treatment with SO_2 caused a bleaching by the formation of a reaction product with anthocyanin, there still was an unfavorable ratio which caused the wines to appear less red and more brown. The aeration definitely decreased the anthocyanin pigment level while the N_2 treatment and the early SO_2 treatment preserved the anthocyanins from this destruction.

Table 3 shows a table of the sensory scores of the six wines from each variety and the significant differences. The wines judged with the best quality were those with SO_2 added either originally or later. The one exception was the blush wine which was judged best with the SO_2 treatment of the must only. This may in part have been influenced by its better color. The wines without SO_2 added had the worst sensory quality, with the nitrogen and aeration samples judged inferior most often. The overall average scores indicated that the SO_2 treated juice plus continued SO_2 wine treatments gave superior wines. Aeration of juice plus SO_2 added to the wine and nitrogen blanketing of the juice plus SO_2 added to the wine was next in quality. The SO_2 treated juice (with no SO_2 added to the wine) was similar to nitrogen plus SO_2 wine treatment. The wines with no SO_2 treatments were definitely inferior in quality.

The judges were asked to determine whether or not a wine was oxidized. Table 4 indicates the decisions made for the experimental wines. Wines which did not have SO_2 added were later judged to be oxidized (even the SO_2 treated juice sample). The wine from the juice treated with nitrogen blanketing was judged more oxidized than the wine made from the juice deliberately oxidized. Once these wines were treated with SO_2, the sensory concept of oxidation was greatly diminished. This concept can be partially attributed to color changes but taste and odor changes were clearly detectable. The work of Bach and Nobis (1985) also shows that juice oxidation is less detrimental than what happens later to the wines.

CONCLUSIONS

SO_2 was essential in making better wine. The best white wines were made using SO_2 both in the juice and in the wine. In many of the tastings, differences appeared to be small as long as the SO_2 was added after the wine was filtered and stabilized.

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Ethanol Stability of Casein Solutions as Related to Storage Stability of Dairy-based Alcoholic Beverages

WILLIAM J. DONNELLY

ABSTRACT

Ethanol stability characteristics of casein in a simulated cream liqeur environment were examined. Stability decreased with increasing concentration of Ca ions and decreasing pH in line with expectations from previous studies with milk. Monovalent cations also had a destabilizing effect while increase in casein concentration improved stability through enhanced Ca chelation. Homogenization with milk fat reduced ethanol stability. Rapid acid production occurred during storage of the simulated system at 55°C when the system contained reducing sugar, leading to a drop in pH and a destabilization of the protein to added ethanol. These characteristics have implications for storage stability of dairy-based alcoholic beverages.

INTRODUCTION

AMONG the most interesting new dairy products to appear in recent years are cream liqueurs. Several product variations exist of which the most common contains approximately 3% casein, 15% milk fat, 14% ethanol (EtOH) and 20% sugar. A shelf life of at least 1 year in temperate climates is normal. With the exception of studies by Banks et al. (1981, 1982), technical data of any kind on these products are not available. These workers described the basic formulation procedure for a typical liqueur and highlighted some compositional and processing factors which were important for shelf life. In particular, fat separation was controlled by homogenization, while protein separation was influenced by the concentration of divalent cations. The latter relationship was expected in view of well established principles for EtOH stability of milk itself. Although casein is probably a universal constituent, not all cream liqueurs contain milk fat and there is a great potential diversity of composition and in the nature of other constituents such as flavorings, carboyhydrate and stabilizers. In spite of this, all must conform to properties expected of casein solutions and, in particular, protein solubility behavior during storage must reflect established properties of casein in the presence of EtOH and other interacting constituents. The present study was therefore undertaken to define the factors which influence EtOH stability of casein solutions in a liqueur-type environment and thereby establish some general principles of importance to storage stability and shelf life of dairy-based alcoholic beverages. A standard composition, applicable to the main type of cream liqueurs was described by Banks et al. (1981). The formulation procedure described by these workers, which presumably resembles industrial practice, involves initial assembly of a base mix, which contains all components with the exception of alcohol and part of the water. The present study centers mainly on the fat-free phase of base mix (FFB).

MATERIALS & METHODS

Materials

Skimmilk was prepared from pasteurized whole milk from the Institute herd by centrifugation at 1000 g for 20 min at 20°C. Sodium caseinate was a commercial product obtained from Kerry Co-Op. Creameries Ltd. Sucrose and glucose were AR grade from BDH Chemicals, Poole, U.K. Maltose was GPR grade from the same supplier.

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Base solutions

Fat-free base (FFB) was made according to the formulation described in Table 1 and used within a few days of preparation. Base mix was made in the same way except that 50g 44% pasteurized cream was used instead of the skimmilk and the system was homogenized essentially as described by Banks et al. (1981). Merthiolate (0.01%) was used as preservative for FFB and base mix.

EtOH stability

EtOH stability measurements were by the method of Horne and Parker (1981). To 1 vol sample (normally 4.5 mL) in a test tube was added 2 vol aqueous EtOH, of gradually increasing EtOH concentration in the range 20–100% (v/v) by increments of 2% (v/v). The solution was immediately swirled and the EtOH stability was defined as the minimum concentration of added EtOH immediately giving rise to particulate material. Adjustments of pH were done by addition of 0.05–0.15M NaOH or HCl to 4.0 mL sample, followed by addition of water to a final sample volume of 4.5 mL. Adjustments of Ca concentration were carried out by addition of 0.5M $CaCl_2$, followed by a pre-determined amount of 0.1M NaOH to maintain constant pH, followed by a final adjustment of vol to 4.5 mL with water. Addition of Ca was done dropwise with stirring at 0–4°C to prevent Ca caseinate or Ca phosphate precipitation and solutions were normally equilibrated by overnight stirring at 4°C. An identical procedure was followed for examination of the effect of the EtOH solution to sample ratio on stability values, except that the volume of added EtOH was varied. The standard error of EtOH stability measurement is estimated to be well within 2% (v/v EtOH) (D.S. Horne, personal communication).

Ultrafiltration

This was carried out at 20°C using an Amicon stirred cell (200 mL capacity) with a PM10 membrane (Amicon Corp., Lexington, MA) discarding the first 10–20 mL eluant.

Storage at 55°C

Caseinate/sugar solutions were distributed in 15-mL aliquots into screw-cap vials (total capacity 30 mL). These were tightly stoppered and placed in an oven at 55°C. Each day's measurement was carried out on a separate vial. (Details of solution composition are given in the legends to Fig. 6 and 7). All compositions are expressed in % w/v.

Compositional analysis

Analysis of Ca, Mg, inorganic phosphate (Pi), citrate (Cit) were carried out as previously described (Donnelly and Horne, 1985).

Table 1—Formulation and calculated final composition of fat-free base (FFB)

Formulation	g	Composition (% w/w)	
Skimmilk	28	Water	61.8
Sucrose	24	Total sugar	32.4
Sodium caseinate	3.5	Total protein	5.2
Water	22.5	(Casein)	(4.9)
pH	6.80–6.90	Salt	0.60
			100

Results

THE COMPOSITION and formulation of FFB is presented in Table 1. This follows essentially the formulation data of Banks et al. (1982) with some minor modifications of an inconsequential nature.

Milk salt equilibria

Distribution of milk salts between protein and protein-free phases of FFB and skimmilk was compared by ultrafiltration at 20°C. Typical results are presented in Table 2. Distribution in skimmilk follows a well established pattern (White and Davies, 1958) and will not be commented on except for purposes of comparison. Distribution in FFB was different from that in skimmilk, with a much greater proportion of cations and a much smaller proportion of Pi associated with protein. An approximate value of 0.88 mM for $[Ca^{2+}]$ in FFB and 1.2 mM for $[Ca^{2+} + Mg^{2+}]$ was calculated from the measured mineral composition of ultrafiltrate (Lyster, 1981).

EtOH stability

The EtOH stability/pH profiles of FFB and the corresponding skimmilk were compared on several occasions in the approximate pH range 6–7. Typical results are shown in Fig. 1. Skimmilk showed a characteristic profile (Horne and Parker, 1981) with maximum and minimum stability plateaus at high and low pH, respectively, and an inflexion in the region of pH 6.6. FFB showed elements of the same pattern; however, the inflexion occurred at much lower pH. Typically, FFB showed a slow decline in stability from above 90% near pH 7 to around 80% near pH 6.2 below which a rapid drop in stability occurred. EtOH stability measurements were also conducted on base mix. Repeatedly, it was observed that the stability of base mix above the inflexion was much lower than that of the corresponding FFB. This is shown in Fig. 1 for base mix derived from the same skimmilk as the FFB described in the figure. A decrease in stability of 16–19% (v/v EtOH) is evident throughout the pH range examined. This is not explained by the presence of fat *per se* in base mix and the consequent change in relative proportions of aqueous sample to added EtOH in the standard stability test. It was separately established that such a change has a negligible influence on the measured EtOH stability values (data not shown), a fact that is further supported by the data described later (Fig. 5).

EtOH stability of sodium caseinate solutions

The contribution of various FFB components to EtOH stability was examined in a simple casein-sugar solution held at constant pH but with variable concentration of added cations. Concentrations of casein and sugar were similar to those in FFB. Figure 2 shows the change in EtOH stability with increasing Ca concentration at different levels of KCl. In general, stability decreased as Ca concentration increased in the range 7–16 mM. A gradual depression of overall stability occurred as KCl concentration increased. EtOH stability/Ca curves were not linear but showed a region of more rapid stability change in the Ca range 10–13 mM. This inflexion occurred at lower Ca concentration and was less pronounced as KCl concentration increased. The Ca concentrations were determined in ultrafiltrates of some of the casein/sugar solutions as a measure of the free calcium ion concentration of these solutions. Results (data not presented) showed that $[Ca^{2+}]$ was at least 2.6 mM in the region of the inflexion and was therefore well above the free divalent cation concentration estimated for FFB. Increasing the casein concentration by 20% stabilized the system to EtOH by removing the inflexion, presumably to beyond the upper level of Ca examined (15 mM).

Stabilization with Pi and Cit

The studies just described were extended to examine the effect of Pi and Cit on EtOH stability. The same basic casein/sucrose system was used, but with addition of KCl to maintain a constant monovalent cation concentration of 78 mM, which approximated the level in FFB. In addition, starting solutions contained either 8 mM Pi or 3 mM Cit reflecting the natural levels of these salts in FFB. Figure 3 shows the effect of added Pi at constant Cit concentration. This increased stability by moving the inflexion to higher Ca concentration. At a Pi concentration of 8 mM, the inflexion was beyond 20 mM Ca, showing that the divalent cation level of FFB (Ca + Mg≈ 15 mm) was well below that required for destabilization to EtOH addition. A similar stabilizing effect was shown by addition of Cit at constant concentration of Pi (Fig. 4). At 3 mM Cit, the approximate level in FFB, high EtOH stability occurred up to 20 mM Ca. Even at 1 mM Cit, good EtOH stability was retained up to approx. 16 mM Ca.

Protein solubility

The above data suggested that the EtOH content and salt composition of cream liqueurs were well removed from the critical levels required for protein coagulation. However, EtOH stability values may not be a measure of protein solubiity *per se*. Evidence for this was obtained from the following experiment. EtOH stability/Ca profiles were constructed for a casein/sucrose solution using three different ratios of EtOH soln. to sample, 2:1, 1:1 and 1:2. Stabilities, as normally expressed, were much closer than expected from the different measurement protocols, implying that the final alcohol content of the mixture at coagulation was method dependent. To quantify this effect, stabilities were expressed as final EtOH concentration rather than the concentration of added EtOH and plotted against Ca concentration. Figure 5 shows that stabilities so expressed, differed markedly by the three methods, moving to lower values as the ratio EtOH soln. to sample decreased. The limits of this effect were not explored, but it is obvious that EtOH stability, as classically determined, is not a measure of absolute protein solubility in casein/EtOH solns.

Acid development

Storage instability of cream liqueur might arise through a drop in pH below the critical value of approx. 6.2, shown in Fig. 1. Various chemical reactions which result in generation of protons are possible in such a system. The effect of storage at 55°C on chemical transformation in liqueur-type systems was, therefore, studied. Preliminary observations indicated that acid production due to protein hydrolysis, protein dephosphorylation and Ca phosphate transformation was slight but that the nature of the sugar in the system had a major influence. This was further investigated in a simplified system consisting

Table 2—Mineral composition of skimmilk, fat-free base (FFB) and their ultrafiltrates

		Component						
							mM	
Sample	pH	Ca	Mg	Pi	Cit	Na	K	Cl[a]
Skim	6.68	30.74	3.88	21.36	8.63	24.79	45.00	—
Skim-UF	6.69	8.39	2.46	11.44	7.62	23.05	40.90	—
FFB	6.85	13.49	1.73	10.23	3.26	51.30	19.11	15
FFB-UF	6.82	3.59	0.63	7.94	3.16	35.22	13.89	14

[a] Estimated

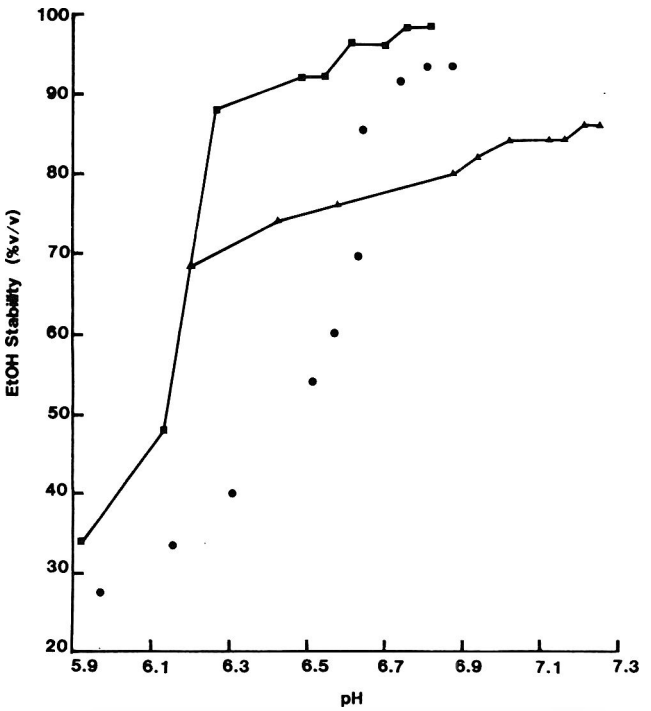

Fig. 1—EtOH stability/pH profiles of various systems: ■, fat-free base (FFB); ▶, base mix; ●, skimmilk as used in formulation of FFB and base mix.

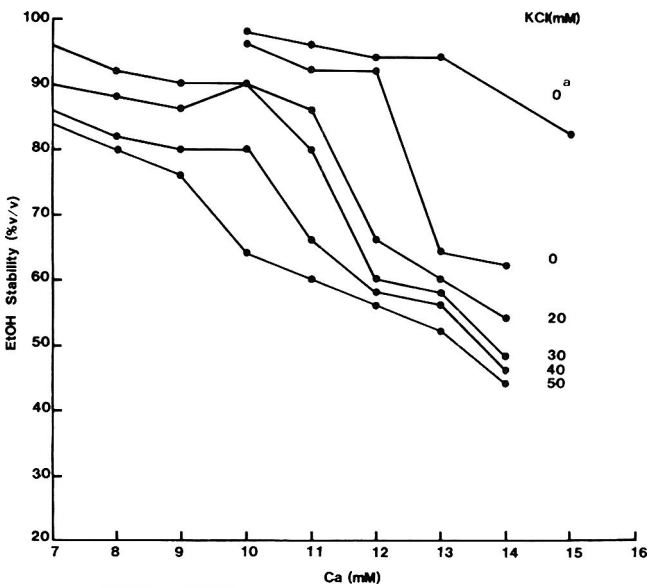

Fig. 2—EtOH stability/Ca profiles of casein/sugar solutions. The basic solution consisted of water, sucrose and sodium caseinate in the proportions 50/23/4.15. Salts were added to 4.0 mL of this solution and after pH adjustment to 6.85 with 0.1M NaOH, the volume was brought to 4.5 mL with water. All salt concentrations refer to the original vol. of 4.0 mL. In addition to the salts shown, the basic solution contained 40 mM Na, added as sodium caseinate. ([a]Casein increased by one-fifth compared to basic solution.)

of sodium caseinate, sugar and milk salts with the approximate composition of liqueur. Figure 6 shows the change in pH at 55°C in solutions containing sucrose or maltose. Rapid acid development occurred in the presence of reducing sugar, resulting in a drop in pH to 6.1 in just 8 days. This was further investigated in liqueur-type systems containing various proportions of glucose and sucrose. These systems also contained EtOH (17% v/v) but this had no significant influence on the

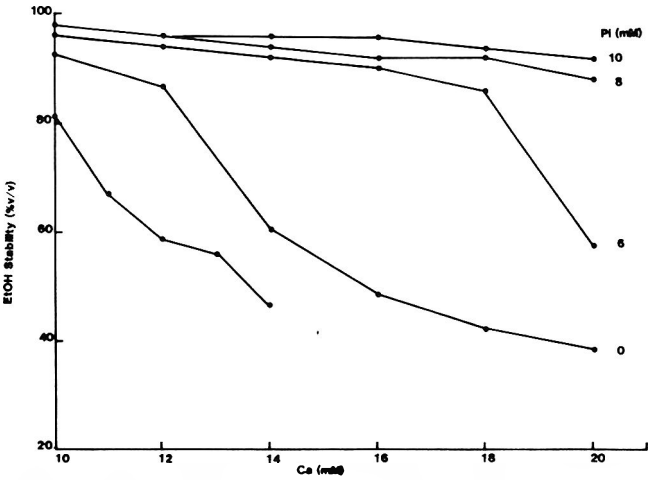

Fig. 3—Effect of addition of Pi on EtOH stability/Ca profiles of casein/sugar solutions. The basic sugar solution was as described in the legend to Fig. 2 but with an additional 3 mM Cit and a level of KCl calculated so that a final constant monovalent cation concentration of 78 mM was reached after adding phosphate salts. Salt addition was as described in the legent to Fig. 2 and concentrations refer to the original sample vol of 4.0 mL. The lower curve is for the basic solutions without any added Cit.

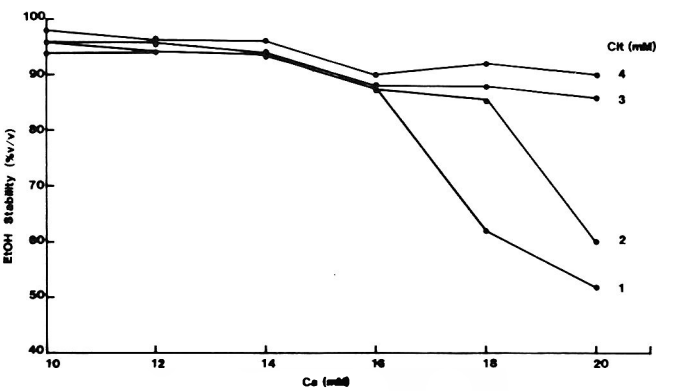

Fig. 4—Effect of addition of Cit on EtOH stability/Ca profiles of casein/sugar solutions. The basic casein/sugar solution was as described in the legend to Fig. 3 except that Cit was replaced by 8 mM Pi. Other parameters are as described in the legend to Fig. 3.

rate of acid development. As the glucose concentration increased from 0 to 20% total sugar, the rate of pH drop increased proportionately (Fig. 7). In FFB, lactose amounted to 6% total sugar analogous to a glucose of 3% total sugar when expressed as reducing equivalent. The data of Fig. 7 indicate that this alone could produce a pH drop of 0.13 units after 9 days at 55°C.

DISCUSSION

EtOH STABILITY of milk is determined by pH and the ionic components of milk serum. Both factors combine to influence protein charge and hence the tendency to casein aggregation when the dielectric strength of the medium is reduced by addition of EtOH (Horne, 1984). Not surprisingly, the present investigations showed that the same general principles applied to sodium caseinate solutions, including base mix, i.e., destabilization by decreasing pH or increasing Ca concentration and stabilization by increasing Pi or Cit. However, differences in the ionic environments of milk and casein solutions were responsible for marked differences in the detailed stability characteristics. In FFB or base mix a complete redistribution

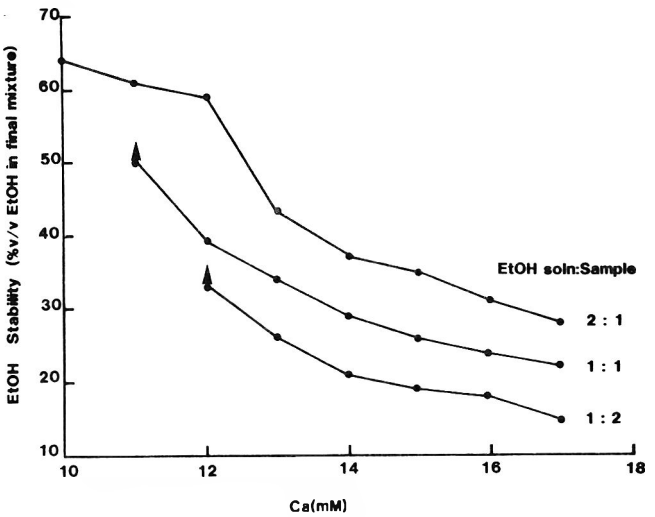

Fig. 5—EtOH stability/Ca profiles of a casein/sugar solution. The basic casein/sugar solution was as described in the legend to Fig. 2. Addition of Ca, pH adjustment and definition of concentrations are also as described in that legend. Arrows denote point at which solutions were stable on addition of 100% EtOH.

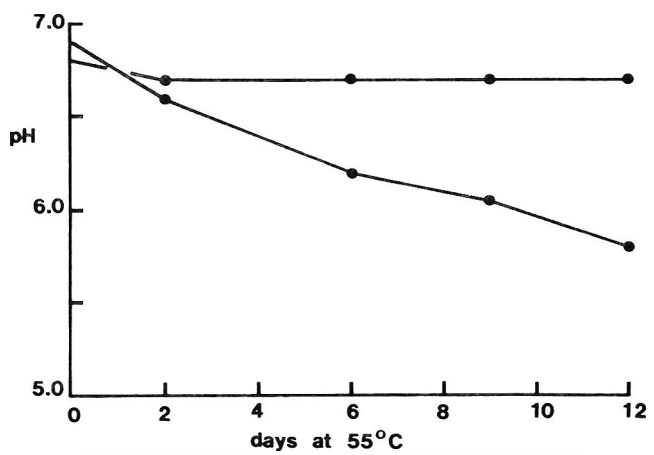

Fig. 6—Change in pH of casein/sugar/salt solutions during storage at 55°C. Solutions consisted of casein, 3.7%; maltose or sucrose 23%; Ca, 18 mM; Pi, 10 mM; Cit, 3 mM; monovalent cations, 78 mM. Upper curve, sucrose solution; lower curve, maltose solution.

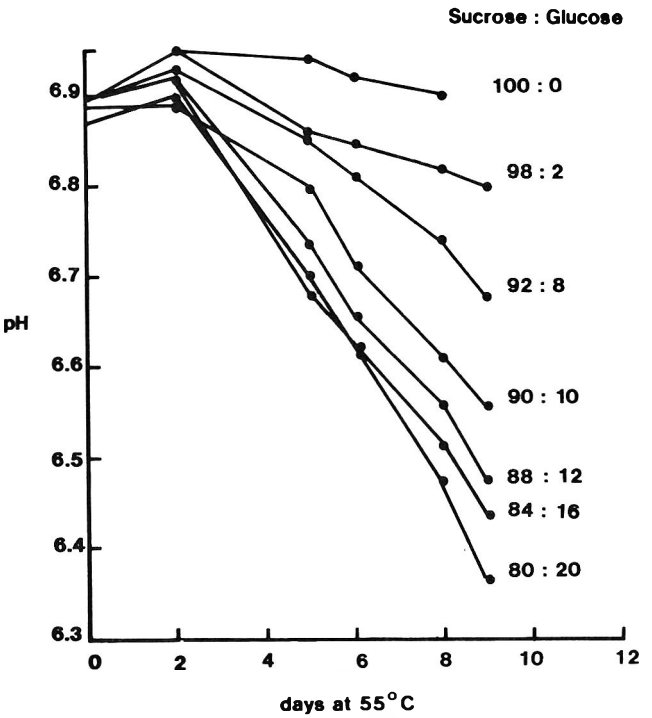

*Fig. 7—**Effect of sucrose**/glucose ratio on change in pH of casein/sugar/salt solutions during storage at 55°C. Solutions were as described in legend to Fig. 6 except that the sugar consisted of various mixtures of sucrose and glucose, and each solution contained 17% (v/v) EtOH.*

occurred of milk salts originating in the skimmilk or cream component primarily due to an increase in the casein/salt ratio compared to milk. This resulted in an increase in casein-bound Ca and a solubilization of colloidal phosphate. The consequent reduction in $[Ca^{2+}]$ stabilized the system to EtOH and shifted the inflexion point of the EtOH stability/pH profile to lower pH. In FFB or base mix the proportion of monovalent cations bound to casein was much greater than in milk reflecting the higher monovalent to divalent cation ratio of the former. Consequently, it may be concluded that monovalent cations have a greater influence on EtOH stability of liqueuer-type systems compared to milk. This influence was not quantified directly, but some general comments can be derived from the data in Fig. 2. These data showed a decrease in EtOH stability of about 3% for each 10 mM increase in KCl in casein solns., where the Ca concentration was 7 mM and where a value for $[Ca^{2+}]$ of 1.7 mM approx. was measured by ultrafiltration. In FFB, where the divalent cation concentration equals 1.2 mM, the influence of KCl on EtOH stability should be even greater. Figure 2 also shows that the influence of KCl on EtOH stability reaches a maximum at the inflexion point of the EtOH stability/Ca profile. However, changes in that region of the profile may not have practical relevance since the inflexion occurs at a divalent cation concentration which is well beyond that in normal liqueur. The stabilizing effect of additional casein (Fig. 2) simply reflects the Ca chelating properties of casein. This contrasts with the destabilizing effect on cream liqueur of increasing total solids (Banks et al., 1981). In that study, however, increase in total solids did not alter the casein/salt ratio and, therefore, did not result in a decrease in $[Ca^{2+}]$. These workers also reported a major effect of pH on shelf life of liqueur which varied from 24 hr to 36 days at 45°C as pH was adjusted from 6.5 to 7.8. Change in shelf life was most rapid in the pH range 7.0–7.5. This appears to be at variance with our results from EtOH stability of base mix or FFB which showed only a slow decline until pH dropped below approx. 6.2. However, in the previous study pH adjustment was by phosphate salt addition at a concentration of 100 mM, thereby, changing fundamentally the Ca/chelate interactions and hence the EtOH stability/pH profile of normal liqueur.

EtOH stability characteristics of FFB and casein/sugar solns. suggested that marked changes in composition would be required to induce protein coagulation in cream liqueurs. One such change could be a drop in pH provided the formulation contained a sufficient amount of reducing sugar and assuming that the present observations at 55°C were relevant to storage at ambient temperatures. In a preliminary investigation it was found that the major proportion of acid production at 55°C required the presence of both reducing sugar and protein, indicating the involvement of Maillard browning reactions. Acid production could occur through decrease in pKa of protein amino groups in the initial complex formation with reducing sugar (Dixon, 1972) or through further conversions leading ultimately to Maillard browning. Indeed, browning was observed in these solutions. The data of Song et al. (1966) yield an approximate Q_{10} value of 2.2 for the initial condensation of protein and reducing sugar. This implies a 10-fold drop in reaction rate at 25°C compared to 55°C and suggests that such reactions could be significant in liqueuer during long-term storage. In this connection buffering capacity of liqueur becoomes

important and the potential role of milk salts as buffer components should not be overlooked. In particular, the suggestion that the cream component might be washed to reduce Ca concentration and thereby extend shelf life (Banks et al., 1981) could promote instability because of loss of buffering salts. In practice, the extent of acid development during storage of commercial liqueurs is unknown as is the concentration of reducing sugars in these products.

Accelerated storage stability studies on liqueur were also carried out by Banks et al. (1981) but at 45°C. In these studies the formulation did not contain added reducing sugar and, therefore, any effect of acid development was eliminated. The main conclusions of that study were that the shelf life of the basic formulation was short, with coagulation of protein occurring after only 8 days, whereas addition of citrate or washing of the cream extended shelf-life to beyond seventy days. These results are difficult to interpret in light of the data of Fig. 3 and 4 which show that the EtOH stability of a standard formulation is high with little scope for improvement through addition of Ca chelators. This difference between EtOH stability values on the one hand and actual storage stability on the other may arise from the effect of homogenization. The marked effect of homogenization on EtOH stability (Fig. 1) may reflect a change in conformation which reduces the energy barrier to protein-protein interaction during storage and poises the system to coagulation. In such a sensitized system, minor changes in pH or salt composition which have only a slight effect on EtOH stability may have an exaggerated effect on shelf life. A similar destabilization effect of homogenization on heat stability of milk (Brunner, 1974) and concentrated milk (Sweetsur and Muir, 1982) has been reported.

The present results do not preclude the involvement of other factors in storage stability of liqueur-type products. For instance the role of protein degradation during storage has not been evaluated. Recent results show that destablization of protein during storage of UHT milk results from plasmin-induced proteolysis (de Koning et al., 1985). No information is available on plasmin activity in liqueur or on the presence of other proteinases, although the extended shelf life of these products provides ample opportunity for enzyme action.

REFERENCES

Banks, W., Muir, D.D., and Wilson, A.G. 1981. Extension of the shelf life of cream-based liqueurs at high ambient temperatures. J. Food Technol. 16: 587.

Banks, W., Muir, D.D., and Wilson, A.G. 1982. Formulation of cream-based liqueurs: a comparison of sucrose and sorbitol as the carohydrate component. J. Soc. Dairy Technol. 35: 41.

Brunner, J.R. 1974. Physical equilibria in milk: the lipid phase. Ch. 10. In "Fundamentals of Dairy Chemistry." 2nd ed., B.H. Webb, A.H. Johnson, and J.A. Alford (Ed.), p. 474. AVI Publishing Company Inc. Westport, CT.

deKoning, P.J., Kaper, J., Rollema, H.S., and Driessen, F.M. 1985. Age thinning and gelation in unconcentrated and concentrated UHT-sterilized skimmilk. Effect of native milk proteinase. Netherlands Milk Dairy J. 39: 71.

Dixon, H.B. 1972. A reaction of glucose with peptides. Biochem. J. 129: 203.

Donnelly, W.J. and Horne, D.S. 1985. The relationship between ethanol stability of bovine milk and natural variations in milk composition. J. Dairy Res. 53: 23.

Horne, D.S. 1984. The ethanol stability of milk. In "Hannah Research," p. 89. The Hannah Research Institute, Ayr, Scotland.

Horne, D.S. and Parker, T.G. 1981. Factors affecting the ethanol stability of bovine milk. 1. Effect of serum phase composition. J. Dairy Res. 48: 273.

Lyster, R.L.J. 1981. Calculation by computer of individual concentrations in a simulated milk salt solution. 11. An extension to the previous model. J. Dairy Res. 48: 85.

Song, Pill-Soon, Chichester, C.O., and Stadtman, F.H. 1966. Kinetic behavior and mechanism of inhibition in the Maillard reaction. 1. Kinetic behavior of the reaction between D-glucose and glycine. J. Food Sci. 31: 906.

Sweetsur, A.W.M. and Muir, D.D. 1982. Natural variation in heat stability of concentrated milk before and after homogenisation. J. Soc. Dairy Technol.: 35, 120.

White, J.C.D. and Davies, D.T. 1958. The relation between the chemical composition of milk and the stability of the caseinate complex. J. Dairy Res. 25: 236.

Ms received 5/19/86; revised 9/17/86; accepted 10/2/86.

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REFERENCES

Amerine, M.A., Kunkee, R.E., Ough, C.S., Singleton, V.L., and Webb, A.D. 1980. Chemistry of fermentation and composition of wines. In "The Technology of Wine Making," (4th ed.), p. 186. Avi Publishing Co., Westport, CT.

Amerine, M.A. and Ough, C.S. 1980. "Methods of Analysis of Musts and Wines" p. 341. J. Wiley and Sons. New York.

Amerine, M.A. and Roessler, E.B. 1983. "Wines. Their Sensory Evaluation." W. H. Freeman and Co., San Francisco, CA.

Bach, H.P. and Nobis, P. 1985. Einfluss der Motoxidation auf den Wein. Weinwirtschaft/Technik 121: 294.

Ough, C.S. 1983. Sulfur dioxide and sulfites. In "Antimicrobials in Foods," A.L. Branen and P.M. Davidson, p. 177. Marcel Dekker, Inc., New York.

Ough, C.S. 1985. Some effects of temperature and SO_2 on wine during simulated transport and storage. Am. J. Enol. Vitic. 36: 18.

Ough, C.S. 1986. Determination of sulfur dioxide in grapes and wines. J. Assoc. Offic. Anal. Chem. 69: 5.

Ribereau-Gayon, J., Peynaud, E., Ribereau-Gayon, P., and Sudraud, P. 1976. Transformations prefermentaire de la ventage. "Traite D'Oenologie. Sciences et Techniques du Vin. Tome 3. Vinifications Transformation du Vin," p. 42. Dunod, Paris.

Select Committee on GRAS. 1985. The reexamination of the GRAS status of sulfiting agents. Life Sci. Res., Offic. Fed. Am. Soc. Expt. Biol. FDA Contract No. 223-83-2020.

White, B.B. and Ough, C.S. 1975. Oxygen uptake studies on grape juice. Am. J. Enol. Vitic. 24: 184.

Yang, W.H. and Purchase, C.R. 1985. Adverse reactions to sulfites. Can. Med. Assoc. J. 133: 865.

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Study of Thermal Properties of Oat Globulin by Differential Scanning Calorimetry

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ABSTRACT

The thermal behavior of oat globulin was studied by differential scanning colorimetry (DSC). The effects of pH, salts, and of various structure perturbants upon thermal characteristics were determined. Raising or lowering pH from near neutrality reduced denaturation temperature (T_d), enthalpy (ΔH) and cooperativity indicated by increase in width at half height ($\Delta T_{1/2}$). The effect of salts on thermal stability was related to their position in the lyotrophic series and suggests involvement of hydrophobic interaction in the thermal stability of oat globulin. Increasing concentrations of urea progressively lowered T_d and ΔH and increased $\Delta T_{1/2}$; sodium dodecyl sulfate (SDS) lowered ΔH without affecting T_d; ethylene glycol (EC) lowered T_d without changing ΔH. Dithiothreitol did not affect DSC characteristics suggesting that disulfide bonds do not contribute to the thermal response of oat globulin.

INTRODUCTION

OAT PROTEIN is recognized to possess good nutritional quality (Hischke et al., 1968) and unique physiochemical and functional properties (Ma, 1983 a,b; Ma and Harwalkar, 1984). These properties may be affected by heat treatments such as cooking, roasting, and steaming, which are routinely used for the processing of cereal grains and products. These thermal treatments could disrupt the forces that maintain the structural integrity of the protein molecules, e.g. hydrophobic and ionic interactions, hydrogen bonds and covalent disulfide bonds. The loss of native structure or denaturation is critical to protein functionality such as gelation, emulsification and foaming (Kinsella, 1976).

Protein unfolding or denaturation is accompanied by enthalpic changes (Privalov and Khechinashviti, 1974) which can be monitored by thermoanalytical techniques such as differential scanning calorimetry (DSC). The enthalpy changes are measured as differential heat flow between sample and reference and recorded as a peak by DSC. The peak analysis enables determination of temperature of transition and enthalpy of denaturation from maximum peak temperature and area of the peak respectively. The sharpness of the peak also indicates the cooperative nature of the transition from native to denatured state. If the rupture of intramolecular bonds occur within a very narrow range of temperature (very sharp peak) the transition is considered highly cooperative. The broader the peak the less cooperative the transition (Wright et al., 1977).

DSC has been used to study thermal denaturation of some food proteins such as muscle proteins (Wright et al., 1977), egg albumin (Donovan et al., 1975), soybean proteins (Hermansson, 1978, 1979), whey proteins (De Wit and Klarenbeck, 1984), β-lactoglobulin (De Wit and Swinkels, 1980; Harwalkar, 1985) and fababean proteins (Arntfield and Murray, 1981). Since samples can be analysed directly without solubilization, DSC is a simple technique particularly useful for studying plant proteins such as oat protein which have poor solubility.

In the present study the thermal behaviour of oat globulin will be examined by DSC under various solvent conditions, pH and ionic strength, and under the influence of various structure perturbing agents, including ethylene glycol, urea, sodium dodecyl sulfate (SDS) and reducing agents. The objective of the work is to study the thermal denaturation characteristics of oat globulin, and to gain some information on the stabilization of the protein structure by covalent and noncovalent forces.

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MATERIALS & METHODS

OATS (*Avena sativa* L., variety Sentinel) were grown at the Central Experimental Farm, Ottawa, Canada. The dehulled oats were ground in a pin mill and defatted by Soxhlet extraction with hexane.

Globulins from oats and other cereals (wheat, barley and rye) were prepared by the Osborne fractionation method as described by Wu et al. (1972) using 0.5M $CaCl_2$.

The thermal behavior of the globulins from oats and other cereal grains was examined with a Perkin Elmer Model DSC-II differential scanning calorimeter with a sensitivity of 0.0025 mcal/sec, and equipped with subambient accessory, Intercooler II, as described previously (Ma and Harwalkar, 1984). Polymer coated Dupont aluminum pans were used for sample and reference. An empty pan sealed with lid was used as a reference. Due to low solubility and difficulty in transferring the aqueous sluries quantitatively the samples (1 mg) were directly weighed onto the pan and 10 μL distilled water or solvent was added. All the samples were prepared at room temperature, and the pans were equilibrated at 300°K. The heating rate was 10°/min over the range of 300–400°K (27–127°C). Indium standards were used for temperature and energy calibration. The enthalpy of denaturation (ΔH) was expressed as calories per gram protein and was calculated using the following equation:

$$\Delta H = \frac{R\,K\,A}{S\,W}$$

where R = control setting; K = calibration constant; S = chartspeed; A = area under peak (cm^2); and W = weight of protein.

The temperature of denaturation (T_d) was determined as the peak maximum temperature. A measure of cooperativity of the thermal transition of globulin was obtained from the width at half peak height ($\Delta T_{1/2}$). The values of T_d and ΔH were an average of at least three separate determinations.

For experiments involving additives, buffer containing the additives was added to the weighed protein in the pan which was then sealed and equilibrated at 300°K (27°C) before DSC analysis. Preliminary tests indicated that incubation of protein with additives up to several hours did not cause significant changes in DSC responses, indicating that the additives exert their effects on the protein in a short time.

RESULTS & DISCUSSION

IN THE DSC THERMOGRAMS of globulins isolated from a number of cereals (Fig. 1), oat globulin showed a sharp endothermic peak with a T_d of 381°K (108°C) and denaturation

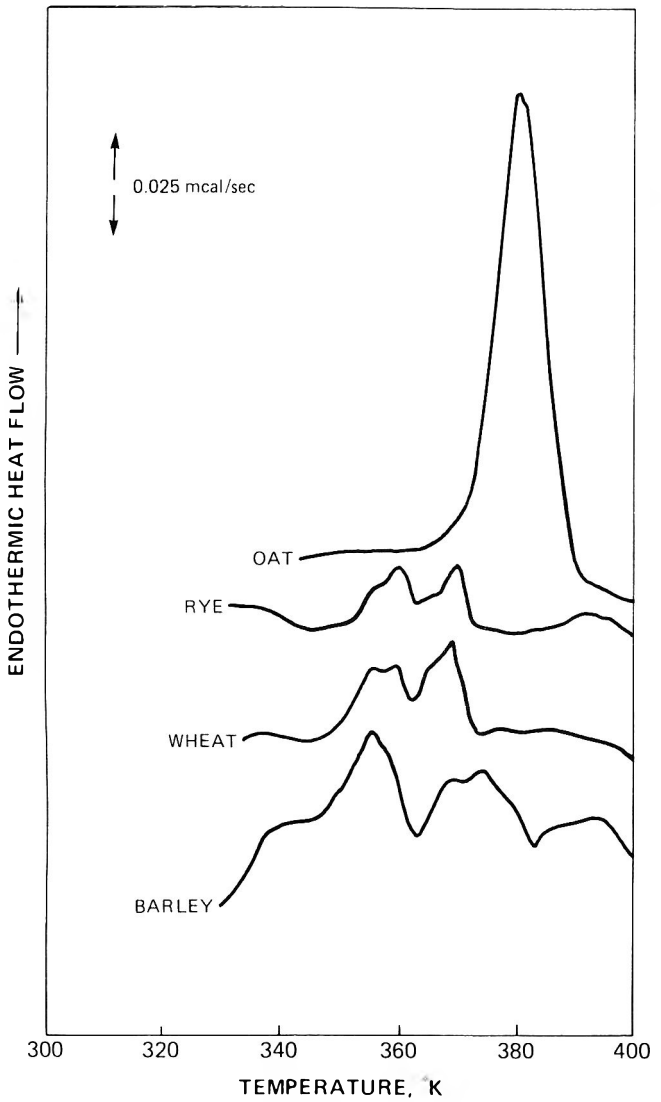

Fig. 1—DSC thermograms of globulins isolated from different cereal grains.

enthalpy ($\triangle H$) of about 6.3 cal/g and $\triangle T_{1/2}$ of 9.6°. Preliminary tests indicate that oat globulin lost its thermal response after heating to 400°K (127°C), and a rescan of the heated samples showed no residual or new peaks. The globulins from the other three cereals (wheat, rye, and barley) showed a broad multipeak thermogram with T_d values of 357–369°K (84–96°C) which were considerably lower than that of oat globulin. The $\triangle H$ values (data not presented) for these globulins were also considerably lower than oat globulin. The results suggest that the oat globulin fraction is comprised of essentially one species of protein, whereas the other cereal proteins are more heterogenous having different T_d values. The high T_d value indicates that oat globulin is very heat stable requiring relatively high temperature to denature.

The salt extraction procedure is a mild process found to cause little or no denaturation to protein (Murray et al., 1981; Arntfield and Murray, 1981). Preliminary tests on oat globulin extracted from nondefated groats also indicate no significant protein denaturation by hexane treatment.

Effect of pH

To examine the effect of pH on heat stability of oat globulin, protein suspensions with the desired pH were prepared by the addition of acid or alkali. The samples were incubated in the sealed pan for about an hour to allow for pH equilibration.

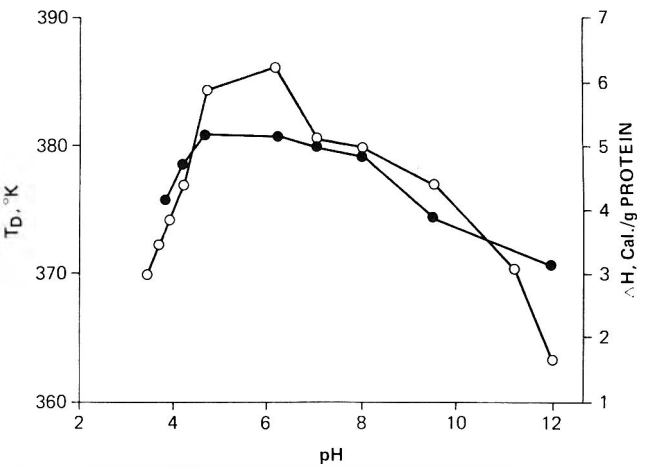

Fig. 2—Effect of pH on the DSC characteristics of oat globulin. ●, transition temperature (T_D); ○, enthalpy ($\triangle H$).

The changes in the T_d and $\triangle H$ of oat globulin heated in the range of pH 3 to 12 are shown in Fig 2. At extremely acidic or alkaline pH T_d decreased indicating reduced stability. The lowering of $\triangle H$ at the extreme acidic or alkaline conditions is indicative of partial denaturation since partially denatured proteins require less heat for complete denaturation. Similar observations on the effect of pH have been made on other plant proteins such as fababean protein (Arntfield and Murray, 1981). The T_d and $\triangle H$ values of the globulin were highest in the pH range 5–7, presumably the isoelectric range. Many proteins have highest thermal stability near their isoelectric pH (Privalov and Khechinashviti, 1974). At pH values far from the isoelectric point the protein was unfolded partially due to intramolecular side-chain charge repulsion leading to rupture of hydrogen bonds and a breakup of hydrophobic interaction.

The pH-induced changes in the conformation may lead to a less cooperative system. The DSC thermograms of oat globulin at pH 3.8, 6.4, and 9.5 are shown in Fig. 3. The T_d values at pH 3.8 and 9.5 were considerably lower than that at pH 6.4, and the transition peaks were broadened. The broadening of peaks indicates that pH-induced changes in the conformation caused the loss of cooperativity. The nonsymmetrical endotherms at pH 3.8 and 9.5 showing shoulders or multiple peaks also suggest partial unfolding of component proteins at acidic or alkaline pH, resulting in a mixed species of different thermal stability.

Effect of salts

The influence of NaCl and $CaCl_2$ on the thermal transition of oat globulins is demonstrated in Table 1. Increasing concentration of NaCl from 0 to 1.0M raised the T_d from 381°K (108°C) to 387°K (114°C), but $\triangle H$ was relatively unchanged; whereas, increasing concentration of $CaCl_2$ in the medium from 0 to 1.0 M lowered both T_d and $\triangle H$ considerably.

The heat stability of proteins is controlled by the balance of polar and nonpolar residues (Bigelow, 1967), such that the higher the proportion of nonpolar residues, the greater is the stability to heat, (i.e., higher temperature required for denaturation). Protein conformation can be perturbed by the addition of salts which influence the electrostatic interaction with the charged groups and polar groups, and affect the hydrophobic interaction via a modification on the structure of water (von Hippel and Schleich, 1969; Damodaran and Kinsella, 1982). The degree to which water structure is affected depends on the nature of the cations or anions. For cations, the extent of water structure breaking effect follows the order $Ca^{++} > Mg^{++} > NH_4^+ > Na^+ > K^+$, which is known as the lyotropic or chaotropic series (Hatefi and Hanstein, 1969). The cations at the higher order of the series (e.g. Ca^{++}) could reduce the free

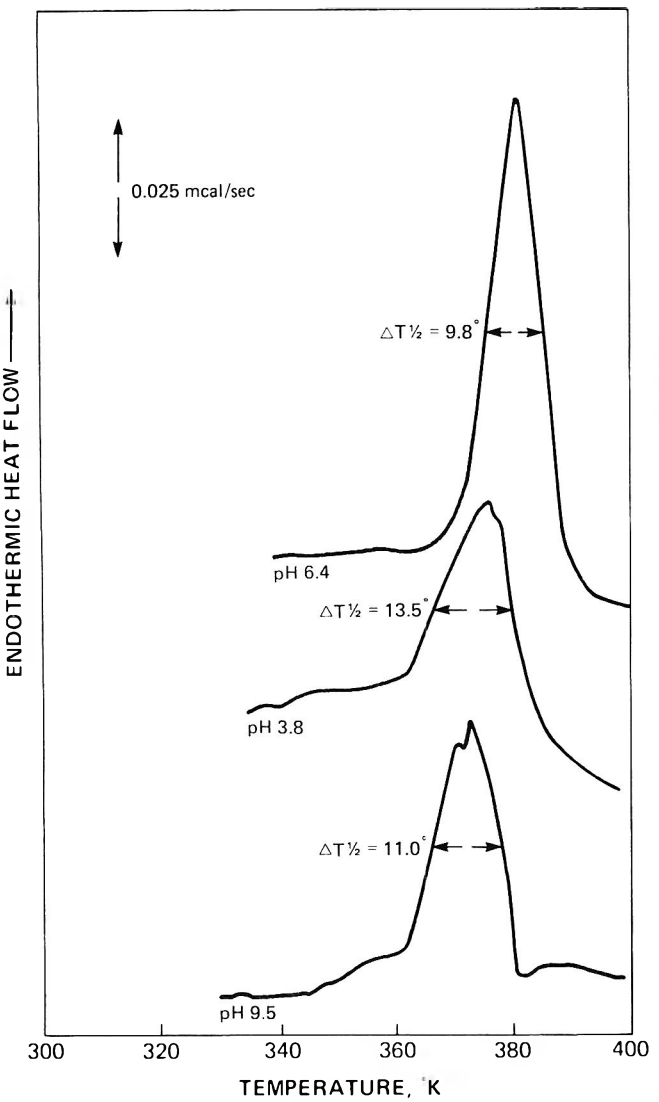

Fig. 3—DSC thermograms of oat globulin at different pH.

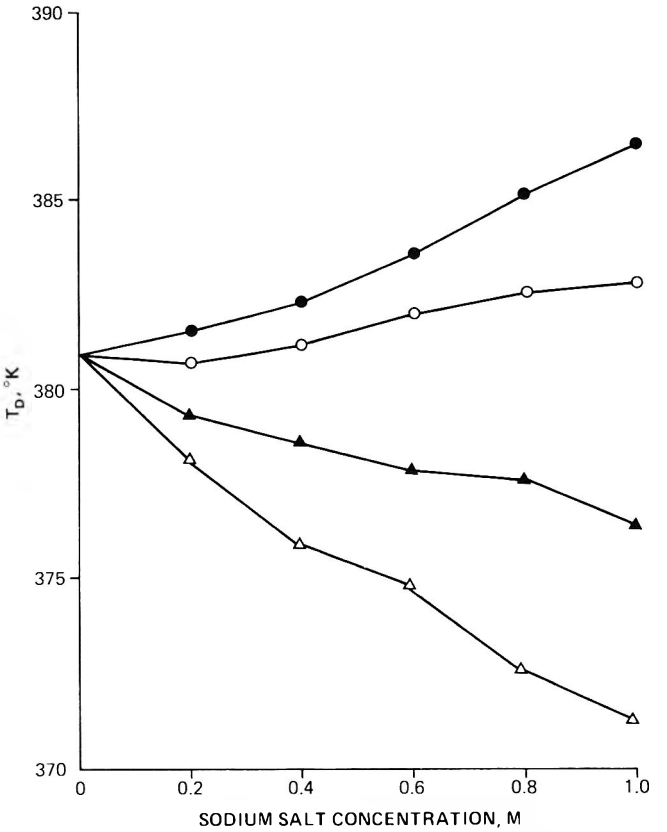

Fig. 4—Effect of different sodium anions on the transition temperature (T_D) of oat globulin. ●, Cl^-; ○, Br^-; ▲, I^-, △, SCN^-.

Table 1—Effect of salt concentration on the thermal stability of oat globulins

Salt conc (M)	NaCl		$CaCl_2$	
	T_d(°K)	ΔH(Cal.g^{-1})	T_d(°K)	ΔH(Cal.g^{-1})
0	380.9	6.25	380.9	6.25
0.1	380.9	6.13	377.2	6.28
0.3	381.7	6.25	—	—
0.5	—	—	372.4	5.01
0.7	384.7	6.24	370.3	4.71
1.0	386.5	6.28	369.0	4.68

[a] Transition temperature
[b] Enthalpy

Table 2—Effect of anions on the thermal stability of oat globulin

Anion	Concentration (M)	T_d(°K)[a]	ΔH(Cal.g^{-1})[b]
Cl^-	1.0	386.5	6.28
Br^-	1.0	382.4	5.19
I^-	1.0	377.0	4.57
SCN^-	1.0	371.0	3.95

[a] Transition temperature
[b] Enthalpy

energy required to transfer the nonpolar groups into water, and could weaken intramolecular hydrophobic interaction and enhance the unfolding tendency of the proteins (von Hippel and Wong, 1965), thus lowering both T_d and ΔH. The data suggest that hydrophobic interaction plays an important role in the thermal stability of oat globulin.

The thermal behavior of oat globulin was also studied under the influence of several anions: Cl^-, Br^-, I^-, and SCN^-. The results (Fig. 4) shows that T_d was increased with increasing concentrations of Cl^- and Br^- ions but was decreased in the presennce of equivalent concentrations of I^- and SCN^- ions. The enthalpy was not significantly affected by the addition of different anions (data not shown) except that at 1.0M concentration, the enthalpy was decreased progressively following the order Cl^-, Br^-, I^- and SCN^- (Table 2). These anions follow the lyotropic series with progressive structure perturbing effect on water following the order $SCN^- > I^- > Br^- > Cl^-$ (Hatefi and Hanstein, 1969). Similar to cations, anions with greater water structure breaking activity could decrease hydrophobic forces and increase the unfolding tendency of proteins (von Hippel and Wong, 1964). The present results show that the relative effectiveness of these anions in perturbing the heat stability (lowering in Td) followed this lyotrophic series. This provides additional support for the involvement of hydrophobic interaction in the heat stability of oat globulins. However, a significant unfolding of the protein with a resulting lowering in ΔH can only be observed at high concentration of the anions.

Effect of ethylene glycol

The data in Fig. 5 show the effect of ethylene glycol on T_d. The T_d value was progressively decreased with increasing concentrations of ethylene glycol. Similar lowering of T_d of other proteins in the presence of ethylene glycol has been observed (Beck et al. 1979; Harwalkar, 1985). Ethylene glycol lowers the dielectric constant of the medium and reduces the force that drives the apolar residues to the interior of the protein molecule, i.e., it weakens the nonpolar interactions of proteins and thereby lowers the thermal stability. The addition of eth-

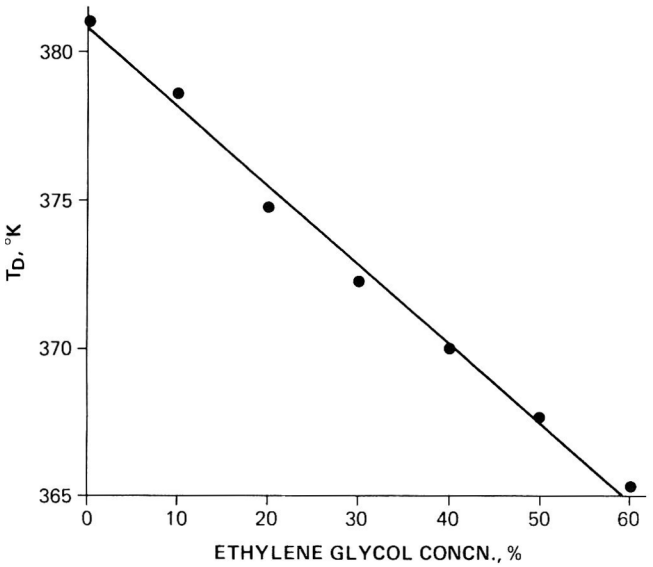

Fig. 5—Effect of ethylene glycol on the transition temperature (T_D) of oat globulin.

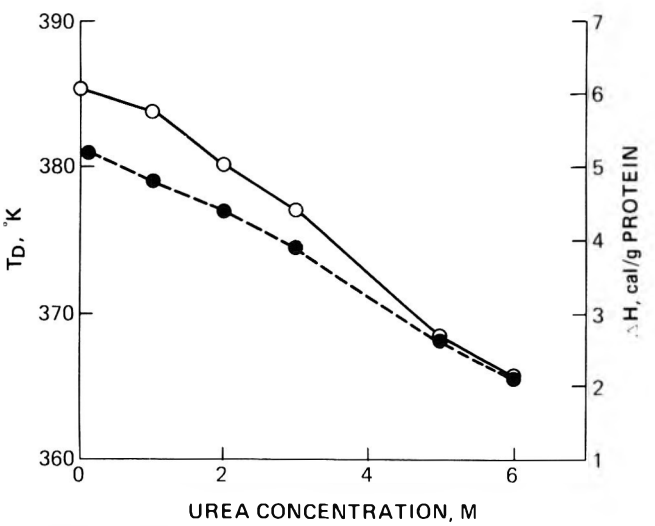

Fig. 6—Effect of urea on the DSC characteristics of oat globulin. ●, transition temperature (T_D); ○, enthalpy (△H).

hylene glycol up to 60% did not cause any change in △H (data not shown). The weakening of hydrophobic interactions by ethylene glycol may not be sufficient to cause significant denaturation of the protein. It has been observed that ethylene glycol at concentration below 50% was unable to dentaure proteins (Tanford, 1968).

Effect of urea

T_d and △H progressively decreased with increasing concentration of urea in the medium (Fig. 6) indicating both a decrease in thermal stability and gradual denaturation of oat globulin by the action of urea. Urea effectively disrupts the H-bonded structure of water and facilitates protein unfolding by weakening the hydrophobic interactions (Kinsella, 1982). Urea also increases the "permitivity" of water (Franks and England, 1975) for the apolar residues causing loss of protein structure and heat stability.

Apart from decreases in T_d and △H, the △T½ value was also affected by urea. The addition of 3.0M urea increased the △T½ value from 9.8 to 12°, indicating a reduction in the cooperativity of the thermal denaturation process. At 8M urea

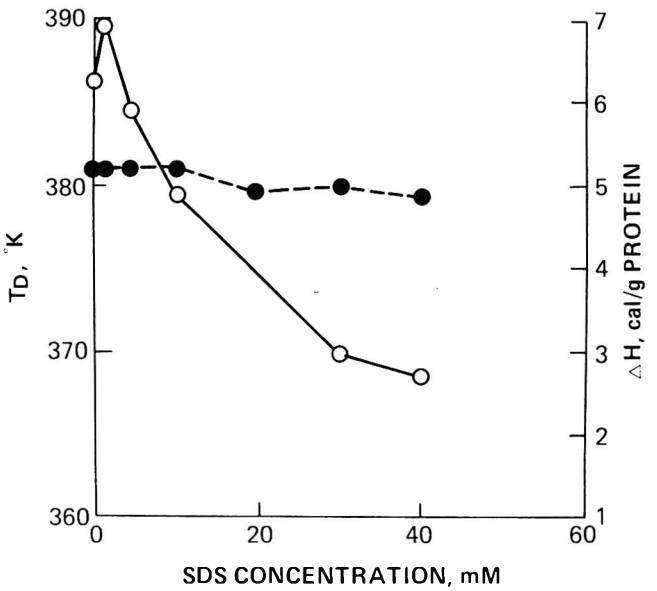

Fig. 7—Effect of sodium dodecyl sulfate on the DSC characteristics of oat globulin ●, transition temperature (T_D); ○, enthalpy (△H).

there was no detectable endothermic response suggesting an insignificant or negligible role for the disulfide bonds in thermal response by DSC, since disulfide bonds are not expected to be cleaved by 8M urea.

Effect of sodium dodecyl sulfate (SDS)

The effect of another protein perturbant, SDS, on the thermal properties of oat globulin is shown in Fig. 7. Initially, addition of small amounts of SDS (1 mM) led to a slight increase in △H without affecting T_d. Further increase in SDS concentration caused no significant change in T_d but lowered △H. The observed increase in △H with very small amounts of SDS is consistent with the observations of changes in protein conformation that are induced by very low concentrations of detergents (Tanford, 1968; 1970). Low concentrations of detergents seem to stabilize protein against denaturation by highly specific interaction between protein and detergent, presumably between cationic groups of protein and anionic groups of SDS (Tanford, 1970). The resulting hydrophobic side chains of SDS may be entropically transferred to the interior of the protein molecule. This could lead to the formation of additional hydrogen bonds and an increase in the △H value. At higher concentrations of SDS, the binding of detergent by globulin, like most proteins, seems to be non-specific, and is accompanied by protein denaturation (Steinhardt, 1975), and thus a decrease in △H.

Effect of reducing agents

To determine the contribution of disulfide bonds to the thermal properties of oat globulin, DSC thermograms in the presence of reducing agent were obtained. Dithiothreitol (DTT) at 10 mM caused a slight decrease in both T_d and △H (Table 2). In combination with 20 mM SDS, DTT caused no significant change in T_d while △H was slightly lowered, when compared to that of 20 mM SDS alone. Mercaptoethanol at 5% (v/v) caused a reduction in △H and a dramatic decrease in T_d (Table 3). In the presence of both mercaptoethanol and SDS, △H was not significantly affected when compared to SDS alone, but T_d was further reduced.

Reductants such as DTT and mercaptoethanol are known to cause the breakage of protein disulfide bonds, particularly in the presence of protein perturbants such as SDS. Oat globulin is an oligomeric protein comprised of six monomers each of

Table 3—Effect of reducing agents on the thermal proteins of oat globulin

Additive	Concentration	T^d(°K)[a]	△H(Cal.g^{-1})[b]
DDT[c]	10 M	381.6	5.5
SDS[d]	20 M	379.6	3.8
DTT	10 M	380.2	3.2
+ SDS	20 M		
Mercaptoethanol	5%	369.7	4.3
Mercaptoethanol	5%	357.5	3.6
+ SDS	20 M		

[a] Transition temperature
[b] Enthalpy
[c] DDT is dithiothreitol
[d] SDS is sodium dodecyl sulfate

which is made up of a basic and an acidic subunit linked by disulfide bonds (Brinegar and Peterson 1982). The present data show that breaking of these bonds by DTT (confirmed by gel electrophoresis in the presence of SDS) did not cause marked change in thermal stability of oat globulin. This is in contrast to the view that disulfide bonds may contribute to heat stability since proteins containing disulfide bonds show higher temperatures and enthalpies of denaturation compared to proteins without disulfide bonds (Kinsella, 1982). The dramatic decrease in T_d by mercaptoethanol and SDS seems to be unrelated to disulfide bond cleavage since mercaptoethanol alone caused a significant reduction in T_d. The decrease in T_d in the presence of mercaptoethanol could have resulted from its action as an alcohol. Monohydric alcohols promote destabilization of proteins by weakening hydrophobic interactions (Brandts and Hunt 1967). DSC studies of lysozyme in alcohol-water mixtures revealed progressive lowering of T_d with increasing alcohol concentration and alkyl chain length (Wright, 1982).

The results of the DSC study indicate that oat globulin has high heat stability and resistance to denaturation as shown by high T_d and △H values. Hydrophobic interactions seems to be a major chemical force contributing to the thermal stability of the oat globulin. Disulfide bonds, though an important covalent linkage in the organization of oat globulin polypeptide, do not appear to be involved in the thermal response.

The present data indicated that thermal stability (measured by T_d) and denaturation (measured by △H) are two parameters not necessarily interrelated. Additives such as ethylene glycol and some neutral salts caused a lowering in thermal stability without affecting △H, while additives such as SDS caused significant denaturation without much change in thermal stability.

CONCLUSION

OATS AND OTHER CEREAL grains are routinely subjected to various heat treatments such as drying, roasting and cooking. Since heat treatments may cause protein denaturation which is generally regarded as detrimental to functionality (Kinsella, 1976), the high thermal stability of oat proteins suggests that functionality can be retained in heat processed oat products. Hence, oat globulin can be used in food formulation requiring high heat stability of protein, e.g. protein-fortified beverages where pasteurization may cuase denaturation and precipitation of unstable protein. However, such stability may limit the use of oat proteins in food systems which require a change of state at processing temperatures lower than the T_d of oat globulin, e.g. heat-induced gelation in baking and meat emulsion. In these cases, physical and chemical treatments may be required to lower the heat stability of oat proteins.

REFERENCES

Arntfield, S.D. and Murray, E.D. 1981. The influence of processing parameters on food protein functionality. I. Differential scanning calorimetry as an indicator of protein denaturation. Can. Inst. Food Sci. Technol J. 14: 289.
Beck, J.F., Oakenfull, D., and Smith, M.B. 1979. Increased thermal stability of proteins in the presence of polyols. Biochemistry 18: 5191.
Bigelow, C.C. 1967. On the average hydrophobicity of proteins and the relationship between it and protein structure. J. Theor. Biol. 16: 187.
Brandts, J.F. and Hunt, L. 1967. The thermodynamics of protein denaturation. III. The denaturation of ribonuclease in water and in aqueous urea and aqueous ethanol mixtures. J. Amer. Chem. Soc. 89: 4826.
Brinegar, A.C. and Peterson, D.M. 1982. Separation and characterization of oat globulin polypeptides. Arch. Biochem. Biophys. 219: 71.
Damodaran, S. and Kinsella, J.E. 1982. Effects of ions on protein conformation and functionality. In "Food Protein Deterioration, Mechanisms and Functionality," ACS Symp. Series 206, p. 327. Cherry, J.P. (Ed.). Amer. Chem. Society, Washington, DC.
De Wit, J.N. and Klarenbeek, G. 1984. Effects of various heat treatments on structure and solubility of whey proteins. J. Dairy Sci. 67: 2701.
De Wit, J.N. and Swinkels, G.A.M. 1980. A differential scanning calorimetric study of the thermal denaturation of bovine α-lactoglobulin — thermal behaviors at temperatures up to 100°C. Biochim. Biophys. Acta 624: 40.
Donovan, J.W., Maxes, C.J., Davis, J.G., and Garbaldi, J.A. 1975. A differential scanning calorimetric study of eggwhite to heat denaturation. J. Sci. Fd. Agric. 26: 73.
Evans, M.T. A., Phillips, M.C., and Jones, M.N. 1979. The conformation and aggregation of bovine α-casein A. II Thermodynamics of thermal association and the effects of changes in polar and apolar interactions on micellization. Biopolymers 18: 1123.
Franks, F. and England, D. 1975. The role of solvent interactions in protein conformation. CRC Crit. Rev. Biochem. 3: 165.
Harwalkar V.R. 1985. The study of thermal denaturation of α-lactoglobulin by differential scanning calorimetry. Proceedings 14th North American Thermal Analysis Society Conference, September 15-18, San Francisco, California, p. 334.
Hatefi, Y. and Hanstein, W.G. 1969. Solubilization of particulate proteins and nonelectrolytes by chaotropic agents. Proc. Natl. Acad. Sci. 62: 1129.
Hermansson, A.M. 1978. Physico-chemical aspects of soy protein structure formation. J. Texture studies 9: 33.
Hermansson, A.M. 1979. Methods of studying functional characteristics of vegetable proteins. J. Am. Oil Chem. Soc. 56: 272.
Hischke, H.H. Jr., Potter, G.C., and Graham, W.R., Jr. 1968. Nutritive value of oat proteins. I. Varietal differences as measured by amino acid analysis and rat growth responses. Cereal Chem. 45: 374.
Kinsella, J.R. 1976. Functional properties of proteins in food: A survey. CRC Crit. Rev. Food Sci. Nutr. 7: 219.
Kinsella, J.E. 1982. Relationship between structure and functional properties of food proteins. In "Food Proteins," P.F. Fox and J.J. Cowden (Ed.). Applied Science Publisher, London.
Ma, C.-Y. 1983a. Chemical characterization and functionality assessment of protein concentrate from oats. Cereal Chem. 60: 36.
Ma, C.-Y. 1983b. Preparation, composition and functional properties of oat protein isolates. Can. Inst. Food Sci. Technol. J. 16: 201.
Ma, C.-Y. and Harwalker, V.R. 1984. Chemical characterization and functionality assessments of oat protein fractions. J. Agric. Food Chem. 32: 144.
Murray, E.D., Myers, C.D., Barker, L.D., and Maurice, T.J. 1981. Functional attributes of proteins — a noncovalent approach to processing and utilization of plant proteins. In "Utilization of Protein Resources," Stanley, D.W., Murray, E.D., and Less, D.H. (Ed.), Food & Nutrition Press Inc. Westport, CT.
Privalov, P.L. 1982. Stability of proteins: proteins which do not present a single cooperative system. Adv. Prot. Chem. 35: 1.
Privalov, P.L. and Khechinashviti, N.N. 1974. A thermodynamic approach to the problem of stabilization of globular proteins: A calorimetric approach. J. Mol. Biol. 86: 665.
Quinn, J.R., Raymond, D.P., and Harwalkar, V.R. 1980. Differential scanning calorimetry of meat proteins. J. Food Sci. 45: 1146.
Steinhardt, J. 1975. The nature of specific and non-specific interactions of detergent with proteins: Complexing and unfolding. In "Protein-Ligand Interactions," p. 412. W. de Gruyter and Co., Berlin.
Tanford, C. 1968. Protein denaturation. Adv. Protein Chem. 23: 122.
Tanford, C. 1970. Protein denaturation. Adv. Protein Chem. 24: 1.
von Hippel, P.H. and Wong, K.Y. 1964. Neutral salts: The generality of their effects on the stability of macromolecular conformations. Science 145: 577.
von Hippel, P.H. and Schleich, T. 1969. The effects of neutral salts on the structure and conformational stability of macromolecules in solution. In "Structure and Stability of Macromolecules," Vol. 2, p. 417. Timasheff, S.N. and Farman, G.D. (Ed.). Marcel-Dekker, New York.
von Hippel, P.H. and Wong, K.Y. 1965. On the conformational stability of globular proteins. J. Biol. Chem. 240: 3909.
Wright, D.J. 1982. Application of scanning calorimetry to the study of protein behavior in foods. In "Developments in Food Proteins-2," p. 61. Hudson, B.J.F. (Ed.) Applied Science Publishers, London.
Wright, D.J., Leach, J.B., and Wilding, P. 1977. Differential scanning calorimetric studies of muscle and its constituent proteins. J. Sci. Fd. Agric. 28: 557.
Wu, Y.V., Saxon, K.R., Cavins, J.R., and Inglett, G.E. 1972. Oats and their dry milled fractions: Protein isolation and properties of four varieties. J. Agric. Food Chem. 20: 757.

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Millet Processing for Improved Stability and Nutritional Quality Without Functionality Changes

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ABSTRACT

Whole millet adjusted to 15% moisture was gradually heated to 97°C over 12 min by passing through a steam-jacketed paddle conveyer to inactive lipid enzymes. Both processed and unprocessed millet were milled to 50% and 80% extraction flours. The 80% flour contained germ fractions, which resulted in much higher protein, lipid, thiamine, riboflavin, niacin, iron, zinc, available lysine, and protein efficiency ratios than the 50% flour. After 49°C storage, peroxide and fat acidity values were lower and flavor scores higher for processed than for unprocessed millet flours. No differences between processed and unprocessed flours were found in birefringence, water absorption and solubility, viscoamylograph values, or in their use in several traditional foods.

INTRODUCTION

MILLET is the name applied to small-seeded annual grasses that were probably first cultivated in Asia or Africa. Major millet types are *Panicum iliaceum* (proso), *Pennisetum americanum* (pearl), *Eleusine coracana* (finger), and *Setaria italica* (foxtail). In the United States and western Europe, millets are grown primarily for hay and pasture or as components of wild birdseed and chick feed mixtures. However, millets are used chiefly as food grains in Africa, eastern Europe, China, India, and other Asiatic countries. Hulled millet grains, either whole or cracked, are cooked into porridge called *kasha* in Russia and Poland. Ground millet is made into a thin pancake known as *kisra* in Sudan (Perten, 1983). Flours for millet can be used to partially replace wheat flour in breads, cookies, and pasta products (Badi et al., 1976; Lorenz and Dilsaver, 1980). In Senegal, millet flour is steam-cooked to prepare *couscous* and consumed as porridge with vegetables and a meat sauce.

Millets can be stored for long periods without substantial quality changes if the kernels remain intact (Kaced, 1982). However, quality rapidly deteriorates after millet is ground into meal (Varriano-Marston and Hoseney, 1983); hydrolytic and oxidative changes occur in lipids (Carnovale and Quaglia, 1973; Lai and Varriano-Marston, 1980). Poor storage quality has been attributed largely to hydrolytic changes associated with the action of lipolytic enzymes (Thiam, 1977).

Ground millet storage stability is improved by dry-milling processes that remove the major lipid-containing portions of the grain (i.e., the germ and covering layers) from the endosperm. Abdelraham et al. (1983) produced low-fat grits from pearl millet that had extended storage life, but yield was only 61%. It has been shown that storage stability of other cereals and oilseeds can be improved by heat-processing methods that inactivate lipid enzymes (Bookwalter, 1983). Cereals and oilseeds can be partially or fully cooked in extruder or roll cookers to inactivate hydrolytic and oxidative enzymes to improve storage stability. Substantial improvement in yield and nutritional quality can be achieved by heat-processing. However, these procedures cause drastic changes in physical characteristics that limit their use in traditional food applications.

The objective of this research was to investigate processing whole millet grain to inactivate deleterious lipid enzymes without changing functionality in order to retain stabilized germ fractions and increase nutritional quality.

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MATERIALS & METHODS

Materials

White proso millet (*Panicum miliaceum*), used in this study, was commercially grown in South Dakota. Millet was screened to remove dust, other grains, weed seeds, and other foreign materials.

Thermal processing equipment

Millet was transported through a continuous two-stage steam-jacketed paddle conveyer (Fig. 1) at a controlled rate of about 60 lb/hr (27 kg/hr). Each stage was 6 ft (1.83m) long and equipped with a 6 in (15.24cm) diameter screw. Screws were linked together with a chain belt driven by a variable speed motor. Millet, with or without added water, was metered into the upper conveyer and exited either at the sample port between conveyers or at the lower conveyer port. Temperatures up to 110°C could be obtained by controlling jacket steam pressure and/or holding time.

Dry milling

Two different pairs of Allis Chalmers corrugated rolls (152 mm × 152 mm) were used to reduce whole millet with and without temper water to desired particle sizes. One pair of rolls with 2.5:1 differential speeds contained 16 corregations per 2.54 cm while the other pair was 1.5:1 differential speeds contained 24 corrugations per 2.54 cm. A laboratory sifter containing various U.S. Standard screens and an aspirator were used to separate milled particles into medium and fine grits, flours, germ, hulls, and shorts prior to either remilling or separating into final products.

Physical tests

Viscosity properties of cooked millet flours at 9% solids were characterized by a Brabender Visco-Amylograph. Birefringence was determined by microscopic observation under polarized light. Color comparisons were made both visually and by measurement with a Hunterlab Model D-25-3 color difference meter. Methods for determining water absorption index and water solubility index have been described by Conway (1971).

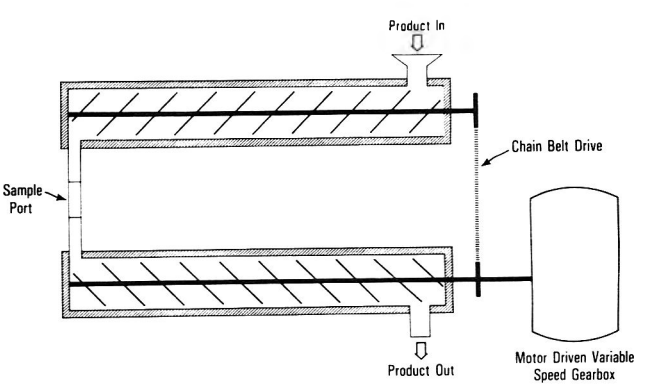

Fig. 1—Continuous two-stage steam-jacketed paddle conveyer.

Table 1—Peroxidase activity and fat acidity values of stored unheated and heat-processed whole and cracked millet

Thermal treatment[a] (°C)	Peroxidase activity	Months' storage at 49°C			
		Fat acidity[b], whole		Fat acidity[b], cracked	
		0	2	0	2
Unheated	Positive	50	70	71	224
68.9	Positive	52	96	73	279
80.0	Positive	41	73	45	205
88.9	Positive	35	45	38	86
98.9	Negative	46	37	43	65

[a] Tempered to 15.0% moisture
[b] Mg KOH/kg sample

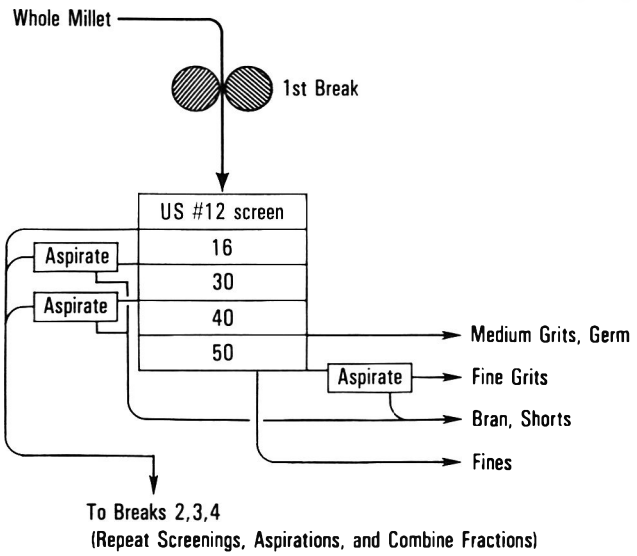

Fig. 2—Four break milling system for millet. High-fat flour combines medium grits, fine grits, fines and germ; bran and shorts diverted to feed use (80% extraction). Low-fat flour combines medium grits, fine grits and fines; bran, shorts and germ diverted to feed use (50% extraction).

Analytical tests

Peroxidase was determined by the method of Bergmeyer et al. (1974) based on color development by decomposition of hydrogen peroxide with guaiacol as hydrogen donor. Peroxide and fat acidity analyses were carried out according to AACC (1976) methods. Peroxides were measured on extracted fat while fat acidity was determined directly. Vitamins, minerals and protein efficiency ratios were assayed by AOAC (1980) methods. The method of Rao et al. (1963) was used to measure available lysine.

Food application

Millet flours were evaluated for their suitability by preparation of *couscous*. Agglomerated particles were formed by blending millet flour with 60% total water during 5 min mixing on low speed in a Hobart mixer. The agglomerates, 2 to 4 mm diameter, were placed on a perforated shelf in a covered double-boiler. The agglomerates were then stirred occasionally to provide uniform steam cooking until tender. Cooking proceeded over 25 min or until disappearance of raw cereal flavor.

Storage stability

Stability tests were made on heat-processed and unprocessed millet flours. Products were packaged in glass containers with screw closures and stored at 49°C for 2 months. Controls were held at −18°C. Withdrawals from storage were made after 0, 1, and 2 months. The samples were evaluated for changes in peroxide values, fat acidity, available lysine, and flavor.

Sensory evaluation

Millet flours were evaluated as 10% gruels after 7 min cooking. Flavor and odor were assessed by a 12-member trained panel, as previously described for soybean products (Warner et al., 1974, 1983). Panelists scored overall flavor quality on a 10-point scoring scale with 1 = bad and 10 = excellent. Statistical evaluations were made on the data after completion of each taste panel series. Analysis of variance, regression analysis (Snedecor and Cochran, 1980) and Duncan's multiple range test (Duncan, 1955) were used to assess experimental results.

RESULTS & DISCUSSION

Thermal processing

Exploratory tests were carried out with the continuous two-stage steam-jacketed paddle conveyer (Fig. 1). Whole millet enzymes were inactivated, as indicated by the peroxidase test, by this processing method. Heat-treatment was most effective after prior addition of water to raise moisture content to 15.0%. Processing was carried out with pass-through times between 4 to 12 min to achieve 68.9° to 98.9°C (Table 1). Peroxidase remained active except with millet heated to 98.9°C. Whole millet, both unheated and heated over 68.9° to 98.9°C, was divided into two portions; one was cracked by coarse grinding and the other was not cracked. Fat acidity values, an indicator of lipase activity, were lower in whole than in cracked millet, both before and after storage. Significantly lower (5% level) fat acidity values were associated with processing at 88.9° and 98.9°C. Lowest fat acidity values and negative peroxidase activity were associated with the 98.9°C heat-treatment. Additional research showed that peroxidase could be inactivated by heat-treating millet containing 15.0% moisture during 12 min heating to attain 97°C.

Dry milling

Milling studies were conducted to provide two product types: one containing minimum germ (low-fat) and the other maximum germ (high-fat). Both types were prepared with the milling system shown in Fig. 2. Each type involved four breaks with corrugated rolls. First and second break rolls contained 16 corrugations/2.54 cm, while third and fourth contained 24 corrugations/2.54 cm. Openings for 1st, 2nd, 3rd, and 4th break rolls were set at 0.1016, 0.0508, 0.0254, and 0.0127 cm, respectively. Each break was sifted through U.S. Standard screens ranging from #12 to #50, followed by three aspirations (Fig. 2) to separate milling fractions. Medium and fine grits and fines were combined for food use, while bran and shorts were combined for feed. Germ fractions could be diverted to either food or feed use, depending on objectives. A low-fat flour was obtained by adjusting millet moisture to 22%, milling within 30 min, followed by air drying. A high-fat flour or germ-containing flour was obtained by milling with no added temper water and limiting inclusion of bran to achieve a high-fat flour with appearance similar to low-fat flour. Product yield was 50% for low-fat and 80% for high-fat flour.

Nutritional aspects

Analyses of unheated and heat-processed low-fat and high-fat flours, along with an unprocessed whole millet flour, are shown in Table 2. Inclusion of germ fractions in high-fat flours resulted in significantly higher protein, fat, ash, and crude fiber values than in low-fat flours. Higher protein and fat levels demonstrated the nutritional advantage of germ retention, and heat-treatment had no adverse effect on levels of these nutrients. Analytical values for whole millet showed the effect of retaining all of the germ and hull as noted by lower proteins and much higher crude fiber, ash, and fat analyses. However, retaining all of the hull, as in whole millet, adversely changed product appearance from a dull yellow to an unpleasant gray.

Vitamin, mineral, and available lysine values and protein efficiency ratios for the various flours are listed in Table 3. The beneficial effect of germ retention was reflected by sig-

Table 2—Analyses[a] of unheated and heat-processed[b] millet flours

Millet flours	Moisture %	Protein %	Fat %	Starch %	Ash %	Crude fiber %
Heated, low-fat	9.6	12.8	1.7	79.0	0.6	1.6
Unheated, low-fat	10.1	12.8	1.8	79.4	0.6	1.5
Heated, high-fat	9.8	14.5	4.3	76.6	1.6	2.8
Unheated, high-fat	10.4	14.4	3.7	77.0	1.6	3.3
Unheated, whole	12.4	13.3	4.8	—	3.6	7.5

[a] Dry matter basis
[b] Paddle conveyer, 15.0% moisture, heated from ambient tc 97°C over 12 min, peroxidase negative
[c] Nitrogen × 6.25

Table 3—Nutritional profile of unheated and heat-processed[a] millet flours

Millet flours	Thiamin (mg/100 g)	Riboflavin (mg/100 g)	Niacin (mg/100 g)	Iron (μg/g)	Zinc (μg/g)	Available lysine (% of protein)	PER[b]
Heated, low-fat	0.11	0.11	3.8	13	17	1.3	0.18
Unheated, low-fat	0.13	0.12	4.0	16	18	1.4	0.24
Heated, high-fat	0.42	0.17	5.4	39	24	1.8	0.43
Unheated, high-fat	0.40	0.17	5.5	42	23	1.7	0.38
Unheated, whole	0.49	0.18	5.6	72	23	1.7	—

[a] See "b" of Table 2.
[b] PER = protein efficiency ratio, rat bioassay, values adjusted to casein at 2.50.

Table 4—Physical characteristics of unheated and heat-processed[a] millet flours

Millet flours	Birefringence[b]	Water absorption index[c]	Water solubility index[d]	Brabender viscosity units		Hunter color values[e]		
				Start	at 95°C	L	a	b
Heated, low-fat	Normal	2.3	1.3	10	290	76.0	−0.9	18.1
Unheated, low-fat	Normal	2.1	1.3	10	310	75.9	−0.7	20.7
Heated, high-fat	Normal	2.3	1.9	10	190	73.8	−0.6	20.4
Unheated, high-fat	Normal	2.2	2.3	10	210	74.5	−0.4	20.3
Unheated, whole	Normal	—	—	—	—	72.7	−1.2	12.2

[a] See "b" of Table 2.
[b] liMicroscopic observation under polarized light
[c] Gel wt, g/g dry sample
[d] Percent dry sample in supernatant
[e] L = lightness; a = + red, − green; b = + yellow, − blue

nificantly higher (5% level) thiamine, riboflavin, niacin, available lysine and protein efficiency ratios in high-fat and whole millet than in low-fat flours. Heat-treatment was shown to have no adverse effect on these nutrients. Iron and zinc levels were also significantly higher in high-fat and whole millet than in low-fat flours. Bran components also are high in minerals such as iron and zinc as reflected by higher ash values (Table 2), and inclusion is unavoidable to maximize germ retention.

Physical properties and food application

Table 4 shows physical characteristics of unheated and heat-processed high- and low-fat and whole millet flours. These products were indistinguishable by microscopic evaluation for birefringence as well as by water absorption and Brabender initial viscosity values. The slightly higher water solubility values of high-fat flours were associated with higher solubles derived from higher protein and fiber content. Higher Brabender viscosity values at 95°C for low-fat flours were associated with higher starch content (Table 2). These data showed that heat-treating millet to 97°C, as described to inactivate lipid enzymes, was not vigorous enough to alter starch and other characteristics that could severely change viscosity, water absorption, water solubility, or other functional properties. Differences in Hunter L, a, b values of high- and low-fat flours were negligible, which was in agreement with their similar visual appearance. However, whole millet flour had the lowest "L"and a much lower "b" value than the low- or high-fat flours. The dull gray visual appearance and Hunter color values of ground whole millet demonstrated the adverse effects of high bran content. These properties certainly will limit the usefulness of ground whole millet.

The performance of various unheated- and heat-processed millet flours was evaluated in *couscous*; results are shown in Table 5. Heat-processed flours required 5 min less time to prepare cooked agglomerated particles than unprocessed flour. *Couscous* prepared with heat-processed millet flours were of higher quality than those from unheated flours as evidenced by more fluffy, discrete, uniform, and more tender particles. *Couscous* prepared with unprocessed millet was slightly sticky, and particles were larger than 4 mm, non-uniform and gritty. Normal cereal cooked flavor was designated for all *couscous* preparations. Color of the various *couscous* was similar except for the slightly lighter colored product made with heat-processed high-fat millet flour.

Storage stability

The effect of heat-processing on stored millet flours is shown in Table 6. Although all peroxide values were within acceptable limits, those of high-fat were significantly lower than of low-fat millet. Higher values for low-fat millet were associated with the air-drying step that followed high moisture tempering to facilitate degermination. Extremely low peroxide values in the high-fat products demonstrate their very low susceptibility to autoxidation. Fat acidity values of unprocessed millet flours were significantly higher, both before and after storage. Slightly lower fat acidity of low-fat flours was associated with degermination. These data demonstrate the effectiveness of the 97°C heat-treatment to inactivate lipase, which minimizes fat hydrolysis.

Table 7 shows mean flavor scores of stored unheated and heat-processed millet flours. There were significant (5% level) flavor declines in both unheated high- and low-fat flours after storage at 49°C for 2 months. These flavor changes in both unprocessed millet flours were associated with significant increases in fat acidity values (Table 6). High lipase activity has previously been associated with bitter flavor development due

Table 5—Performance[a] of unheated and heat-processed[b] millet flour in couscous

Millet flours	Preparation time (min)	Agglomeration characteristics[c]	Visual color[d]	Texture and flavor
Heated, low-fat	20	Fluffy, uniform particles	99.5	Tender, normal cereal
Unheated, low-fat	25	Sl. sticky, nonuniform particles	99.5	Chewy, normal cereal
Heated, high-fat	20	Fluffy, normal discrete particles	100	Tender, normal cereal
Unheated, high-fat.	25	Sl. sticky, large particles	99.5	Gritty, normal cereal

[a] 60% moisture basis
[b] See "b" of Table 2
[c] Normal particle size: 2-4 mm dia
[d] 100 = light tan; 99.5 = tan

Table 6—Peroxide and fat acidity values of stored unheated and heat-processed[a] millet flours

	Months' storage at 49°C					
	Peroxide values (meq/kg fat)			Fat acidity (mg KOH/kg sample)		
Millet flours	0	1	2	0	1	2
Heated, low-fat	15	20	13	24	58	68
Unheated, low-fat	14	25	17	95	259	268
Heated, high-fat	2.2	3.3	1.8	37	66	90
Unheated, high-fat	2.2	7.0	2.7	69	330	378

[a] See "b" of Table 2

Table 7—Mean flavor scores[a] of stored unheated and heat-processed[b] millet flours

	Months' storage at 49°C		
Millet flours	0	1	2
Heated, low-fat	6.7	6.5	6.9
Unheated, low-fat	7.5	6.5	6.5
Heated, high-fat	7.2	7.0	7.1
Unheated, high-fat	7.2	6.8	6.5

[a] 10-point scoring scale: 1 = bad, 10 = excellent
[b] See "b" of Table 2

to hydrolytic rancidity. However, there were no significant flavor changes in heat-processed high- or low-fat millet flours, which illustrates the beneficial effect of this treatment.

CONCLUSIONS

HEAT-PROCESSING whole millet at 97°C in a steam-jacketed conveyer inactivated lipid enzymes and extended storage life. Germ fractions could be retained in the final milled product to improve both nutritional quality and yield. The degree and method of heat-processing or the level of retained germ fractions resulted in no adverse changes in functionality or performance in selected traditional foods.

REFERENCES

AACC. 1976. "Cereal Laboratory Methods," 7th ed. American Association of Cereal Chemists, St. Paul, MN.
Abdelrahman, A., Hoseney, R.C., and Varriano-Marston, E. 1983. Milling process to produce low-fat grits from pearl millet. Cereal Chem. 60: 189.
AOAC. 1980. "Official Methods of Analysis," Horwitz, W. (Ed.), 13th ed. Association of Official Agricultural Chemists, Washington, D.C.
Badi, S.M., Hoseney, R.C., and Finney, P.L. 1976. Pearl millet: II. Partial characterization of starch and use of millet flour in bread making. Cereal Chem. 53: 733.
Bergmeyer, H.V., Gawehn, K., and Gradal, M. 1974. Enzymes as biochemical reagents. In "Methods of Enzymatic Analysis," Bergmeyer, H.V. (Ed.), Vol. 1, 2nd ed., p. 474. Academic Press, Inc., New York.
Bookwalter, G.N. 1983. World feeding strategies utilizing cereals and other commodities. Cereal Foods World 28: 507.
Carnovale, E. and Quaglia, G.B. 1973. Influence of temperature and humidity controlled preservation on the chemical composition of milling products from millet. Ann. Technol. Agric. 22: 371.
Conway, H.F. 1971. Extrusion cooking of cereals and soybeans. Food Prod. Dev. 5(2): 27.
Duncan, D.B. 1955. Multiple range and multiple F tests. Biometrics 11: 1.
Kaced, I. 1982. Storage stability of pearl millet. Masters thesis, Kansas State University, Manhattan.
Lai, C.C. and Varriano-Marston, E. 1980. Changes in pearl millet during storage. Cereal Chem. 57: 275.
Lorenz, K. and Dilsaver, W. 1980. Rheological properties and food application of proso millet flours. Cereal Chem. 57: 21.
Perten, H. 1983. Practical experience in processing and use of millet and sorghum in Senegal and Sudan. Cereal Foods World 28: 680.
Rao, S.R., Carter, F.L., and Frampton, B.L. 1963. Determination of available lysine in oilseed meal proteins. Anal. Chem. 35: 1927.
Snedecor, G.W. and Cochran, W.G. 1980. "Statistical Methods," 7th ed. Iowa State University Press, Ames, IA.
Thiam, A.A. 1977. Contribution to the study of the biochemical phenomena of millet and sorghum flour determination. Tropical Products Institute Conference Papers, p. 69. Institut Technologie Alimentaire. Dakar, Senegal.
Varriano-Marston, E. and Hoseney, R.C. 1983. Barriers to increased utilization of pearl millet in developing countries. Cereal Foods World 28: 392.
Warner, K., Ernst, J.O., Boundy, B.K., and Evans, C.D. 1974. Computer handling of taste panel data. Food Technol. 28: 42.
Warner, K., Mounts, T.L., Rackis, J.J., and Wolf, W.J. 1983. Sensory characteristics and gas chromatographic profiles of soybean protein products. Cereal Chem. 60: 102.
Ms received 10/6/86; accepted 10/27/86.

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The Neutral Detergent Fiber, Acid Detergent Fiber, Crude Fiber, and Lignin Contents of Distillers' Dried Grains with Solubles

FAYE M. DONG and BARBARA A. RASCO

ABSTRACT

Distillers' dried grains with solubles (DDGS) were prepared from two cultivars of soft white winter wheat, a blend of red wheats and corn. DDGS made from the three whole wheat grains and corn contained, on a dry weight basis, 30–55% neutral detergent fiber (NDF), 10–14% acid detergent fiber, 7–9% crude fiber, and 2–4% lignin, an increase of 3–4 times over that contained in the unconverted whole grains. The significantly higher concentration of DDGS NDF isolated without sodium sulfite (vs with sodium sulfite) in the detergent solution was not due entirely to increased levels of residual protein.

INTRODUCTION

DISTILLERS' DRIED GRAINS with solubles (DDGS) are the major by-products of fermentation of whole grains to alcohol. Corn is the most popular grain used commercially to manufacture fuel alcohol, although during World War II, wheat was used as a carbohydrate source (Prescott et al., 1959). Recently, there has been renewed interest in using wheat as the fermentable material (Wu et al., 1984). In our laboratory, a process for making DDGS with improved flavor has been developed with the intent of producing wheat DDGS that are suitable for human food (Rasco et al., 1987). Since a large percentage of the grain starch is converted to ethanol during the fermentation process, the remaining components, notably protein and crude fiber, increase in concentration by approximately three-fold (Rasco et al., 1987; Bookwalter et al. 1984).

The purposes of the present study were to: (1) quantitatively and qualitatively characterize the fiber in DDGS produced from white wheat (high and low protein varieties), red wheat, white wheat (pastry) flour and corn; (2) examine the effect of adding sodium sulfite to the detergent solution used in the neutral detergent fiber (NDF) assay; (3) calculate and compare the caloric contents of DDGS to those of the unconverted grains.

MATERIALS & METHODS

Preparation of DDGS

DDGS were prepared using a process developed in our laboratory (Rasco et al., 1987). Hill 81 cultivar, a soft white winter wheat, was a gift from St. John Grain Growers (St. John, WA). Corn (Grade #1 yellow dent), red wheat (Grade #1 baker's blend of ⅔ Weston, a variety of hard red winter wheat, and ⅓ hard red spring wheat from the varieties Fremont, Pilot or Bannock), Tyee cultivar, a soft white winter club wheat, and pastry flour (made from soft white wheat) were purchased locally (Natural Foods Warehouse, Lynnwood, WA). All grains were from the 1985 crop year. Whole grains were ground to approximately 40 mesh. The ground whole grains and pastry flour were individually suspended in water (25–33% w/v) and sequentially treated with thermostable alpha-amylase (Takatherm L-340: Miles Laboratories, Inc., Elkhart, IN) for 1.5 hr at 90°C, then amyloglucosidase (Diazyme L-200: Miles Laboratories, Inc.) for 4 hr at 60°C. Following saccharification, yeast (Red Star Distiller's Active Dry Yeast; Universal Foods Corp., Milwaukee, WI) was added. After fermentation, the mash was concentrated by filtration and evaporation, then dried to approximately 5–10% moisture.

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Incubation of unconverted grains with alpha-amylase

Neutral detergent fiber (NDF) was measured in the starting grain and finished DDGS products according to the method of Goering and Van Soest (1970) with modifications suggested by McQueen and Nicholson (1979). A 0.50-g sample of each ground grain was suspended in 25 mL 0.067M sodium phosphate buffer, pH 6.5, containing 0.02% (w/v) sodium azide (McQueen and Nicholson, 1979) and 0.003% (w/v) alpha-amylase (Takatherm L-340; Miles Laboratories, Inc.; optimum temperature, 90°C; optimum pH 6.0–6.5). The suspension was incubated in a boiling water bath for 1 hr to liquefy the starch, allowing for an improved filtration rate after extraction in the detergent solution and thus a more accurate measurement of NDF. Filtration problems were more pronounced in the unconverted grain samples than in the DDGS products. Initial filtration problems with DDGS were improved by decreasing the sample size from 1.0g to 0.20–0.25g. The NDF content of DDGS was sufficiently high so that a reliable weight of NDF (approximately 80–100 mg) was recovered per 0.20—0.25g sample. Therefore, since filtration problems were generally not serious and since a 1½ hr treatment with alpha-amylase had been performed during the production of DDGS, the DDGS samples were not routinely subjected to pretreatment with alpha-amylase before NDF analysis. The measured NDF content of DDGS samples treated with alpha-amylase before NDF extraction was the same as in untreated samples. The addition of diatomaceous silica (Product #D3877, Sigma Chemical Co., St. Louis, MO) did not improve the filtration rates of extracted whole grain and pastry flour pretreated with alpha-amylase.

Extraction of NDF, ADF, crude fiber and lignin

The NDF content of DDGS and of the grain samples pre-treated with alpha-amylase was measured using the method of Goering and Van Soest (1970), except that the addition of decahydronaphthalene was omitted as suggested by McQueen and Nicholson (1979). The effect of adding 40 mM sodium sulfite to the neutral detergent solution on the amount of NDF recovered were also examined. Residual nitrogen was measured by the official AOAC (1984) Kjeldahl method, section 14.026. A conversion factor of 5.7 for wheat or 6.25 for corn was used to convert % nitrogen to % protein.

Acid detergent fiber (ADF) was measured by the method outlined in AOAC (1984), sections 7.074-7.076, with some modifications. The DDGS sample size was reduced to 0.50g to facilitate filtration. The ADF samples were ashed for 3 hr at 550°C before the final weighing. The weight of the ADF was calculated by subtracting the weight of the ash from the weight of the extracted sample. Crude fiber (CF) was analyzed according to the AOAC (1984) method, sections 7.071-7.073. Lignin was assayed by the AOAC (1984) method, sections 7.077. Since no problems with filtration occurred without the addition of asbestos, the latter was omitted from the procedure.

Statistical analysis

Significance was determined by the Student's t-test (two-tailed), one way analysis of variance, and Duncan's new multiple range test (Steel and Torrie, 1960).

RESULTS

THE PERCENTAGES OF NDF (with and without sodium sulfite added to the neutral detergent solution), ADF, CF and lignin are shown in Table 1. The NDF, ADF, CF, and lignin levels in DDGS were significantly greater than the respective levels in the starting grains. On the average, all fiber fractions in the DDGS increased 3-4 times over that in the unfermented

Table 1—Neutral detergent (NDF), acid detergent (ADF), crude fiber (CF), and lignin contents of Hill 81, red and Tyee wheats, pastry flour, corn and distillers' dried grains with solubles[a]

	NDF[b]		ADF	CF	Lignin
	(% dry weight basis, $\bar{X} \pm$ S.D.)[c]				
	$+Na_2SO_3$	$-Na_2SO_3$			
Hill 81 wheat	11.8 ± 0.2 (7)[d]	14.5 ± 2.7 (6)*	2.7 ± 0.2	2.1 ± 0.2	1.2 ± 0.0
DDGS	31.3 ± 1.8 (7)	48.9 ± 2.7 (3)***	9.9 ± 0.2	8.0 ± 0.1	3.0 ± 0.3
Red wheat	10.9 ± 0.4 (4)	10.9 ± 0.0 (2)	2.9 ± 0.1	2.1 ± 0.2	1.0 ± 0.1
DDGS	34.2 ± 2.6 (5)	54.4 ± 5.3 (4)***	10.5 ± 0.2	7.9 ± 0.3	3.9 ± 0.2
Tyee wheat	11.7 ± 0.5 (4)	13.2 ± 0.7 (4)**	2.8 ± 0.3	2.5 ± 0.4	1.0 ± 0.2
DDGS	39.5 ± 1.1 (4)	37.8 ± 1.9 (5)	13.9 ± 1.0	9.0 ± 0.1	3.6 ± 0.3
Pastry flour	1.9 ± 0.2 (4)	2.2 ± 0.8 (4)	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.0
DDGS	12.6 ± 0.6 (4)	–[e]	5.3 ± 0.6	3.3 ± 0.2	0.8 ± 0.1
Corn	14.4 ± 1.1 (4)	16.4 ± 2.2 (4)	3.9 ± 0.3	2.1 ± 0.1	0.9 ± 0.2
DDGS	44.3 ± 2.7 (5)	46.1 ± 2.3 (6)	12.4 ± 1.2	6.7 ± 0.2	1.9 ± 0.4

[a] See text for complete names of grains.
[b] See "Materials & Methods" for a description of the NDF procedure. ± NA_2SO_3 indicates addition or omission of 40 mM Na_2SO_3 to neutral detergent solution.
[c] % NDF, ADF, CF and lignin in each type of DDGS were significantly greater than the respective percentages in the original grains at the level of $p < 0.001$, except for corn/DDGS lignin ($p<0.02$), pastry flour/DDGS lignin ($p < 0.01$); Student's t-test.
[d] Number of replicates in parenthesis. ADF, CF and lignin were assayed in triplicate.
[e] Filtration difficulties prevented completion of analysis.
* $p < 0.05$, ** $p < 0.02$, *** $p < 0.001$ — Significance of difference between NDF measured with and without Na_2SO_3 (Student's t-test).

grain. The only exception was pastry flour DDGS, which contained much higher levels of fiber than the unconverted flour. NDF represented the largest fiber fraction, followed by ADF, CF, and then lignin. Although no particular starting grain or DDGS contained the highest concentrations of all fiber fractions, pastry flour and DDGS made from pastry flour consistently had the lowest concentrations of all fiber fractions measured.

The omission of sodium sulfite resulted in significant increases in the measured NDF concentration in DDGS made from Hill 81 and red wheat and in the unconverted Tyee and Hill 81 wheat (Table 1). In all other samples, the omission of sodium sulfite had no significant effect ($p > 0.05$; Student's t-test).

The effects of sodium sulfite were further investigated by measuring residual nitrogen in the NDF samples isolated from DDGS and correcting the NDF values by subtracting the concentration of residual protein (Table 2). Percent residual nitrogen was significantly higher in the NDF of Hill 81 and red wheat DDGS isolated without sodium sulfite than with sodium sulfite. However, the Hill 81 and red wheat DDGS NDF levels corrected for residual protein were still significantly higher in the absence of sodium sulfite than the protein-adjusted NDF levels in the presence of sodium sulfite (Table 2). Tyee DDGS NDF, which was not significantly affected by sodium sulfite, contained nitrogen levels that were not significantly different. The corrected NDF level without sulfite was *lower* than the level with sulfite. The relationship between the values for corn DDGS NDF with and without sulfite were not affected by the correction for residual protein.

The caloric content of DDGS was calculated from proximate analysis data obtained for another study (Rasco et al., 1987; Table 3). Because of the increase in concentration of NDF and decrease in concentration of non-NDF fermentable carbohydrate in DDGS, the calculated caloric content was from 15% (pastry) to 50% (red wheat) lower in DDGS compared to the starting grain.

DISCUSSION

THE METHODS employed with the modifications outlined in the present study produced reproducible results. The coefficients of variation were usually ≤ 10% for all fiber fractions isolated from either DDGS or the whole grains. The data were more variable for pastry flour due to the low fiber levels which were at the limits of detection of the methods and thus difficult to measure accurately.

Published methods for measuring NDF vary considerably in the reagents and chemicals (*e.g.* sodium sulfite) added to the neutral detergent solution prior to sample treatment, the temporal sequence of alpha-amylase treatment (*i.e.* before, during, or after boiling the sample in the neutral detergent solution), and the length of time needed to perform the procedure (Goer-

Table 2—Neutral detergent fiber (NDF) contents of distillers' dried grains with solubles (DDGS) made from Hill 81, red and Tyee wheats and from corn: residual nitrogen in NDF and % NDF corrected for residual protein

	$+Na_2SO_3$[a]		$-Na_2SO_3$	
	Residual N[b]	Corrected for residual protein	Residual N	Corrected for residual protein
	% dwb, $\bar{X} \pm$ S.D. (n)			
Hill 81 DDGS	1.02 ± 0.10	25.5 ± 1.8 (7)	1.60 ± 0.14**	39.8 ± 2.7 (3)***
Red wheat DDGS	1.05 ± 0.10	28.2 ± 2.6 (5)	2.07 ± 0.57*	42.6 ± 5.3 (4)***
Tyee DDGS	2.08 ± 0.04	27.6 ± 1.1 (4)	2.54 ± 0.32	23.3 ± 1.9 (5)**
Corn DDGS	1.59 ± 0.30	34.4 ± 2.7 (5)	2.08 ± 0.10	33.1 ± 2.3 (6)

[a] See "Materials & Methods" for a description of the NDF procedure. ± Na_2SO_3 refers to the addition or omission of 40 mM Na_2SO_3 to the neutral detergent solution.
[b] % N in NDF. Measured in triplicate by Kjeldahl analysis.
[c] % protein was calculated by multiplying % residual N by 5.7 for wheat or by 6.25 for corn. The % protein was subtracted from the values used to calculate the corresponding averages reported in Table 1.
* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ — Significance of difference between either % residual N or "corrected NDF" measured with and without Na_2SO_3 (Student's t-test).

Table 3—Proximate analysis[a] and calculated caloric contents of Hill 81, red and Tyee wheats, pastry flour, corn, and distillers' dried grains with solubles (DDGS)[b]

		Ash	Lipid	Protein % dwb	NDF[c]	Non-NDF carbohydrate[d]	Calculated[e] calories
	% H_2O			[$\bar{X}$ ± S.D. (n)]			100g (dwb)
Hill 81 wheat	9.9 ± 0.5 (6)	1.8 ± 0.1 (6)	1.6 ± 0.1 (6)	14.3 ± 0.3 (3)	14.5 ± 2.7 (6)	67.8	343
DDGS	6.6 ± 0.4 (3)	7.1 ± 0.1 (3)	3.9 ± 0.1 (3)	41.3 ± 1.4 (3)	48.9 ± 2.7 (3)	0	200
Red wheat	9.1 ± 0.1 (3)	1.5 ± 0.0 (3)	1.7 ± 0.3 (4)	13.9 ± 1.4 (5)	10.9 ± 0.0 (2)	72.0	359
DDGS	13.1 ± 0.3 (6)	6.0 ± 0.1 (6)	2.9 ± 0.4 (3)	38.7 ± 3.0 (4)	54.4 ± 5.3 (4)	0	181
Tyee wheat	9.6 ± 0.1 (5)	1.6 ± 0.2 (3)	1.4 ± 0.3 (3)	6.3 ± 0.4 (3)	13.2 ± 0.7 (4)	77.5	348
DDGS	6.8 ± 0.7 (5)	6.8 ± 0.2 (5)	6.0 ± 0.0 (3)	22.1 ± 1.0 (3)	37.8 ± 1.9 (5)	27.3	252
Pastry flour	11.1 ± 0.1 (3)	0.6 ± 0.0 (3)	1.0 ± 0.0 (3)	9.1 ± 1.2 (3)	2.2 ± 0.8 (4)	87.1	393
DDGS	8.7 ± 0.1 (3)	7.6 ± 0.2 (3)	2.7 ± 0.0 (3)	30.5 ± 2.4 (3)	12.6 ± 0.6 (4)	46.6	333
Corn	10.2 ± 0.1 (3)	1.2 ± 0.1 (3)	3.8 ± 0.1 (3)	7.5 ± 0.8 (3)	16.4 ± 2.2 (4)	71.1	349
DDGS	8.9 ± 0.1 (3)	12.0 ± 0.2 (3)	11.0 ± 0.9 (3)	24.7 ± 1.7 (3)	46.1 ± 2.3 (6)	6.2	223

[a] Proximate analysis data are from a study by Rasco et al. (1987). See Rasco et al. (1987) for methodology (H_2O, ash, lipid, protein).
[b] See text for complete names of grains.
[c] Neutral detergent fiber measured without sodium sulfite in the neutral detergent solution, except for pastry flour DDGS, which was assayed with sodium sulfite. Data taken from Table 1.
[d] Non-NDF carbohydrate = 100 − (% ash + % lipid + % protein + % NDF).
[e] Caloric content based on 9 Cal/g lipid, 4 Cal/g protein, 4 Cal/g non-NDF carbohydrate.

ing and Van Soest, 1970; Robertson and Van Soest, 1981; McQueen and Nicholson, 1979; AACC, 1983). We decided to use the conventional neutral detergent fiber procedure of Goering and Van Soest (1970), to pretreat whole grain and flour samples with a thermostable, microbial alpha-amylase with low hemicellulase activity, and to eliminate the addition of decahydronaphthalene. Because of the high protein content of some DDGS products, we felt that the addition of sodium sulfite was needed to aid protein hydrolysis. However, the effect of not adding sulfite on the measurable NDF was questionable. Therefore, we isolated NDF both with and without sodium sulfite present. Omission of sodium sulfite resulted in very significant increases ($p < 0.001$) in the NDF fraction isolated from red wheat DDGS and high protein white wheat DDGS (Table 1). These two products each contained approximately 40% protein (Table 3). Conceivably, the NDF fraction isolated without sodium sulfite from DDGS with a higher protein content may have retained more residual protein than did the NDF fraction from the DDGS with a low protein content. Although sodium sulfite significantly reduced the level of residual nitrogen in NDF, it did *not* account for the entire difference between sulfite and nonsulfite NDF concentrations. One effect of sodium sulfite may be to increase lignin loss (Hartley, 1972); however, the lignin concentration of 3–4% in wheat DDGS is not high enough to account for the effect of sulfite. Perhaps sodium sulfite solubilizes other cell wall constituents in DDGS.

The values we obtained for NDF were comparable to those reported by Wu and Stringfellow (1982) for corn DDG (no solubles), McQueen and Nicholson (1979) for whole wheat and corn, and Asp et al. (1983) for whole wheat. Our results for NDF concentrations are also similar to the nonstarch polysaccharide levels in white and whole wheat flours reported by Englyst et al. (1982). The NDF content in white wheat and pastry flour found in the present study were comparable to the levels of "total dietary fiber" (TDF) reported by Prosky et al. (1984, 1985) who used the enzymatic, gravimetric procedure. Although analysis of some foods such as wheat flour may result in similar NDF and TDF values, analysis of other foods may show significantly different levels.

Our corn DDGS CF levels were similar to those reported by Bookwalter et al. (1984). Our CF values for whole wheat and pastry flour were comparable to those obtained by Wu et al. (1984).

The level of all fiber fractions increased 3–4 times in DDGS compared to the unconverted grains, similar to the increase in protein concentration (Table 3). No fiber fraction appeared to be preferentially increased or decreased by the procedure to manufacture DDGS. When the levels of the four fiber fractions are compared among the five starting materials and five DDGS products, the least amount of fiber was isolated from pastry flour and DDGS made from pastry flour. This is not surprising since pastry flour has had the bran removed during milling.

The estimations of hemicellulose content as the difference of NDF minus ADF, and of cellulose as the difference of ADF minus lignin have limitations due to the variable degrees of extraction of soluble and insoluble fiber fractions (Robertson and Van Soest, 1981). With those limitations in mind, we calculated the difference of % NDF minus % ADF, which indicated that DDGS made from corn or the whole grains contained approximately 20–40% hemicellulose (dwb). Our hemicellulose values for corn of 11–15% and white wheat of 8–12% agree with those reported by McQueen and Nicholson (1979) who also calculated hemicellulose by difference. Cellulose calculated as the difference of ADF minus lignin is estimated to be 2-3% in corn and the whole grain flours, and 7–11% in the DDGS made from corn and the whole grains.

The procedure for manufacturing DDGS resulted in a substantial decrease in the concentration of non-NDF carbohydrate and a significant increase in the concentration of NDF. DDGS contained a lower caloric and higher fiber density than the original grain contained (Table 3). Two slices of bread (weighing a total of approximately 2 ounces or 56g) made with all-purpose flour will contain approximately 1g NDF. Replacing 25% by weight of the all-purpose flour with whole wheat flour in a conventional bread formulation will increase the dietary fiber content to approximately 3g in two slices. However, the level of dietary fiber can be increased to 4–7g per two slices of bread if the bread formulation contains 25% DDGS from either whole red or white wheats and 75% all-purpose flour. Clearly, the incorporation of whole grain DDGS in foods commonly consumed could make a significant contribution to an individual's daily dietary fiber intake. Partial substitution of DDGS for all-purpose or whole wheat flour in appropriate food products appears to be nutritionally advantageous for people desiring to increase their dietary fiber intake.

CONCLUSION

FOUR FIBER FRACTIONS of DDGS made from five grain sources were measured. DDGS contained an average of 3–4 times the NDF, ADF, CF and lignin concentration measured in the grains used for the fermentation. DDGS made from Hill 81, Tyee, red wheat, and corn contain approximately 20–40% NDF on a dry weight basis. The addition of sodium sulfite to the NDF extraction procedure resulted in significantly lower NDF levels in DDGS made from high protein wheats. This decrease in NDF was not completely due to solubilization of protein and lignin. It can be concluded that DDGS manufac-

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Functional-Properties of Raw and Heat Processed Brown Bean (*Canavalia rosea* DC) Flour

B.W. ABBEY and G.O. IBEH

ABSTRACT

The proximate composition and the functional properties of the flour of brown bean (*Canavalia rosea* DC) were investigated. Brown bean flour had 27.1 g/100g dry weight (DW) crude protein and 7.6 g/100g DW ether extractives. The brown bean flour had good water and oil absorption but poor gelation properties. Solubility of the brown bean protein was minimal at pH 4.0 but increased at pH 10. Foaming capacity of the flour could be improved by increasing concentration as well as by adding NaCl and was influenced by pH. Emulsion capacity was dependent on pH and salt concentration. Heat processing generally reduced the foamability and emulsification capacity of the brown bean flour.

INTRODUCTION

THE SEEDS of brown bean (*Canavalia rosea* DC) are consumed by both man and animals and form an important source of dietary protein especially in West Africa and Nigeria in particular. In Nigeria it is widely cultivated in the southeastern region. Malnutrition is usually prevalent where too few different plant foods are available. A wider variety of food legumes can complement existing foods by providing additional protein and vitamins (NAS, 1979).

The use of this edible bean as food deserves increased study and promotion. The authors are not aware of any published work on this bean particularly on the functional properties. In this investigation, the proximate composition of the beans and the functional properties of the raw and heat processed flour were determined.

MATERIALS & METHODS

Flour samples

Brown beans (*Canavalia rosea*) obtained from different commercial sources were used. The brown bean flour used for the functional studies was made from seeds which were dehulled, ground with a domestic grinder and defatted using n-hexane. The brown bean flour was also autoclaved for 15 min at 121°C and air dried at 28°C overnight to a mositure of 15% (heat processed).

Proximate analysis

Proximate analyses for moisture, protein (N × 6.25), ether extractives and ash were carried out in triplicate according to AOAC (1975) methods and reported on a dry weight basis.

Protein solubility

Method of Sathe and Salunkhe (1981) as described by Abbey and Ibeh (1986) was employed. Analyses were performed in triplicate.

Water and oil absorption capacity

Water and oil absorption capacities were determined in triplicate on a dry weight basis according to the method described by Beuchat (1977).

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Table 1—Proximate composition of brown bean flour

Components	Defatted flour		Undefatted flour	
	Raw	Heat processed	Raw	Heat processed
Dry matter %	90.2[a]	90.0[a]	91.8[a]	90.8[a]
Crude protein %	27.1[a]	26.9[a]	27.3[a]	27.6[a]
Ether extract %	0.4[a]	0.5[a]	7.6[a]	7.2[a]
Total ash %	4.4[a]	4.6[a]	4.9[a]	4.3[a]
Crude fiber %	1.9[a]	1.9[a]	1.9[a]	1.6[a]
N free extractives %	69.2[a]	66.1[a]	58.3[a]	59.3[a]

[a] Mean of triplicate determinations on dry weight basis. Means with the same letters in the same row of defatted or undefatted flour are not significantly different at 5% level.

Table 2—Protein solubility of raw and heat processed brown bean flour

pH	Sample (μg protein/mL)	
	Raw flour	Heat processed flour
2	218[b]	64[d]
4	184[c]	54[d]
6	252[a]	79[c]
8	216[b]	90[b]
10	252[a]	144[a]
12	228[ab]	64[d]

[a-d] Mean of triplicate determinations. Means with different letters in the same column are significantly different at the 5% level.

Table 3—Water and oil absorption capacities of brown bean flour

Sample	Water absorbed (g/g)	Oil absorbed (g/g)
Raw flour	2.7[b]	2.9[b]
Heat processed flour	3.4[a]	3.4[a]

[a,b] Mean of triplicate analyses. Means with different letters in the same column are significantly different at 5% level.

Gelation capacity

Gelation capacity was measured by the method described by Coffman and Garcia (1977). The least gelation concentration was taken as that concentration when the sample from the inverted test tube did not fall or slip.

Emulsion properties

Emulsions (2%, w/v) were prepared according to the method of Beuchat (1977). The details have been described earlier (Abbey and Ibeh, 1986). The emulsification capacity of the sample was considered to be the point of discontinuation of oil addition. Stability of the emulsions was evaluated for up to 60 hr at room temperature (25°C) by noting the separation of water in graduated cylinders. Effects of concentration were evaluated at 2, 4, 6, 8, and 10% (w/v). Effects of pH on emulsion capacity were evaluated on 2% (w/v) slurries by adjusting the pH to a desired value with HCl or NaOH prior to preparing emulsions. All experiments were conducted in triplicate at room temperature (25°C).

Foaming properties

Foaming capacities and stability were studied according to the method of Coffman and Garcia (1977). Effects of concentration of flour, salt (NaCl) and pH on foaming properties were investigated following the method described by Abbey and Ibeh (1986). All experiments were conducted at room temperature (25°C) in triplicate. Statistical analyses were done using PROC ANOVA procedures (SAS, 1985).

Table 4—Emulsion capacity and stability of raw and heat processed brown bean flour

Sample	Oil emulsified (g/g)	Initial volume of emulsion (mL)	Volume (mL) of water separated at 25°C after time (hr)								
			0	1	2	3	18	25	40	45	60
Raw flour	92	160	50[e]	64[d]	66[d]	70[c]	72[bc]	74[b]	84[a]	84[a]	84[a]
Heat processed flour	50	142	60[d]	70[c]	76[b]	76[b]	78[ab]	78[ab]	78[ab]	80[a]	80[a]

[a-e] Mean of triplicate determinations. Means with different letters in the same row are significantly different at the 5% level.

Table 5—Effects of pH on emulsion capacity of brown bean flour

pH	Sample (emulsion capacity, g/g)	
	Raw flour	Heat processed flour
2	98[d]	48[f]
4	102[c]	54[e]
6	116[b]	60[d]
8	118[b]	71[c]
10	124[a]	80[b]
12	126[a]	85[a]

[a-f] Mean of triplicate determinations. Means with different letters in the same column are significantly different at 5% level.

Table 6—Effect of salt (NaCl) on emulsion capacity of brown bean flour

Salt conc %	Sample (emulsion capacity, g/g)	
	Raw flour	Heat processed flour
0	92[b]	50[a]
0.2	94[b]	25[b]
0.4	100[a]	15[c]
0.6	98[a]	10[c]
0.8	84[c]	9[d]
1.0	72[d]	9[d]

[a-d] Mean of triplicate determinations. Means with different letters in the same column are significantly different at 5% level.

Table 7—Foaming capacity and stability of raw and heat processed brown bean flour

Sample	Volume after whipping (ml)	%Volume increase	Volume (mL) at 25°C after time (hr)						
			0.5	1	2	2.5	3	5	36
Raw flour	118[a]	18	112[b]	110[c]	110[c]	110[c]	110[c]	110[c]	110[c]
Heat processed flour	118[a]	18	102[b]	100[c]	100[c]	100[c]	100[c]	100[c]	100[c]

[a-c] Mean of triplicate determinations. Means with different letters in the same row are significantly different at 5% level.

RESULTS & DISCUSSION

Proximate composition

The proximate composition of defatted and undefatted brown bean flour is shown in Table 1. Crude protein of brown bean flour was slightly higher than that of cowpea flour. Furthermore, the ether extractives (7.6 g/100g DW) was significantly higher than the value reported for cowpea flour (2.4 g/100g DW) (Abbey and Ibeh, 1986).

Protein solubility

Values for protein solubility (Table 2) of the raw and heat processed brown bean flour indicated that the minimum protein solubility was at pH 4. This value was not significantly different from those of pH 2 and 12. Protein solubility decreased in heat processed brown bean flour at every pH studied. At pH 2 protein solubility of 64 g/ml was observed compared to 218 g/ml for raw brown bean flour. The pattern observed for the brown bean flour was similar to that obtained with the raw and heat processed cowpea flour (Abbey and Ibeh, 1986).

Water and oil absorption capacity

Results of water and oil absorption capacities are shown in Table 3. Heat processed brown bean flour had significantly higher water absorption capacity 3.4 g/g flour than the raw flour, 2.7 g/g. Heat processing also significantly increased the fat absorption capacity of brown bean flour from 2.9 g/g flour to 3.4 g/g flour. This increase could be due to the dissociation of the proteins that might occur on heating and also to denaturation which would unmask the non-polar residues from the interior of the protein molecule. The water and oil absorption capacities of raw and heat processed brown bean flour were found to be similar to the values obtained for cowpea flour (Abbey and Ibeh, 1986).

Gelation capacity

Least gelation concentration for the raw and heat processed brown bean flour were 18% and 20% (w/v), Orespectively.

Table 8—Effect of concentration on foaming capacity of raw and heat processed brown bean flour

Conc % (w/v)	Raw cowpea flour		Heat processed flour	
	Final foam volume (mL)	% Volume increase	Final foam volume (mL)	% Volume increase
2	118[e]	18	118[d]	18
4	140[d]	40	132[c]	32
6	145[c]	45	136[b]	36
8	152[b]	52	138[ab]	38
10	160[a]	60	140[a]	40

[a-e] Mean of triplicate determinatins. Means with different letters in the same column are significantly different at the 5% level.

The least gelation concentration for the raw brown bean flour was higher than values obtained for raw cowpea (*Vigna unguiculata*), 16% (w/v) (Abbey and Ibeh, 1986), but similar to that for winged bean flour, 18% (w/v) (Sathe et al., 1982).

The least gelation concentration for the heat processed brown bean flour was not significantly different from that of the heat processed cowpea flour (Abbey and Ibeh, 1986). Hermansson (1979) has reported that gelation was due to an aggregation of denatured molecules involving the formation of a continuous network which exhibited a certain degree of order. It has been suggested that the presence of seed coat fractions in legumes can interfere with the formation of such a continuous network (Sathe et al., 1982). The high gelling values obtained in this work can be attributed to the presence of seed coat fractions.

Furthermore, the nature of the protein in the flour may have an effect on the gelling properties. It has been reported that considerably higher protein concentration was usually required for the gelation of globular proteins (Schmidt, 1981).

Emulsion properties

Emulsion capacity of raw brown bean flour and the heat processed brown bean flour was 92 g/g and 50 g/g, respectively (Table 4). Heat processing decreased the emulsification capacity of brown bean flour at every pH studied (Table 5). At pH 2 the emulsification capacity of heat processed brown bean flour was 48 g/g flour compared to 98 g/g flour for raw flour. At pH 6 an emulsification capacity of 60 g/g flour was observed for heat-processed flour as against 116 g/g for the raw flour.

Table 9—Effect of pH on foaming capacity and stability of raw and heat processed brown bean flour

Sample	pH	Volume after whipping (mL)	% Volume increase	Volume (mL) at 25°C after time (hr)								
				0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	36
Raw flour	2	148[a]	48	146[b]	146[b]	140[c]	138[d]	134[e]	130[f]	130[f]	128[g]	110[h]
	4	129[a]	29	128[a]	128[a]	128[a]	124[b]	120[c]	120[c]	116[d]	110[e]	110[e]
	6	126[a]	26	125[ab]	124[b]	124[b]	116[c]	116[c]	114[d]	112[e]	110[f]	110[g]
	8	126[a]	26	125[a]	118[b]	118[b]	118[b]	118[b]	118[b]	118[b]	116[c]	110[d]
	10	132[a]	32	125[b]	118[c]	118[c]	118[c]	116[d]	114[e]	112[f]	112[f]	110[g]
	12	148[a]	48	146[b]	146[b]	144[c]	142[d]	138[e]	136[f]	134[g]	134[g]	110[h]
Heat processed flour	2	110[a]	10	102[b]	100[c]	100[c]	100[c]	100[c]	100[c]	100[c]	100[c]	100[c]
	4	114[a]	14	108[b]	100[c]	100[c]	100[c]	100[c]	100[c]	100[c]	100[c]	100[c]
	6	124[a]	24	112[b]	102[c]	100[c]	100[c]	100[c]	100[c]	100[c]	100[c]	100[c]
	8	116[a]	16	106[b]	100[c]	100[c]	100[c]	100[c]	100[c]	100[c]	100[c]	100[c]
	10	116[a]	16	104[b]	100[c]	100[c]	100[c]	100[c]	100[c]	100[c]	100[c]	100[c]
	12	130[a]	30	110[b]	104[c]	100[c]	100[c]	100[c]	100[c]	100[c]	100[c]	100[c]

[a-h] Mean of triplicate analyses. Means with different letters in the same row are significantly different at 5% level.

Table 10—Effect of salt concentration on foaming capacity of raw and heat processed brown bean flour

Salt Conc %	Raw flour		Heat processed flour	
	Final foam volume (mL)	% Volume increase	Final foam volume (mL)	% Volume increase
0.0	118	18[h]	118	18[e]
0.2	134	34[c]	136	36[d]
0.4	138	38[b]	142	42[b]
0.6	140	40[a]	148	48[a]
0.8	132	32[d]	140	40[bc]
1.0	128	28[e]	138	38[c]
1.5	126	26[f]	142	42[b]
2.0	124	24[g]	140	40[bc]

[a-h] Mean of triplicate determinations. Means with different letters in the same column are significantly different at the 5% level.

Depending on the concentration, NaCl increased or decreased the emulsification capacity of raw brown bean flour (Table 6). At concentrations of 0.4% and 0.6%, there was an increase in the emulsification capacity while at concentrations beyond this, emulsification capacity decreased steadily. A similar effect was observed for cowpea flour (Abbey and Ibeh, 1986).

In the case of the heat processed brown bean flour, addition of 0.2% NaCl caused a significant change in emulsification; an emulsification capacity of 25 g/g flour was recorded compared to 50 g/g flour for raw flour. NaCl at all the concentrations studied decreased the emulsification capacity of heat processed brown beann flour compared to raw flour.

Foaming properties

Results of foaming capacity and stability of raw and heat processed brown bean flour are presented in Table 7. After 36 hr standing at room temperature (25°C), the foam for raw brown bean flour did not collapse completely (110 mL volume) unlike that of the heat processed brown bean flour which collapsed completely after 1 hr standing. Heat processing considerably lowered the foam stability of brown bean flour. The foaming capacity of both the raw and heat processed brown bean flours was found to be the same. Foaming was concentration dependent increasing with higher concentration of brown bean flour (raw and heat processed) with 60% and 40% increase in volume at 10% (w/v) concentration, respectively (Table 8).

Heat processing considerably lowered the foaming capacity of brown bean flour at pH up to 10 (Table 9). At pH 6 a volume increase of 24% was observed for heat processed flour compared to 26% ($P < 0.05$) for raw flour. At pH 2 the foaming capacity values were 110 and 148 for heat processed and raw flour, respectively. It may be noted that all these effects were significant but statistical significance of the means in the same column are not shown in Table 9, only the significance in the same row are presented. Heat processed brown bean flour gave the highest percent foam increase at pH 12 (30%) and 48% increase for raw flour at pH 12.

At a concentration of 0.6% NaCl foaming capacity of raw and heat processed brown bean flour increased to a maximum (Table 10). Beyond this point (0.6%), there was no further increase. The volume increase at 0.6% NaCl concentration was 40% and 48% for raw and heat processed brown bean flour, respectively.

REFERENCES

Abbey, B.W. and Ibeh, G.O. 1986. Functional properties of raw and heat processed cowpeas (*Vigna unguiculata* Walp). Submitted for publication.

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

Beuchat, L.R. 1977. Functional and electrophoretic characteristics of succinylated peanut flour proteins. J. Agric. Food Chem. 25: 258.

Coffman, C.W. and Garcia, V.V. 1977. Functional properties and amino acid content of a protein isolate from mung bean flour. J. Food Technol. 12: 473.

Hermansson, A.W. 1979. Functional properties of proteins for foods: Flow properties. J. Texture 5: 425.

NAS. 1979. Tropical legumes: Resources for the future. Report on an Ad hoc Committee on Technology Innovation Board on Science and Technology for International Development, Commission on International Relations and National Research Council. National Academy of Sciences, Washington, DC.

SAS. 1985. "SAS User's Guide: Basic Version, 5 Edition." SAS Institute, Inc., Cary, NC.

Sathe, S.K. and Salunkhe, D.K. 1981. Functional properties of the Great Northern bean (*Phaseolus vulgaris* L.) proteins: emulsion, foaming, viscosity and gelation properties. J. Food Sci. 46: 74.

Sathe, S.K., Desphande, S.S., and Salunkhe, D.K. 1982. Functional properties of winged bean (*Psophocarpus tetragonobulus* [L] [DC]) proteins. J. Food Sci. 47: 503.

Schmidt, R.H. 1981. Gelation and coagulation. In "Protein Functionality in Foods." (Ed.) Cherry, K.J.J.P., p. 131. ACS Symp. Ser. 147, Amer. Chem. Soc., Washington, DC.

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Comparison of AOAC Method and Fluorogenic (MUG) Assay for Enumerating *Escherichia coli* in Foods

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ABSTRACT

The fluorogenic 4-methylumbelliferone glucuronide (MUG) test for identifying and enumerating *Escherichia coli* was compared with the standard most probable number method described in the *Official Methods of Analysis* of the Association of Official Analytical Chemists. Pecan nutmeats, frozen headless shrimp, and chicken pot pies were seeded with high, medium, and low numbers of viable *E. coli* organisms ranging from 15 to 500 CFU/g. Twelve replicate units of each food at each seeding level were analyzed by both methods. A statistical evaluation indicated that the MUG method yielded significantly ($\alpha = 0.05$) higher recovery in three of nine comparisons. The MUG method ranged from 22% lower to 92% higher than the AOAC geometric mean. The MUG method has the potential for reducing analytical time, labor, and media usage.

INTRODUCTION

ENZYME PROFILES to enumerate and identify microorganisms have attracted the attention of investigators seeking rapid, reliable, and economical methods (Pickett and Pederson, 1968; Huber and Mulanax, 1969; Peterson and Hsu, 1978). One of the most promising approaches for enumerating *Escherichia coli* is a fluorogenic most probable number (MPN) method (Feng and Hartman, 1982; Robison, 1984; Alvarez, 1984; Koburger and Miller, 1985) that is based on the synthesis of β-glucuronidase by *E. coli*. Incorporation of 4-methylumbelliferyl-β-D-glucuronide (MUG) into various media, including lauryl tryptose (LST) broth (Feng and Hartman, 1982; Robison, 1984; Alvarez, 1984) results in the enzymatic breakdown and release of fluorogenic 4-umbelliferone (Dahlén and Linde, 1973). This fluorogenic moiety is observed in cultures under UV light, wavelength 366 nm. Although some other bacteria, including *Salmonella* and *Shigella*, sometimes synthesize glucoronidase (Feng and Hartman, 1982; Kilian and Bülow, 1976) and about 3% of *E. coli* organisms are glucuronidase-negative (Feng and Hartman, 1982), the value of the MUG method does not appear to be substantially reduced. Other precautions include the presence of endogenous glucuronidase in some seafoods (Koburger and Miller, 1985) and the potential for suppression by competing organisms (Feng and Hartman, 1982); in addition, some types of glassware may fluoresce. Organisms such as *Aeromonas, Enterobacter, Vibrio*, and *E. hermanii* that may be mistaken for *E. coli* by the common biochemical schemes are differentiated from *E. coli* by the MUG method.

The objective of this study was to compare the standard MPN method (AOAC, 1984) with the MUG method for enumerating *E. coli* in foods using a statistical design to compare methods.

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METHODS & MATERIALS

Bacterial strain

E. coli 286C-2 was obtained from D.B. Shah, Food & Drug Administration, Cincinnati, OH.

Media and reagents

Media and reagents were obtained as follows: dehydrated LST, from GIBCO Diagnostics (Madison, WI): Levine eosin methylene blue agar, from BBL (Cockeysville, MD); EC medium, from Difco Laboratories (Detroit, MI); and MUG, from Sigma Chemical Company (St. Louis, MO). LST-MUG was prepared by adding 50 μg MUG/mL LST. LST and LST-MUG were prepared as dual batches with the same date of preparation and a common manufacturer's lot number. Butterfield's buffer (AOAC, 1984) was used as diluent. The *E. coli* inoculum was cultured in yeast extract-tryptone broth (Schleif and Wensink, 1985).

Procedure

Pecan nutmeats, frozen headless shrimp, and chicken pot pies purchased at local supermarkets were seeded with high, medium, and low concentrations (15 to 500/g) of *E. coli*. Twelve 50-g portions of each food were weighed into sterile Oster blenders at each seeding level. After blending for 2 min at 1800 rpm, serial dilutions were prepared with 90 mL buffer per dilution for the standard 3-tube MPN method (AOAC, 1984). Two replicates from each blender were analyzed by both the AOAC and the MUG method. Uninoculated foods were analyzed as controls by both methods with each set of samples. Both positive (inoculated) and negative (uninoculated) media controls were included in the analysis of each set of samples.

Statistical methods

The counts/g were transformed to $\log_{10}$ counts to ensure normality of the data. An analysis of variance (Ostle and Mensing, 1975) was used to compute the tests of significance and to calculate the replicate error or repeatability variance. Counts reported as <3 were set to equal 1. Two values reported as >11,000 were deleted from the analysis.

RESULTS & DISCUSSION

THE DATA obtained from the MPN values were transformed to $\log_{10}$ counts/g for statistical analysis (Table 1). The geo-

Table 1—Summary of E. coli geometric means per gram for chicken pot pies, frozen shrimp, and pecans by the AOAC and MUG methods

Product	Seeding levels	Method[a] Geometric mean/g AOAC	MUG	Difference, %
Chicken pot pie	370	357	434	21.6
	160	268	283[b]	5.6
	15	18	14	−22.2
Frozen shrimp	500	485	641[b]	32.2
	75	60	115	91.7[c]
	15	18	31	72.2[c]
Pecans	450	147	145	−1.4
	73	31	40	29.0
	40	16	27	68.7[c]

[a] Twenty-four observations, except as noted.
[b] Twenty-three observations.
[c] Significant at the $\alpha = 0.05$ level.

metric mean/g is shown for each method and concentration. Three of the tests for methods comparison were significant at the $\alpha = 0.05$ level. The MUG method yielded significantly higher results at the $\alpha = 0.05$ level. Seven of nine means were higher by the MUG assay. The percent differences of the MUG method ranged from 22% lower to 92% higher than the AOAC method. Estimates of replicate error are shown in Table 2. The overall pooled error, as expressed in $\log_{10}$ counts/g, was higher for the MUG than for the AOAC method (0.08958 versus 0.06988). However, these values did not differ at the $\alpha = 0.05$ significance level. Both estimates are lower than the value of 0.11252 reported (Peeler et al., 1980) for the AOAC method in mashed potatoes. Error terms are expressed as repeatability standard deviations, and percent coefficients of variance are expressed as $\log_{10}$ counts/g (Table 3).

The MUG method was equivalent to the AOAC method for chicken pot pies and recovered significantly more *E. coli* from one pecan and two frozen shrimp seeding levels. Both methods recovered *E. coli* in concentrations expected from the initial inoculation levels for chicken pot pies and frozen shrimp. The recovery of *E. coli* from pecans ranged from 33 to 55% for the MUG method and 33 to 42% for the AOAC method. The variation in the two methods cannot be shown to differ. This study shows that the MUG method is a useful alternative to the current AOAC method; it has the potential for reducing analytical time, labor, and media usage.

Table 2—Estimates of error variances for the AOAC and MUG methods

Product	Method	
	AOAC	MUG
Chicken pot pies	0.07735 (36)[a]	0.10158 (35)
Frozen shrimp	0.07218 (35)	0.08667 (36)
Pecans	0.06011 (36)	0.08075 (36)
Overall pooled replicate error	0.06988 (108)	0.08958 (106)
F-tests	1.28	

[a] Degrees of freedom are shown in parentheses.

Table 3—Precision estimates in terms of $\log_{10}$ counts/g

Sample	Mean		Repeatability, std. dev.		Coefficient of variation, %	
	AOAC	MUG	AOAC	MUG	AOAC	MUG
			Chicken pot pies			
1	2.55304	2.63757	0.17863	0.26771	7.0	10.1
2	1.24452	1.12904	0.35975	0.39122	28.9	34.7
3	2.42846	2.45159	0.26595	0.27939	11.0	11.4
			Shrimp			
1	1.78097	2.06156	0.25440	0.38687	14.3	18.8
2	1.26505	1.48891	0.24185	0.15675	19.1	10.5
3	2.68540	2.80699	0.30550	0.29271	11.4	10.4
			Pecans			
1	21.6598	2.16273	0.30609	0.23822	14.1	11.0
2	1.21333	2.42505	0.21772	0.27686	17.9	19.4
3	1.48803	1.60532	0.19812	0.32991	13.3	20.6

REFERENCES

Alvarez, R.J. 1984. Use of fluorogenic assays for the enumeration of *Escherichia coli* from selected seafoods. J. Food Sci. 49: 1186.

AOAC. 1984. "Official Methods of Analysis," 14th ed. Association of Official Analytical Chemists, Arlington, VA.

Dahlén, G. and Linde, A. 1973. Screening plate method for detection of bacterial β-glucuronidase. Appl. Microbiol. 26: 863.

Feng, P.C.S. and Hartman, P.A. 1982. Fluorogenic assays for immediate confirmation of *Escherichia coli*. Appl. Environ. Microbiol. 43: 1320.

Huber, D.M. and Mulanax, M.W. 1969. Identification of plant-pathogenic fungi via aminopeptidase profiles. Phytopathology 59: 1032.

Kilian, M. and Bülow, P. 1976. Rapid diagnosis of Enterobacteriaceae: 1. Detection of bacterial glycosidases. Acta Pathol. Microbiol. Scand. Sect. B 84: 245.

Koburger, J.A. and Miller, M.L. 1985. Evaluation of a fluorogenic MPN procedure for determining *Escherichia coli* in oysters, J. Food Prot. 48: 244.

Ostle, B. and Mensing, R.W. 1975. "Statistics in Research," 3rd ed. Iowa State University Press, Ames, IA.

Peeler, J.T., Messer, J.W., Leslie, J.E., and Houghtby, G.A. 1980. Variation in food microbiology tests used to evaluate analyst performance. J. Food Prot. 43: 729.

Peterson, E.H. and Hsu, E.J. 1978. Rapid detection of selected gram-negative bacteria by aminopeptidase profiles. J. Food Sci. 43: 1853.

Pickett, M.J. and Pederson, M.M. 1968. Characterization of bacteria by their degradation of amino acids. Appl. Microbiol. 16: 1591.

Robison, B.J. 1984. Evaluation of a fluorogenic assay for detection of *Escherichia coli* in foods. Appl. Environ. Microbiol. 48: 285.

Schleif, R.F. and Wensink, P.C. 1985. "Practical Methods in Molecular Biology." Springer-Verlag, New York.

Ms received 7/28/86; revised 9/2/86; accepted 10/7/86.

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tured from whole grain could provide a significant source of dietary fiber.

REFERENCES

AACC. 1983. "Approved Methods of the American Association of Cereal Chemists," 8th ed. American Association of Cereal Chemists, St. Paul, MN.

AOAC. 1984. "Official Methods of Analysis of the Association of Official Analytical Chemists," 14th ed. Association of Official Analytical Chemists, Inc., Arlington, VA.

Asp, N-G, Johansson, C-G, Hallmer, H., and Siljestrom, M. 1983. Rapid enzymatic assay of insoluble and soluble dietary fiber. J. Agric. Food Chem. 31: 476.

Bookwalter, G.N., Warner, K., Wall, J.S., and Wu, Y.V. 1984. Corn distillers' grains and other by-products of alcohol production in blended food. II. Sensory, stability and processing studies. Cereal Chem. 61: 509.

Englyst, H., Wiggins, H.S., and Cummings, J.H. 1982. Determination of the non-starch polysaccharides in plant foods by gas-liquid chromatography of constituent sugars as alditol acetates. Analyst 107: 307.

Goering, H.K. and Van Soest, P.J. 1970. "Forage Fiber Analyses (Apparatus, Reagents, Procedures and Some Applications)." U.S. Dept. Agric. Agricultural Handbook No. 379, U.S. Govt. Printing Office, Washington, DC.

Hartley, R.E. 1972. p-Coumaric and ferulic acid components of cell walls of rye grass and their relationships with lignin and digestibility. J. Sci. Food Agr. 23: 1347.

McQueen, R.E. and Nicholson, J.W.G. 1979. Modification of the neutral-detergent fiber procedure for cereals and vegetables by using alpha-amylase. J. Assoc. Anal. Chem. 62: 676.

Prescott, S.C., and Dunn, C.H. 1959. "Industrial Microbiology." McGraw Hill Book Co. New York, NY.

Prosky, L., Asp, N-G., Furda, I., DeVries, J.W., Schweizer, T.F., and Harland, B.F. 1984. Determination of total dietary fiber in foods, food products and total diets: interlaboratory study. J. Assoc. Off. Anal. Chem. 67: 1044.

Prosky, L., Asp., N.-G., Furda, I., DeVries, J.W., Schweizer, T.F., and Harland, B.F. 1985. Determination of total dietary fiber in foods and food products: collaborative study. J. Assoc. Off. Anal. Chem. 68: 677.

Rasco, B.A., Dong, F.M., Hashisaka, A.E., Gazzaz, S.S., Downey, S.E., and San Buenaventura, M.L. 1987. Chemical composition of distillers' dried grains with solubles (DDGS) from white wheat, red wheat, and corn. J. Food Sci (in press).

Robertson, J.B. and van Soest, P.J. 1981. The detergent system of analysis and its application to human foods. Ch. 8 In "The Analysis of Dietary Fiber in Food," W.P.T. James and O. Theander (Ed.), p. 123. Marcel Dekker, Inc., New York.

Steel, R.G.D. and Torrie, J.H. 1960. "Principles and Procedures of Statistics." McGraw Hill Book Co., New York, NY.

Wu, Y.V. and Stringfellow, A.C. 1982. Corn distillers' dried grains with solubles and corn distillers' dried grains: dry fractionation and composition. J. Food Sci. 47: 1155.

Wu, Y.V., Sexson, K.R., and Lagoda, A.A. 1984. Protein-rich residue from wheat alcohol distillation: fractionization and characterization. Cereal Chem. 61: 423.

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Purification and Properties of an Antimicrobial Substance Produced by *Lactobacillus bulgaricus*

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ABSTRACT

Media taken from cultures of *Lactobacillus bulgaricus* were found to contain an antibiotic substance different from lactic acid. This antibiotic substance was active against *Pseudomonas fragi* and, to a lesser extent, *Staphyloccus aureus*. The optimum pH for activity was approximately 4.0. At this pH the activity was stable at 6°C for 18 days and for at least 1 hr at 100° C. Active substance was isolated from the growth medium and purified by reverse phase high performance liquid chromatography procedures after removal of proteins by ethanol precipitation. Chromatography was performed first on an uncapped C-8 column, then on a fully capped C-18 column. A negative Biuret test indicated that the purified substance was not a polypeptide. UV absorption and mass spectra were consistent with a structure having an aromatic moiety and a molecular weight ≤ 700.

INTRODUCTION

THERE ARE REPORTS that *Lactobacillus bulgaricus* produces one or more antimicrobial substances active against several microorganisms (Dahiya and Speck, 1968; Gilliland and Speck, 1975; Gilliland and Martin, 1980; Abdel-Bar and Harris, 1984). Part of this antimicrobial activity has been attributed to production of hydrogen peroxide, organic acids, or both. Reddy et al. (1983), however, reported that *L. bulgaricus* DDS14 produces an antibiotic substance, called bulgarican, that was different from hydrogen peroxide and lactic acid.

Both the crude and purified antimicrobial material may have potential for controlling undesirable microbial growth and extending shelf life of food products such as milk, fish, meat, and pharmaceuticals. The use of cell-free material offers obvious advantages over the use of whole cells as a food additive in terms of minimizing texture and flavor changes. Although the crude fermentation liquor would have greater potential for use than whole cells, the purified antimicrobial material would be most desirable. Reddy et al. (1983) partially purified and characterized an antimicrobial substance elaborated by *L. bulgaricus*, but did not attempt to identify the chemical structure of the compound. The present study was designed to further purify and more completely characterize antimicrobial substance(s) produced in cultures of this organism by more sophisticated techniques than were used previously.

MATERIALS & METHODS

Microorganisms

Freeze-dried cultures of *Lactobacillus bulgaricus* 7994, *Staphylococcus aureus* 9144, and *Pseudomonas fragi* 4973 were obtained from the American Type Culture Collection. *L. bulgaricus* was rehydrated in 10% skim milk (w/v) and incubated at 37°C for 72 hr. *S. aureus* was rehydrated in trypticase soy broth and was incubated at 37°C for 24 hr. *P. fragi* was rehydrated in nutrient broth and incubated at 22°C for 24 hr. Cultures were maintained at 6°C in the above media. Stock cultures were activated by three successive transfers at 24 hr intervals.

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Bioassays

Antimicrobial activity was quantitated by a disk assay procedure (Branen et al., 1975; Pulusani et al., 1979; Reddy et al., 1983). Sterile nutrient agar was mixed with 0.2% by volume of a 24 hr culture of *P. fragi* and 15 mL aliquots were poured into 150 mm culture plates. Plates were used immediately after the agar solidified or stored at 6°C for a few hours. Uniform paper assay discs 12.7 or 6.35 mm in diameter were placed on the agar, then wetted with 30 or 10 μL of sample, respectively. These volumes were determined to be appropriate for near saturation of the discs. The larger disks were used for experiments concerning the test organisms and the stability of the antimicrobial activity in crube extracts (Tables 1 and 2). The smaller discs were used for assays of column fractions. Plates were kept at 25 ± 2°C for 1 hr to allow the test material to diffuse into the agar, then incubated at 22°C for 24 hr. Antimicrobial activity was determined from the diameter of the clear zone around each disc.

Fermentation by L. bulgaricus and isolation of crude extract

Sterile 10% (w/v) skim milk was inoculated with 5% by volume of an actively growing culture of *L. bulgaricus* and subsequently incubated for 96 hr at 37°C. Crude extract of *L. bulgaricus* medium (LCE) was obtained by centrifuging the fermented milk (pH = 3.85) at 20,000 × *g* for 15 min to remove the curd and microbial cells. Whey protein was precipitated from the supernate by addition of one volume of 95% ethanol. The mixture was stored at 6°C for 20 hr.

Table 1—Inhibitory effect of L. bulgaricus crude extract (LCE) and control crude extract (CCE)[a] toward P. fragi and S. aureus

Test organism	Inhibition zones[b] (mm): Lactobulgarican crude extract (LCE)	Inhibition zones[b] (mm): Control crude extract (CCE)	Difference in sizes of inhibition zones[c] (mm)
P. fragi	34.67	27.5	7.17
S. aureus	29.5	24.5	5.0

[a] LCE and CCE were concentrated to 1/5 of the original volume.
[b] Each measurement was an average of three plates. 12.7 mm sterile discs were used.
[c] *P. fragi* was significantly more susceptible than *S. aureus* at $P \leq 0.05$.

Table 2—Stability of Antimicrobial Substance in Crude L. bulgaricus *Extract*[a]

Treatment		Inhibition zones (mm)
	Temp (°C) Time (min)	
25	1 (control)	33.43
25	60	33.80
60	60	33.50
100	60	33.36
121	15	32.10[b]
121	30	31.80[b]
121	60	29.90[b]
	Storage time (days) at 6°C	
0	(control)	32.50
5		32.56
10		32.27
15		32.61
18		32.31
19		28.60[b]
20		26.90[b]

[a] Antimicrobial activity was tested against *P. fragi* using 12.70 mm sterile discs. Each measurement was an average of three determinations.
[b] Significantly different ($p \leq 0.001$) from the control.

then centrifuged as above. The resulting supernate was dried under vacuum using a 55°C water bath and a rotary evaporator, then redissolved in one-fifth the original volume of 50% methanol in water or water alone.

A control crude extract (CCE) was prepared using the same procedure as above except that the skim milk was not inoculated with *L. bulgaricus* and the sterile skim milk was subsequently adjusted to pH 3.85 (the pH of LCE) with a few drops of 85% reagent grade lactic acid (Eastman Kodak Co., Rochester, NY) prior to incubation. The control group was included to determine the inhibitory effect of lactic acid. Differences in the diameters of inhibition zones between LCE and CCE samples were taken to represent the inhibitory effect of antimicrobial substances produced by *L. bulgaricus*.

Statistical annalyses

A one-way analysis of variance was used to test the effect of temperature and pH on activity of the LCE. A student t-test was used to determine which organism was most susceptible to the inhibitory effect of LCE and to test the inhibition zones attributed to LCE versus those attributed to CCE.

Chromatographic purification of antimicrobial substance

A highly purified antimicrobial substance was isolated using two reverse phase chromatographic steps. Approximately 20 ml aliquots of crude extract in water (LCE) were applied to a Lobar prepacked LiChroprep RC-8 column (310 × 25 mm, containing 40–63 μm particles, from EM Reagents). The column was eluted successively with 50 mL water; a 200 mL gradient of 0% to 60% methanol in water; 50 mL of 100% methanol; and 50 mL of 100% isopropanol. Fractions (100 drops) were collected automatically using a Gilson drop-counting fraction collector. Methanol and water were removed by evaporation to dryness at 75°C in a shaker water bath, then samples were rehydrated to 1/20 the original volume with 0.05M sodium phosphate buffer (pH 4.0) prior to disk assays. Pooled active fractions were concentrated by evaporation, then 200 μl aliquots were further purified by high performance chromatography on a Bio-Rad ODS-5 column (25 × 4 mm) eluted isocratically at a flow rate of 1 mL/min for 15 min with phosphate buffer (0.05M sodium phosphate, pH 4.0), then for 30 min with a linear gradient of 0% to 50% methanol in phosphate buffer using a Varian LC-5000 gradient former with variable wavelength detector set at 210 nm. One ml fractions were collected and disc assayed after evaporation to dryness and dissolution in 1/5 volume phosphate buffer as described above.

Characterization of purified antimicrobial substance

Biuret tests were performed according to the method of Oser (1965). Catalase treatment was used to determine whether the antimicrobial activity was due to contaminating hydrogen peroxide. Bovine liver catalase (Sigma Chem. Co., St. Louis, MO; 35300 units/mg) was added to an aliquot of purified antimicrobial substance at a final concentration of 70 units/mL. A control group was treated with catalase inactivated by heating at 100°C for 5 min. Mixtures were incubated for 1 hr at 37°C, then disc assayed. Ultraviolet absorption spectra were recorded using a Cary 219 UV/Vis recording spectrophotometer. Mass spectra were obtained using a Finnigan 4510 GC-EI/CI automated mass spectrometer system at 86 eV, 140°C ion-source temperature. Negative and positive chemical ionization spectra were obtained using isobutane at 0.4 torr.

RESULTS & DISCUSSION

Relative activities towards P. fragi and S. aureus

Inhibition zones obtained towards both *P. fragi* and *S. aureus* due to antimicrobial activity produced by *L. bulgaricus* are summarized in Table I. Growth of both organisms was inhibited, but *P. fragi* was affected significantly ($P \leq 0.05$) more than *S. aureus*. *P. fragi* was used as the test organism for all further assays of animicrobial activity.

The finding that antimicrobial activities produced by lactic acid bacteria were more effective towards *Pseudomonas* than *Staphylococcus* species is supported by other observations. Angelo et al. (1980) noted that *Streptococcus* species were more inhibitory towards *P. fluorescens* than *S. aureus*. *S. diacetilactis* is active against both *S. aureus* and *P. fragi*, but more active against the latter organism (Reddy and Ranganathan, 1983). *P. aeruginosa* is resistant to many antibiotics, but is sensitive to a proteinaceous inhibitor produced by *L. acidophilus* (Mehta et al., 1983).

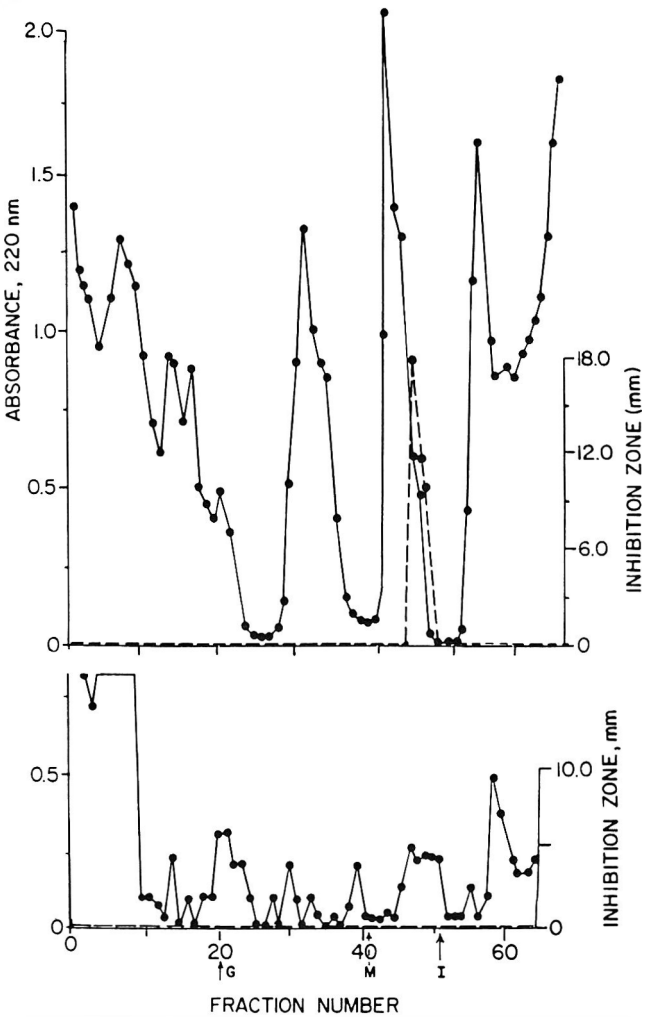

Fig. 1—Elution profile of L. bulgaricus *crude extract (top) and control extract (bottom) from LiChroprep RC-8 column. The letters G, M, and I indicate the fraction number at which the methanol gradient, 100% methanol, and 100% isopropanol were applied to the column, respectively. Solid lines indicate the absorbancies of fractions, and dotted lines indicate the sizes of inhibition zones.*

Stability of antimicrobial activity

pH Effects. Samples of LCE and CCE were adjusted to pH values in the range 1.0–12.0 using 1.0N HCl or 1.0N NaOH. Antimicrobial activities were then measured after storage at 6°C for 24 hr. Examination of inhibition zones showed that pH 5.0–12.0 resulted in complete inactivation of LCE. A one-way analysis of variance showed differences among inhibitory samples to be significant ($p < 0.01$) and a graphical multiple comparison of treatment means indicated that significant differences existed between pH 4.0 and any other pH value (data not shown). Furthermore, pH 4 proved to be the optimum pH as the largest difference (4.17 mm) in sizes of inhibition zones were observed at that pH for LCE compared for CCE. Reddy et al. (1983) reported that the activity of bulgarican was affected by pH. Bulgarican was active at acidic pH, but exhibited reduced activity at pH 6.0 and no activity above pH 7.0. Acidic pH optima have also been observed for an antimicrobial substance produced by *Lactobacillus acidophilus* (Hamdan and Mikolajcik, 1974; Mehta et al., 1983).

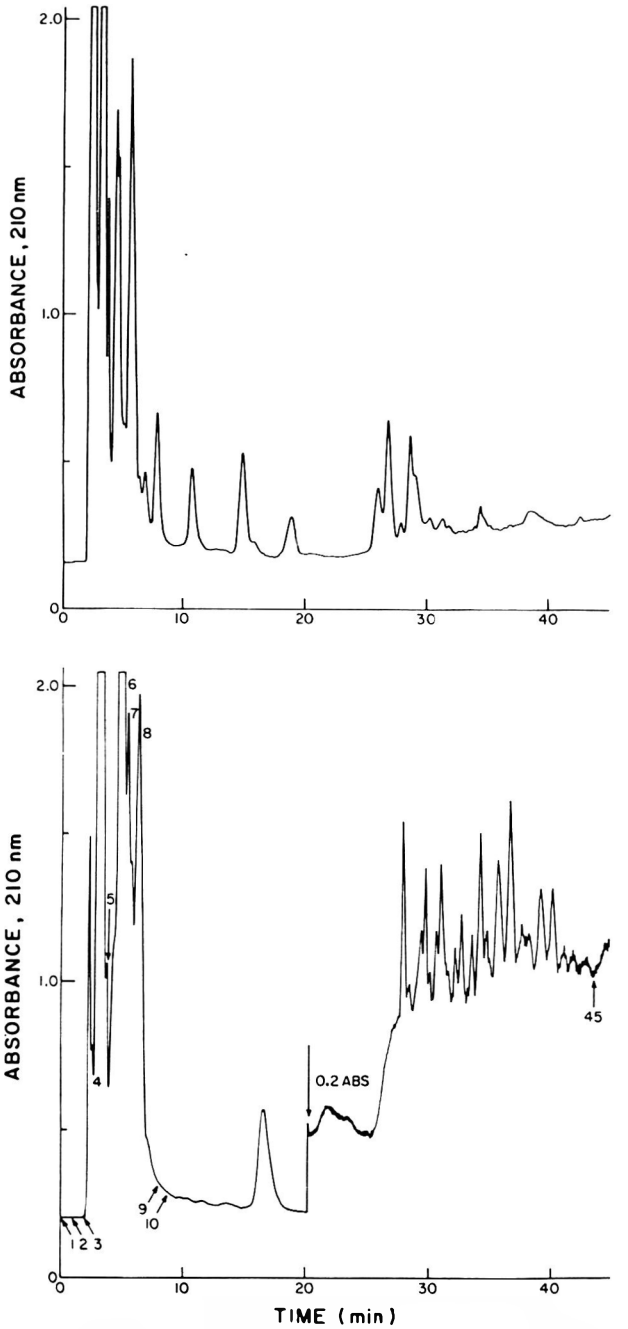

Fig. 2—Reverse phase HPLC chromatogram of L. bulgaricus *crude extract (top) and active material recovered from the RC-8 column (bottom). Activity was recovered mainly in fraction 4.*

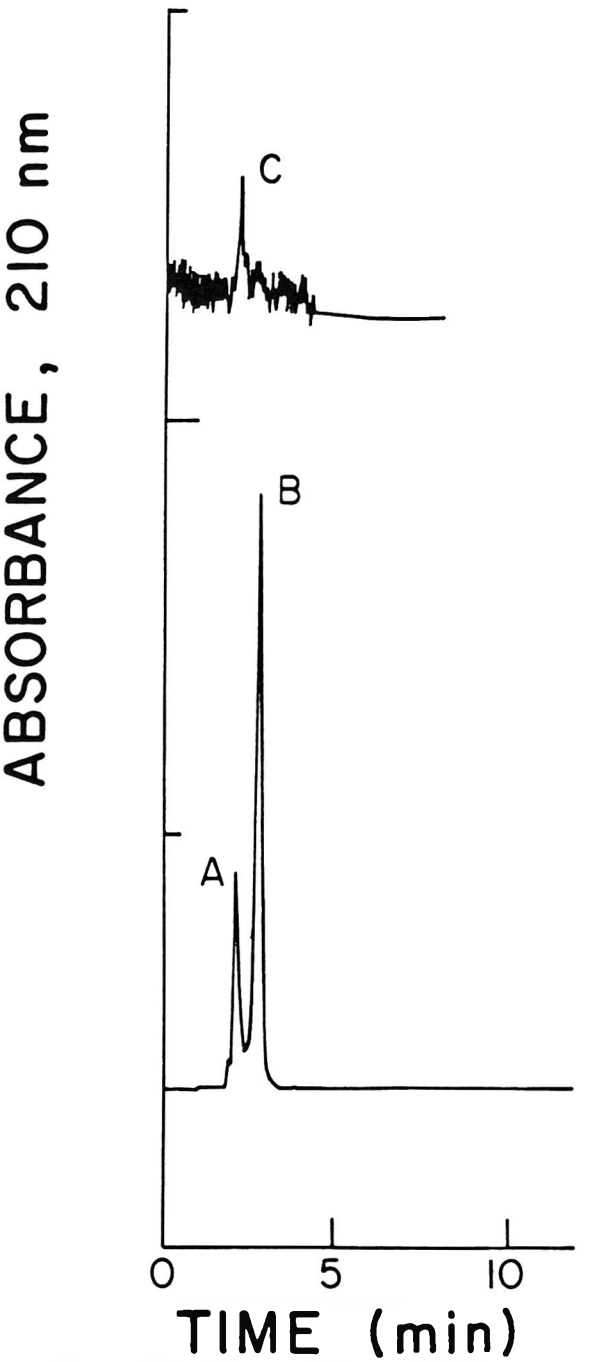

Fig. 3—Rechromatography of purified antimicrobial substance (bottom) and phosphate buffer alone (top). Activity was recovered in peak B only. Peak A was the phosphate buffer front.

Thermal stability. One milliliter samples LCE, adjusted to pH 4.0, were incubated at different temperatures (25°, 60°, 100°, and 121°C) for increasing lengths of time up to 1 hr to examine the thermal stability of the antimicrobial activity. Activity was stable for at least 1 hr at 100°C, but significant activity was lost by autoclaving (121°C) (Table 2). Samples stored under refrigeration (6°C) were stable for at least 18 days, after which significant loss of activity was observed (Table 2). This behavior is similar to that of acidophilin (stable for 30 days at 5°C, Shahani et al., 1977) and bulgarican (stable for 9 days at room temperature, Reddy et al., 1983).

Purification of antimicrobial substance from crude extract

Preparative reverse phase chromatography on the RC-8 column was effective in resolving multiple LCE components. Antimicrobial activity was localized only in fractions eluting after application of 100% methanol, significantly behind the major extract components (Fig. 1). No antimicrobial activity was detected in corresponding fractions obtained upon identical chromatography of control extract (CCE) containing lactic acid (Fig. 1), hence the active fraction recovered from LCE contained a fermentation product other than lactic acid. This finding is in agreement with Hamdan and Mikolajcik (1974) and Reddy et al. (1983) who reported that acidolin and bulgarican, respectively, did not contain lactic acid.

Contiguous active fractions were pooled and concentrated. High performance chromatography on a fully capped ODS-5 reverse phase column showed that the applied sample was still very complex. Active substance eluted rapidly from the column and was predominantly recovered in a one tube corresponding to a single, strong peak in the chromatogram (Fig.

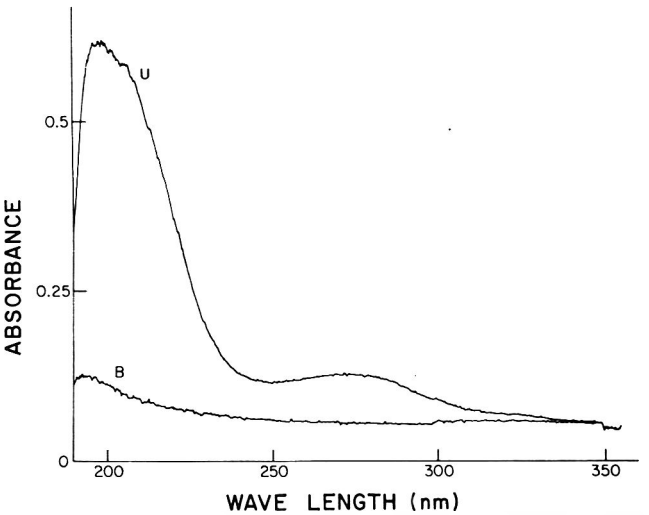

Fig. 4—Ultraviolet absorption spectrum of purified antimicrobial substance (U) and phosphate buffer (B).

2). Rechromatography of the concentrated active fraction under isocratic conditions (phosphate buffer) revealed two peaks; only the second peak (B) was active (Fig. 3). Injection of sample buffer alone identified the first peak as the sample buffer front (Fig. 3). Chromatography of several 200 μL aliquots yielded sufficient highly purified antimicrobial substance for preliminary characterization.

Partial characterization of purified antimicrobial substance

Results of Biuret tests were negative, indicating the antimicrobial substance was not a polypeptide longer than 2–3 residues. The result is also consistent with the early elution of active substance from the ODS-5 column, since proteins and oligopeptides are strongly retained under the conditions used (Rill and H. Henricks, unpublished observations). This finding is similar to that of Pulanski et al. (1979), who reported that antimicrobial substance produced by *Streptococcus* were amines of low molecular weights. Treatment with catalase did not diminish antimicrobial activity, ruling out possible contributions from hydrogen peroxide.

The absorption spectrum of the purified substance exhibited a broad band in the 255–290 nm region, with maximal absorbance at approximately 274 nm, and an intense band below 200 nm (Fig. 4). The long wavelength band is consistent with an aromatic moiety.

Prominent mass peaks were observed at M/E = 91.1 and 179.2 in the negative chemical ionization mass spectrum of the antimicrobial substance, and at M/E = 91.0, 135.1, 163.1, and 235.1 in the positive chemical ionization mass spectrum (Fig. 5). The mass spectrum was similar to that reported for acidolin by Hamden and Mikolajcik (1974), who detected peaks

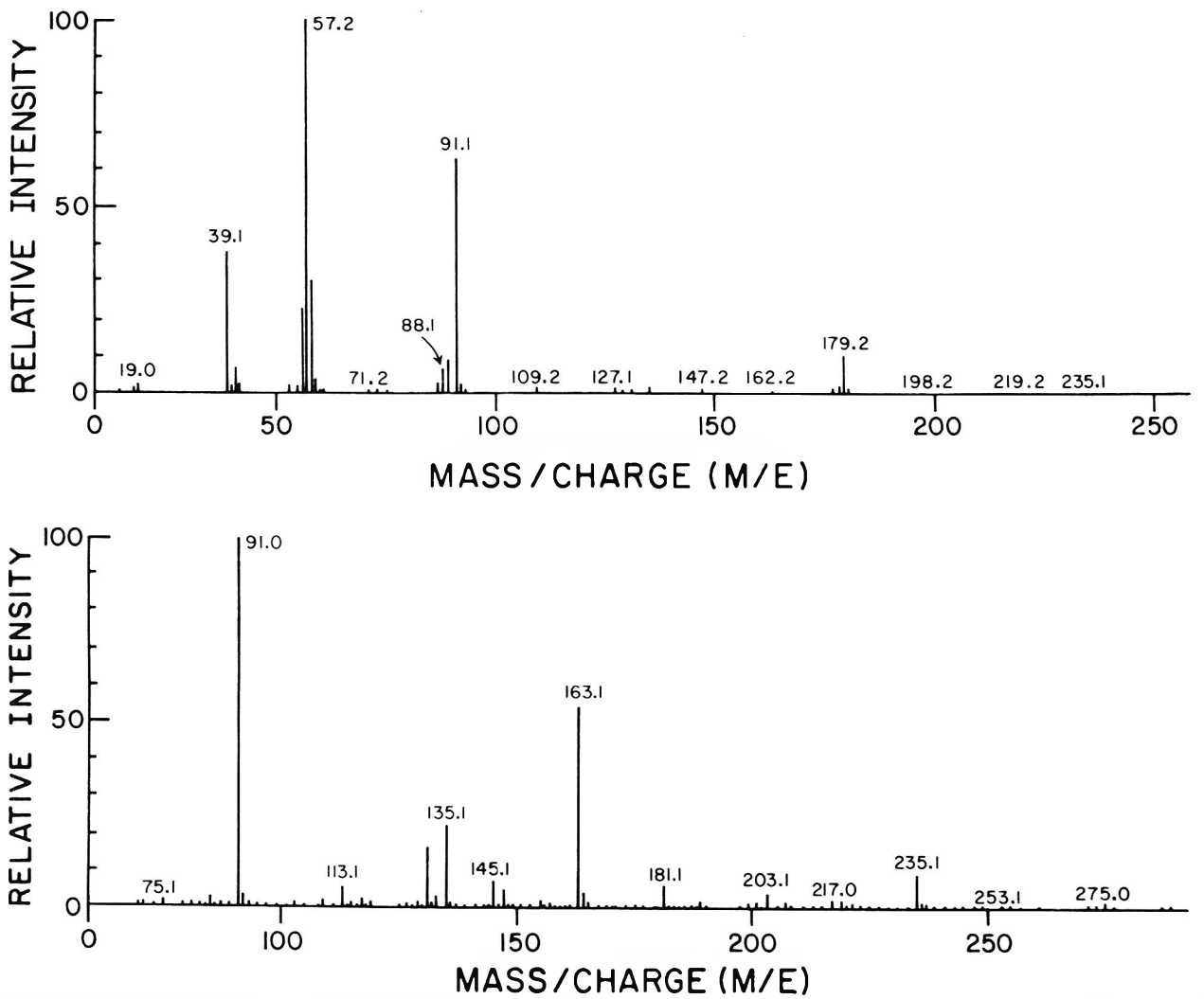

Fig. 5—Mass spectra of purified antimicrobial substance. Top spectrum: negative chemical ionization. Bottom spectrum: Positive chemical ionization. The peak at 57.2 is an artifact (C_4H_9) from isobutane.

at m/e = 52, 77, and 91 and suggested the presence of a cyclic function. Both the 91 m/e species and the UV spectral data for the antimicrobial substance isolated from *L. bulgaricus* in the present study are consistent with the presence of a benzyl moiety (C_7H_7). The available data were not sufficient to make a unique assignment of this or other mass peaks, however. No significant peaks of higher mass were observed, even at very low ionization potentials. An upper molecular weight limit of approximately 700 was suggested by the sum of molecular weights of major species observed in the positive chemical ionization mass spectrum. Further analyses must be done to ascertain the purity of the active substance after ODS-5 chromatography; however, the simplicity of the mass spectra observed indicated that levels of contaminants were small.

Although antimicrobial substances produced by a variety of lactic acid producing bacteria have been studied, very little work has been reported on the chemical nature of the inhibitory substance produced by *L. bulgaricus*. Reddy et al. (1983) found only one of three strains of *L. bulgaricus* tested produced antimicrobial activity. In a crude purication procedure using silica gel chromatography, they found that most of the fractions exhibited antimicrobial activity. They pooled three of the most active fractions, termed the active substance bulgarican and used the material for studies of activity and stability. Results of the present study of optimum pH for antimicrobial activity agree with those of Reddy et al. (1983) in that the substance is active at acid pH levels; however, the antimicrobial substance examined in the present study was inactivated at pH 5. The optimum pH of the material studied here was pH 4, while Reddy et al. (1983) reported a pH optimum of 2.2 for bulgarican. Both bulgarican and the antimicrobial substance described in the present study appear highly stable. Bulgarican is reportedly stable for 9 days at pH 2.2 and activity was retained after autoclaving at 120°C for 60 min. The substance described here was stable at 6°C for 18 days and was moderately stable to heating up to 100°C (Table 2). Reddy et al. (1983) made no attempt to identify the chemical structure of the antimicrobial substance.

In summary, the nonlactic acid antimicrobial activity produced by *L. bulgaricus* occurred in the extracellular medium, was water soluble, was a relatively low molecular weight compound, and probably contained an aromatic group. It was active against both *S. aureus*, a Gram-positive organism, and *P. fragi*, a Gram-negative organism, but expressed greater activity towards the latter. At pH 4.0, the pH of optimal activity, the activity was stable to storage at 6°C for at least 18 days and moderately stable to heating up to 100°C. Although the present study has provided new information regarding the nature of an antimicrobial substance produced by *L. bulgaricus*, more work is needed for a complete chemical characterization of the substance. Studies are also needed which address the mechanisms of inhibition of other microbes by lactic acid bacteria.

REFERENCES

Abdel-Bar, N.M. and Harris, N.D. 1984. Inhibitory effect of *Lactobacillus bulgaricus* on psychotrophic bacteria in associative cultures and in refrigerated foods. J. Food Prot. 47: 61.

Angelo, J.A., Shahani, K.M., and Ayelo, A.D. 1980. Estimation of antimicrobial activity of lactic cultures. J. Dairy Sci. 63 (Suppl): 52.

Branen, A.L., Go, H.C., and Genske, R.P. 1975. Purification and properties of antimicrobial substances produced by *Streptococcus diacetilactis* and *Leuconostoc citrovorum*. J. Food Sci. 40: 446.

Dahiya, R.SD. and Speck, M.L. 1968. Hydrogen peroxide formation by lactobacilli and its effect on *Staphylococcus aureus*. J. Dairy Sci. 51: 1568.

Gilliland, S.E. and Martin, D.R. 1980. Inhibitory action of *Lactobacillus bulgaricus* toward psychotrophic bacteria from raw milk. Miscellaneous publication, Oklahoma Agricultural Exp. Sta. No. 107: 45.

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

Hamdan, I.Y. and Mikolajcik, E.M. 1974. Acidolin: An antibiotic produced by *Lactobacillus acidophilus*. J. Antibiotics. 27: 631.

Mehta, A.M., Patel, K.A., and Davee, P.J. 1983. Purification and properties of the inhibitory protein isolated from *Lactobacillus acidophilus* AC1. Microbiology 38: 73.

Oser, B.L. 1965. In "Hawk's Physiological Chemistry," 14th ed., p. 179. McGraw-Hill Book Co., New York.

Pulusani, S.R., Rao, D.R., and Sunki, G.R. 1979. Antimicrobial activity of lactic cultures: Partial purification and characterization of antimicrobial compounds(s) produced by *Streptococcus thermophilus*. J. Food Sci. 44: 575.

Reddy, N.S. and Ranganathan, B. 1983. Preliminary studies on antimicrobial activity of *Streptococcus lactic* subsp. *diacetylactis*. J. Food Prot. 46: 222.

Reddy, G.V., Shahani, K.M., Friend, B.A., and Chandan, R.C. 1983. Natural antibiotic activity of *Lactobacillus acidophilus* and *bulgaricus:* III: Production and partial purification of bulgarican from *Lactobacillus bulgaricus*. Cult. Dairy Prod. J. 18: 15.

Shahani, K.M., Vakil, J.F., and Kilara, A. 1977. Natural antibiotic activity of *Lactobacillus acidophilus* and *bulgaricus*. II. Isolation of acidophilin from *L. acidophilus*. Cult. Dairy Prod. J. 12: 8.

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Serological Reactivity and In Vivo Toxicity of *Staphylococcus aureus* Enterotoxins A and D in Selected Canned Foods

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ABSTRACT

Staphylococcal enterotoxins A (SEA) and D (SED) were added to infant formula and to cream of celery soup to determine the persistence of their serological and biological activities during simulated commercial canning. When both food products were retorted at 123.9°C with F_0 values of approximately 3 and 8, the enterotoxins were reduced or inactivated serologically; however, they were found to be biologically active when injected into kittens. It was concluded that SEA and SED remain active under some canning conditions and are potential hazards to health.

INTRODUCTION

THE ENTEROTOXINS produced by some strains of *Staphylococcus aureus* are responsible for numerous cases of foodborne disease. Although most outbreaks of staphylococcal food poisoning are caused by foods that do not receive stringent heat treatment, canned foods have occasionally been incriminated in illness that displays the symptoms of staphylococcal enterotoxin intoxication. For example, the symptoms of several persons who used boiled goat's milk in their coffee were typical of food poisoning; these symptoms were also observed in a monkey fed the incriminated product (Drysdale, 1950). Other foods in which staphylococci were rendered nonviable by heat but yet caused typical intoxications include cooked sausage (Tatini, 1976), spray-dried milk (Armijo et al., 1957; Anderson and Stone, 1955), lobster bisque (CDC, 1976) and processed baby formula (Bennett, 1982). Because these cases involved heat-treated foods, interest has again focused on the protein that is the enterotoxin and on factors such as menstruum, pH, presence or absence of salt or sugar, time-temperature heating parameters and the animal systems, chemical alterants (Tatini, 1976) and serological tools (Bennett, 1978) used in their study.

Staphylococcal enterotoxin B (SEB) is used most frequently in experimentation, probably because of its availability. Studies of other enterotoxins have generally involved high temperatures in heating menstruums: distilled water (Denny et al., 1966), veronal buffer (Read and Bradshaw, 1966; Hilker et al., 1968; Stinson and Troller, 1974), sodium acetate (Soo et al., 1973; Tatini, 1976), phosphate buffers, phosphate-buffered saline (Tatini, 1976; Reichert and Fung, 1976; Chu et al., 1966) and bacteriological broth media (Jordan et al., 1931; Bergdoll et al., 1951; Denny et al., 1966; Jamlang et al., 1971; Fung et al., 1973). Only a limited number of studies have been conducted in actual food substrates or food extracts (Read et al., 1965; Denny et al., 1971; Humber et al., 1975; Soo et al., 1973; Tatini, 1976; Lee et al., 1977).

Studies on the effect of retort temperatures on the biological and serological activities of staphylococcal enterotoxins A (SEA) (Bennett et al., 1977) and D (SED) (Bennett and Bradshaw, 1980) used 0.25% bovine serum albumin as a heating menstruum to simulate the protein content of foods. These studies showed that a retention in biological activity follows reduced serological activity. Bovine serum albumin has also been used in other studies (Stelma et al., 1980) of thermal inactivation of SEA.

To evaluate the potential hazard of staphylococcal enterotoxins in foods retorted for canning, small amounts of SEA or SED were added to infant formula and condensed cream of celery soup before retorting at 123.9°C with F_0 values of approximately 3 and 8.

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MATERIALS & METHODS

Organisms

S. aureus strain FDA 743 was used to produce SEA: strain "Japan" was used to produce SED.

Enterotoxin production

Crude toxins were produced in flasks of brain heart infusion broth (pH 3.5) on a gyratory shaker (Casman and Bennett, 1963). Culture broths containing toxin were collected by centrifugation, filtered and concentrated by the Amicon method and/or by osmotically forced dialysis in 30–50% polyethylene glycol (PEG) 20,000 (Carbowax 20 M, Union Carbide Corp., New York, NY). Enterotoxins in the concentrates were determined by the microslide gel double diffusion test (Bennett and McClure, 1976).

Product preparation

Infant formula and condensed cream of celery soup were prepared on the day of canning. Water, instant nonfat dry milk, granulated sugar, soybean oil, an emulsifier and a stabilizer were used to prepare infant formula with a proximate analysis similar to a 135 kcal/100 mL concentrated infant formula (Berry and Kohnhorst, 1984). SEA or SED were added to the formula at respective rates of 1.2 and 2.4 μg toxin/100g of separate split batches of product. The formula was handfilled by weight (450 ± 0.5g) into 300 × 407 double-seamed cans and placed in a water bath to equilibrate to a temperature of 10°C before processing.

Condensed cream of celery soup was prepared from a commercial formula by using water, frozen celery, a dry premix, cream and soybean oil (Berry and Bradshaw, 1980). SEA and SED were added to the soup at a rate of 2.5 μg/100g of product. To ensure uniform distribution, the toxins were added to the soup while the ingredients were being mixed at 49°C but before the product thickened during heating to 88°C. The soup was poured immediately into 300 × 407 cans (420g) and equilibrated to 79°C in a water bath before processing. The fill weights used for the two products were chosen to give a gross headspace of 6.0 mm, similar to commercial processes.

Unheated control cans, with and without enterotoxin, were filled and handled identically until processing began. Cans containing 1.6-mm diameter, copper-constantan thermocouples (CNS-Eklund Custom Thermocouples, Cape Coral, FL) for measuring temperature at the geometric center of the cans were filled under the same conditions (three cans for each batch) so that the degree of thermal processing could be calculated from the temperature/time data.

Thermal processing

The cans were processed in a laboratory model Steritort (FMC Corp., Processing Machinery, Madera, CA) that simulates the continuous-agitating FMC Sterilmatic retort. The product is agitated as the cans roll freely on their rims around the periphery of the horizontal pressure vessel. Each batch of cans was processed in steam at 123.9°C with a Steritort reel rotational speed of 9 rpm. The cans containing thermocouples were connected via slip rings to an electronic data acquisition system (Berry and Bradshaw, 1980) that recorded the temperature/time data on magnetic tape. The tapes were later used to automate data reduction to parameters useful in describing the heating process and the sterilization value (F_o). (The F_o is the equivalent process time in minutes, at a temperature of 121.1°C, received by the slowest heating point in the can.) F_o values were calculated by the general method (Stumbo, 1973), assuming a z value of 10°C.

The two batches of infant formula containing SEA and SED were split and processed for 3.2 and 5.9 min; these times were sufficient for intended F_o values of approximately 3 and 8 (Berry and Kohnhorst, 1984). Such F_o values are representative of minimal and normal values for a commercial product. The infant formula processing was preceded by a two-stage, 12 min preheat which increased the retort and product temperature to 100°C.

The celery soups containing SEA and SED were mixed and processed separately for intended F_o values of 3 and 8 (4 batches). The Steritort was preheated to 123.9°C before the cans of celery soup were installed to minimize the effect of the retort's come-up time (about 45 sec) on the heating of the product. Because the viscosity of each batch of celery soup differed somewhat and affected the heating rates, the F_o was calculated during processing to determine the required processing time for each run. This was done at 30 sec intervals, using a hand-held calculator. For an intended F_o of 3, the necessary processing time was 10.2 min for both batches containing SEA and SED. For an intended F_o of 8, the processing times were 14.6 and 16.0 min, respectively.

At the end of heating, the steam was turned off and the cans were spray-cooled with tap water in the retort until the product temperature was <35°C. The unheated control cans of celery soup and infant formula were cooled in cold water, with intermittent shaking, at the same time the retort run began.

Extraction and chromatography of food

Samples of unheated and heated infant formula and soup were extracted and toxins were separated from food proteins by ion exchange chromatography, using carboxymethyl cellulose (CMC) physical variant 22. These studies were conducted by the method of Casman and Bennett (1965) as modified by Bennett and McClure (1980). The eluates were placed in dialysis tubing, concentrated completely in 30% polyethylene glycol and then lyophilized. Lyophilized CMC food eluates not containing either SEA or SED were eluted from the columns under the same conditions as those containing the enterotoxins.

Serological assay for the enterotoxins

Heated and unheated enterotoxins in the chromatographed eluates were studied serologically by the microslide gel double diffusion test (Casman et al., 1969) described in detail by Bennett and McClure (1980).

Bioassay for enterotoxin activity

Eluates that were lyophilized and chromatographed from the retorted products containing SEA and SED were rehydrated in physiological saline and injected intravenously into kittens. The kittens were observed for emesis, which would indicate that the heated enterotoxin had retained its biological activity.

RESULTS & DISCUSSION

F_o VALUES FOR SEA and SED in retorted infant formula and condensed celery soup are shown in Table 1. The intended and delivered F_o values are compared for the two products and processing times at 123.9°C in the Steritort. The infant formula cans were heated rapidly and uniformly, and single F_o values (3.3 and 8.2) describe the thermal process delivered to each can for the two levels of processing. Heating rates for the cans of celery soup were much slower and varied—a normal occurrence for viscous products like condensed soup. The range of delivered F_o values describes (for each batch processed) the fastest- and slowest-heating of the three cans containing thermocouples at their geometric centers. In general, delivered F_o values were higher for the celery soup than for the infant formula for the same intended F_o value (3 or 8). Some of the extra sterilization value occurred during cooling, which took considerably longer for the celery soup. Also, the process was not ended until the desired F_o value was delivered to the slowest-heating can. The exception was the celery soup with SED heated to an F_o value of 3 when the same processing time (10.2 min) was used for both enterotoxins in lieu of calculating F_o during processing.

Table 1—Sterilization values (F_o) of staphylococcal enterotoxins A and D in infant formula and cream of celery soup

Product	Enterotoxin serotype	Processing time (min in Steritort at 123.9°C)	F_o value (equiv. min at 121.1°C) Intended	Delivered
Infant formula	A, D	3.2	3.0	3.3
	A, D	5.9	8.0	8.2
Celery soup	A	10.2	3.0	3.7- 4.6[a]
	A	14.2	8.0	9.5- 9.8
	D	10.2	3.0	2.5- 3.1
	D	16.0	8.0	8.0-10.9

[a] Values for slowest- and fastest-heating cans.

Table 2—Comparative serological fate of staphylococcal enterotoxins A (SEA) and D (SED) in retorted infant formula and condensed cream of celery soup

Product	F_o value (equiv. min at 121.1°C) for 123.9°C steritort process	Serological activity in chromatographed eluates[a] SEA 1.0 mL	SEA 0.25 mL	SED 1.0 mL	SED 0.25 mL
Infant formula	3	+[b]	NT[c]	–[d]	–
	8	–	–	–	–
Celery soup	3	–	–	–	–
	8	–	–	–	–

[a] Foods extracted and processed by ion exchange chromatography.
[b] Serologically detectable enterotoxin.
[c] NT, not tested serologically.
[d] No serologically detectable enterotoxin.

Table 2 compares qualitative data on the serological activity of SEA and SED in retorted infant formula and celery soup subjected to F_o values of 3 and 8 at 123.9°C. Although some SEA survived when the infant formula was retorted at an F_o value of 3, it was completely inactivated when the retort time was extended to an F_o value of 8. In contrast, SED was serologically inactivated at both F_o values (3 and 8). Under the conditions of this study, SEA was slightly more resistant to the effects of heat than SED at an F_o value of 3. Table 2 also shows representative data on the serological fate of SEA and SED in retorted condensed cream of celery soup processed at the same sterilization times. In contrast to the retention of serological activity by SEA in retorted infant formula at an F_o value of 3, SEA in retorted soup was serologically inactivated when both the chromatographed eluates were tested at the 1.0 mL and 0.25 mL levels of the eluates. Similarly, in the celery soup, SEA was serologically inactivated at an F_o value of 8, and SED was inactivated at both F_o values.

Kittens were injected with heated chromatographed toxins to determine whether there was a direct correlation of serological and biological activities. Table 3 shows the biological response in kittens to SEA in retorted infant formula. The SEA in this product retained some serological activity when subjected to an F_o value of 3 and produced emetic responses in 4 of the 6 kittens injected. In contrast, SEA in infant formula which was heated to an F_o value of 8 was serologically inactivated, although this toxin also produced emesis in 4 of the 6 kittens injected.

The biological activity of SED in retorted infant formula is

Table 3—Biological activity of staphylococcal enterotoxins A (SEA) and D (SED) in retorted infant formula and condensed cream of celery soup

Product-toxin serotype	F_o value (equiv. min at 121.1°C) for 123.9°C steritort process	Biological activity in kittens: Serological activity	Biological activity in kittens: Emetic response / No. injected
Infant formula			
SEA	3	+[a]	4/6
SEA	8	−[b]	4/6
Infant formula			
SED	3	−	4/6
SED	8	−	3/6
Infant formula			
control[c]	3	ND[d]	0/6
control	8	ND	0/6
Celery soup			
SEA	3	−	5/6
SEA	8	−	3/6
Celery soup			
SED	3	−	1/6
SED	8	−	4/6
Celery soup			
control	3	ND	0/6
control	8	ND	0/6

[a] +, Serological activity present.
[b] −, No serological activity.
[c] Product contained no SEA or SED.
[d] ND, not done.

shown in Table 3. SED was serologically inactivated at the minimal F_o value of 3 and the maximum F_o value of 8. However, when injected with the chromatographed SED in infant formula, 4 of 6 and 3 of 6 kittens produced emetic responses with the F_o values of 3 and 8, respectively. Similar results were obtained for the biological activity of SEA in retorted condensed cream of celery soup, which received intended F_o values of 3 and 8 min for the 123.9°C process. Five of six kittens produced emetic responses from SEA in soup heated for an F_o value of 3, whereas 3 of 6 demonstrated biological activity from the effects of the enterotoxin with an F_o value of 8. This table also summarizes the response of kittens injected with SED contained in retorted cream of celery soup. Only 1 of 6 kittens produced an emetic response to SED heated to an F_o of 3, whereas SED heated for a longer time (F_o of 8) caused 4 of 6 injected kittens to become ill. These data suggest that fewer biological determinants were exposed with the F_o of 3 than with the F_o of 8 process and that there is less biological activity in the product treated at the F_o of 3. Product controls without SEA and SED showed no emetic response in kittens upon injection of the chromatographed eluates.

It was concluded that serologically reduced or inactivated enterotoxin can be biologically active, i.e., toxic. Consequently, staphylococcal enterotoxins that contaminate food products before canning may survive certain heat processes and be a potential hazard to health.

REFERENCES

Anderson, P.H.R. and Stone, D.M. 1955. Staphylococcal food poisoning associated with spray-dried milk. J. Hyg. 53: 387.

Armijo, R., Henderson, D.A., Timothee, R., and Robinson, H.B. 1957. Food poisoning outbreaks associated with spray-dried milk—an epidemiologic study. Am. J. Public Health 47: 1093.

Bennett, R.W. 1978. Antibody production and antigenicity of heat altered staphylococcal enterotoxin. American Society for Microbiology, Abstract P20, 1978: 189.

Bennett, R.W. 1982. Staphylococcal foodborne illness. In "Microbiological Safety of Foods in Feeding Systems," ABMPS Report No. 125, National Research Council, National Academy Press, Washington, DC.

Bennett, R.W. and Bradshaw, J.G. 1980. Serological and biological activities of staphylococcal enterotoxin type D. American Society for Microbiology, Abstract P12, 1980: 132.

Bennett, R.W., Bradshaw, J.G., and Amos, W.T. 1977. Biological and serological activities of heated staphylococcal enterotoxin A. American Society for Microbiology, Abstract P18, 1977: 257.

Bennett, R.W. and McClure, F. 1976. Collaborative study of the serological identification of staphylococcal enterotoxins by the microslide gel double diffusion test. J. Assoc. Off. Anal. Chem. 59: 594.

Bennett, R.W. and McClure, F. 1980. Extraction and separation of staphylococcal enterotoxin in foods: Collaborative study. J. Assoc. Off. Anal. Chem. 63: 1205.

Bergdoll, M.D., Kadavy, J., Surgalla, M., and Dack, G.M. 1951. Partial purification of staphylococcal enterotoxin. Arch. Biochem. 33: 259.

Berry, M.R., Jr. and Bradshaw, J.G. 1980. Heating characteristics of condensed cream of celery soup in a Steritort: Heat penetration and spore count reduction. J. Food Sci. 45: 869.

Berry, M.R., Jr. and Kohnhorst, A.L. 1984. Heating characteristics of homogeneous milk-based formulas in cans processed in an agitating retort. J. Food Sci. 50: 209.

Casman, E.P. and Bennett, R.W. 1963. Culture medium for the production of staphylococcal enterotoxin A. J. Bacteriol. 86: 18.

Casman, E.P. and Bennett, R.W. 1965. Detection of staphylococcal enterotoxin in food. Appl. Microbiol. 13: 181.

Casman, E.P., Bennett, R.W., Dorsey, A.E., and Stone, J.E. 1969. The micro-slide gel double diffusion test for the detection and assay of staphylococcal enterotoxins. Health Lab. Sci. 6: 185.

CDC. 1976. Foodborne and waterborne disease outbreaks—Annual summary, 1975. Centers for Disease Control, Atlanta, GA.

Chu, F.S., Thadhani, K., Schantz, E.J., and Bergdoll, M.S. 1966. Purification and characterization of staphylococcal enterotoxin A. Biochemistry 5: 3281.

Denny, C.B., Humber, J.Y., and Bohrer, C.W. 1971. Effect of toxin concentration on the heat inactivation of staphylococcal enterotoxin A in beef bouillon and in phosphate buffer. Appl. Microbiol. 21: 1064.

Denny, C.B., Tan, P.L., and Bohrer, C.W. 1966. Heat inactivation of staphylococcal enterotoxin A. J. Food Sci. 31: 762.

Drysdale, A. 1950. *Staphylococcus aureus* food poisoning. An account of an outbreak of Khartoum. J. Trop. Med. 53: 12.

Fung, D.Y.C., Steinberg, D.H., Miller, R.D., Kurantnick, M.J., and Murphy, T.F. 1973. Thermal inactivation of staphylococcal enterotoxins B and C. Appl. Microbiol. 26: 938.

Hilker, J.B., Heilman, W.R., Tan, P.L., Denny, C.B., and Bohrer, C.W. 1968. Heat inactivation of enterotoxin A from *Staphylococcus aureus* in veronal, buffer. Appl. Microbiol. 16: 308.

Humber, J.Y., Denny, C.B., and Bohrer, C.W. 1975. Influence of pH on the heat inactivation of staphylococcal enterotoxin A as determined by monkey feeding and serological assay. Appl. Microbiol. 30: 755.

Jamlang, E.M., Bartlett, M.L., and Snyder, H.E. 1971. Effect of pH, protein concentration and ionic strength on heat inactivation of staphylococcal enterotoxin B. Appl. Microbiol. 22: 1034.

Jordan, E.O., Dack, G.M., and Woolpert, O. 1931. The effect of heat and storage and chlorination on the toxicity of *Staphylococcus* filtrates. J. Prev. Med. 5: 383.

Lee, I.C., Stevenson, K.E., and Harmon, L.G. 1977. Effect of beef broth protein on the thermal inactivation of staphylococcal enterotoxin B. Appl. Environ. Microbiol. 33: 341.

Read, R.B., Jr. and Bradshaw, J.G. 1966. Thermal inactivation of staphylococcal enterotoxin B in veronal buffer. Appl. Microbiol. 14: 130.

Read, R.B., Jr., Prichard, W.L., Bradshaw, J., and Black, L.A. 1965. In vitro assay of staphylococcal enterotoxins A and B in milk. J. Dairy Sci. 48: 411.

Reichert, C.A. and Fung, D.Y.C. 1976. Thermal inactivation and subsequent reactivation of staphylococcal enterotoxin B in selected liquid foods. J. Milk Food Technol. 39: 516.

Soo, H.M., Tatini, S.R., and Bennett, R.W. 1973. Thermal inactivation of staphylococcal enterotoxins A and D. American Society for Microbiology Abstract E2, 1973: 1.

Stelma, G.N., Jr., Bradshaw, J.G., Kauffman, P.E., and Archer, D.L. 1980. Thermal inactivation of mitogenic and serological activities of staphylococcal enterotoxin A at 121°C. IRCS Med. Sci. Microbiol. Parasitol. Infect. Dis. 8: 629.

Stinson, J.V. and Troller, J.A. 1974. Characterization of the thermal destruction of enterotoxin B at frying temperatures. Work documents, topic 4C: Human infections and intoxications due to microbes associated with foods. IV International Congress of Food Science and Technology, Madrid, Sept. 22–27, 1974.

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

Tatini, S.R. 1976. Thermal stability of enterotoxins in food. J. Milk Food Technol. 39: 432.

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Experimental Errors Associated with the Estimation of Thermal Diffusivity from Thermal Process Data

JOHN W. LARKIN and JAMES F. STEFFE

ABSTRACT

An increase in the accuracy and precision of thermal diffusivity (α) was observed when estimated from thermal process data using the analytical heat conduction equation as compared to using the process f_h value. Differences in α for the two methods ranged from 2.4 to 3.5%. Correcting for the presence of the thermocouple probe became more important with smaller can sizes. The analytical solution to the heat conduction equation proved to be a very sensitive model with regard to prolonged retort come-up time and heat conduction along the thermocouple probe.

INTRODUCTION

AS PROCESS SIMULATION activity increases, there will be greater demands for accurate physical property data. One key physical property for thermal process simulation is thermal diffusivity. This parameter is required to predict time-temperature relationships that may cause changes in nutrients, texture, etc. Since thermal process data are easy to obtain and routinely collected, use of this information for estimating thermal diffusivity is desirable. However, experimentally obtained thermal process data have a number of inherent errors that may cause problems in estimating thermal diffusivity. Larkin and Steffe (1982) reviewed some of the problems (position of thermocouple probe, can dimensions, measurement of time and temperature, and assumptions concerning the surface heat transfer coefficient) using computer simulation to elucidate their effects. However, other factors (physical presence of thermocouple, head space, retort come-up time, and homogeneity of food product) are best investigated from experimental data.

The objective of this paper was to use a model food system (sodium-calcium alginate gel) to investigate the following: (1) compensation for heat conduction along a thermocouple probe, (2) can head space, and (3) retort come-up time. In addition, the influence of these factors in estimating thermal diffusivity using temperature matching (nonlinear regression) and the regular regime method was considered.

Measuring thermal diffusivity

In comparison to the thermal conductivity data available for food (Woodams and Nowrey, 1968; Polley et al., 1980), thermal diffusivity (α) has been a neglected parameter. Methods of estimating α can be grouped into four general categories (Nesvadba, 1982a; Singh, 1982): (1) heat pulse or heat source method, (2) direct use of temperature profiles, (3) temperature matching, and (4) regular regime method. Heat pulse and heat source methods usually entail a known heat source, either applied to the sample through the outside of the sample container or by the use of a probe inserted into the sample. The second group of estimation methods involves the use of an individual point of the data in conjunction with the analytical solution to the problem. Methods that fall into the category of temperature matching may include one of the other three methods; however, the principle attribute of the procedure is that of minimizing the difference between the measured and predicted temperatures. The regular regime method involves the estimation of a α from heat conduction data taken over long time periods, where the heat conduction curve follows a regular pattern, (straight line on semilog graph paper).

The heat pulse or heat source method (Moysey et al., 1977; Rao et al., 1975; Suter et al., 1975; Baghe-Khandan and Okos, 1981; Choi and Okos, 1983) of estimating α does not lend itself to thermal process data. A critical limitation when using temperature profiles (Flambert, 1974; Nesvadba, 1982b) to obtain α is that they rely on a single point (maximum or minimum) of temperature resulting in less precise estimates of α than alternative methods.

Nonlinear regression has become the most popular (Matthews and Hall, 1968; Ross et al., 1969; Albin et al., 1979; Narayana and Krishna Murthy, 1981) method of the different temperature matching calculation procedures. Others have relied on alternative calculation procedures (Hayakawa, 1971; Hayakawa and Bakal, 1973; Lenz, 1977; Young et al., 1983). Thermal process data has been used by few researchers, Lenz (1977) and Young et al. (1983), to estimate α. It should be noted that nonlinear regression is a method for data analysis, not data collection; thus, any data collection procedure can be used. For example, the line heat source method uses nonlinear regression when estimating both thermal conductivity and thermal diffusivity at the same time (Nix et al., 1967, 1969).

The regular regime method has received the most attention (Hicks, 1961; Teixeira et al., 1969; Annamma and Rao, 1974; Teixeira et al., 1975a, b; Bhowmik and Hayakawa, 1979; Ohlsson, 1980; Rizvi et al., 1980; Uno and Hayakawa, 1980; Peterson and Adams, 1983). Gaffney et al. (1980) made an extensive review of the use of the regular regime for estimating α, which also took into account the possibility of estimating finite surface heat transfer coefficients. A rather specialized regular regime method was developed by Dickerson (1965). In this method, a constant change in temperature is applied to an infinite cylinder, and α is estimated from a simplified solution to the transient heat conduction equation. Dickerson's procedure has not received a lot of attention, but was used by Rizvi et al., (1980) and Luna and Bressan (1985).

Theoretical development

Since 1923 when Ball published the "formula" method for thermal process calculations it has become a standard by which all other methods are compared. Olson and Jackson (1942) correlated the analytical solution to the heat conduction problem with parameters of the formula method and showed that f_h is directly related to α after long periods of heating. This correlation is commonly called the Olson and Jackson equation:

$$f_h = \frac{1}{\alpha}\left[\frac{0.398}{(1/R^2) + (0.4267/L^2)}\right] \quad (1)$$

Even though this equation is easy to use, it is limited by assumptions: (1) infinite surface heat transfer coefficient and constant surrounding temperature, (2) constant thermal prop-

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erties, and (3) use of just the first term of an infinite series approximation. A number of authors (Hicks, 1961; Teixeira et al., 1975a) found that estimating α from f_h results in estimation differences of 5% to 13%.

An alternative to the regular regime method of estimating α involves matching experimental temperatures (Moré et al., 1980) to the analytical solution to Fourier's heat conduction equation of an infinite slab (2) and an infinite cylinder (3):

$$\frac{d^2\theta}{dx^2} = \frac{1}{\alpha}\frac{d\theta}{dt} \tag{2}$$

$$\frac{1}{r}\frac{d\theta}{dt} + \frac{d^2\theta}{dr^2} = \frac{1}{\alpha}\frac{d\theta}{dt} \tag{3}$$

Equations (2) and (3) have been solved (Özişik, 1980) employing the following assumptions: (1) temperature independent physical and thermal properties, (2) homogeneous and isotropic materials, (3) heating by pure conduction, (4) uniform initial temperature, (5) environmental changes are instantaneous and remain constant (i.e., no lag time in retort come-up, (6) surface resistance will consist of apparent h values associated with external surface convection, conduction in container material and internal surface convection, and (7) there is no phase change in the product during heating. Both the temperature matching and regular regime methods involve only the heating phase when estimating α.

Error associated with physical presence of a thermocouple is primarily due to heat conduction along the thermocouple probe (Yoshida et al., 1982). This error increases in significance as the thermal conductivity of the probe increases over that of the food product. The difference in thermal conductivity causes the probe to act like a pin fin on a heat exchanger, where the probe tip becomes partially heated by heat transfer along the probe itself. Mathematical solution for conduction along a thermocouple probe only exists for special configurations (Jaeger, 1955) and, with the small size of probe, finite difference and finite element solutions require an extensive grid structure and computation time (Yoshida et al., 1982). When heat conduction along the thermocouple is assumed to be in a quasi-steady state condition (heat conduction within the probe equilibrates swiftly, compared to the slowly changing boundary conditions, so the heat conduction along the thermocouple can be modeled as a steady state problem), the temperature distribution of a radially mounted probe can be described (Larkin, 1984) as

$$\frac{d^2T(r,t)}{dr^2} - m^*\left(T(r,t) - T_c(r,t)\right) = 0 \tag{4}$$

where m*, the probe factor, is equal to hP/(kA). The analytical solution to Eq. (4) with r = R (center of can) was determined by Larkin (1984):

$$\theta^*(t) = \theta(t)\left\{1 + \frac{2e^{-m^*R}}{(m^*R)^2}\left[\tanh(m^*R) - 1\right] + \frac{2}{m^*R}\left[\frac{1}{m^*R} - \tanh(m^*R)\right]\right\} \tag{5}$$

When the probe factor is large (m* > 100), Eq. (5) is approximated by

$$\theta^*(t) = \theta(t)\left\{1 + \frac{2}{m^*R}\left[\frac{1}{m^*R} - 1\right]\right\} \tag{6}$$

Using Eq. (5) or (6) and the analytical solution to Fourier's heat conduction equation ($\theta(t)$), the probe factor can be estimated from a thermal process for a product with known α. When the probe factor is known, α can be estimated from the thermal process data of specific food products.

Table 1—Diffusivity values, estimated using analytical heat conduction equation, for repeated thermal processes of the same 2% KELSET-water sample

Run	Diffusivity (m^2/hr)
1	0.000592
2	0.000594
3	0.000595
4	0.000622

Table 2—Can sizes used for data collection

Can number	Radius (R) (m)	Half height (L) (m)	L/R
303 × 406	0.0383	0.05250	1.370
307 × 409	0.0417	0.05575	1.340
401 × 411	0.0516	0.05650	1.141

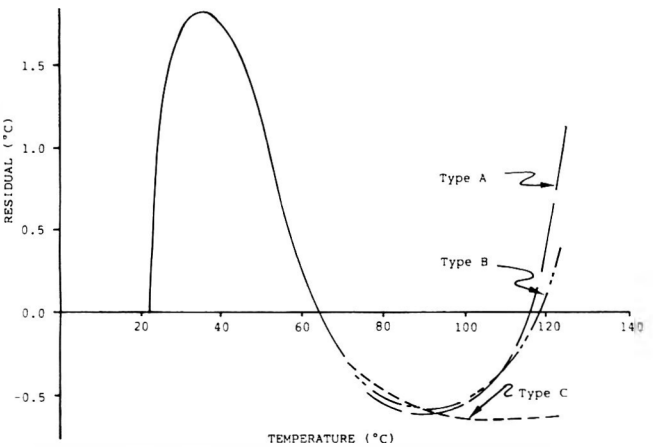

Fig. 1—Three types of residual error found when estimating thermal diffusivity from thermal process data using Eq. (2) and (3).

MATERIALS & METHODS

The model food system

In considering thermal process data, a product with known thermal properties must be used to quantify the effect of heat conduction along the thermocouple probe, the error associated with neglecting finite surface heat transfer coefficients, and the effect of inherent process variations on the estimation of α. Since many foods are largely composed of water, the thermal properties of these materials are going to be similar to water; however, to use water as a model system, a convection heating inhibitor must be added.

This study required a water based model food system that was pourable. It was discovered that a 2% solution of a commercial gum — KELSET (sodium-calcium alginate distributed by KELCO, a division of Merck and Company, Inc.)—would produce a highly viscous system where convection heating was eliminated. The material was chosen because it has a high viscosity with a low solids content and retains much of its viscous character at high temperatures. It was found that multiple process (3 or more heating-cooling cycles) caused KELSET to break down and decrease in viscosity. This caused an increase in convention heating and a resulting increase in apparent α (Table 1). Because of this, KELSET mixtures were never heated for more than one cycle.

KELSET solutions were prepared by measuring a volume of deionized water to which 3% (by volume) 2N solution of hydrochloric acid (HCl) was added. the HCl prevented the KELSET from thickening during mixing. The low pH water was stirred with a rotary mixer at a high speed during which the KELSET was added (2% by weight) very slowly, to prevent lumping. Then an equal amount of 2N sodium hydroxide was added to neutralize the solution causing it to thicken to a

Table 3—Average estimated diffusivity values for 2% KELSET solutions[a]

Can size	$\alpha_1 \times 10^3$ (m^2/hr)	MSE (C)	$\alpha_2 \times 10^3$ (m^2/hr)	m* (m^{-1})	MSE (C)	$\alpha_3 \times 10^3$ (m^2/hr)
	Estimation method					
	Fo		Fo and m*			f_h
303 × 406						
$\bar{x}$	0.6023	0.0632	0.5987	5001	0.0295	0.6016
$S_{\bar{x}}$	0.00502	0.0274	0.00595	1153	0.0313	0.0188
307 × 409						
$\bar{x}$	0.6107	0.3267	0.5973	1884	0.00294	0.6009
$S_{\bar{x}}$	0.00677	0.2460	0.00471	488	0.00328	0.0178
401 × 411						
$\bar{x}$	0.5989	0.0987	0.5918	3864	0.0108	0.6106
$S_{\bar{x}}$	0.00448	0.0960	0.00398	1680	0.0277	0.0233

[a] α_1, estimated using Eq. 2 and 3; α_2, estimated using Eq. 2, 3, and 5; α_3, estimated using Eq. 1.

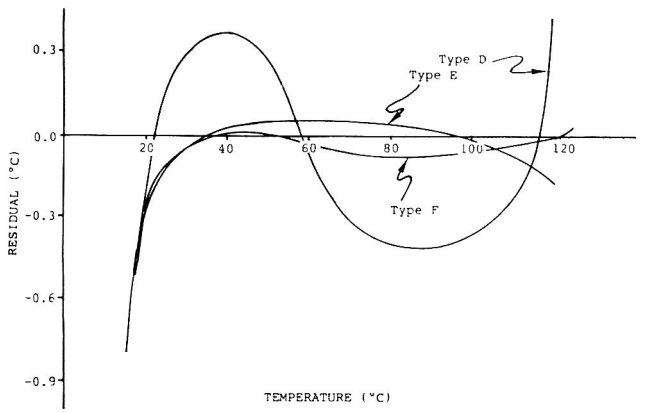

Fig. 2—Three types of residual error found when estimating thermal diffusivity from thermal process data using Eq. (2) and (3) and a correction for heat conduction along the thermocouple probe.

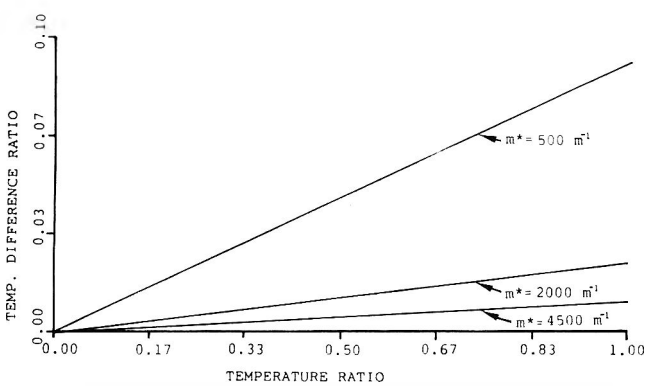

Fig. 3—Difference in temperature ratio resulting from correction of heat conduction along the thermocouple probe for three different probe factors.

pourable gel. By using the acid and the base, the amount of high speed mixing was reduced; thus the incorporation of air was minimized.

Experimental procedure

Temperature measurements for a thermal process are usually taken using the "nonprojecting" Ecklund thermocouples (Ecklund, 1949). Heat conduction errors due to the presence of the Ecklund thermocouple were demonstrated by Ecklund (1955) and alluded to by Teixeira et al. (1975b).

To investigate the influence of conduction related errors on the estimation of α, temperature measurements were conducted with both an Ecklund thermocouple (part no. C-1, 39.69mm length) and a mineral insulated probe (type T thermocouple, LOVE Controls Corporation, part no. 1818-57). The probe sheath (1.59mm diameter) was made of 304 stainless steel and allowed for an exposed junction. The mineral insulated thermocouple was mounted on the can with the use of a nylon fitting ($\alpha = 1.136 \times 10^{-7}$ m^2/sec) made specially for this research project. After mounting, and before can filling, the probe was positioned in the center of the can. Cans were filled as full as possible to eliminate head space effects.

Three cans sizes (Table 2) were utilized throughout the study. The 303 x 405 was used in conjunction with the Ecklund thermocouple and the mineral insulated thermocouple was used in the 307 x 409 and 401 x 411 cans. All experiments were performed in a mini-retort where the come-up time was less than 90 sec. Initial sample temperatures varied from 20°-25°C and the retort temperature was always 121°C.

Data were collected with a Hewlett Packard Model 3045 DL data acquisition system, at 45 sec intervals for the duration of a test (70-85 min). A test run was completed when the temperature ratio difference between the retort and the product dropped below 0.10. Data were stored on a magnetic tape and later uploaded to a main-frame CDC computer for analysis using programs described by Larkin and Steffe (1982). Only data within a temperature ratio (θ) range of 0.15 to 0.85 were considered in estimating α.

The can surface heat transfer coefficient (h) was determined using a finite copper cylinder the size of the 303 x 406 can. The Biot Number of the cylinder was estimated with the same programs used for estimating α. Before placement into the retort, the cylinder was kept in a water bath for over two hrs to establish a uniform initial temperature. Immediately, upon transfer from the water, the retort was sealed and started. Data were collected every 5 sec.

To establish an estimate of precision in estimating α from thermal process data, 25 runs for each can where completed using the KELSET model system. These runs, a total of 75, were also used to estimate the probe factor associated with the experimental thermocouples. Two additional runs each were completed for an 8 min come-up time with a known can head space of 1.27 cms.

RESULTS & DISCUSSION

Errors in estimating thermal diffusivity

To verify the assumption of an infinite surface heat transfer coefficient, the surface resistance (h) was estimated for a steam environment using solid copper cylinder with known dimensions and thermal properties, along with the assumption of lumped-capacity heating. Since the time required to place the copper cylinder in the retort, seal it, and establish pressure (15 psig) took 20 to 25% of the total process time,simulation of a step-change in surface temperature was not possible. Thus, any estimate of the surface resistance would be low. An average surface resistance of about 3700 W/m^2C was measured for a number of runs. This corresponds to a lumped-capacity value (h(V/A)/k) of 85.0 for the 303 x 406 can. Considering the fact that this value is lower than the true value and that Ramaswamy et al. (1983) reported h values of 11,000 W/m^2C for condensing steam, the surface heat transfer coefficient was considered infinite for all experimental runs.

The trends of a typical residual (actual temperature minus the calculated temperature) plot when estimating α using nonlinear regression are presented in Fig. 1. The amount of scatter varied with each of the 75 test runs, but in all cases definite trends in the residuals are apparent. Three general trends (A,B,C) were observed. Each demonstrated a large underestimation of temperature at low time, which decreased to an overestimation after longer time periods. Differences in the type of plots were present only at the end of heating. Type A plots had a large underestimation in temperature at the end of the

Table 4—Average difference between diffusivity values of 2% KELSET solutions[a]

Can size	$\alpha_2-\alpha_1 \times 10^3$ (m^2/hr)	%difference $(\alpha_2-\alpha_1)/\alpha_2$	$\alpha_2-\alpha_3 \times 10^3$ (m^2/hr)	%difference $(\alpha_2-\alpha_3)/\alpha_2$
303 × 406				
$\bar{x}$	−0.0037	−0.612	−0.0029	−0.463
$S_{\bar{x}}$	0.0026	0.436	0.0142	2.34
307 × 409				
$\bar{x}$	−0.0134	−2.25	−0.00367	−0.603
$S_{\bar{x}}$	0.00431	0.715	0.0143	2.40
401 × 411				
$\bar{x}$	−0.0062	−1.05	−0.0188	−3.17
$S_{\bar{x}}$	0.00363	0.614	0.0209	3.52

[a] α_1, estimated using Eq. 2 and 3; α_2, estimated using Eq. 2, 3, and 5; α_3, estimated using Eq. 1.

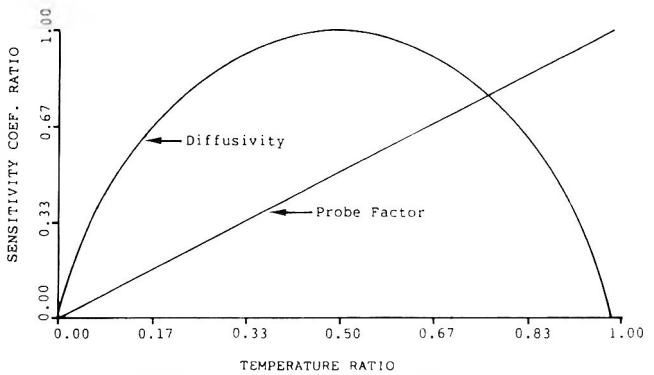

Fig. 4—Sensitivity coefficient ratio as a function of temperature ratio for diffusivity and probe factor estimated simultaneously from thermal process data.

heating period, type B plots had only slight underestimation and the overestimation for type C plots remained relatively constant until the end of heating.

When residuals show a very strong correlation, they depend on each other and two factors can cause this dependence (Beck and Arnold, 1977). First, the model may not adequately describe the data and second, the data may be correlated. Schisler (1979) showed that transient heat conduction data collected rapidly, over a short period of time, has autocorrelation in the residuals. Since our sampling rate was low, 1 data point every 45 sec, any autocorrelation due to sampling can be neglected. The data shows that the lag period is much shorter than expected, indicating that the model does not properly describe the data. This lack of lag time resulted in an underestimation of temperatures, 1.0 to 3.0°C at the beginning of heating and may be the result of: (1) internal convection heating, (2) temperature dependent thermal properties, or (3) heat conduction along the thermocouple. A finite external surface heat transfer coefficient was ruled out because it would cause the lag time to increase — not decrease.

To determine if internal convection heating was present during the thermal process, a very thick and nonflowable food product (commercially prepared pumpkin puree) was processed. General residual trends, identical to Fig. 1, were obtained for ten different runs. These results, in addition to showing that the trend in residuals is not due to convection heating, demonstrated that the 2% KELSET solution is very adequate in preventing convection heating of water.

If temperature dependent thermal properties were the cause of the large temperature underestimations at the beginning of heating, the thermal property values would have to start high and decrease with heating. Gaffney et al. (1980) and Choi and Okos (1983) presented data and models for the thermal diffusivity of water at different temperatures. Both diffusivity models indicated that the α of water increased with temperature, which is the opposite trend needed to account for the short lag time.

In related work, Beverloo and Weldring (1969) (Cowell et al., 1959; Hostetler and Dutson, 1977) demonstrated that the error associated with heat conduction along a radially mounted thermocouple rapidly increased from time zero to a maximum and then asymptotically returned to zero. The shape of their error curve measured for 16 different thermocouples is the same shape as the type C curve in Fig. 1.

Correcting for errors in temperature measurement

Table 3 lists the average α values (of 25 runs) for each can size. Taking into account only the temperatures of a thermal process between the θ range of 0.15 to 0.85, the average α value for water is $.5915 \times 10^{-3}$ m^2/hr. Thermal diffusivity was consistently estimated to be higher than water when using only the analytical solution of Fourier's heat conduction equation (α_1, in Table 3). By incorporating a correction factor (m*) for the heat conduction along the thermocouple probe, a noticeable reduction in the estimated α values can be seen (α_2, Table 3). Considering a 95% confidence interval ($t_{0.95}(25) = 2.06$) for α_2, the α for water fell well within the confidence region for each can size (303 x 406, $0.586 < \alpha_2 < 0.611$; 307 x 409, $0.586 < \alpha_2 < 0.607$; 401 x 411, $0.584 < \alpha_2 < 0.600$). In comparison, the 95% confidence region for α_1 included the α value for water (303 x 406, $0.592 < \alpha_1 < 0.613$; 307 x 409, $0.597 < \alpha_1 < 0.624$; 401 x 411, $0.589 < \alpha_1 < 0.607$) for the 401 x 411 can only.

A two way (can sizes and estimation method) analysis of variance of the data showed that there was no significant (PR > F = 0.36) variation in α for the different can sizes, but there was a significant (PR > F = 0.0001) difference in α for the different estimation methods. When a Tukey's studentized range test was done for the estimation method, α_1 and α_3 were not significantly (alpha = 0.05) different from each other, however α_2 was significantly different from both α_1 and α_3. The difference of α_2 from α_1 and α_3 can be seen from Table 3 because a reduction in the estimated value of α (α_1 to

Table 5—Estimated diffusivity values for duplicate runs of 2% KELSET solutions having a head space of 1.27 cm and a come-up time of 8 mins[a].

Can size	$\alpha_1 \times 10^3$ (m^2/hr)	MSE (C)	$\alpha_2 \times 10^3$ (m^2/hr)	m* (m^{-1})	MSE (C)	$\alpha_3 \times 10^3$ (m^2/hr)
			Estimation method			
	Fo		Fo and m*			f_h
Head Space (1.27 cm)						
307 × 409						
1	0.6020	0.1186	0.5936	2733	0.00105	0.5786
2	0.6002	0.1186	0.5919	2754	0.00441	0.5785
401 × 411						
1	0.5928	0.0612	0.5869	3120	0.00309	0.5670
2	0.5930	0.0306	0.5888	4400	0.000672	0.5812
Come-up Time (8 min)						
307 × 409						
1	0.5625	0.4883	0.5625	∝	0.4962	0.5869
2	0.5617	0.8410	0.5617	∝	0.8550	0.5931

[a] α_1, estimated using Eq. 2 and 3; α_2, estimated using Eq. 2, 3, and 5; α_3, estimated using Eq. 1.

α_2) was accompanied by a reduction in the mean square error (MSE) of the estimate. The three general types of residual trends that were observed when estimating both α_2 and m* are shown in Fig. 2. Scatter was dependent on the test run and the size of the scatter either increased or remained the same as that for the curves in Fig. 1. The type D curve (Fig. 2) resulted from an unchanged residual plot of Fig. 1 (type A) and an unchanged MSE value. When the MSE was unchanged, the probe factor (m*) was large and could be neglected. The type D curve (Fig. 2) appeared a number of times (9 out of 25) for the 303 x 406 can (which used the Ecklund thermocouple) and a few times (5 out of 25) for the larger 401 x 411 can. All of the type C curves (Fig. 1) were reduced to type F curves (Fig. 2) with the incorporation of m*. The intermediate B curves (Fig. 1) resulted in mostly type E to F curves when m* and α were estimated. The significance of m* in smoothing out the residual plot was directly related to the size of the upward curved portion of Fig. 1 at the end of the heating period.

Even with the incorporation of the probe factor there remained an error between the predicted and actual temperature at the beginning of the heating period (Fig. 2). These errors were a result of the quasi-steady state assumption used in deriving Eq. (5). The assumption is not true over short time periods where Eq. (5) will cause the model to predict temperatures larger than the actual values. This overestimation of temperature at the beginning of heating does not influence the estimate of α because the assumption remains valid for the thermal process data that falls between the θ range of 0.15 to 0.85.

The temperature difference compensated for by using Eq. (5) is presented in Fig. 3 The temperature difference ratio was calculated as the difference between the corrected temperature ratio (Eq. 2, 3, and 5) and the temperature ratio calculated using just the analytical equations (Eq. 2 and 3). The slope of the lines are dependent on both the m* value and the dimensions of the can. However, for the three can sizes evaluated (Table 2), differences between the plots were insignificant. Curves in Fig. 3 are straight lines, meaning that Eq. (5) will tend to compensate on a linear basis; thus, the best compensation is found for type C plots (Fig. 1) over long time periods.

Table 4 presents the percentage difference in α when estimated with and without Eq. (5). The correction (2.25%) is largest for the 307 x 409 can. Since a 2.25% variation in estimated thermal properties of food items is not unreasonable, the importance of correcting for heat conduction along the thermocouple probe seems small even though incorporating m* caused a significant reduction in the MSE of the estimate. However, when α is being estimated from a thermal process using a radially mounted probe, the magnitude of the error associated with the probe should be assessed. m* is particularly important for small cans and for cans that have a large L/R ratio. When a can similar to or larger than the 401 x 411 can is used, correcting for heat conduction along the thermocouple probe (for the type of probes used in this study) becomes unnecessary.

Sensitivity coefficients (Beck and Arnold, 1977) of Eq. (2), (3), and (5) were analyzed, to investigate the ease of estimation and any possible correlation of the estimated parameters. Sensitivity coefficients are calculated by taking the first derivative of the model $\theta(\alpha, Bi)$ with respect to one of the independent variables (α, Bi):

$$\text{Sensitivity coefficient of } \alpha = \alpha \frac{d\theta(\alpha,Bi)}{d\alpha} \qquad (7)$$

Sensitivity coefficients, indicate the change in the response (in this case the temperature ratio) as a function of the variable. There are two important qualities of the model that can be learned from studying sensitivity coefficients. The first is the correlation that the variables may have with each other and the second concerns the magnitude of the sensitivity coefficient with respect to the response. Areas where small changes in the response cause large changes in the independent variables correspond with large sensitivity coefficients and locations where precise estimates of the independent variables can be obtained. Thus, the parameter with the larger sensitivity coefficients will be the parameter that is estimated more precisely from the nonlinear regression analysis.

Sensitivity ratios (SR) plotted against θ for the model consisting of Eq. (2), (3) and (5) are shown in Fig. 4. These plots were made for each can size used in the experiments with m* ranging from 1000 to 5000 m^{-1} (a total of 9 plots). Each plot is essentially the same and the coefficients are not correlated (Fig. 4): correlation exists if the two curves differ by a constant. Magnitudes of the sensitivity coefficients differ on the order of 1 (α) to 17 (m*) for m* equal to 1000 (m^{-1}) and 1 to 85 for m* equal to 5000 (m^{-1}). Therefore, when estimating both α and m* simultaneously, the estimate of m* will probably have a larger standard deviation than that of α. Considering the small influence that m* has on α, the importance of estimating m* is low. therefore, even though residual plots like those in Fig. 1 may exist, corrections in estimating α (i.e., incorporation of a probe factor) may not be justified.

Errors from deviant thermal processes

A few thermal process runs were made with a long come-up time (8 min, a typical come-up time of a production scale retort) and for cans containing a 1.27 cm head space (measured from the lip of the can before sealing). The 307 x 409 cans demonstrated a slight (0.65 to 1.57% difference for both α_1 and α_2) change in α for a 1.27 cm head space when estimated using the analytical solution (Tables 3 and 5). The large L/R ratio caused most of the heat to penetrate radially. Diffusivity, when estimated from f_h (α_3) showed a larger decrease in the estimate (3.73 to 5.98% difference). The results for all three estimates in Table 5 are within two standard deviations of the results in Table 3.

The 8 min. come-up time caused an appreciable reduction (8.0 and 5.9%) in the estimation of α_1 and α_2 (Tables 3 and 5). This reduction was accompanied by residuals that were very different from the previous experiments indicating the model was poorly fitting the data. One may infer that the data collected did not meet the assumptions associated with the model. Hence, commercial retorts, which typically have long come-up times should not be used when collecting thermal process data for estimating α.

The plots (used to estimate α_3) for the runs with a 1.27 cm head space or an 8 min come-up time did not show any deviation from normal (normal being a linear portion at long time), even though there were reductions in α_3 of 1.8 to 6.0%. With no deviation, the possibility of incorrectly assuming the results are accurate is greatly increased. Just because the plot has a linear portion in the curve, one cannot assume the thermal process data used to estimate α_3 meet all the assumptions associated with the estimation.

Estimating diffusivity from Olson and Jackson equation

Even though the estimation of α_3 (α calculated from the Olson and Jackson equation—Eq. 1) did not show a significant difference from α_1 (Table 3, α calculated using the analytical solution), estimates of α_3 did have a standard deviation 3 to 5 times that found for α_1. The larger standard deviation resulted in coefficient of variance (C_v) values of 3.0 to 3.8% where the C_v values for α_1 ranged from 0.7 to 1.1.

To date α has been determined for very few food products. An analysis of mayonnaise (Larkin, 1984) resulted in an estimate of α ($\bar{x}$ = 0.4101 x 10^{-3} m^2/hr, $s_{\bar{x}}$ = 0.602 × 10^{-5} m^2/hr) that consisted of residual plots demonstrating a strong lack-of-fit of Eq. (2) and (3). The plots indicate combined convection and conduction heat transfer taking place during the heating making the estimated value highly questionable. In turn, when α ($\bar{x}$ = 0.3187 × 10^{-3} m^2/hr, $S_{\bar{x}}$ = 0.613 x 10^{-5} m^2/hr) was estimated using f_h, the time-temperature plot was very linear at prolong heating times indicating no deviation from normal. A difference of over 20% exists between the two estimation methods! In both cases, a plot of Eq. (2) and (3) using the estimated α value was not consistent with the measured time-temperature data.

When there is no deviation from normal when estimating α from f_h, one should verify the process conditions imposed on the system by Eq. 1. On the other hand, when using Eq. (2) and (3) to estimate α, one can verify the process conditions imposed by the equations through the analysis of the estimation itself.

SUMMARY & CONCLUSIONS

TEMPERATURE measurement errors due to the presence of a thermocouple will cause the residuals of estimated α values to be autocorrelated. Compensating for heat conduction down the thermocouple probe will reduce residuals along with a reduction in autocorrelation. The difference in α estimated with and without compensating for temperature measurement errors depends on the size and shape of the can. Larger cans with small L/R ratios will yield more accurate temperature measurements due to the reduced influence of heat flowing radially into a can and down the thermocouple. Estimations of α with Ecklund thermocouples having metal fittings do not appreciably differ from α values estimated using data collected with a small diameter probe having nylon fittings.

Thermal diffusivity estimated from thermal process data using the regular regime method [Eq. (1)] will result in estimates having larger standard deviations than those found using nonlinear regression and the analytical solution to the heat conduction equation [Eq. (2) and (3)]. Unsatisfied boundary conditions, will be more evident when α is estimated using Eq (2) and (3) than when using Eq. 1. When thermal process data is used in conjunction with Eq. (2) and (3), there are a number of processing conditions that must be maintained to make the estimate of α accurate and precise. Some are not important when estimating α from f_h: position of thermocouple, measurement of time zero, and calibration of the temperature measuring equipment (Gaffney et al., 1980). When estimating α from thermal process data, the following guidelines, in addition to those of Larkin and Steffe (1982) are recommended: (1) Use a 401 x 411 or larger can to reduce errors associated with heat conduction down the thermocouple probe; (2) Maintain a minimum head space in the can, especially for cans with a small L/R ratio; (3) Keep the come-up time of the retort to a minimum. Less than 2 minutes is adequate when considering canned food products of a typical size such as those used in this study; (4) Investigate residuals of α for trends indicating nonconformity to the boundary conditions imposed on the model. This is particularly helpful in determining if convection heating is present; (5) Estimate α from Eq. (2) and (3) using nonlinear regression and, if necessary, correct for the presence of the thermocouple probe.

NOMENCLATURE

- A = Cross sectional surface area of probe (m^2)
- Bi = Biot number
- C_v = Coefficient of variance ($s_{\bar{x}}/\bar{x}$)*100)
- f_h = The inverse slope of the linear portion of the heating curve (sec)
- Fo = Fourier number
- h = Surface heat transfer coefficient between the probe and the food item or the can and the surrounding media ($W/m^2°C$)
- k = Thermal conductivity of product ($W/m°C$)
- L = Height of the can (m)
- m^* = Probe factor hP/kA (m^{-2})
- MSE = Mean Square Error of the estimate (Sum of temperature difference)/(No. data points − 1)
- r = Distance from the outside surface; r = R at the tip of the probe (m)
- R = Radius of the can (m)
- P = Perimeter of probe (m)
- $S_{\bar{x}}$ = Standard deviation of the mean
- SR = Sensitivity coefficient ratio, sensitivity coefficient/maximum sensitivity coefficient value
- t = Process time (h)
- $T_c(r,t)$ = Temperature of food item surrounding the probe at position r and time t (°C)
- $T(r,t)$ = Temperature of probe at position r and time t (°C)
- T_i = Initial temperature of the food system (°C)
- T_∞ = Temperature of the surrounding media (°C)
- V = Volume of the can (m^3)
- $\bar{x}$ = Mean of the population

Greek letters

- α = Thermal diffusivity (m^2/hr)
- $\theta(t)$ = Temperature ratio $((T - T_\infty)/(T_i - T_\infty))$ of food item at center and time t calculated from the analytical solution to Fourier's heat conduction equation
- $\theta^*(t)$ = $\theta(t)$ adjusted using the probe factor, m*

REFERENCES

Albin, F.V., Badari Narayana, K., Srinivasa Murthy, S., and Krishna Murthy, M.V. 1979. Thermal diffusivities of some unfrozen and frozen food models. J. Food Technol. 14: 361.

Annamma, T.T. and Rao, C.V.N. 1974. Studies on thermal diffusivity and conductivity of fresh and dry fish. Fishery Technol. 9: 28.

Baghe-Khandan, M.S. and Okos, M.R. 1981. Effect of cooking on the thermal conductivity of whole and ground lean beef. J. Food Sci. 45: 1302.

Ball, C.O. 1923. Thermal process time for canned foods. Bull. 37 Vol. 7 Part 1, National Research Council, Washington, DC.

Beck, J.V. and Arnold, K.J. 1977. "Parameter Estimation in Engineering and Science." John Wiley and Sons, New York, NY.

Beverloo, W.A. and Weldring, J.A.G. 1969. Temperature measurements in cans and the consequences of errors for the process calculation. Lebensm-Wiss u. Technol. 2: 9.

Bhowmik, S.R. and Hayakawa, K. 1979. A new method for determining the apparent thermal diffusivity of thermally conductive food. J. Food Sci. 44: 469.

Choi, Y. and Okos, M.R. 1983. The thermal properties of tomato juice concentrates. Trans. ASAE. 26: 305.

Cowell, N.D., Evans, H.L., Hicks, E.W., and Mellor, J.d. 1959. Conduction errors in thermocouples used for heat penetration measurements in foods which heat by conduction. Food Technol. 14: 425.

Dickerson, Jr., R.W. 1965. An apparatus for the measurement of thermal diffusivity of foods. Food Technol. 19: 880.

Ecklund, O.F. 1949. Apparatus for the measurement of the rate of heat penetration in canned foods. Food Technol. 3: 231.

Ecklund, O.F. 1955. Correction factors for heat penetration thermocouples. Food Technol. 10: 43.

Flambert, C.M.F. 1974. Nouvelle methode de determination de la diffusivite thermique — Transfert conductif. Lebensm-Wiss u. Technol. 7: 299.

Gaffney, J.J., Baird, C.D., and Eshleman, W.D. 1980. Review and analysis of the transient method for determining thermal diffusivity of fruits and vegetables. ASHRAE Trans. 86: 261.

Hayakawa, K. 1971. Development of a procedure for determining apparent thermal diffusivity of viscous liquid food by using a cylindrical cell. Presented at 11th International Thermal Conductivity Conference, Albuquerque, NM, Sept. 28-Oct. 1.

Hayakawa, K. and Bakal, A. 1973. New computational procedure for determining the apparent thermal diffusivity of a solid body approximated with an infinite slab. J. Food Sci. 38: 623.

Hicks, E.W. 1961. Uncertainties in canning process calculations. J. Food Sci. 26: 218.

Hostetler, R.L. and Dutson, T.R. 1977. Effect of thermocouple wire size on cooking times of meat samples. J. Food Sci. 42: 845.

Jaeger, J.C. 1955. Conduction of heat in a solid in contact with a thin layer of a good conductor. Quart. J. Mech. and Applied Math. 8: 101.

Larkin, J.W. 1984. Thermal diffusivity estimation from thermal process data. Ph.D. dissertation, Michigan State Univ.

Larkin, J.W. and Steffe, J.F. 1982. Error analysis in estimating thermal diffusivity from heat penetration data. J. Food Process Eng. 6: 135.

Lenz, M.K. 1977. The lethality-fourier number method. Its use in estimating confidence intervals of the lethality or process time of a thermal process and in optimizing thermal processes for quality retention. Ph.D. thesis, Univ. of Wisconsin, Madison, WI.

Luna, J.A. and Bressan, J.A. 1985. Heat transfer during bringing of cuartirolo Argentino cheese. J. Food Sci. 50: 858.

Matthews, Jr., F.V. and Hall, C.W. 1968. Method of finite differences used to relate changes in thermal and physical properties of potatoes. Trans. ASAE. 11: 558.

Moysey, E.B., Shaw, J.T., and Lampman, W.P. 1977. The effect of temperature and moisture on the thermal properties of rapeseed. Tarns. ASAE. 20: 461.

Moré, J.J., Garbow, B.S., and Hillstrom, K.E. 1980. User Guide for MINPACK-1, Argonne National Lab., Argonne, IL.

Narayana, K.B. and Krishna Murthy, M.V. 1981. Heat and mass transfer characteristics and the evaluation of thermal properties of moist food materials. Trans. ASAE. 24: 789.

Nesvadba, P. 1982a. Methods for the measurement of thermal conductivity and diffusivity of foodstuffs. J. Food Eng. 1: 93.

Nesvadba, P. 1982b. A new transient method for the measurement of temperature dependent thermal diffusivity. J. Phys. D., Appl. Phys. 15: 725.

Nix, G.H., Lowery, G.W., Vachon, R.I., and Tanger, G.e. 1967. Direct determination of thermal diffusivity and conductivity with a refined line-source technique. Progress in Astronautics & Aeronautics 20: 865.

Nix, G.H., Vachon, R.I., Lowery, G.W., and McCurry, T.A. 1969. The line-source method: Procedure and iteration scheme for combined determination of conductivity and diffusivity. In "Thermal Conductivity, Proceedings of the 8th Conference," C.Y. Ho and R.E. Taylor (Ed.), p. 999. Plenum Press, New York.

Ohlsson, T. 1980. Optimal sterilization temperature for sensory quality in cylindrical containers. J. Food Sci. 45: 1517.

Olson, F.C.W. and Jackson, J.M. 1942. Heating curves theory and practical application. Indust. Eng. Chem. 34: 337.

Özişik, M. 1980. "Heat Conduction." John Wiley and Sons, New York.

Peterson, W.R. and Adams, J.P. 1983. Water velocity effect on heat penetration parameters during institutional size retort pouch processing. J. Food Sci. 48: 457.

Polley, S.L., Snyder, O.P., and Kotnour, P. 1980. A compilation of thermal properties of foods. Food Technol. 34(11): 76.

Poulsen, K.P. 1982. Thermal diffusivity of foods measured by simple equipment. J. Food Eng. 1: 115.

Ramaswamy, H.S., Tung, M.A., and Stark, R. 1983. A method to measure surface heat transfer from steam/air mixtures in batch retorts. J. Food Sci. 48: 900.

Rao, M.A., Barnard J., and Kenny, J.F. 1975. Thermal conductivity and thermal diffusivity of process variety squash and white potatoes. Tarns. ASAE. 18: 1188.

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Comparing Flame and Mechanical Deaeration of High Vacuum Canned Green Beans and Apple Slices

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ABSTRACT

Mechanical deaeration in a vacuum closing machine and flame deaeration achieved comparable results in removing noncondensible gases from low-liquid packs of canned green beans and apple slices. Residual headspace oxygen was calculated from experimental measurements of pressure, void volume, and percent oxygen. Prolonged (> 5 sec) exposure of hot filled samples to mechanical vacuum prior to seaming did not result in lower residual oxygen. Sample changes through 9 mo storage were not influenced by deaeration method; consequently, residual oxygen or vacuum in cooled samples immediately after sampling may be used to assess adequacy of air removal.

INTRODUCTION

RECENT WORK on high vacuum flame sterilization (HVFS) has demonstrated the potential for this low liquid process to improve product quality (Carroad et al., 1980; Heil, 1983; Heil et al., 1983; Leonard et al., 1983, 1984a; O'Mahony et al., 1983; Schweingruber et al., 1981; Seet et al., 1983). Among the products which were successfully high vacuum flame sterilized (Heil, 1983), green beans with enclosed pockets of gas, and apples with their gas-laden, heat-sensitive tissues were particularly difficult to deaerate using a pilot HVFS processing system with flame deaeration, followed by flame sterilization for low acid foods. In one study (Leonard et al., 1984a), mechanical-vacuum deaeration of peaches and pears in a vacuum chamber was satisfactorily substituted for flame deaeration.

In the above studies, air removal by flame deaeration was measured directly by final gauge vacuum in the processed can of product and indirectly by color changes in light colored fruits (e.g., peaches and pears) known to be very sensitive to oxidative discoloration, even in the processed state. However, measurement of residual headspace oxygen levels offers a potentially more useful assessment of deaeration effectiveness since oxygen is the main concern in product quality degradation. To date, no determinations of residual oxygen in high vacuum canned products have been reported.

The purpose of the present work was to provide direct comparisons of the effectiveness of flame deaeration and mechanical deaeration of cut green beans and sliced apples, and to document performance in terms of both can vacuum levels and the actual headspace oxygen content, as estimated by gas chromatography.

MATERIALS & METHODS

Processing

For each product, there were two parts of the study: (1) A preliminary test to determine what residence time under mechanical vacuum would achieve air removal comparable to that obtained in established flame deaeration processes, and (2) a storage test to see if initial deaeration effectiveness would be maintained over time.

Beans. Green beans were cut to 2.5 cm length, held on ice until blanced in water 5 min at 79.4°C, and hot filled (blanced equivalent of 340g raw beans) at 75.3 ± 0.8°C into 303 × 406 inside enameled cans containing 50 mL 0.6% NaCl brine. In the preliminary test, the hot open cans were instantaneously exposed to 635 mm Hg vacuum for residence times of 0, 3, 5, 7, 10 or 15 sec in a vacuum closing machine. For zero dwell, no vacuum was used in the closing-machine chamber. Cans were thermally processed as described below, and were then immediately cooled and analyzed.

For the main storage study, hot filled cans from the blancher were alternately flame or mechanically deaerated. For flame deaeration, cans had lids clinched in a loose first seaming operation and were rotated at 50 rpm for 25 sec over each of three burners on an angular deaerator (Heil et al., 1983) followed by 5 min in a vertical deaerator prior to completion of the double seam. Can heating rates (measured by temperature rise in cans of water) were 24.3, 15.5, and 15.2 kJ/min from the three 15 cm long burners of the angular deaerator and 10.1 kJ/min from the burner of the vertical deaerator.

Conditions for mechanical deaeration were 3 or 5 sec under 635 mm Hg. Since mechanical deaeration caused product cooling, the sealed cans were reheated 3 min in atmospheric steam at 98.8°C at 45 rpm can rotation before flame sterilization.

All sealed cans of flame or mechanically deaerated green beans received the same flame sterilization process: 2 min rising, 2 min holding followed by cooling to < 38°C. Can heating rates were 29.2 kJ/min during rising and 6.3 kJ/min during holding, with 45 rpm can rotation. The adequacy of the process was monitored by measuring can surface temperatures with infrared sensors at the critical points during flame sterilization (Leonard et al., 1984b). Based on inoculated pack and infrared sensor reading relationships, cans that reached 126.7°C surface temperature at the end of rising were considered commercially sterile.

Apples. Golden Delicious apples were peeled using a live-knife peeler and hand cut into eighths with a corer-slicer. The slices were steam blanched for 4 min at 93.3°C and hot filled at 79.9 ± 0.4°C (blanced equivalent of 340g raw apples) into 303 × 406 plain tin cans containing 50 mL water.

For the storage samples, alternate hot filled cans were mechanical-vacuum deaerated or flame deaerated. Mechanical vacuum samples were held for 5 or 7 sec under 508 mm Hg prior to seaming. Because of evaporative cooling of the product, sealed cans of mechanically-deaerated apples were heated 3 min in 98.8°C steam with 45 rpm can rotation to complete enzyme inactivation and sterilization. The flame deaerated samples were heated 20 sec over each of three burners on the angular deaerator and 3 min on the vertical deaerator. Burner inputs to cans were 21.3, 15.5, and 15.2 kJ/min on the angular and 7.9 kJ/min in the vertical deaerator. After flame deaeration, the apples, an acid product (pH 3.8), were commercially sterile; thus they were immediately sealed and cooled to < 38°C.

Sample analysis

Cans were equilibrated at room temperature (25 ± 2°C). Absolute pressure was measured using a strain gauge transducer (Model EPS-2032-15AW, Entran Corp.) described by Hadjiyianni (1982). The headspace gases were sampled with a gas-tight syringe and mole percent oxygen determined in a gas chromatograph (Aerograph Model A-90-C) using He gas as carrier at 60 mL/min, in a 3.05m long, 4.57 mm i.d. stainless steel column with 13×, 30/60 mesh molecular sieve packing, and thermal conductivity detector with 250 mA filament current. The column was regenerated as necessary by flushing with He at elevated temperatures for several hours. Temperature of the product was taken and varied only ±1.3K for beans (< 1% total variation on Kelvin Scale) and ±2.2K for apples (< 1.5% variation). Void volume (total headspace) was measured by displacement, i.e. weighing the water needed to fill the non-product space in the can.

Moles (n) of residual oxygen in the headspace were estimated from the ideal gas law:

$$n = X_{O_2} P_g V/RT \qquad (1)$$

using experimental values of the oxygen mole fraction on a dry basis

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Table 1—Green beans. Effects of dwell time under 635 mm Hg chamber vacuum on oxygen removal. (Beans were blanched in water 5 min before filling. Filling temperature: 75.3 ± 0.8°C)

Factors evaluated	Dwell time (sec) 0	3	5	7	10	15
Moles of residual oxygen in headspace (μmol)	220.9 ± 26.1[d]	36.4 ± 7.6[c]	14.9 ± 1.0[a]	21.7 ± 6.6[a,b]	33.9 ± 13.6[b,c]	26.3 ± 5.5[b,c]
Oxygen concentration (mol %)	12.52 ± 0.54[c]	18.94 ± 1.13[d]	7.94 ± 0.34[a]	8.82 ± 2.61[a,b]	12.96 ± 4.80[b,c]	9.84 ± 1.62[b]
Absolute pressure (mm Hg)	492.1 ± 9.8[a]	52.9 ± 7.1[b]	54.3 ± 5.9[b]	56.6 ± 1.3[b]	58.6 ± 2.3[b]	58.9 ± 3.5[b]
Void volume (mL)	69.8 ± 5.4[a]	122.5 ± 6.2[b]	119.8 ± 10.8[b]	144.0 ± 4.0[c]	143.4 ± 1.8[c]	146.3 ± 4.6[c]

[a,b,c,d] Values in a row with letters in common did not differ significantly ($p > 0.05$)

Table 2—Apple slices. Effects of dwell time under 508 mm Hg chamber vacuum on oxygen removal. (Apple slices were blanched in steam 4 min before filling. Filling temperature: 79.9 ± 0.4°C)

Factors evaluated	Dwell time (sec) 0	3	5	7	10	15
Moles of residual oxygen in headspace (μmol)	277.8[1]	29.6 ± 34.2	19.7 ± 7.8	34.2 ± 7.0	18.5 ± 7.0	22.0 ± 15.5
Oxygen concentration (mol %)	18.76[1]	4.82 ± 0.41	9.24 ± 3.11	5.49 ± 1.48	8.39 ± 1.33	11.91 ± 5.50
Absolute pressure (mm Hg)	666.1[1]	176.5 ± 176.4	47.0 ± 11.6	111.8 ± 54.4	48.4 ± 12.0	43.2 ± 7.6
Void Volume (mL)	42.9[1]	79.6 ± 11.4[a]	198.1 ± 8.9 [c,d]	157.7 ± 23.4[b]	188.7 ± 7.0[c]	201.0 ± 2.2[d]

[a,b,c,d] Values in a row with no letters or with letters in common did not differ significantly ($p > 0.05$).

[e] Measurements were complete on only one sample. Values are given for reference but not included in statistical test.

(X_{O_2}), absolute pressure (P_g), void volume (V), and sample temperature (T). Since the mole fractions obtained from the chromatographic analysis were fractions of the dry noncondensible gases (assumed to be only nitrogen and oxygen). P_g in Eq. (1) is the measured total pressure (P) minus the vapor pressure of water at the temperature of sampling. Water vapor pressure ranged between 22–26 mm Hg for green beans and 20–26 mm Hg for apple slices.

For the preliminary dwell-time studies, three cans were analyzed for each residence time. In the storage studies, five samples per process condition were evaluated at each storage time during 9 mo at ambient temperature. Zero-storage-time samples were analyzed within 3 hr of processing. Inherent difficulties in taking measurements, especially obtaining headspace gas samples under high vacuum, led to loss of a few samples and, therefore, to variable sample sizes. Results are reported as averages ± one std. deviation, representing a minimum of three measurements. When analysis of variance indicated significant differences, Student's "t" test for small samples was used to test differences between sample means.

RESULTS & DISCUSSION

Preliminary tests to establish dwell times

The effect of vacuum chamber residence time on void volume, absolute pressure, oxygen concentration and calculated residual headspace oxygen levels are given in Table 1 for green beans and Table 2 for apples. The void volume in the cans increased initially as the 50 mL of added water and other surface water flashed during the first 3 to 5 sec of dwell time. Random variations in void volume were caused by variations in fill weight (typically ± one piece) and were, therefore, larger for apples than for beans.

Within experimental error, residual headspace oxygen and absolute pressure were minimized after 5 sec of mechanical deaeration for both beans and apples. Neither variable changed systematically as dwell time was increased to 7, 10, and 15 sec. The random variability in moles of oxygen after 5 sec dwell time appeared to be due to random variations in the experimental oxygen concentration results. Errors could have occurred in the chromatographic analysis or from random leaks during sampling through the septum. A possible systematic error, introduction of oxygen from air in the bore of the syringe needle, was calculated to be no more than 1.58×10^{-8} moles, less than 1% of the values reported.

If air removal from the can were the only process occurring during "deaeration", one would expect the headspace composition to remain approximately equal to that of atmospheric gases, i.e., about 21 mol % oxygen. Since the concentration of oxygen after deaeration was of the order of 10% on a dry basis, the results in Tables 1 and 2 appear to be consistent with the following hypothetical mechanism: Ebullition of free water reduced air in the can to a very low level within 5 sec so that the headspace gas composition at the time of double seaming was very likely close to that of pure water vapor. After seaming, cooling reduced the vapor pressure of water to 20–26 mm Hg, and the residual noncondensible gases within the food tissue partitioned between the pieces and the headspace. Since respiration of fruits and vegetables consumes oxygen (Kader, 1986), it is reasonable to assume that the residual oxygen percentage within the heated bean and apple particles was at a sub-atmospheric level; therefore, at equilibrium, lower-than-atmospheric percentages of oxygen were observed in the headspaces of the processed cans.

Because of the substantial time required for headspace gas analysis and overall data acquisition, the preliminary tests were performed separately from the storage tests, using separate sources of raw material. Since the raw materials were different, data obtained from the preliminary tests should not be compared directly to storage test results.

Comparing mechanical and flame deaeration

Green beans. Based on results of the dwell time experiments, samples of canned green beans were processed to study headspace gas composition, as affected by methods of deaeration and storage (Table 3). To compensate for possible differences caused by raw material variations and response to storage, two dwell times (3 and 5 sec) were selected to ensure

Table 3—Green beans. Effect of deaeration method and storage on headspace in high vacuum flame sterilized green beans

Storage (days)	Deaeration method: Mechanical (3 sec)	Mechanical (5 sec)	Flame
	Moles of residual oxygen (μmol)		
0	8.8 ± 2.9[a]	23.2 ± 10.6[b]	6.9 ± 6.3[a]
1	10.6 ± 3.2	10.4 ± 4.1	7.8 ± 1.8
7–8	7.6 ± 4.8[a]	20.6 ± 9.4[b]	10.1 ± 2.7[a]
14	13.7 ± 4.2[a]	25.1 ± 7.5[b]	20.2 ± 1.8[b]
33–35	16.8 ± 2.7[a]	31.0 ± 8.4[b]	27.8 ± 4.4[b]
39–43	19.8 ± 1.6[a]	37.2 ± 10.9[b]	34.0 ± 2.6[b]
57	29.9 ± 11.6	39.3 ± 6.8	33.9 ± 4.2
270	19.0 ± 2.6[a]	27.7 ± 4.0[b]	32.0 ± 3.0[b]
	Oxygen concentration in headspace (mol %)		
0	9.66 ± 2.28[a]	9.67 ± 1.82[a]	19.48 ± 2.75[b]
1	11.60 ± 3.59[b]	6.55 ± 1.51[a]	14.21 ± 3.04[b]
7–8	12.18 ± 2.58	8.50 ± 1.80	11.12 ± 7.47
14	9.78 ± 3.81[a]	10.80 ± 4.08[a,b]	14.52 ± 0.41[b]
33–35	9.99 ± 1.72[b]	7.54 ± 0.55[a]	14.57 ± 2.17[c]
39–43	10.42 ± 1.27[b]	8.20 ± 0.82[a]	16.40 ± 1.27[c]
57	14.64 ± 3.66[b]	8.07 ± 0.46[a]	17.81 ± 0.82[b]
270	8.52 ± 0.82[a]	7.47 ± 1.14[a]	13.23 ± 0.52[b]
	Absolute pressure (mm Hg)		
0	38.8 ± 5.1[b]	55.8 ± 5.2[c]	28.9 ± 6.8[a]
1	38.5 ± 1.1[b]	47.0 ± 3.3[c]	34.9 ± 2.5[a]
7–8	33.1 ± 3.4[a]	62.2 ± 9.3[b]	55.3 ± 30.7[a,b]
14	43.9 ± 3.6[a]	53.6 ± 2.4[b]	47.3 ± 2.2[a]
33–35	48.2 ± 5.1[a]	76.5 ± 11.8[c]	54.9 ± 1.4[b]
39–43	52.3 ± 3.2[a]	82.1 ± 18.4[c]	57.5 ± 2.7[b]
57	54.3 ± 16.8[a]	84.2 ± 6.4[b]	56.6 ± 2.1[a]
270	57.3 ± 0.5[a]	77.0 ± 4.3[c]	67.1 ± 1.2[b]
	Void volume (mL)		
0	124.0 ± 9.4[b]	132.3 ± 18.3[b]	100.6 ± 8.0[a]
1	127.3 ± 9.6[b]	134.1 ± 15.0[b]	110.8 ± 6.2[a]
7–8	126.7 ± 3.6[b]	123.4 ± 26.1[a,b]	100.6 ± 13.2[a]
14	123.8 ± 9.6[b]	139.5 ± 5.1[c]	108.4 ± 6.3[a]
33–35	122.2 ± 10.1[b]	143.1 ± 14.2[c]	109.2 ± 6.4[a]
39–43	122.7 ± 3.6[b]	145.9 ± 10.7[c]	114.3 ± 4.9[a]
57	128.8 ± 8.4[b]	148.1 ± 4.6[c]	101.9 ± 12.2[a]
270	133.3 ± 4.2[b]	138.3 ± 5.1[b]	109.3 ± 3.1[a]

[a,b,c] Values in a row with no letters or with letters in common did not differ significantly ($p > 0.05$).

Table 4—Apple slices. Effect of deaeration method and storage on headspace in high vaccum flame sterilized apple slices

Storage (days)	Deaeration method: Mechanical (5 sec)	Mechanical (7 sec)	Flame
	Moles of residual oxygen (μmol)		
0	157.9 ± 23.2	159.8 ± 8.8	202.1 ± 49.7
1	—	—	236.0 ± 29.1
3	106.5 ± 30.8	76.2 ± 16.6	—
7–8	36.4 ± 9.5	38.2 ± 9.8	28.8 ± 8.8
14–21	41.6 ± 9.2	46.8 ± 10.3	80.0 ± 40.3
28–35	43.4 ± 3.4	45.0 ± 4.6	48.5 ± 16.2
43–49	40.0 ± 8.4[a]	40.8 ± 4.7[a]	65.5 ± 18.5[b]
270	34.0 ± 4.6	41.3 ± 9.7	49.3 ± 24.9
	Oxygen concentration in headspace (mol %)		
0	15.97 ± 0.82	15.58 ± 0.52	18.14 ± 2.73
1	—	—	—
3	9.65 ± 2.06	6.82 ± 1.52	17.59 ± 5.98
7–8	3.52 ± 0.83	3.37 ± 0.86	2.62 ± 0.58
14–21	3.56 ± 0.48	3.82 ± 0.76	6.06 ± 3.00
28–35	4.07 ± 0.31	4.03 ± 0.26	3.68 ± 0.78
43–49	3.55 ± 0.71	3.59 ± 0.59	4.95 ± 1.53
270	2.61 ± 0.17	3.34 ± 0.44	3.30 ± 1.40
	Absolute pressure (mm Hg)		
0	142.4 ± 20.4	150.6 ± 17.9	164.0 ± 37.5
1	—	—	244.5 ± 33.9
3	177.8 ± 30.4	167.0 ± 15.6	—
7–8	157.6 ± 20.6	176.9 ± 29.2	191.5 ± 29.3
14–21	173.8 ± 21.9[a]	179.3 ± 6.2[a]	221.6 ± 17.6[b]
28–35	144.2 ± 14.7[a]	157.3 ± 26.0[a]	218.2 ± 26.1[b]
43–49	156.7 ± 11.5[a]	162.4 ± 34.3[a]	231.6 ± 24.7[b]
270	196.6 ± 10.9[a]	185.7 ± 34.7[a,b]	220.4 ± 5.7[b]
	Void volume (mL)		
0	153.4 ± 14.9	150.2 ± 14.7	151.6 ± 20.4
1	—	—	124.6 ± 16.4
3	140.0 ± 15.8	147.8 ± 10.5	—
7–8	148.7 ± 16.3	139.0 ± 10.3	118.2 ± 21.2
14–21	139.6 ± 7.6[a]	142.6 ± 2.3[a]	124.6 ± 7.5[b]
28–35	162.0 ± 6.6[a]	155.4 ± 14.8[a]	124.2 ± 16.1[b]
43–49	153.2 ± 4.5[a]	154.4 ± 18.3[a]	118.9 ± 6.1[b]
270	143.7 ± 0.6	150.7 ± 13.3	140.7 ± 12.0

[a,b] Values in a row with no letters or with letters in common did not differ significantly ($p > 0.05$).

that at least one would provide a level of oxygen removal comparable to flame deaeration.

First, compare 3 sec mechanical deaeration with flame deaeration at zero storage time in Table 3. The amount of residual oxygen was comparable based on calculations from measured values of P, V, and percent oxygen. Mechanical deaeration resulted in significantly higher void volume (probably indicating greater water removal) and significantly lower oxygen concentration in the headspace. However, the absolute pressure in the mechanically deaerated cans was significantly higher than in the flamed cans.

Surprisingly, 5 sec dwell time did not give better deaeration than 3 s in these experiments. Void volume and percent oxygen were not statistically different at zero storage time, but absolute pressure was unexplainably higher, causing a higher calculated residual amount of headspace oxygen.

Changes through 9 mo storage did not substantially alter the comparisons. Void volumes did not change during storage, and the variations in percent oxygen which were statistically significant showed no pattern with time. Statistical comparison of the effect of storage on absolute pressure within each deaeration method indicated a statistically significant increase with storage time for all methods of deaeration. The pressure increase was not caused by microleaks in cans or double seams since percent oxygen did not increase. It may indicate that the beans released additional gases during storage or that gases were generated from product-container interaction. Neither event could be confirmed by the GC analysis because the column used was not suitable for the detection of gases other than N_2 and O_2.

Product quality was informally assessed. No differences in bean wholeness, texture, color or flavor were observed among the packs. Although the actual void volumes were different, apparent headspaces, reflecting product shrinkage, were comparable for all packs regardless of the method of deaeration. This implies that a larger amount of brine may have been lost when the beans were mechanically deaerated. The excessive loss would indicate too much flashing caused by using excessive chamber-vacuum for the filling temperature used. However, no product "explosion" was observed during the preliminary dwell time tests, and therefore chamber-vacuum adjustments were not considered during the preparation of the samples.

In summary, for the single fill temperature chamber vacuum used, the bean data show that, within statistical variation of the small number of samples tested, mechanical deaeration was comparable or better than flame deaeration both initially and throughout storage. Furthermore, since pressure changes directly reflected changes in calculated moles (n) of residual oxygen in the cans, measuring can vacuum may be an adequate indication of degree of deaeration; however, neither measuring can vacuum nor reporting n alone adequately indicates the lower concentration of oxygen achieved by the mechanical deaeration technique.

Apple slices. Low vacuum levels for 3 sec dwell samples in the preliminary tests (Table 2) prompted use of 5 and 7 sec dwell times for preparing apple storage samples (Table 4). The 5 sec apple packs showed significantly lower vacuum levels than green beans with comparable deaeration time; such differences would be expected (1) because apples contain large amounts of cellular gases whereas green beans have more open structural voids filled with gases, and (2) because the chamber

vacuum was lower resulting in less flashing and possibly less air removal. In general, the 5 and 7 sec dwell times gave results that by statistical analysis were not significantly different.

Based on zero storage time (measured within 3 hr of processing), flame and mechanical deaeration gave results which were not significantly different for all variables measured. Partly, this reflects variability in the data. The apple slices were very high in aromatic volatiles which often appeared to interact with the molecular sieve chromatograph packing. Although the columns were regenerated daily, variations still persisted. Apple volatiles may also have affected the pressure transducer. Further variability was introduced in the void volume by packing to $\pm$ 1 apple slice.

The effect of storage on the apple slices was more complicated than for green beans. The concentration of oxygen and the total amount of residual oxygen in the apples gradually decreased through storage. This may have resulted from oxygen reacting with the tin lining of the can, and/or oxygen redissolving in the food during storage. Since oxygen dissolution would have come to equilibrium early in the storage period, whereas the percent oxygen in the headspace continued to change over the duration of the storage study, reaction with the tin lining appears to be the more plausible explanation.

The changes that occurred during storage did not substantially alter the conclusion that measurements made at zero storage time were adequate for ascertaining comparable product deaeration. When all of the samples are considered there is some evidence that the mechanical vacuum conditions used resulted in somewhat higher void volumes, lower absolute pressures, equal headspace oxygen concentration and lower moles of residual oxygen, compared to flame deaeration. The larger void volumes suggest that mechanical deaeration removed larger amounts of water and this resulted in better air removal (lower pressure, less residual oxygen) than by flame deaeration. As with the beans, pressure in each can correlated reasonably well with residual oxygen, indicating that measurements of can vacuum could be used as a rough indication of degree of deaeration.

Apparent headspace and product quality factors such as wholeness, texture, color, and flavor were comparable in all packs of apple slices, regardless of the deaeration method.

CONCLUSIONS

THIS WORK CONFIRMS that mechanical vacuum deaeration can be as effective as flame deaeration for producing high vacuum canned foods. Quality of the mechanically deaerated products was as good as that of flame deaerated products through 270 days of storage. Either vacuum measurements or residual oxygen determinations on the cooled cans immediately after processing may be used to compare deaeration levels in both deaeration procedures. Increasing dwell times beyond 5 sec (up to 15 sec) for the fill temperatures and chamber vacuums used did not clearly enhance oxygen removal. Additional work is needed to determine the optimum combination of dwell time, fill temperature and chamber vacuum needed for each mechanically deaerated product.

REFERENCES

Carroad, P.A., Leonard, S.J., Heil, J.R., Wolcott, T.K., and Merson, R.L. 1980. High vacuum flame sterilization: Process concept and energy use analysis. J. Food Sci. 45: 696.

Hadjiyianni, A.H. 1982. Analysis of headspace gases in vacuum-packed foods. M.S. thesis, Dept. Food Sci. & Technol., Univ. of California, Davis, CA.

Heil, J.R. 1983. High vacuum flame sterilization of particulate foods: Improved quality and economics. In "Proceedings of Future-Pak '83." Ryder Assoc. Inc., Whippany, NJ.

Heil, J.R., Carroad, P.A., Merson, R.L., and Leonard, S. 1983. Development of high vacuum flame processes for sliced peaches and pears. J. Food Sci. 48: 1106.

Kader, A.A. 1986. Biochemical and physiological basis for effects of controlled and modified atmospheres on fruits and vegetables. Food Technol. 40(5): 99.

Leonard, S.J., Heil, J.R., Carroad, P.A., Merson, R.L., and Wolcott, T.K. 1983. High vacuum flame sterilized fruits: Storage study on sliced Clingstone peaches, sliced Bartlett pears and diced fruit. J. Food Sci. 48: 1484.

Leonard, S.J., Heil, J.R., Carroad, P.A., Merson, R.L., and Wolcott, T.K. 1984a. High vacuum flame sterilized fruits: Influence of can type on storage stability of vacuum packed peach and pear slices. J. Food Sci. 49: 263.

Leonard, S., Osaki, K., and Heil, J. 1984b. Monitoring flame sterilization processes. Food Technol. 38(1): 47.

O'Mahony, M., Buteau, L., Klapman-Baker, K., Stavros, I., Alford, J., Leonard, S.J., Heil, J., and Wolcott, T.K. 1983. Sensory evaluation of high vacuum flame sterilized clingstone peaches, using ranking and signal detection measures with minimal cross-sensory interference. J. Food Sci. 48(6): 1626.

Schweingruber, P.J., Carroad, P.A., Leonard, S.J., Heil, J.R., Wolcott, T.K., O'Mahony, M., and Wilson, A. 1981. Evaluation of instrumental methods for firmness measurements of fresh and canned Clingstone peaches. J. Texture Studies. 12: 389.

Seet, S.T., Heil, J.R., Leonard, S.J., and Brown, W.D. 1983. High vacuum flame sterilization of canned diced tuna: Preliminary process development and quality evaluation. J. Food Sci. 48: 364.

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Rizvi, S.S.H., Blaisdell, J.L., and Harper, W.J. 1980. Thermal diffusivity of model meat analog systems. J. Food Sci. 45: 1727.

Ross, I.J., Rudnick, Jr., A.W., and Fox, J.O. 1969. The thermal diffusivity of ice cream at cryogenic temperatures. Paper No. 69-881, presented at the Winter Meeting of ASAE, Chicago, IL Dec. 15.

Schisler, I.P. 1979. ARIMA statistical models of transient heat conduction errors for Aitken's confidence region. Ph.D. thesis, Michigan State Univ., East Lansing.

Singh, R.P. 1982. Thermal diffusivity in food processing. Food Technol. 36: 87.

Suter, D.A., Agrawal, K.K., and Clary, B.L. 1975. Thermal properties of peanut pods, hulls and kernels. Trans. ASAE. 18: 370.

Teixeira, A.A., Dixon, J.R., Zahradnik, J.W., and Zinsmeister, G.E. 1969. Computer optimization of nutrient retention in the thermal processing of conduction-heated foods. Food Technol. 23: 845.

Teixeira, A.A., Stumbo, C.R., and Zahradnik, J.W. 1975a. Experimental evaluation of mathematical and computer models for thermal process evaluation. J. Food Sci. 40: 653.

Teixeira, A.A., Zinsmeister, G.E., and Zahradnik, J.W. 1975b. Computer simulation of variable retort control and container geometry as a possible means of improving thiamine retention in thermally processed foods. J. Food Sci. 40: 656.

Uno, J. and Hayakawa, K. 1980. A method of estimating thermal diffusivity of heat conduction food in a cylindrical can. J. Food Sci. 45: 692.

Woodams, E.E. and Nowrey, J.E. 1968. Literature values of thermal conductivities of foods. Food Technol. 22(4).

Yoshida, H., Yamamoto, S., and Yorizane, M. 1982. Temperature measurement error by radial insertion of thermocouple in thermal conductivity measuring device. Chem. Eng. Commun. 15: 151.

Young, K.E., Steffe, J.F., and Larkin, J.W. 1983. Product temperature prediction in hydrostatic retorts. Trans. ASAE. 26: 316.

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Water Activity Calculation by Direct Measurement of Vapor Pressure

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ABSTRACT

An improved method for calculation of water activity from vapor pressure manometric data, which takes into account the volume expansion of nonadsorbing gases, the initial pressure in the desiccant flask and the pressure exerted by the desiccant, was developed. The water activity of pure water was measured over a range of nonadsorbing gas pressures and initial pressures in the desiccant flask. Standard deviations in a_w of pure water calculated using no corrections, corrections for volume expansion only, and full corrections were 0.221, 0.011, and 0.005, respectively. Correction only for volume expansion may under some conditions result in larger errors than when no corrections are made at all. Based on the improved equation, an experimental modification which reduces loss of water from samples during measurement was proposed.

INTRODUCTION

WATER ACTIVITY (a_w) measurement and control are of fundamental importance in many food research and production situations. Of the many methods available to measure water activity, direct measurement of the vapor pressure of foods is considered the most reliable, as it measures the quantity of interest directly and, unlike hygrometers, requires no calibration with standard solutions. Direct measurement techniques have long been used in the determination of water activity (Makower and Meyers, 1943). The method has undergone several improvements over time (Taylor, 1961; Sood and Heldman, 1976), and its latest variants have been found to be superior to other commonly used methods for water activity determination in most food systems (Labuza et al., 1976). More recnetly, electronic transducers have replaced the traditional oil manometers for pressure measurement (Troller, 1983). This development has made vapor pressure measurement devices more compact, which in turn makes temperature control less problematic, as it is easier to eliminate temperature gradients in a small volume than in a large one.

The application of this method of water activity determination to biological and food systems remains limited to foods which do not adsorb or contain large amounts of gases other than water vapor, since the equations for calculation of a_w from manometric data in their present form do not correctly account for these. The purpose of this work was to show why the current methods of calculation of water activity from vapor pressure manometric data may not give accurate results and to present an equation which accounts for non-water, non-adsorbing gas pressures precisely. A modification of current methodology which reduces water loss from samples during the measurement stage will also be discussed.

THEORETICAL ANALYSIS

A SCHEMATIC DIAGRAM of a vapor pressure capcitance manometer apparatus typically used in a_w determination is shown in Fig. 1. It consists of two flasks, one for sample and the other for desiccant, connected by way of valves V2 and V3 to a tubing manifold, which leads through V1 to a pressure transducer PT and through valve V6 to a vacuum pump. Valves V4 and V5 connect the sample flask and tubing manifold sections to the atmosphere, respectively. The entire apparatus is contained in a temperature-controlled environment. The procedure for measuring a_w described in the literature (Troller, 1983) using this type of system essentially involves (a) placing the sample in the sample flask and filling the desiccant flask with a desiccant material (usually $CaSO_4$), (b) evacuating the desiccant flask and tubing manifold (the sample flask being isolated) to below the vapor pressure of the desiccant (usually, but not always, below 0.20 Torr), (c) evacuating the sample flask of nonwater vapors (with the desiccant flask isolated) for 30 sec to 2 min, (d) isolating the vacuum pump and allowing the pressure to develop in the tubing manifold and sample flask until a steady pressure reading at constant temperature is obtained, (e) isolating the sample flask and opening the desiccant flask valve, and (f) adsorbing the water vapors onto the desiccant material until there is no change in pressure with time.

To date, the pressure of water vapor over a sample has been obtained by subtracting the pressure reading taken in step f from that taken in step d above, and then dividing that difference by the vapor pressure of pure water at the temperature of the experiment. Estimates of water activity obtained in this manner are good only to the extent that the pressure measurements taken in steps b and f above are close to zero. Otherwise, water activity estimates turn out to be inaccurate. To obtain a more correct water activity estimate in the presence of nonadsorbing gases, one must follow the state of the gases in contact with the transducer.

The initial preessure in the desiccant flask measured in step b above is contained in volume V_d of void space in the des-

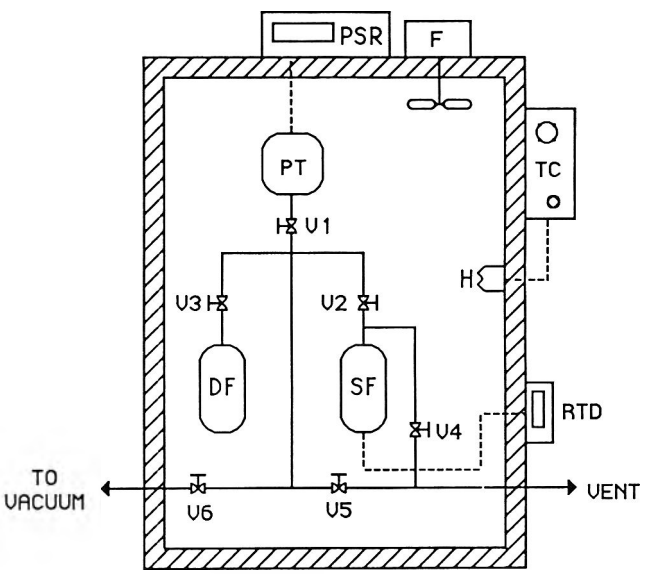

Fig. 1—Schematic representation of the capacitance manometer apparatus used. PT = pressure transducer; PSR = power supply readout; TC = temperature controller; H = heater; RTD = platinum resistance thermometer; F = Fan, Vx (x = 1 – 6) valves; SF = sample flask; DF = desiccant flask.

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iccant flask up to the desiccant flask valve. It consists of (a) nonadsorbing gases which remain in the desiccant flask after its evacuation ($P_d°$) and (b) a combination of water vapor and nonadsorbing gases which emanate from the pores of the desiccant particles. Partially hydrated desiccant materials exert a water vapor pressure dependent on the amount of water which has been adsorbed or absorbed. In addition, highly porous granular desiccants also tend to retain some nonadsorbing gases in them unless exposed to strong vacuum for extended periods of time. In this work we refer to the total vapor pressure exerted by the desiccant material as P*, regardless of its composition. It is a low but measurable quantity which is relatively constant unless the desiccant is exposed to extended, strong vacuum.

Once the pressure reading from the sample flask in step d above is stable, the valve leading to that flask is closed and the valve leading to the desiccant flask is opened. Between these two steps, the pressure against the transducer is exerted by n_1 moles of water vapor and non-adsorbing gases in tubing manifold volume V_t consisting of the tubing between the transducer, the sample flask valve, the desiccant flask valve, the vent valve and the vacuum pump valve. The equation of state at low pressures is then

$$P_1 V_t = n_1 R_g T \quad (1)$$

Recalling that the vapor consists of both water (n_{1w} moles) and nonadsorbing gases (n_{1i} moles), Dalton's law states that the pressure read by the transducer is given by

$$P_1 = P_{1w} + P_{1i} \quad (2)$$

In the final step, the valve leading to the desiccant flask is opened. The desiccant flask originally contains non-adsorbing gas as well as a certain amount of vapor corresponding to the vapor pressure of the desiccant.

After opening the valve leading to the desiccant flask, we assume that the water vapor in the tubing manifold is entirely adsorbed onto the desiccant, leaving in the gas phase only the non-adsorbing material and the vapor pressure of the desiccant. This gas is contained in a volume $V_2 = V_t + V_d$. Assuming ideal behavior, the final mass balance may be written in terms of pressure and volume (at constant temperature) as

$$P^* (V_d + V_t) + P_d°V_d + P_{1i}V_t = P_2 (V_d + V_t) \quad (3)$$

where P_2 is the final transducer reading taken in step f after sorption of water in the tubing manifold onto the desiccant. P_2 has conventionally been taken to be the non-adsorbing gas pressure (Lewicki et al., 1978). In fact, the pressure which truly corresponds to the nonadsorbing gas pressure over the sample is P_{1i}. Rearrangement of Eq. (3) gives P_{1i} as

$$P_{1i} = P_2 (1 + R) - P_d° R - P^* (1 + R) \quad (4)$$

where $R = V_d/V_t$. Thus the relation for water activity in systems where there are non-adsorbing gases involved is

$$a_w = \frac{P_1 - P_{1i}}{P^{sat}} = \frac{P_1 - (P_2 - P^*)(1 + R) + P_d° R}{P^{sat}} \quad (5)$$

Benado and Rizvi (1985) and Nunes et al. (1985) simultaneously recognized that the calculation of water activity from vapor pressure manometric data requires a correction for volume expansion of the non-adsorbing gases upon exposure to the desiccant. The latter authors proposed a correction for volume expansion which includes provision for the first term in equation (4) but not for the second or third. Their appraoch is valid if the desiccant used is in powdered form and exerts extremely low vapor pressure (such as P_2O_5). However, many granular desiccants behave in such a manner that they may exert a significant vapor pressure especially if the desiccant is not very fresh. In these cases, as well as in cases where the void volume in the desiccant flask (up to the desiccant flask valve) V_d is much larger than the volume of the tubing manifold, the initial pressure in the desiccant flask must be taken into account.

To obtain the ratio of desiccant flask to manifold tubing volumes, Eq. (5) may be applied to dry air ($a_w = 0.0$). If P^* is known, a measurement of $P_d°$, followed by isolation of the desiccant flask, insertion of an amount of dry air to give a nonadsorbing gas pressure P_{1i}, and re-opening the desiccant flask valve to obtain P_2, yields all the required information. The ratio R may be computed from Eq. (5) as

$$R = \frac{P_1 - P_2 + P^*}{P_2 - P_d° - P^*} \quad (6)$$

The formula for R is again similar to that of Nunes et al. (1985) except that the equation given herein includes terms for $P_d°$ and P^*. It is seen from Eq. (6) tht if P^* is small with respect to the other parameters (as indeed it often is), then the equation amy be reduced to the form

$$R = \frac{P_1 - P_2}{P_2 - P_d°} \quad (7)$$

It is also noted that in cases where P^* may not be measured with accuracy (such as in many oil manometer systems), Eq. (7) provides a good approximation for R. Eq. (5) behaves as it should in the limits of pressure ranges. If, for example, all nonadsorbing gases are removed from the desiccant headspace ($P_d° = 0.0$), and no nonadsorbing gas is present on the sample side of the system, ($P_{1i} = 0.0$), then the water activity manometer would give $P_2 = P^*$, the right hand side of Eq. (4) becomes zero, and Eq. (5) gives $a_w = P_1/P^{sat}$.

MATERIALS & METHODS

THE VAPOR PRESSURE manometer apparatus used was typical of those used in this application and is shown in Fig. 1. The flasks consisted of 2.54 cm o.d. tubes sealed off at one end and connected to the system by means of Ultratorr™ fittings. The pressure transducer (Datametrics 600 Electronic Transducer, 1400 Power Supply/Readout, Datametrics, Wilmington, DE) had a linear range of 0–100 Torr absolute. Vacuum conditions down to approximately 2 microns were established by means of a vacuum pump (Welch Duo-Seal 1402, Skokie, IL). Temperature was monitored on the surface of the sample flask using a Platinum RTD (Omega, 199B, Stamford, CT) and the chamber temperature was controlled to within 0.1 °C using a proportional temperature controller TC (Oven Industries 5GX220P Mechanicsburg, PA).

To prove the validity of the equations developed, vapor pressure data were obtained for pure water using rather extreme values of nonadsorbing gas pressure and initial pressure in the desiccant flask. By obtaining data for cases where the previously used equations fail, and by showing that the equations developed in this work hold for these conditions, the applicability of the method described will be demonstrated.

The procedure used was similar to that described previously with the following modifications: (a) Samples consisted of pure water and the desiccant used was Drierite™ ($CaSO_4$); (b) After the evacuation of the desiccant and tubing manifold sections, the vacuum pump was isolated and the pressure monitored with time until it attained a constant level. This reading, less the previously measured value of P*, was taken to be the initial nonadsorbing gas pressure in the desiccant flask ($P_d°$); and (c) The sample flask was evacuated for such a time that the pressure was well within the range of accuracy of the transducer. This took between 5 and 10 sec.

The pressure exerted by the desiccant material P* was obtained by pulling vacuum on the desiccant material for about 30 min, isolating the vacuum pump and measuring the pressure until it had achieved a constant level. Air was then admitted into the desiccant fllask externally to prove the validity of the equations developed. The increase in pressure due to admitted air was taken as $P_d°$. In actual practice, it is rather difficult to differentiate P* from $P_d°$. Our data showed that results were almost equally good if the pressure read in step 2 was used as P* or $P_d°$ as long as no air was externally admitted into the

desiccant flask. It is important that the initial pressure in the desiccant flask be accounted for.

Occasionally, when the time required for water vapor to adsorb onto the desiccant flask (i.e. when P_{1i} or P_d^o) was very large, the measurements were split up into separate procedures for water and non-adsorbing gas pressure measurements as follows: once a value for P_1 was obtained for a water vapor/nonadsorbing gas mixture, the desiccant flask was opened and the water vapor allowed to adsorb onto the desiccant. The desiccant flask valve was then closed and more nonadsorbing gas was permitted to enter the tubing manifold section through the vent. The pressure after this step was noted, the desiccant flask valve was opened again, and the pressure was allowed to attain a final value, P_2. The value of P_1 for a_w measurements where this alternative procedure was used was taken to be the sum of pressures of the original water/non-adsorbing gas mixture and the additional nonadsorbing gas permitted to enter the tubing manifold section after the water vapors had been adsorbed onto the desiccant. The alternative procedure yields equivalent results to having a much higher non-adsorbing gas pressure in the sample chamber, without the need to wait a long time for diffusion of water to take place across the small orifice desiccant flask valve, as discussed below.

RESULTS & DISCUSSION

A TEST for validity of Eq. (5) is that R should be constant regardless of the values of P_1, P_2, P_d^o and P*. To obtain values of R over an entire range in pressure, several repetitions of the aforementioned procedure using dry air were carried out, each with different values of each of the above parameters. Table 1 shows the behavior of the value of R as a function of these parameters using very fresh desiccant (P* = 0.0) as calculated by Eq. (6) and (7) and the equation of Nunes et al. (1985). The ratio of desiccant to manifold tubing volumes was approximately constant for P_1 and $P_2 >> P_d^o + P^*$. Under these conditions, the value of R was the same regardless of whether it was calculated using Eq. (6), (7) or the equation of Nunes et al. (1985). Table 1 shows, however, that when P_d^o is not insignificant with respect to P_1 and P_2, the equation of Nunes et al. (1985) breaks down. This latter condition is achieved in practice when a) the water activity of the sample is very low, b) the desiccant used in the apparatus is partially spent (P* > 0), and c) the desiccant flask is not fully evacuated between sample measurements ($P_d^o > 0$). Overall, the variation in R for 25 independent measurements using a wide range of values of P_d^o, P_1 and P_2 may be seen from Table 2. Equation (6) represents a significant improvement over Nunes et al. (1985) equation. The tables show that this was especially true when the value of P_d^o approached P_1. When $P_1 >> P_d^o$, the resulting values of R had little variability and turned out to be very similar for all equations.

Table 1—Calculated values of the ratio of volume of desiccant flask to volume of tubing manifold R = V_d / V_t

P_d^o (Torr)	P_1 (Torr)	P_2 (Torr)	R[a]	R[b]
0.012	13.29	8.240	1.220	1.221
0.018	7.400	3.340	1.216	1.222
0.019	31.09	13.97	1.225	1.227
0.022	18.07	8.160	1.214	1.217
0.109	6.309	2.902	1.174	1.220
0.207	1.882	0.961	0.958	1.221
0.459	2.542	1.395	0.822	1.225
1.395	11.153	5.787	0.927	1.222

[a] Nunes et al. (1985)
[b] This work.

Table 2—Overall variability in measurement of the ratio volume of desiccant flask to volume of manifold tubing R = V_d/V_t

	Eq. (6)	Partially corrected equation[a]
Number of measurements	25	25
Average value of R	1.221	0.976
Maximum value of R	1.238	1.225
Minimum value of R	1.213	0.130
Standard Deviation	0.005	0.305
Standard Error	0.001	0.061

[a] Equation of Nunes et al. (1985)

The repercussions of the above arguments on the practical measurement of water activity can be estimated from a few simple calculations. Table 3 shows the effect of various non-adsorbing gas pressures and initial pressures in the desiccant flask on the calculated a_w at 10°C of pure water ($a_w = 1.0$), with the value of P* = 0.05 Torr. The first three columns in Table 3 show the a_w which would be estimated using the calculation method of Lewicki et al. (1978), and the last two columns in the table show the a_w which would be estimated using the calculation method of Nunes et al. (1985) and Eq. (5) if P* were assumed to be 0. Applicaton of Eq. (5) including P* = 0.05 Torr terms resulted in calculated $a_w = 1.0$. Table 3 demonstrates the clear inability of the uncorrected equation to account for significant amounts of non-adsorbing gases in the sample chamber, resulting in large errors in calculated values of a_w. It also shows that this problem is usually reduced somewhat by applying the correction of Nunes et al. (1985). Their correction applies only to the nonadsorbing gases in the sample, i.e., to P_{1i}, not to P* or P_d^o. It can be seen that the equation of Nunes et al. (1985) considerably reduces errors in calculation of a_w when large amounts of non-adsorbing gas exist in the sample.

When little or no nonadsorbing gas is present in the sample flask and P_d^o is significant, however, Table 3 shows that the correction of Nunes et al. (1985) results in greater error than would result from using no correction for volume expansion at all. This is due to the multiplication by (1 + R) of the "measured" nonadsorbing gas pressure (P_2), which in this case would actually be a combination of P* and P_d^o, resulting in the substraction of a larger number from the measured water plus nonadsorbing gas (P_1) pressure than should be the case. The table shows that the use of Eq. (5) without the correction for P* (but with the correction for P_d^o) results in lower errors than is the case for both the uncorrected equation and the equation of Nunes et al. (1985).

Table 4 summarizes the results of a_w measurements on pure water at approximately 37°C also made under a variety of nonadsorbing gas pressures in the sample and desiccant flasks. The table shows that although use of Eq. (5) with the inclusion of P* gives the most accurate and precise results, application of Eq. (5) lumping both P* and P_d^o into P_d^o still improves the accuracy and precision of estimated a_w as compared to the equation of Nunes et al. (1985), and, especially, as compared to the uncorrected equation.

Application of Eq. (5) to systems which use electronic transducers for pressure measurement permit some simple and useful changes in methodology to be applied for accurate a_w determination of samples whose water contents are low yet whose a_w is high. Lewicki et al. (1978) showed that during the sample flask evacuation step (approximately 30 sec in du-

Table 3—Effect of nonadsorbing gas pressures over water and desiccant on a_w as calculated using various methods

	P_{1i} (Torr)				
P_d^o (Torr)	0.0	4.0	8.0	Partial correction[a]	Eq. (5)
0.0	0.995	1.237	1.480	0.998	0.995
0.2	0.982	1.225	1.468	0.960	0.995
0.4	0.970	1.213	1.456	0.932	0.995
0.6	0.958	1.200	1.444	0.905	0.995
0.8	0.946	1.189	1.431	0.877	0.995
1.0	0.933	1.177	1.420	0.850	0.995

[a] Equation of Nunes et al. (1985)

Table 4—Variability in measured a_w values of pure water

	Uncorrected equation	Partial correction[a]	Eq. (5)	Eq. (5) (P* = 0)
Average a_w	1.206	0.983	0.999	0.992
Std Dev	0.221	0.011	0.005	0.009
Std Err	0.064	0.003	0.001	0.002

[a] Equation of Nunes et al. (1985)

ration), large amounts of water may be removed from high a_w samples, thereby reducing sample moisture (and thus a_w). Measured a_w of samples therefore do not correspond to the moisture level of samples as originally placed in the manometric device, but of samples as they are withdrawn from it. This necessitates moisture content determination after a_w measurement. One method which has been used to get around this problem has been to freeze the sample flask in liquid nitrogen before evacuation. This has disadvantages in that (a) it takes a rather long time for samples to warm up from liquid nitrogen to experimental temperatures and (b) the structure of a food might be damaged during the freezing process, thereby changing the a_w-moisture content behavior of the product.

The methodology used in this work reduces water loss from samples during the sample flask evacuation step because the sample is exposed to vacuum only for such time as requried to achieve a pressure reading within the range of the transducer (between 5–10 sec). This leaves a large amount of nonadsorbing gas in the vapor space above the food sample, but the equations developed are capable of quantitating these gases in a fundamentally sound manner. Another advantage of this methodology is that the temperature drop during sample flask evacuation decreases, due to vaporization of a smaller quantity of water in this step. Thus, the sample reaches the temperature of the surroundings more rapidly using this methodology than it does using conventional methods.

Allowing large amounts of nonadsorbing gases to remain in the vapor space above the sample carries with it some disadvantages as well. The biggest disadvantage is the long diffusion time necessary for water vapor to diffuse across the desiccant flask valve and onto the desiccant. When little or no nonadsorbing gas is present, adsorption of water vapor onto desiccant occurs relatively rapidly because the desiccant, by adsorbing water, creates a pressure gradient across the desiccant flask valve. This causes pressure-induced flow to occur in the direction of the desiccant. When large amounts of nonadsorbing gases are present, the first thing to happen upon opening the desiccant flask valve is an equalization of pressure. This is followed by adsorption of water vapor. The speed of adsorption of water vapor adsorption is lower because the flow of water vapor across the desiccant flask valve is induced mostly by concentration differences (diffusion), not by pressure differences. At low total pressure, the diffusion coefficient of water vapor is roughly inversely proportional to the pressure. Diffusion times for adsorption of water vapor therefore increase linearly with increasing total pressure. This problem is severely compounded by the fact that inexpensive positive sealing valves tend to have relatively small orifice sizes. Bi-directional diffusion through a small orifice connecting two rather large reservoirs can be extremely slow. If a new a_w measurement system is to be designed with the proposed procedure in mind, flanged valves with orifice sizes on the order of centimeters can greatly reduce required measurement times and are strongly recommended.

CONCLUSION

VAPOR PRESSURE DATA for pure water obtained for a wide variety of nonadsorbing gas pressures in the sample as well as in the desiccant flasks demonstrated that the application of Eq. (5) to vapor pressure manometric data resulted in improved a_w estimation than did other calculation methods. Use of Eq. (5) in systems which use pressure transducers also permitted modification of the experimental methodology resulting in reduced water loss from samples. Application of the new methodology to vapor pressure manometer apparatus requires the use of large orifice valves; however, because in the presence of large amounts of nonadsorbing gases, diffusion of water becomes the slowest step in the measurement process and can severely reduce the speed of measurement. Even without permitting very large amounts of nonadsorbing gases to remain in the sample flask, however, the data lead to the conclusion that Eq. (5) is a fundamentally sound representation of the experimental sequence and is an improvement over uncorrected and partially corrected calculation methods, especially for high values of V_d/V_t, as well as at low a_w and temperature, where vapor pressure is low.

REFERENCES

Benado, A.L. and Rizvi, S.S.H. 1985. Thermodynamic properties of water on rice as calculated from reversible and irreversible isotherms. J. Food Sci. 50: 101.

Labuza, T.P., Acott, K., Tatini, S.R., and Lee, R.Y. 1976. Water activity determination: a collaborative study of different methods. J. Food Sci. 41: 910.

Lewicki, P.P., Busk, G.C., Peterson, P.L., and Labuza, T.P. 1978. Determination of factors controlling accurate measurement of water activity by the vapor pressure manometer technique. J. Food Sci. 43: 244.

Makower, B. and Myers, S. 1943. A new method for determination of moisture in dehydrated vegetables. Proc. Inst. Food Technol. 4th Conference, p. 156.

Nunes, R.V., Urbicain, M.J., and Rotstein, E. 1985. Improving accuracy and precision of water activity measurements with a water vapor pressure manometer. J. Food Sci. 50: 148.

Sood, V.C. and Heldman, D.R. 1976. Analysis of a vapor pressure manometer for measurement of water activity in nonfat dry milk. J. Food Sci. 39: 1011.

Taylor, A.A. 1961. Determiantion of moisture equilibria in dehydrated foods. Food Technol. 15: 536.

Troller, J.A. 1983. Water activity measurements with a capacitance manometer. J. Food Sci. 48: 739.

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Relationship Between Water Activity and Freezing Point Depression of Food Systems

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ABSTRACT

A simplified freezing point depression (FPD) equation was derived for calculating water activity (a_w) of food systems. The a_w values as calculated by FPD data agreed with literature data for a variety of foods to within ± 0.01 a_w units. The FPD equation was found particularly useful for calculating a_w values of frozen foods at temperatures between 273.15–233.15 °K (0 to −40°C).

INTRODUCTION

WATER ACTIVITY (a_w) is the most widely used parameter to indicate the availability of water in food systems for predicting food stability. The effects of water activity on microbial growth, chemical reactions and enzyme activity have been studied extensively (Troller and Christian, 1978; Karel, 1975; Labuza, 1975; Scott, 1957).

The a_w values of aqueous solutions and many liquid food systems have been correlated to the thermodynamic expressions in terms of freezing point depression by many researchers (Ferro-Fontan and Chirife, 1981; Lerici et al., 1983). The values of water activity calculated from the thermodynamic expressions have been shown to closely agree with the data measured by the electric hygrometer at room temperature in the range of 0 to 40% total solids (Lerici et al., 1983).

The a_w values of various frozen foods including meats, fish, fruit and vegetables have been reported by several researchers (Dyer et al., 1966; Hill and Sunderland, 1967; Storey and Stainsby, 1970; Fennema and Berny, 1974). The vapor pressure measurement which is commonly used for frozen foods is prone to errors, care must be exercised in using the reported values. It is preferable to use the calculated a_w values from the well-accepted values of vapor pressure of ice divided by vapor pressure of supercooled water at the same temperature (Fennema, 1981).

On the basis of thermodynamic principles, Andrews (1976) stated that in any equilibrium system, regardless of whether it involves phase transition or it contains solutes, all equilibria are maintained locally, in the small region of the equilibrium, by the equality of chemical potential of the solvent. By accepting this principle, the freezing point depression equation should be generally applicable to food or biological systems at both above-freezing and subfreezing temperatures.

The purpose of this work was to evaluate a_w values of foods at both above-freezing and subfreezing temperatures with the aid of the freezing point depression equation.

THEORY

FOR IDEAL SOLUTIONS using Raoult's law, the water activity can be expressed as:

$$a_w = \frac{W_w/M_w}{W_w/M_w + W_{so}/M_s} = \frac{W_w}{W_w + EW_{so}} \quad (1)$$

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in which W_w is weight of water, M_w molecular weight of water (= 18 kg/kg mole), W_{so} weight of dissolved solids, M_s effective molecular weight of dissolved solids and $E = M_w/M_s$. It is convenient to express a_w in terms of mass fraction by dividing the right side of Eq. (1) by total weight of solution:

$$a_w = \frac{W_w/W_T}{W_w/W_T + EW_{so}/W_T} \quad (2)$$

where $W_T = W_w + W_{so}$. Denoting $X_1 = W_{so}/W_T$, Eq. (2) can be expressed as:

$$a_w = \frac{1 - X_1}{(1 - X_1) + EX_1} \quad (3)$$

in which the value of E can be calculated from the determination of M_s by using the following equation (Daniels et al., 1970):

$$M_s = \frac{1000\ K_f W_{so}}{W_w\ \Delta T} = \frac{1000\ K_f X_1}{(1 - X_1)\ \Delta T} \quad (4)$$

In this equation ΔT is the difference between the freezing point of pure water and that of the solution and is the observed freezing point depression (FPD) caused by the dissolved solids. A constant K_f is called the freezing point constant or molal depression constant which may be calculated by the following Van't Hoff's equation (Daniels et al., 1970):

$$K_f = \frac{RT^2}{1000\ L_f} \quad (5)$$

in which R is the ideal gas constant, T the freezing point of the solution, L_f the heat of fusion per kg. For water using R = 1.987 kcal/kg-mole °K, T = 273.15°K and L_f = 79.75 kcal/kg the constant K_f has a value of 1.86 kg °K/kg-mole. Recall $E = \frac{18}{M_s}$ and from Eq. (4) one can obtain:

$$E = \frac{18\ (1 - X_1)\ \Delta T}{1000\ K_f\ X_1} \quad (6)$$

For aqueous solutions using K_f = 1.86, Eq. (6) yields:

$$E = \frac{0.0097\ (1 - X_1)\ \Delta T}{X_1} \quad (7)$$

where the constant 0.0097 $\left(= \frac{M_w L_f}{RT^2}\right)$ has a unit $°K^{-1}$. By substituting Eq. (7) into Eq. (3), the water activity can be expressed as:

$$a_w = \frac{1}{1 + 0.0097\ \Delta T} \quad (8)$$

In this equation, water activity is expressed in terms of the observed freezing point depression.

It should be noted that Eq. (4) used in the derivation of Eq. (8) is applicable to an ideal dilute solution only, in order to

Table 1—Water activity and vapor pressure of ice-water system at various temperatures[a]

Temperature		Vapor pressure P		Water activity			
ΔT (°K)	T (°K)	Liquid water (mm Hg)	Ice (mm Hg)	$a_w = \frac{P_{ice}}{P_{water}}$	a_w^x	a_w^y	a_w^z
0	273.15	4.579	4.579	1.000	1.000	1.000	1.000
5	268.15	3.163	3.013	0.953	0.954	0.953	0.953
10	263.15	2.149	1.950	0.907	0.912	0.907	0.908
15	258.15	1.436	1.241	0.864	0.873	0.864	0.865
20	253.15	0.943	0.776	0.823	0.838	0.824	0.823
25	248.15	0.607	0.476	0.784	0.805	0.785	0.784
30	343.15	0.382	0.286	0.749	0.775	0.749	0.746
40	233.15	0.142	0.0966	0.681	0.720	0.681	0.675

[a] Fennema (1981); Fennema and Berny (1974).
[x] a_w calculated from Eq. (8).
[y] a_w calculated from Eq. (9).
[z] a_w calculated from Eq. (10).

Table 2—A comparison between experimental and calculated a_w values for various pure reagents

Solute	Conc (% by wt.)	FPD[a] (°C)	Water activity a_w^x	a_w^y	a_w^z
NaCl	1	0.6	0.994	0.994	0.994
	5	3.1	0.968	0.971	0.970
	10	6.6	0.931	0.940	0.938
	15	10.9	0.891	0.904	0.900
	20	16.5	0.844	0.862	0.852
Fructose	10	1.2	0.991	0.988	0.988
	15	1.9	0.985	0.982	0.982
	30	4.7	0.961	0.956	0.955
	40	7.7	0.935	0.931	0.928
Sucrose	5	0.3	0.996	0.997	0.997
	10	0.6	0.992	0.994	0.994
	15	1.0	0.988	0.990	0.990
	20	1.4	0.983	0.987	0.986
	30	2.8	0.971	0.974	0.973
	40	4.7	0.955	0.956	0.955
Lactose	1	0.1	0.999	0.999	0.999
	3	0.3	0.997	0.997	0.997
	5	0.4	0.995	0.996	0.996
	8	0.6	0.992	0.994	0.994
Glycerol	5	1.0	0.991	0.990	0.990
	10	2.5	0.981	0.976	0.976
	20	5.4	0.958	0.950	0.949
	30	9.8	0.927	0.913	0.909
	40	15.5	0.887	0.869	0.860

[a] Experimental data of FPD (Lerici et al., 1983).
[x] Measured by electric hygrometer (Lerici et al., 1983)
[y] a_w calculated from the FPD using Eq. (8).
[z] a_w calculated from the FPD using Eq. (9).

Table 3—A comparison between experimental and calculated a_w values for some liquid foods

Products	Total solids (% by wt)	FPD[a] (°C)	Water activity a_w^x	a_w^y	a_w^z
Freeze-dried skim milk	10	0.6	0.997	0.994	0.994
	20	1.6	0.990	0.985	0.985
	30	2.3	0.982	0.978	0.978
	40	4.1	0.971	0.962	0.961
Freeze-dried coffee beverage	5	0.3	0.996	0.997	0.997
	10	0.6	0.991	0.994	0.994
	20	1.3	0.990	0.988	0.987
	30	2.3	0.978	0.978	0.978
	40	3.7	0.964	0.965	0.965
Concentrated grape juice	10	1.2	0.986	0.988	0.988
	20	2.9	0.976	0.973	0.972
	30	4.4	0.962	0.959	0.958
	8	1.1	0.993	0.989	0.989
juice	10	1.3	0.989	0.988	0.987
	20	2.3	0.985	0.978	0.978
Dried tomato juice	5	0.5	0.998	0.995	0.995
	10	0.9	0.989	0.991	0.991
	15	1.6	0.985	0.985	0.985
	20	2.0	0.980	0.981	0.981

[a] Experimental data of FPD (Lerici et al., 1983).
[x] a_w measured by electric hygrometer (Lerici et al., 1983).
[y] a_w calculated from the FPD using Eq. (8).
[z] a_w calculated from the FPD using Eq (9).

Table 4—A comparison between experimental and calculated a_w values for glycerol

Solute	Conc (% by wt)	FPD[a] (°C)	Water activity a_w^x	a_w^y	a_w^z
Glycerol	5	1.0	0.991	0.990	0.990
	10	2.5	0.981	0.977	0.976
	20	5.4	0.958	0.953	0.949
	30	9.8	0.927	0.923	0.909
	40	15.5	0.887	0.882	0.859

[a] Experimental data of FPD (Lerici et al., 1983).
[x] Measured by electric hygrometer (Lerici et al., 1983).
[y] a_w calculated from the FPD using Eq. (9) with $C = -0.00012$.
[z] a_w calculated from the FPD using Eq. (10).

compensate the deviation from ideality at higher concentration, the following semi-empirical modification was assumed.

$$a_w = \frac{1}{1 + 0.0097 \Delta T + C \Delta T^2} \quad (9)$$

Using data for ice-water system (Fennema and Berny, 1974), the value of $C = 5 \times 10^{-5} K^{-2}$ was determined.

Eq. (9) was compared with the well-known freezing point depression equation which was derived for ice-water system (Ferro-Fontan and Chirife, 1981; Robinson and Stokes, 1965):

$$-\ln a_w = 27.622 - 528.373 (1/T) - 4.579 \ln T \quad (10)$$

RESULTS & DISCUSSION

FENNEMA AND BERNY (1974) have shown that at subfreezing temperatures, the equilibrium vapor pressures of plant and animal tissues and simple solutions did not differ significantly from the equilibrium vapor pressure of ice. Thus, the a_w values of frozen foods and solutions can be calculated by the ratio of vapor pressure of ice and supercooled liquid water at comparable temperatures. Eq. (8), (9), and (10) provide useful estimates of a_w from the measurements of freezing points. The results in Table 1 show a comparison between experimental and calculated a_w values of ice-water system for the range of temperatures between 0 and −40°C (Fennema and Berney, 1974). In the range of temperatures between 0° and −15°C the differences between a_w values measured and calculated using Eq. (8), (9) and (10) were less than 0.01. With increasing FPD values, the deviations from the measured values became greater for those calculated using Eq. (8) but only small deviations for those calculated using Eq. (9) and (10). It is evident that Eq. (9) provides a simple and accurate relationship

for calculating a_w values for frozen foods at all temperatures within the range of 0 to -40°C.

It should be noted that empirically modified Eq. (9) with $C = 5 \times 10^{-5}$ agrees closely with the well-known thermodynamic Eq. (10) for ice-water systems. The differences between the calculated results of Eqs. (9) and (10) are less than 0.01 but Eq. (9) is simpler to use. Therefore, Eq. (9) can be used to replace Eq. (10) for calculation.

Lerici et al. (1983) have reported experimental FPD and a_w values for a number of aqueous solutions and liquid food systems. Their results were calculated and presented in Tables 2 and 3. Their measured FPD values were used for calculating a_w values using Eq. (8) and (9) for a comparison with a_w values measured by electric hygrometer.

Table 2 shows a comparison between experimental and calculated a_w values for various pure substances. In the range of FPD values between 0° and 5°C, the differences between a_w values measured and calculated using either Eq. (8) or (9) were less than 0.01 for all the substances. At higher FPD values greater deviations were observed.

Table 3 shows a comparison between experimental and calculated a_w values for some liquid foods. The differences between the a_w values measured and calculated using either Eq. (8) or (9) were within less than 0.01 a_w units. It should be noted that the FPD values were in the range between 0° to 5°C. The good agreements could be expected due to the low range of FPD values observed.

On the basis of Eq. (8), it can be shown (Mickley et al., 1957) that the effect of measurement errors of FPD on the calculated values of a_w can be estimated by:

$$a_w = \frac{-0.0097}{(1 + 0.0097\ \Delta t)^2}\ \Delta\ (\Delta t)$$

$$\simeq -0.01\ \Delta\ (\Delta t) \qquad (11)$$

For example, the measurement errors in temperature $\pm$ 0.2°C would account for approximately $\pm$ 0.002 variations in a_w values. The errors in a_w greater than 0.01 were considered deviations from ideal solution laws. To quantify the deviations, Eq. (9) with different empirical constants were used. For example, the experimental data of glycerol in Table 2 were fitted to $\pm$ 0.005 a_w by Eq. (9) with $C = -0.00012$. A comparison of calculated data using Eq. (9) and (10) is presented in Table 4. It should be pointed out that Eq. (9) requires only one empirical constant to characterize the deviation from the ideal equation (8) but it cannot be extrapolated beyond the observed range.

By rearranging Eq. (4), the FPD can be expressed as a function of concentration and molecular weight of soluble solids as:

$$\Delta T = \frac{1000\ K_f X_1}{(1 - X_1)\ M_s} \qquad (12)$$

It is seen that the ΔT value decreases as the molecular weight of solute increases and it increases as the concentration increases. Since a_w is inversely related to the second degree polynomial equation of ΔT by Eq. (9), the a_w value increases as the molecular weight of solute increases and it decreases as the concentration increases. On the basis of these relationships, it is evident that the variations of a_w and ΔT are due to both composition and concentration of soluble solids in the food systems. Therefore, Eq. (4) coupled with Eq. (9) should be useful for studying food components for control of a_w in foods.

CONCLUSIONS

BECAUSE THE DERIVATION of Eq. (9) was based on the well-established thermodynamic relationships, it should be generally applicable to food systems at both above-freezing and subfreezing temperatures. It provides accurate and simple relationship for calculating a_w of frozen foods at various subfreezing temperatures. It can also be used to replace the widely used freezing point depression equation for all practical purposes.

REFERENCES

Andrews, F.C. 1976. Colligative properties of simple solutions. Science 194: 567.

Daniels, F., Alberty, R.A., Williams, J.W., Cornwell, C.D., Bender, P., and Harriman, J.E. 1970. "Experimental Physical Chemistry," 7th ed. McGraw-Hill Book Co. Inc., New York.

Dyer, D.F., Carpenter, D.K., and Sunderland, J.E. 1966. Equilibrium vapor pressure of frozen bovine muscle. J. Food Sci. 31: 196.

Fennema, O. 1981. Water activity at subfreezing temperatures. In "Water Activity: Influences on Food Quality." Academic Press. New York.

Fennema, O. and Berry, L.A. 1974. Equilibrium vapor pressure and water activity of food at subfreezing temperatures. Proc. 4th Intern. Congr. Food Sci. Technol. 2: 27.

Ferro-Fontan, C. and Chirife, J. 1981. The evaluation of water activity in aqueous solutions from freezing point depression. J. Food Technol. 16: 21.

Hill, J.E. and Sunderland, J.E. 1967. Equilibrium vapor pressure and latent heat of sublimation for frozen meats. Food Technol. 21: 1276.

Karel, M. 1975. Stability of low and intermediate moisture foods. In "Freeze-Drying and Advanced Food Technology." Academic Press, London.

Labuza, T.P. 1975. Oxidative changes in foods at low and intermediate moisture levels. In "Water Relations of Foods." Academic Press, London.

Lerici, C.R., Piva, M., and Rosa, M.D. 1983. Water activity and freezing point depression of aqueous solutions and liquid foods. J. Food Sci. 48: 1667.

Mickley, H.S., Sherwood, T.K., and Reed, C.E. 1957. "Applied Mathematics in Chemical Engineering," 2nd ed. McGraw-Hill, New York.

Robinson, R.A. and Stokes, R.M. 1965. "Electrolyte Solutions," 2nd ed. Butterworth Scientific Publications, London.

Scott, W.J. 1957. Water relations of food spoilage microorganisms. Adv. J. Biol. Sci. 6: 549.

Storey, R.M. and Stainsby, G. 1970. The equilibrium water vapor pressure of frozen cod. J. Food Technol. 5: 157.

Troller, J.A. and Christian, J.H.B. 1978. "Water Activity and Food," Academic Press, New York.

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A Graphical Interpretation of Time-Temperature Related Quality Changes in Frozen Food

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ABSTRACT

Frozen food quality change was presented graphically as a response parameter to the storage variables time and temperature. Computer graphics were used to portray sensory changes in frozen hamburger during storage. The resulting three-dimensional surface (a "quality-response surface") was simplified by contour plotting and the responses of both full- and partial-history time-temperature indicators were superimposed. Examples of the resulting indicator action diagram were developed for frozen hamburger, and possible applications to frozen inventory management were discussed.

INTRODUCTION

FROZEN FOOD QUALITY is a complex topic which must include the considerations of consumptive safety and sensory properties, product composition and physical properties, chemical and enzymatic reactions, and microbiological interaction and growth. Many of the physical, chemical, enzymatic, and microbial changes which contribute to frozen food quality are highly dependent on storage conditions, namely, the combined effects of time and temperature. It is well known that abusive temperature conditions during storage and handling may lead to frozen products of inferior quality.

The factors contributing to the maintenance of frozen food quality during cold storage include storage temperature, initial product composition and quality, processing techniques (both pre-freezing and freezing treatments) and the product packaging process and materials. Research results from frozen food studies have been reviewed in books by Van Arsdel et al. (1969) and Jul (1984). Singh and Wang (1977) published a comprehensive review on quality changes in frozen foods during storage and noted several physical, chemical and sensory quality changes which were related to time-temperature exposure. A general review of quality changes in perishable and semi-perishable foods (including frozen foods) can be found in a book by Labuza (1982).

Various interpretations of the relationship between time and temperature, and frozen food quality changes have been discussed under the loosely defined heading of "shelf-life kinetics." These interpretations include mathematical models using the Arrhenius relationship, a model using an approach analogous to thermal death time, and a variety of empirical relationships specific to given sets of data. Generally, these mathematical models are somewhat complicated and often require use of a small computer or programmable calculator to obtain results.

A time-temperature indicator is a device which registers a response according to some combined effect of time and temperature. Review papers by Schoen and Byrne (1972) and Kramer and Farquhar (1976) provide information on patents and commercially developed indicators that monitor temperature changes during storage. Wells and Singh (1985) have developed an indicator classification scheme based on a particular indicator's response, and its independence of (or dependence on) a threshold temperature. The classification specifies that if a time-temperature indicator can respond only after a present threshold temperature is exceeded, it is considered to be a "partial-history" indicator; while those devices which respond independent of a threshold temperature are said to be "full-history" indicators. Examples of full- and partial-history indicators are the I-POINT Time-Temperature Monitor (I-POINT Technologies, Malmo, Sweden), and the 3M MonitorMark (3M MonitorMark, Packaging Systems Division/3M, St. Paul, MN), respectively.

Recent investigations conducted by Mistry and Kosikowski (1983), Zall et al. (1984), and Singh et al. (1984, 1986) have demonstrated the use full-history time-temperature indicators as food quality monitors. These researchers have presented evidence that full-history time-temperature indicators can be used to monitor food quality changes in a variety of refrigerated and frozen foods. Manske (1983) pointed out that no direct correlation between food quality change and partial-history indicator response should be made, as the intended design of most partial-history indicators was to identify abusive temperature conditions.

The objective of this paper was to introduce a graphical interpretation of food quality change and discuss the integration of this presentation into a food quality management procedure using time-temperature indicators.

Theoretical considerations

Several researchers have discussed the use of mathematical models to describe quality changes in foods. Labuza (1979, 1984), Saguy and Karel (1980) and Lai and Heldman (1982) each discuss models which can be used to describe the effect of time and temperature on food quality. Food systems are highly complex, and precise time-temperature related quality change mechanisms are not fully understood. Because of this, all time-temperature induced quality changes cannot be included within one mathematical model. Generally, only a single index of quality is considered in the modeling procedure.

The index of quality may be any physical, chemical, enzymatic, sensory or microbial characteristic which is detectable and predominant within the food and has importance in relation to product definition and acceptance. Since foods are destined for human consumption, it can be argued that the index of quality is most appropriately defined with sensory perception. It is important, however, that an index of quality be defined in terms of an analytically quantifiable attribute and not as an integrated subjective response.

Time-temperature related changes in food quality, or the quality-response to storage and distribution conditions, can be modeled with concepts of chemical kinetics. The change in an index of quality monitored under isothermal storage conditions is modeled with the differential equation:

$$\frac{d[Y]}{dt} = -k[Y]^n \qquad (1)$$

where Y = index of quality; t = time; k = reaction rate constant; n = reaction order (a curve-fitting parameter).

The quality-response of most foods has been observed to follow either a zero-order (n = 0) or a first-order (n = 1) reaction model. The values of k are determined by regression anal-

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ysis of quality change data obtained at constant temperature storage, fitted to the integrated form of Eq. (1).

The temperature dependence of the rate constant is often given by the Arrhenius relationship. The form of the Arrhenius equation is:

$$k = k_o \exp \quad (-E_A/R_gT) \qquad (2)$$

with k_o = constant, independent of temperature (also referred to as the preexponential factor); E_A = activation energy; R_g = ideal gas constant; T = absolute temperature.

When the transformed form of equation 2 is plotted as ln(k) versus 1/T, the result is a straight line. Since

$$\ln(k) = \ln(k_o) - \frac{E_A}{R_g}\left(\frac{1}{T}\right) \qquad (3)$$

is linear in form, the values of k_o and E_A may then be calculated from the regression intercept and slope, respectively. Thus, with estimates of k_o and E_A, the index of quality, (Y), can be predicted for combinations of storage time (t) and temperature (T).

MATERIALS & METHODS

Frozen hamburger storage study

The frozen hamburger data discussed in the following example were reported previously by Singh et al. (1984) and by Singh and Wells (1985). Sensory measurements of rancidity changes in hamburger were made at 14-day intervals from three constant temperature storage conditions. Sensory panel members were trained in the recognition of hamburger rancidity and were asked to evaluate the difference between a reference sample and the experimental treatment samples. Perceived differences were rated as deviation-from-reference on a 10-cm unstructured, anchored scale. The scale midpoint was identified as the reference and corresponded to a score of 50.

Data analysis

The sensory data were analyzed using the SAS Statistical Software Package (SAS, 1982). Mean sensory scores for each evaluation session were compared according to the multiple-means comparison techniques of the Scheffe MSD test and the Fisher LSD test, under the restriction of a significant treatment effect for a 3-way analysis of variance (temperature treatment, judge, replication). Graphical modeling of sensory data was conducted with the software package SAS/GRAPH (SAS, 1985).

RESULTS & DISCUSSION

Storage study results

The plot of hamburger rancidity scores for −12°C and −35°C (reference) storage is shown in Fig. 1. For the same data, Singh and Wells (1985) reported that hamburger samples stored at −12°C were found to be significantly different from reference samples held at −35°C from day 103 of storage onward. This finding is highly conservative since comparisons were made according to the Scheffe MSD test, a highly conservative, less powerful means comparison technique. Using the Fishers LSD test for comparison, significant differences in rancidity scores between −12°C and −35°C samples were found from day 61 of storage onward. Most likely, a noticeable difference in hamburger rancidity scores would be observed at an intermediate storage time, say 84 days. From examination of the −12°C scores at 84 days, it would be expected that a noticeable change (from reference) in hamburger rancidity would occur at a sensory score of approximately 58.

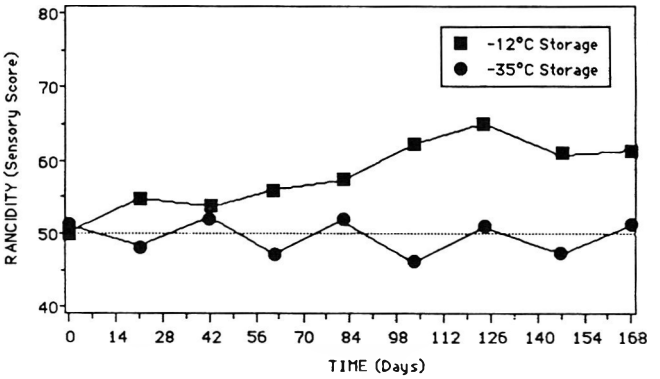

Fig. 1—Sensory measurements of hamburger rancidity during frozen storage.

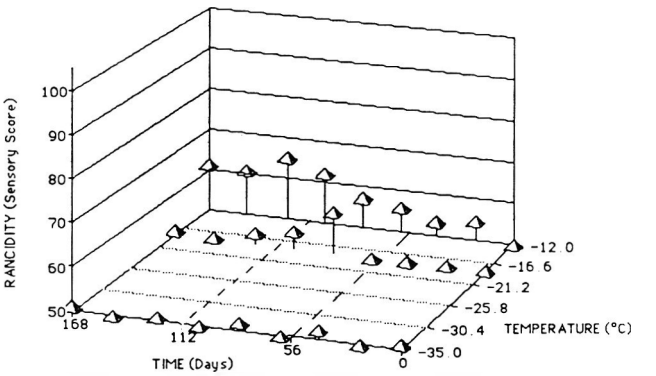

Fig. 2—Three-dimensional scatter diagram of frozen hamburger rancidity score.

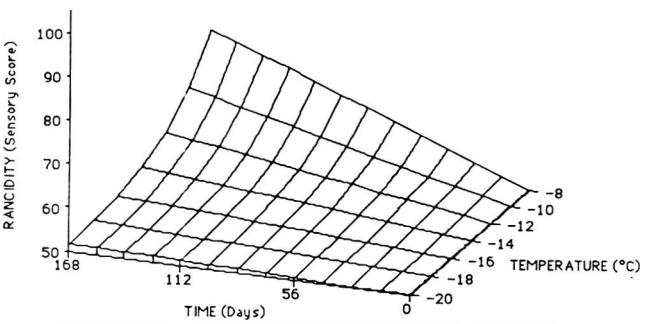

Fig. 3—"Quality-response surface" for frozen hamburger rancidity.

Quality-response surface: A graphical representation

The time-temperature related quality-response for the frozen hamburger rancidity data is given in the three-dimensional scatter plot shown in Fig. 2. By modeling changes in hamburger rancidity as a zero-order reaction, the resulting pre-exponential factor (k_o) and activation energy (E_A) were equal to $8.482 \times 10.^{24}$ rancidity score/day and 31,000 cals/mole, respectively. A graphical representation of the time-temperature, quality relationship of hamburger rancidity is shown in Fig. 3. In this three-dimensional representation, the hamburger rancidity sensory scores predicted by the zero-order reaction and the Arrhenius equation are depicted as a surface, the so-called "quality-response surface."

The term "quality-response surface" as defined here means a graphical mapping of food quality measurements which correspond to a time-temperature combination. (The term "quality-response surface" should not be confused with "response surface methodology," which is a sophisticated statistical tech-

nique in experimental design.) A "quality-response surface" is intended to represent a product quality-change, or quality-response, resulting from the temperature conditions encountered during frozen storage and distribution. A "quality-response surface" could evolve from independent measurements of a product characteristic during a constant temperature storage study consisting of several temperature treatments; or could be generated using a mathematical model.

There are three elements which are essential in depicting a "quality-response surface:" (1) lines of constant time, (2) lines of constant temperature,and (3) contours of constant quality. The lines of constant time and constant temperature result from the projection of the time-temperature axes grid onto the "quality-response surface," and the contours of constant quality arise by tracing discrete quality levels across the surface. A three-dimensional visualization of the "quality-response surface" marked with lines and contours is somewhat difficult. A planar depiction of the "quality-response surface" and an application for this graphical presentation is discussed below.

Quality contour diagram

Contour plotting is a technique used in descriptive geometry by which a three-dimensional surface can be shown in two-dimensions. The height of the "quality-response surface" is represented by projecting the contours of constant quality onto the plane defined by the time and temperature axes. The quality contour diagram for the hamburger rancidity "quality-response surface" is shown in Fig. 4. The contours of constant quality are shown on this diagram along with the corresponding sensory scores for hamburger rancidity. Lines of constant time are shown as horizontal lines extending from the time axis across the plot. Similarly, lines of constant temperature are drawn vertical from the temperature axis.

The axes of the quality contour diagram and the contours of constant quality provide a complete visual relationship between time, temperature and quality. Given the measures of any two of the three parameters, the third one can be determined. For example, hamburger stored for 70 days at $-12°C$ would give rise to sensory rancidity score of 55. Time and temperature combinations which yield scores between quality contours can be estimated by interpolating between neighboring contours. In addition, the spacing of the contour lines provides information about the rate at which hamburger rancidity scores change. Contours which appear at closely spaced intervals (when moving along lines of constant temperature) represent the steepest portions of the "quality-response surface." Steeper portions of the "quality-response surface", as seen in the bottom right hand portion of Fig. 4, correspond to the temperature conditions which result in more rapid quality changes.

Time-temperature indicators and the "quality-response surface"

The use of time-temperature indicators as food quality monitors is most appropriately suited to frozen foods which are highly uniform. In general, frozen fruits and vegetables are less uniform in product definition and character than are certain manufactured or prepared foods. Because of the process control involved in the manufacture of prepared products, the initial composition and physical properties are reasonably well defined and consistent. This uniformity in initial product quality, coupled with known and uniform packaging characteristics, reduce the variables which affect product quality change during storage and distribution. The primary variables which remain to contribute to the maintenance of product quality during frozen storage are time and temperature.

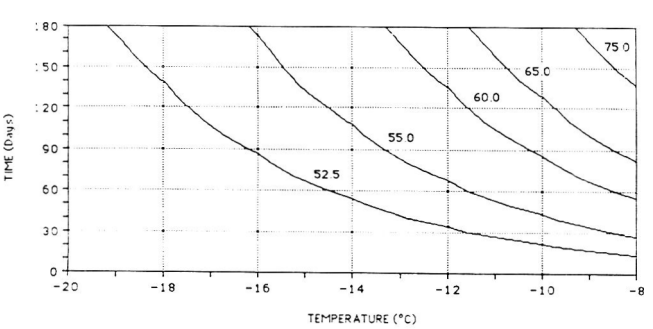

Fig. 4—Quality contour diagram for frozen hamburger rancidity.

To utilize the "quality-response surface" expression of food quality, and integrate a quality contour diagram with an application of time-temperature indicators, it is advantageous to define an "action threshold" for the quality characteristics described by the "quality-response surface." The "action threshold" is that amount of quality change above which distribution action need be taken (e.g., product shipment, product recall, lower pricing). The "action threshold" could represent a consumer level determination of quality change which leads to product rejection; or could represent a quality change which is noticeably different from a reference sample. The "action threshold" would correspond to a contour on the quality contour diagram and would partition the diagram into regions of "action" and "non-action." The "action region" would be in the upper right hand portion on the quality contour diagram and would indicate the time-temperature combinations which require distribution action. Time-temperature combinations which fall into the "nonaction region" would not require distribution action.

For the data considered in this study, to insure a product of no noticeable rancidity change, distribution action should correspond to a hamburger rancidity score somewhat below the 58 value (a score which was noticeably different from reference during the storage study). A reasonable choice for distribution action would be a rancidity score of 55. Thus, time-temperature combinations which result in sensory rancidity scores above 55 (the "action threshold") would fall in the "action region."

"Indicator action diagram"

Once a quality parameter has been fully defined in terms of a "quality-response surface" with action threshold, an application of time-temperature indicator as food quality (single characteristic) monitor is straightforward. A full-history indicator would be selected to closely mimic the entire "quality-response surface" or a partial-history indicator would be chosen to mimic the surface above the line of constant temperature which corresponds to the partial-history indicator's response temperature.

By superimposing indicator response values on the time and temperature scale of the quality contour diagram the "indicator action diagram" is formed. An "indicator action diagram" is a direct result of the combination of the quality contour diagram of the defined "quality-response surface" and the indicator response scale. Consider two examples of "indicator action diagrams," which result from considering the hamburger rancidity quality contour diagram given in Fig. 4 (with "action region" specified), and the I-POINT Time-Temperature Monitor and 3M MonitorMark time-temperature indicators are shown in Fig. 5 and 6, respectively. To interpret these "indicator action diagrams" one must understand the meaning of the response for each type of indicator.

A full-history time-temperature indicator will indicate a response over the range of all temperatures. The response of the full-history indicator will approximate, to some degree, a quality contour and can be directly related as quality response. Thus, a full-history indicator response can be thought of as changing across contours of constant quality. The I-POINT Time-Temperature Monitor has a response scale of 0, 1, 2,

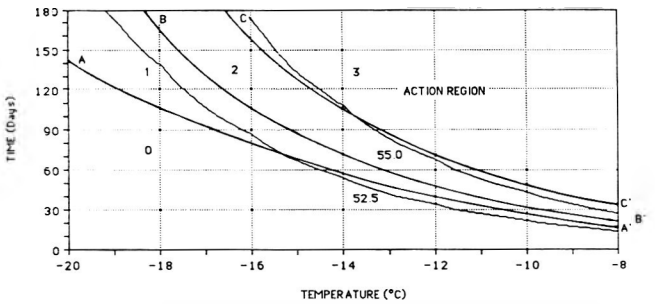

Fig. 5—Full-history time-temperature indicator action diagram for frozen hamburger rancidity and I-Point Time-Temperature Monitor Model 3015.

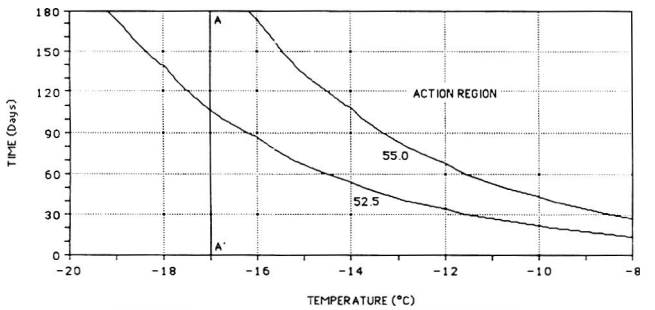

Fig. 6—Partial-history time-temperature indicator action diagram for frozen hamburger rancidity and 3M MonitorMark Model L17.

and 3. Upon activation the 0 response is given until a specific time-temperature combination has been achieved, then the indicator will respond with 1. The discrete indicator responses, 0, 1, 2, or 3, correspond to predetermined combinations of time and temperature.

The response boundaries for the I-POINT Time-Temperature Monitor model 3015, shown as segments AA', BB', and CC' in Fig. 5, were determined from published manufacturer data. The full-history indicator action diagram shows the indicator response and quality-response correspondence. A full scale reading of the I-POINT Time-Temperature Monitor model 3015 (scale reading 3) approximately corresponds to the "action region" of the quality contour diagram, as it overlays the 55 contour on the quality contour diagram. A full scale reading for I-POINT model 3015 would indicate that distribution action need to be taken as noticeable changes in hamburger rancidity are likely to have occurred.

A partial-history time-temperature indicator will register a response only when a threshold temperature has been exceeded. Thus, a partial-history indicator response can be thought of as changing across lines of constant temperature on the quality contour diagram. The 3M MonitorMark model L17 will respond to temperature exposures above −17°C. The −17°C line of constant temperature is shown in Fig. 6 as segment AA'. If this threshold temperature is not exceeded, no indicator response is registered (a zero response). A zero response cannot be interpreted as no quality change but infers that the rate of quality change has been no more rapid than that which would occur at −17°C, the indicator threshold temperature. However, if temperature conditions exist which raise the temperature above −17°C, an indicator response will be seen and the product will experience a faster rate of quality change.

Several models of the 3M MonitorMark, each with different temperature thresholds, could be used to segment the quality contour diagram. The lines of constant temperature corresponding to the threshold temperature of each indicator model would serve as a segment boundary. The combination of the indicators' responses and storage time would specify a partitioned section on the quality contour diagram. The combination of indicator response and action threshold would indicate a range of storage times, the minimum of which would guarantee a product quality change less than that which would require distribution action. In effect, a partial-history indicator action diagram shows the reduction in storage time (the length of time before distribution action needed to be taken), due to improper temperature maintenance. A limitation of the partial-history indicator action diagram is that once a full scale indicator reading is achieved, and improper temperature maintenance continues, a further reduction in storage time cannot be determined.

Potential use of the "indicator action diagram"

In developing an application of time-temperature indicators to food quality monitoring, one must consider how to define and monitor food quality in a quantitative manner; how to integrate the expression of food quality with time-temperature indicator response; and, how to simply and effectively apply the procedure. The use of a "quality-response surface" expressed as a quality contour diagram is one possible solution to these requirements. However, research to determine the important food characteristics, the "quality-response surface" of these characteristics, and their action thresholds, must be conducted before time-temperature indicators can be fully utilized.

The concept of an "indicator action diagram" can be applied to full- or partial-history time-temperature indicators as a valuable quality assurance aid to frozen food manufacturers and distributors. This graphical portrayal of food quality change and time-temperature indicator response can simplify complicated mathematical models, presenting them in a visual format. The "indicator action diagram" provides a means by which product inventory could be managed based on estimated quality levels, and would establish a framework to justify taking corrective actior to expedite product distribution and/or remedy any deficient handling procedures. The use of time-temperature indicators, as frozen food quality monitors, has a potentially important application to inventory management. The implications of improved management techniques for frozen food inventories could result in a higher quality product being delivered to the consumer.

CONCLUSIONS

The specific conclusions related to this research are: (1) Results of food quality change modeling may be presented graphically with the aid of SAS/GRAPH plotting procedures. (2) An "Indication Action Diagram" can be used to determine the need for distribution action of frozen hamburger.

NOMENCLATURE

Y	index of quality (sensory score)
t	time (days)
k	reaction rate constant (score/day)
n	reaction order (a curve-fitting parameter)
k_o	pre-exponential factor, independent of temperature (score/day)
T	absolute temperature (K)
E_A	activation energy (cals/mole)
R_g	ideal gas constant (cals/mole-K)

REFERENCES

Jul, M. 1984. "The Quality of Frozen Foods," Academic Press, New York.
Kramer, A. and Farquhar, J.W. 1976. Testing of time-temperature indicating and defrost devices. Food Technol. 30(2): 50.
Labuza, T.P. 1979. A theoretical comparison of losses in food under fluctuating temperature sequences. J. Food Sci. 44: 1162.
Labuza, T.P. 1982. "Shelf-Life Dating of Foods," Food & Nutrition Press, Westport, CT.
Labuza, T.P. 1984. Application of chemical kinetics to deterioration of food. J. Chem. Educ. 61: 348.

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Phytate × Calcium/Zinc Molar Ratios: Are They Predictive of Zinc Bioavailability?

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ABSTRACT

The utility of the phytate/zinc and phytate × calcium/zinc molar ratios for predicting zinc bioavailability from processed soybean foods was investigated. Weight gain and bone zinc accumulation in rats fed various soy protein products were plotted against the calculated molar ratios. The phytate × calcium/zinc ratio was a better predictor of zinc bioavailability in similarly processed products than was the phytate/zinc ratio. However, in some cases the phytate × calcium/zinc ratio was not effective since some processing procedures apparently altered binding of phytic acid to minerals and other food components.

INTRODUCTION

IT IS WIDELY RECOGNIZED that zinc is generally less bioavailable from foods of plant origin than from foods of animal origin or from soluble inorganic zinc salts. Phytic acid found in cereals and legumes is thought to be a major contributor to the reduced availability of zinc from these foods (Erdman, 1981). Further, it has been shown that high dietary calcium accentuates the effect of phytate on zinc bioavailability (Likuski and Forbes, 1965; Oberleas, 1975; Oberleas et al., 1966; Oberleas and Harland, 1981; O'Dell, 1969, 1980; Davies and Ried, 1979; Davies and Olpin, 1979; Morris and Ellis, 1980a; Ellis et al., 1982a; Forbes et al., 1984; BaFundo et al., 1984). The formation of Zn-Ca-phytate complexes or other Zn-phytate complexes in the upper gastrointestinal tract of monogastric animals is believed to be a major mechanism by which phytate reduces dietary zinc bioavailability. The prediction of mineral bioavailability from phytate-containing foods is complicated by the complex interactions between minerals and phytic acid contained in foods, phytase activities in the meal and/or in the intestine, previous food processing conditions (especially pH), digestibility of the foods as well as the physiological status of the consumer of the foods (Erdman, 1979).

The phytate/zinc [(P)/(Z)] molar ratio has been suggested as an indicator of zinc bioavailability (Oberleas, 1975; Oberleas and Harland, 1981; Davies and Olpin, 1979; Morris and Ellis, 1980a, b). However, it can be shown that the (P)/(Z) molar ratio is a poor indicator as it does not take into account the well-known aggravating effects of calcium on zinc absorption in phytic acid-containing diets (Forbes et al., 1983, 1984).

Recently, it has been suggested that the phytate X calcium/zinc [(P)(C)/(Z)] molar ratio is a better predictor of zinc utilization (Davies et al., 1985; Ellis et al., 1985; Bindra et al., 1986). The objective of the current work was to investigate the (P)(C)/(Z) molar ratio as an accurate predictor of zinc bioavailability by examining a series of studies published from this laboratory (Forbes and Parker, 1977; Weingartner et al., 1979; Erdman et al., 1980; Forbes et al., 1983, 1984; Ketelsen et al., 1984). In these investigations zinc bioavailability was evaluated from various soy foods produced under carefully-controlled processing conditions. Relative zinc bioavailability was evaluated by comparing weight gain or bone zinc mineralization in rats fed diets containing test soy proteins with diets containing egg white protein with added zinc carbonate, an excellent source of available zinc.

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MATERIALS & METHODS

SEVERAL previously published studies were used as our data source to evaluate the effect of dietary calcium, zinc and phytate on zinc bioavailability (Forbes and Parker, 1977; Weingartner et al., 1979; Erdman et al., 1980; Forbes et al., 1983, 1984; Ketelsen et al., 1984). The standard slope ratio technique was used to evaluate zinc bioavailability in several of the studies (Forbes and Parker, 1977; Forbes et al., 1979, 1983, Erdman et al., 1980). Briefly, isonitrogenous, isoenergetic diets were fed to groups of male weanling rats. Control diets containing 18% or 20% egg white protein were supplemented with one of several levels of zinc as zinc carbonate. To test for the availability of zinc in the soy product, several levels of zinc were added to the egg white-based diet by substituting the soy product for egg white on an equivalent protein basis. To provide a measure of relative zinc bioavailability, the data for the weight gain and for total bone (tibia or femur) zinc, after 21 days *ad libitum* feeding, were statistically analyzed by regression analysis to compare the slopes of the linear portions of the lines relating response per unit of added mineral. Differences in weight gain and bone zinc were evaluated by t-tests.

In a study by Ketelsen et al. (1984) the bioavailability of ^{65}Zn from intrinsically and extrinsically labeled soy products was evaluated in rats by feeding radioactively-labeled test meals identical in composition to the previous diet except that the soy product was either intrinsically or extrinsically labeled with ^{65}Zn. In another study (Forbes et al., 1984) young male weanling rats were fed egg white-based diets containing 12 ppm zinc as $ZnCO_3$ with varying levels of sodium phytate and calcium carbonate in a 21-day *ad libitum* feeding period. To provide a measure of zinc bioavailability, weight gain and total tibia zinc were plotted against the (P)/(Z) molar ratio.

The data from the above studies were statistically analyzed using the broken-line model fitted by the method of least squares (Robbins, 1986). The usefulness of the (P)/(Z) and (P)(C)/(Z) molar ratios for predicting the bioavailability of zinc from these processed soybean products was tested by comparing the calculated molar ratios to absolute weight gain and bone zinc accumulation.

It should be noted that Davies et al. (1985) expressed (P)(C)/(Z) molar ratios in terms of moles/kg of diet while Bindra et al. (1986) presented their ratios in millimoles intake/day. In the present work the former expression was used (calcium, zinc and phytate were expressed as moles/kg of diet) in part because each group of rats was fed a single defined dry diet *ad libitum* for the duration of the study. Expressing (P)(C)/(Z) in terms of millimoles intake/day is not desirable because increasing the intake of a specific diet will increase the calculated molar ratio. Comparison between groups of differing caloric intake is not possible unless adjustment is made to place all diets on the same calorie intake basis. Expression of data on a millimole/1000 Kcal intake basis, in contrast, seems to be a reasonable approach. To utilize moles/kg diet, as is utilized in the current study, one must calculate all molar ratios on a dry basis.

RESULTS & DISCUSSION

IN THE STUDY by Forbes et al. (1984), dietary (P)/(Z) molar ratios were varied from 0 to 50 while the (P)(C)/(Z) molar ratios (expressed as moles/kg diet) varied from 0 to 8. All rats received 12 ppm zinc, the NRC requirement for the rat (NAS, 1978). Figure 1 shows the results of plotting weight gain (g) versus the (P)(C)/(Z) molar ratio. Including the calcium level in the predictive equation results in declining weight gain as the (P)(C)/(Z) molar ratio increases. As shown in the diagram

Table 1—Comparison of the relative zinc bioavailability in soy foods with various phytate/zinc [(P)/(Z)] and phytate X calcium/zinc [(P)(C)/(Z)] molar ratios

Product	Reference[a]	(P)/(Z)	(P)(C)/(Z)	Relative bioavailability %	
				Wt gain	Tibia zinc
Egg white-$ZnCO_3$[b]	(1)-(4)	0	0	100	100
Soy isolate-acid	(2)	35	5.1	106	64
Soy flour-$ZnCO_3$[b]	(1)	24.6	3.5	94	94
Soy conc-acid	(2)	66	9.5	93	48
Soy isolate-neutral	(2)	34.1	4.9	85	45
Soy conc-neutral-$ZnCO_3$[b]	(4)	52	7.5	77	70
Soy conc-neutral	(2)	57	8.2	66	83
Soy beverage	(4)	26	3.8	63	40
Soy flour	(1)	24.6	3.5	55	34
Mg-tofu	(3)	26.1	4..6	52	40
Ca-tofu	(3)	27.6	4.8	51	36
Soy conc-neutral	(4)	52	7.5	41	20

[a] References: (1) Forbes and Parker (1977); (2) Erdman et al. (1980); (3) Forbes et al. (1983); (4) Forbes et al. (1979).
[b] $ZnCo_3$ added to soy product or egg white control diet

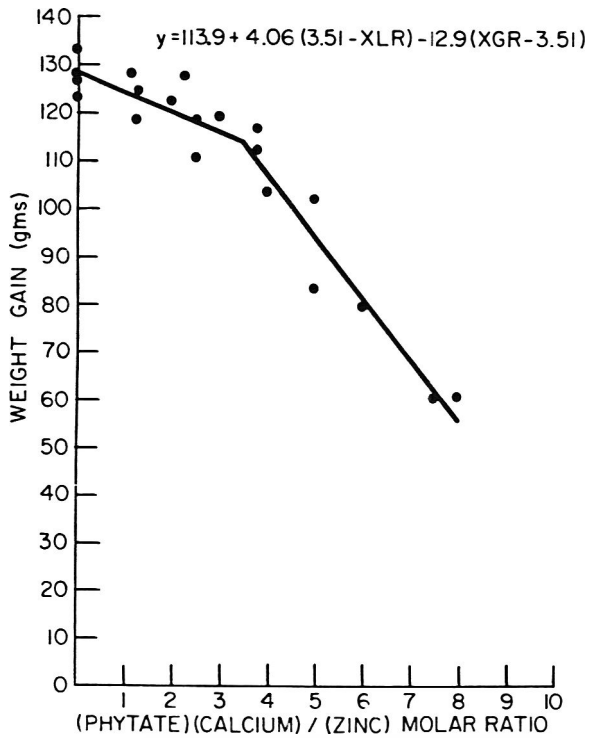

Fig. 1—Fitted broken line model for mean weight gain of groups of rats (n = 6) fed diets with various (phytate)(calcium)/(zinc) molar ratios.

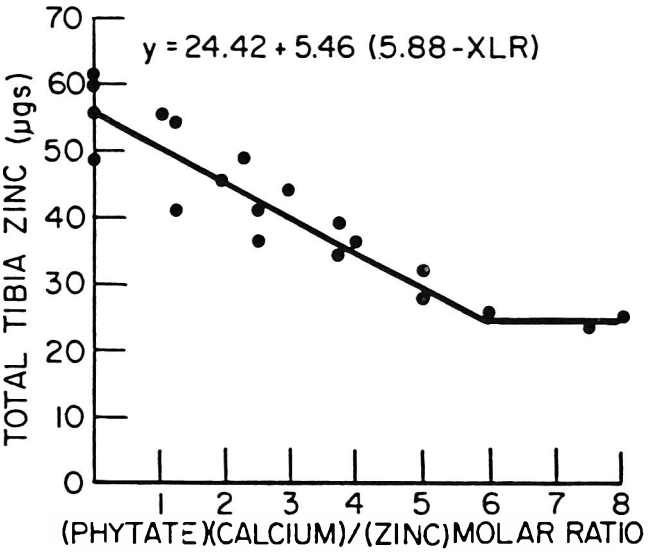

Fig. 2—One-slope broken line model for mean total tibia zinc of groups of rats (n = 6) fed diets with various (phytate) (calcium)/(zinc) molar ratios.

(Fig. 1), there is a breakpoint in the curve indicating that there was a (P)(C)/(Z) molar ratio at which the weight gain response changed. The fitted broken line model was gain = 113.9 + 4.06(3.51 − XLR) − 12.9(XRG-3.51). In this equation X = diet molar ratio; XLR = diet molar ratios less than 3.51 (i.e., R is the breakpoint in the curve); and XGR = diet molar ratios greater than 3.51. At X ≥ 3.51, (3.51 − XLR) = zero; and at X ≤ = 3.51, (XRG − 3.51) = zero. This model accounted for 81.4% of the total variation and 94.5% of the variation due to the diet treatment. Growth depressing effects were approximately 3 times more severe when the molar ratio exceeded 3.51 than when the ratio fell below 3.51. For each 1 unit increase in molar ratio above 3.51, weight gain was reduced by 12.9 ± 1.175g. For each unit increase in molar ratio between 0 and 3.51, weight gain was depressed 4.06 ± 1.175g. At a molar ratio of 3.51, rats gained 113.9 ± 4.36g.

In recalculating data from their studies, Davies et al. (1985) selected a molar ratio of 3.5 (moles/kg) as the critical (P)(C)/(Z) ratio above which weight gain of rats was depressed significantly. This ratio represents the point on the regression line which was 3 standard deviations from average weight gain of their control rats receiving phytate-free diets. By comparison, the breakpoint of the curve above which point weight gain was significantly depressed in the current study was at 3.51 (moles/kg). It is curious that the two methods agree upon the same molar ratio although Davies et al. (1985) appear to have chosen the number 3.5 somewhat arbitrarily and the ratio 3.51 moles/kg from this study was estimated objectively by least squares regression analysis.

Figure 2 shows the results of plotting total tibia zinc versus the (P)(C)/(Z) molar ratio. Total tibia zinc decreased as the (P)(C)/(Z) molar ratio increased. The simple linear regression equation, tibia zinc = 54.98 − 4.577X, accounted for 86% of the variation due to treatment and indicated that tibia zinc decreased 4.577 µg for every 1 unit increase in molar ratio. However, the one-slope broken line equation, tibia zinc = 24.42 + 5.461(5.875-XLR), accounted for significantly ($p < 0.01$) more treatment variation than did the simple linear regression equation, thus indicating that the broken-line more accurately describes the response. In these equations X = diet molar ratio; XLR = diet molar ratios less than 5.88. The expression (5.875 − XLR) is evaluated as zero at values of X ≥ 5.88. The one-slope broken line accounted for 81.6% of the total variation and 88.6% of the variation due to diet treatment. The broken-line analysis indicated that tibia zinc remained constant at 24.42 ± 1.250 µg when the diet molar ratio was equal to or exceeded 5.88 ± 0.330. For every 1 unit increase in diet molar ratio between 0 and 5.88, tibia zinc decreased 5.46 ± 0.319 µg. Plotting weight gain or total tibia

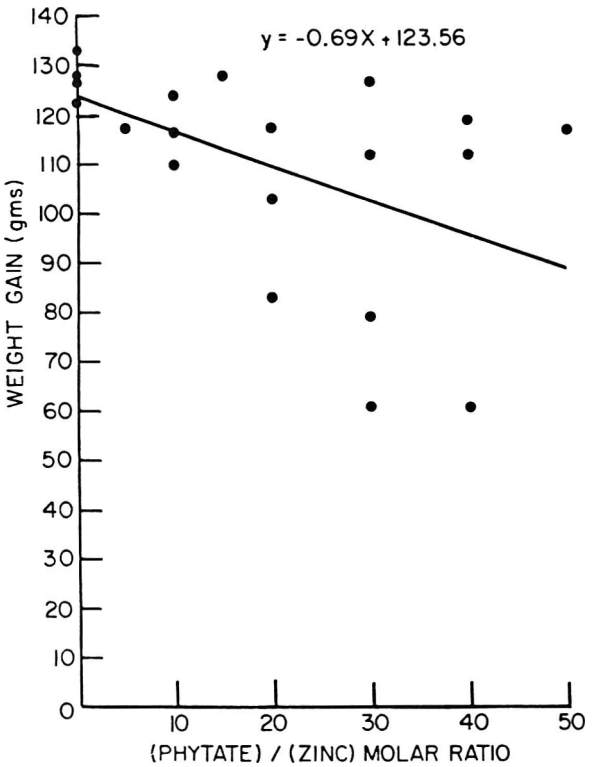

Fig. 3—Linear regression model for mean weight gain of groups of rats (n = 6) fed diets with various (phytate)/(zinc) molar ratios.

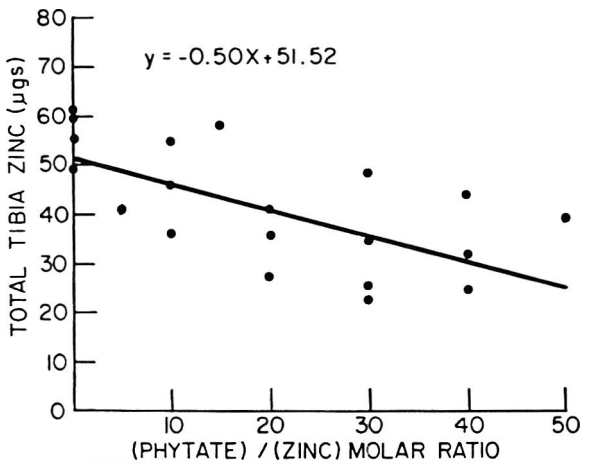

Fig. 4—Linear regression model for mean total tibia zinc of groups of rats (n = 6) fed diets with various (phytate)/(zinc) molar ratios.

zinc (Fig. 3, 4) versus the (P)/(Z) molar ratio results in highly scattered diagrams which are not useful in predicting zinc bioavailability because the effect of differing levels of dietary calcium is not accounted for.

It can be noted that the breakpoints in Fig. 1 and 2 differ. For weight gain the breakpoint is at 3.51 and for bone zinc it is at 5.88. Moreover, above the molar ratio 3.51 the weight gain is far more severely impacted than below 3.51. Thus, the impact of increasing the (P)(C)/(Z) molar ratio is more deleterious on weight gain than it is for bone zinc deposition. This may be the result of the role of zinc as a coenzyme for an estimated 200 enzymes, many of which affect protein synthesis and thus growth (O'Dell, 1984). The plateau reached at high (P)(C)/(Z) ratios for tibia zinc in the fitted 1-slope broken-line equation suggested that bones contained a small amount of zinc necessary for bone metabolism. As zinc became more available, i.e., lower (P)(C)/(Z) ratios, it then may have been deposited in bone matrix and total bone zinc increased.

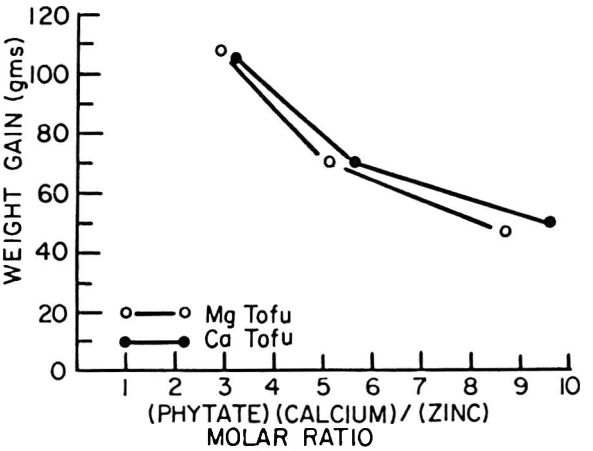

Fig. 5—Relationship of mean weight gain of groups of rats (n = 6) fed magnesium- or calcium-precipitated tofu-based diets with three different levels of total dietary calcium.

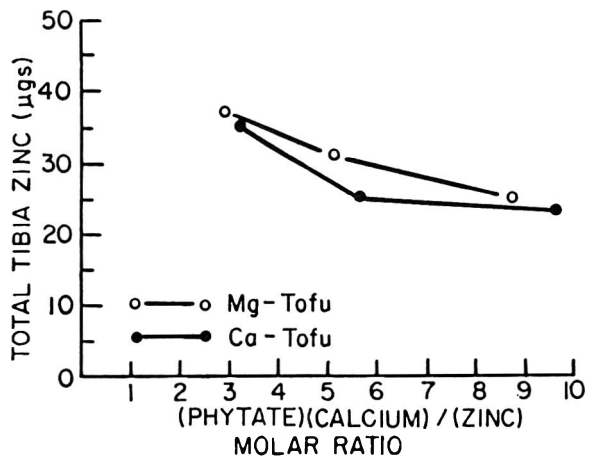

Fig. 6—Relationship of mean total tibia zinc of groups of rats (n = 6) fed magnesium- or calcium-precipitated tofu-based diets with three different levels of total dietary calcium.

It should be noted that these experiments investigated the effects of added sodium phytate and not the effects of naturally occurring dietary phytate on zinc bioavailability. Therefore, these results must be interpreted with some caution since sodium phytate will react differently than would phytic acid endogenous to a cereal or legume. Being highly soluble and ionizable over a wide range of pH, the sodium phytate may readily interact with other minerals to form insoluble phytates under intestinal conditions, thus rendering them "unavailable." Naturally occurring phytic acid, on the other hand, especially after processing, may already be in the form of insoluble calcium or magnesium salts or bound to protein and, therefore, may be incapable of reacting with other endogenous or added minerals and thus have less adverse affects on availability (Cheryan, 1980).

In another study by Forbes et al. (1983) the bioavailability of zinc contained in calcium- and in magnesium-precipitated tofu was compared to zinc supplied as $ZnCO_3$ in egg white diet. Zinc was supplied at 9 ppm in all diets while the calcium level was varied at 0.4, 0.7 and 1.2% calcium. The results (Fig. 5, 6) demonstrate that as the (P)(C)/(Z) molar ratio increased from about 3.0 in the 0.4% calcium-containing soy diets to about 9.0 in the 1.2% calcium-containing diets, the weight gain and bone zinc of the rats fed over a 21-day period markedly decreased.

Table 1 shows the relative bioavailability of zinc vs the (P)(C)/(Z) molar ratio for weight gain or total tibia zinc from various processed soybean products (Forbes and Parker, 1977; Forbes et al., 1979, 1983; Erdman et al., 1980). Slope ratio techniques, as described in the materials and methods section, were utilized to evaluate zinc bioavailability for all products listed in Table 1. In these products the calculated (P)(C)/(Z) molar ratios were less predictive of zinc bioavailability suggesting that the predictive value of the (P)(C)/(Z) molar ratio may have been confounded by the effects of the processing procedures. For example, when acid-precipitated and neutralized soy concentrates were produced in this laboratory using the same lot of soybeans (Erdman et al., 1980; Ketelsen et al., 1984), zinc bioavailability of the two products with similar (P)(C)/(Z) molar ratios were quite different. In the study by Erdman et al. (1980), two soy concentrates, one acid-precipitated and the other neutralized, but both containing essentially identical levels of zinc, phytic acid and calcium, resulted in quite different zinc relative bioavailabilities, i.e., 93% and 66%, respectively, for weight gain and 48% and 29%, respectively for total tibia zinc as related to a control egg white $ZnCO_3$ diet. Similar results were found when acid-precipitated and neutralized soy isolates were compared.

The bioavailability of ^{65}Zn from intrinsically and extrinsically labeled acid-precipitated and neutralized soy concentrate was evaluated in rats (Ketelsen et al., 1984). These findings confirmed results of previous feeding studies showing that neutralization of soy concentrate without altering zinc, calcium or phytic acid reduces zinc bioavailability to the rat (Forbes et al., 1979; Cheryan, 1980; Erdman et al., 1980).

It appears then that when food processing procedures are modified, the value of the predictive equation may be reduced. The reduced bioavailability of zinc in neutralized soy products as compared to those acid-precipitated products may be the result of the formation of stable protein-phytic acid-zinc complexes in the dried neutral product (Erdman et al., 1980, 1983; Erdman and Forbes, 1981). Since protein-phytic acid-mineral associations occur in solution at a neutral pH, these associations may form more tightly-bound complexes during the drying of the soy protein. In the digestive tract, these stable complexes may inhibit the complete digestion of protein to free amino acids. These complexes would be resistant to digestion and, consequently, inefficient absorption of zinc would occur.

Despite the shortcomings of the (P)(C)/(Z) ratio for predicting zinc bioavailability, one would clearly expect poor zinc availability for the rat if the ratio exceeded 3.5. But what about the predictability of the equation for man? Davies et al. (1985) suggested that (P)(C)/(Z) ratios exceeding 0.5 may adversely affect zinc balance in man. Perhaps, the human is less able than the rat to digest and absorb zinc from insoluble complexes in the gastrointestinal tract. The rat's propensity to coprophagize may be one factor that enables the rat to adjust to higher (P)(C)/(Z) molar ratios.

Since humans clearly consume a variety of diets, it is appropriate to consider the intake of phytic acid, calcium and zinc from three different groups of people based on their typical dietary pattern. First there is the average American diet which is low in phytic acid, low in calcium and contains about 12 mg zinc per day (the adult RDA). Secondly, is a vegetarian whose diet contains calcium sulfate precipitated tofu, whole grain cereals and legumes. This second population consumes a diet high in phytic acid and calcium. Finally, there is that large group of women now supplementing their diet with calcium to reduce the risks of osteoporosis.

Harland and Peterson (1981) determined that the average American consumes about 750 mg phytic acid per day with the intake ranging from 300–1300 mg per day. Oberleas and Harland (1981) and Ellis et al. (1982b) calculated the phytic acid and zinc in a vegetarian and a nonvegetarian menu. Oberleas and Harland (1981), for example, found that the vegetarian menu contains 2575 mg phytic acid and 11.2 mg zinc daily and the nonvegetarian menu contains 290 mg phytic acid and 11.4 mg zinc per day.

Using the above data and assuming an average dietary intake of 2500 Kcals and 500g dry food, we estimated that the typical American diet contains about 700 mg calcium, 12 mg zinc, and 750 mg phytic acid per day which results in a (P)(C)/(Z) molar ratio of about 0.2 M/kg dry diet or about 43 mM/1000 Kcals. The vegetarian diet including calcium sulfate precipitated-tofu contains about 2575 mg phytic acid and 1500 mg calcium, resulting in a molar ratio of about 1.6 M/kg diet or about 316 mM/1000 Kcal. And, a woman on the typical American diet but taking 1.5g calcium daily (1.0g supplemented and 500 mg in diet) consumes a diet with a molar ratio of about 0.46 M/kg diet or about 92 mM/1000 Kcal. Recalculation of data provided by Morris (Morris, 1986; Ellis et al., 1985) suggests (P)(C)/(Z) molar ratios of about 0.27, 0.52, 0.43 and 0.48 M/kg dry diet for nonvegetarian Americans, vegetarian Americans, vegetarian Asian Indians and lactating Nepalese vegetarians, respectively.

From these calculations it is clear that Americans may consume a (P)(C)/(Z) ratio high enough to pose a risk for reduced zinc bioavailability. The tofu-consuming vegetarian is clearly at risk if Davies et al. (1985) estimate of 0.5 M/kg diet as a cut off is correct.

Worldwide, certain populations may jeopardize zinc status due to high phytic acid intakes. Thus, some caution should be exercised in use of high levels of calcium supplements when the diet is also high in phytic acid. Under these conditions supplements may cause reduced zinc absorption.

Recently, Bindra et al. (1986) evaluated the diets and zinc status of 112 Punjabi Sikh Canadian immigrants who had lived in Canada for at least 2 years (range 2.2–15.5) and who were consuming predominantly lacto-ovo vegetarian diets. Due to frequent consumption of milk and milk products, especially yogurt, the Punjabi diets contained adequate calcium (568 vs 422 mg/1000 Kcal diet: Punjabi vs omnivorous diets, [n = 30]). The zinc concentration was 4.5 vs 5.1 mg/1000 Kcal and phytate was 788 vs 423 mg/1000 Kcal diet for the Punjabi and omnivorous diets, respectively. The average daily total caloric intake was 1892 Kcal. Recalculation of their data results in estimated (P)(C)/(Z) molar ratios of about 250 mM/1000 Kcal and about 90 mM/1000 Kcal for the Punjabi and omnivorous diet groups, respectively. A crude estimate of (P)(C)/(Z) molar ratio expressed on a M/kg dry diet basis would give ratios of about 0.9 and 0.3 M/kg for Punjabi and omnivorous, respectively. Strikingly, 32% of the Punjabi immigrants had low serum zinc values (< 70 μg/dL), suggesting less than optimum zinc status of this population.

The possible adverse effects of magnesium upon zinc bioavailability in phytate-containing diets was also investigated by Forbes et al. (1984). They found that magnesium caused a far less pronounced effect on zinc utilization than did calcium.

In summary, the efficiency of the (P)(C)/(Z) molar ratio for predicting zinc bioavailability was tested. It was found that the (P)(C)/(Z) molar ratio was predictive of zinc bioavailability within a study if calcium was varied with a given (P)/(Z) molar ratio or if phytic acid was varied with a given (C)/(Z) molar ratio. However, some processing procedures alter the interactions of zinc with other components within the soy product thus reducing the predictive value of the (P)(C)/(Z) molar ratio. For the rat (P)(C)/(Z) molar ratios above 3.5 M/kg dry diet or above 800 mM/1000 Kcal would predict poor zinc status. For humans, ratios above 0.5 M/kg dry diet or 200 mM/1000 Kcals may be cause for concern.

REFERENCES

BaFundo, K.W., Baker, D.H., and Fitzgerald, P.R. 1984. Zinc utilization in the chick as influenced by dietary concentrations of calcium and phytate and by *Eimeria acervulina* infection. Poultry Sci. 63: 2430.
Bindra, G.S., Gibson, R.S., and Thompson, L.U. 1986. [Phytate][Calcium]/

[Zinc] ratios in Asian immigrant Lacto-ovo vegetarian diets and their relationship to zinc nutriture. Nutr. Res. 6: 475.
Cheryan, M. 1980. Phytic acid interactions in food systems. CRC Crit. Rev. Food Sci. Nutr. 13: 297.
Davies, N.T., Carswell, A.J.P., and Mills, C.F. 1985. The effects of variation in dietary calcium intake on the phytate-zinc interaction in rats. In "Trace Elements in Man and Animals-TEMA 5." (Ed) Mills, C.F., Bremmer, I., and Chesters, J.K., p. 456. Aberdeen, Scotland.
Davies, N.T. and Olpin, S.E. 1979. Studies on the phytate:zinc molar contents in diets as a determinant of Zn availability to young rats. Br. J. Nutr. 41: 591.
Davies, N.T. and Reid, H. 1979. An evaluation of the phytate, zinc, copper, iron and maganese contents of, and availab:lity from, soya-based textured-vegetable-protein meat substitutes or meat-extenders. Br. J. Nutr. 41: 579.
Ellis, R., Morris, E.R., and Hill, A.D. 1982a. Bioavailability to rats of iron and zinc in calcium-iron-phytate and calcium-zinc-phytate complexes. Nutr. Res. 2: 319.
Ellis, R., Morris, E.R., Hill, A.D., and Smith, J.C.,Jr., 1982b. Phytate:zinc molar ratio, mineral, and fiber content of three hospital diets. J. Am. Diet. Assn. 81: 26.
Ellis, R., Reynolds, R.D., Kelsey, J.L., and Morris, E.R. 1985. Phytate:zinc and Phytate X Calcium:Zinc molar ratios of adult subjects consuming self-selected diets. Fed. Proc. 44: 1506 (Abstract #6405).
Erdman, J.W. Jr. 1979. Oilseed phytates: Nutritional implications. J. Am. Oil Chem. Soc. 56: 736.
Erdman, J.W. Jr. 1981. Bioavailability of trace minerals from cereals and legumes. Cereal Chem. 58:21.
Erdman, J.W. Jr. and Forbes, R.M. 1981. Effects of soya protein on mineral bioavailability. J. Am Oil Chem. Soc. 58: 489.
Erdman, J.W. Jr., Forbes, R.M., and Kondo, H. 1983. Zinc bioavailability from processed soybean products. In "Nutritional Bioavailability of Zinc." (Ed) Inglett, G.E. ACS Symposium #210, p. 173. Washington, DC.
Erdman, J.W. Jr., Weingartner, K.E., Mustakas, G.C., Schmutz, R.D., Parker, H.M., and Forbes, R.M. 1980. Zinc and magnesium bioavailability from acid-precipitated and neutralized soybean protein products. J. Food Sci. 45: 1193.
Forbes, R.M., Erdman, J.W. Jr., Parker, H.M., Kondo, H.M., and Ketelsen, S.M. 1983. Bioavailability of zinc in coagulated soy protein (tofu) to rats and effects of dietary calcium at a constant phytate:zinc ratio. J. Nutr. 113: 205.
Forbes, R.M. and Parker, H.M. 1977. Biological availability of zinc in and as influenced by whole fat soy flour in rat diets. Nutr. Reports Intern. 15: 681.
Forbes, R.M., Parker, H.M., and Erdman, J.W. Jr. 1984. Effects of dietary phytate, calcium, and magnesium levels on zinc bioavailability to rats. J. Nutr. 114: 1421.
Forbes, R.M., Weingartner, K.E., Parker, H.M., Bell, R.R., and Erdman, J.W., Jr. 1979. Bioavailability to rats of zinc, magnesium and calcium in casein, egg and soy protein-containing diets. J. Nutr. 109: 1652.
Harland, B.F. and Peterson, M. 1978. Nutritional status of lacto-ovo vegetarian Trappist monks. J. Am. Diet. Assn. 72: 259.
Ketelsen, S.M., Stuart, M.A., Weaver, C.M., Forbes, R.M., and Erdman, J.W. Jr. 1984. Bioavailability of zinc to rats from defatted soy flour, acid-precipitated soy concentrate and neutralized soy concentrate as determined by intrinsically and extrinsically labelling techniques. J. Nutr. 114: 536.
Likuski, H.J.A. and Forbes, R.M. 1965. Mineral utilization in the rat. III. Effects of calcium and phytic acid on the utilization of dietary zinc. J. Nutr. 85: 230.
Morris, E.R. 1986. Personal communication.
Morris, E.R. and Ellis, R. 1980a. Effect of dietary Phytate/Zinc molar ratio on growth and bone zinc response of rats fed semipurified diets. J. Nutr. 110: 1037.
Morris, E.R. and Ellis, R. 1980b. Bioavailability to rats of iron and zinc in wheat bran: Response to low-phytate bran and effect of the Phytate/Zinc molar ratio. J. Nutr. 110: 2000.
NAS. 1978. "Nutrient Requirements of Laboratory Animals." National Academy of Sciences, Washington, DC.
Oberleas, D. 1975. Factors influencing availability of minerals. In "Proceedings Western Hemisphere Nutrition Congress IV." p. 156.
Oberleas, D., and Harland, B.F. 1981. Phytate content of foods: Effect on dietary zinc bioavailability. J. Am. Diet. Assn. 79: 433.
Oberleas, D., Muher, M.E., and O'Dell, B.L. 1966. Dietary metal-complexing agents and zinc bioavailability in the rat. J. Nutr. 90: 56.
O'Dell, B.L. 1969. Effects of dietary components upon zinc availability. Am. J. Clin. Nutr. 22: 1315.
O'Dell, B.L. 1984. History and status of zinc in nutrition. Fed. Proc. 43: 2821.
Robbins, K.R. 1986. A method, SAS program, and example for fitting the broken line to growth data. Univ. Tenn. Expt. Sta., Research Report No. 86-09.
Weingartner, K.E., Erdman, J.W. Jr., Parker, H.M., and Forbes, R.M. 1984. Effects of soy hull upon the bioavailability of zinc and calcium from soy flour-based diets. Nutr. Rep. Intern. 19: 223.
Ms received 6/19/86; revised 10/24/86; accepted 10/25/86.

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Lai, D.J. and Heldman, D.R. 1982. Analysis of kinetics of quality change in frozen foods. J. Food Proc. Eng. 6(1982): 179.
Manske, W.J. 1983. The application of controlled fluid migration to temperature limit & time temperature integrators. IIR Commission C2, Preprints. 16th International Congress of Refrigeration. p. 632.
Mistry, V.V. and Kosikowski, F.V. 1983. Use of time-temperature indicators as quality control devices for market milk. J. Food Protection. 46(1): 52.
SAS. 1982. "SAS User's Guide: Statistics." SAS Institute, Inc., Cary, NC.
SAS. 1985. "SAS/Graph User's Guide," 5 ed. SAS Institute, Inc., Cary, NC.
Saguy, I. and Karel, M. 1980. Modeling of quality deterioration during food processing and storage. Food Technol. 34(2): 78.
Schoen, H.M. and Byrne, C.H. 1972. Defrost indicators. Food Technol. 26(10): 46.
Singh, R.P., Barrett, E.L., Wells, J.H., Grisius, R.C., and Marum, W. 1986. Critical evaluation of time-temperature indicators for monitoring quality changes in perishable and semiperishable foods. Report prepared for U.S. Army Natick Research & Development Center, Natick, MA. January.
Singh, R.P. and Wang, C.Y. 1977. Quality of frozen foods-A review. J. Food Proc. Eng. 1(1977): 97.
Singh, R.P. and Wells, J.H. 1985. Use of time-temperature indicator to monitor quality of frozen hamburger. Food Technol. 39(12): 42.
Singh, R.P., Wells, J.H., Dolan, K.D., Gonnet, E.J., and Muñoz, A.M. 1984. Critical evaluation of time-temperature indicators for monitoring quality changes in stored subsistence. Report prepared for U.S. Army Natick Research & Development Center, Natick, MA. September.
Van Arsdel, W.B., Copley, M.T., and Olson, R.L. 1969. "Quality and Stability of Frozen Foods," John Wiley & Sons, New York.
Wells, J.H. and Singh, R.P. 1985. Performance evaluation of time-temperature indicators for frozen food transport. J. Food Sci. 50: 369.
Zall, R., Chen, J., and Fields, S.C. 1984. Evaluation of automated time-temperature monitoring systems in measuring freshness of UHT milk. Paper presented at the Ninth Annual Eastern Research Highlights Conference, Washington, DC. November.
Ms received 4/5/86; revised 9/15/86; accepted 11/15/86.

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Oral and Nonoral Perception of Solution Viscosity

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ABSTRACT

Solution viscosity, like many other textural properties of foods, can be perceived by more than one sensory modality or method of sensory exploration. The present study compared judgments of solution viscosity obtained by oral (tactile/kinesthetic) and nonoral (visual and tactile/kinesthetic) modes of sensing. The slopes of the functions describing physical and perceptual viscosity were similar for the different methods but solutions were perceived as more viscous when judged in the mouth.

INTRODUCTION

TEXTURAL PROPERTIES of foods may be perceived by more than one sensory modality and by more than one method of sensory exploration. For example, solution viscosity can be perceived by the visual and somesthetic senses when liquids are poured and subsequently consumed. When liquids are stirred and then consumed they are perceived by the same sensory modalities (somesthetic and kinesthetic) but by different methods of sensory exploration (hereafter termed sensory strategy): stirring with the hand and oral manipulation.

When food texture is perceived via two or more sensory channels, an important issue is whether perception is significantly different in the different channels. There is a tendency to attach singular importance to oral evaluations of food texture, but if other sensory cues are available then their potential impact on overall texture perception should be assessed. Few studies have compared the discriminative abilities of different sensory regions with regard to textural stimuli (but see Stevens and Guirao, 1964; Moskowitz, 1972; Anderson et al., 1973; Shama and Sherman, 1973a,b; Christensen and Vickers, 1981) although basic studies, for example of the skin senses, suggest there are significant regional differences in sensitivity (Weinstein, 1968).

A potential difficulty in studies of food texture is that the physical properties of texture are often defined by parameters of shear rate, shear stress, or amount of deformation. This dependence of physical measures of texture on the conditions of deformation can complicate the interpretation of results when different sensory modalities or strategies are used; observed perceptual differences could be due to differences in the physical stimulus produced by different patterns of deformation as opposed to physiological and psychological processes associated with different sensory assessments of texture. Viscosity is a textural property amenable to studies comparing perception by different sensory methods because the physical stimulus can be held constant. When viscous solutions exhibit Newtonian behavior (shear rate proportional to shear stress), then solution viscosity is independent of shear conditions. Consequently, any differences in the perception of viscosity can be attributed to the different sensory modes.

Only one previous study compared the perception of Newtonian solutions when different sensory modalities or strategies were used. Stevens and Guirao (1964) had subjects use magnitude estimation to judge a series of Newtonian oils ranging in viscosity from 10–95,000 centipoises (cps). Subjects judged viscosity either visually by shaking tubes containing the thickened solutions, or by stirring the solutions while blindfolded or not blindfolded. Although different sensory modalities were used (visual and somesthetic/kinesthetic), log-log plots of perceived versus physical viscosity yielded similar slopes (0.4) for the different methods of sensory assessment.

The present study compared oral (somesthetic/kinesthetic) and non-oral (visual or somesthetic/kinesthetic) methods of assessing viscosity. Oral judgments may be different from other sensory judgments because of the presence of saliva, which could dilute as well as warm the solutions. The net result may be that solutions in the mouth are perceived as thinner. The present study allowed comparisons not only of the steepness of the functions relating physical and perceptual measures of viscosity (slopes) but also comparisons of differences in the magnitude of perceived viscosity as a function of sensory modality or strategy.

MATERIALS & METHODS

TWO EXPERIMENTS were performed that were similar in overall design. In the first, subjects judged solution viscosity using three different modes of sensing with each presented as a separate task [the design of Stevens and Guirao (1964)]. In the second experiment, judgments of viscosity by different sensory modes were intermixed so that a comparison could be made of differences in the magnitude of perceived viscosity produced by different methods of sensory assessment. Differences between the two experiments are described in the separate Methods sections.

Subjects

In the first experiment, 20 male and female subjects between the ages of 18-35 years were paid to participate. Subjects were excluded if they reported any food allergies. In the second experiment, 16 additional subjects with similar characteristics were tested.

Test solutions

In both experiments, deionized water was thickened to 3, 9, 27, 81, 243, 729, 1093, and 2187 cps (± 10%) with a food-grade gum, sodium alginate (Kelco-Gel LV, Kelco Corp., Chicago, IL); water (1 cps) was also used as a test solution. This thickening agent was chosen because it exhibited nearly Newtonian behavior in the shear rate range (10-300 sec^{-1}) thought to approximate human evaluation of solution thickness (Shama and Sherman, 1973 a,b; Christensen, 1979). There are manufacturing-related differences in the degree of Newtonian behavior exhibited by these gums so that preliminary testing of different lot numbers of the same gun was required. Physical viscosities were determined with a Wells-Brookfield micro viscometer (Model RVT). The solutions were prepared by slowly adding the gum (0.08 g - 3.92 g/100 ml H20) to water while it was being blended at high shear. Solution viscosities were found to be stable 24-96 hr following preparation, and so human testing was performed during this period. To mask visual differences in the appearance of the solutions, a small amount (.0125 g/100 ml solution) of a brown, food-grade dye was added to the solutions 1 hr before human testing. The dye reduced the long-term stability of solution viscosities, and therefore was added only before use.

Sensory testing

Experiment 1. Subjects participated in a single session lasting approximately 1 hr that included three separate sensory tests (each separated by a five-min rest period). Subjects judged the thickness of the same series of solutions in the three tasks but used a different mode

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of sensing for each. A total of 18 solutions were presented in each task (9 solutions × 2 presentations) with all but the first solution randomly presented; the first solution was always 243 cps. The order in which subjects were tested with the three sensory tasks was also randomized.

Subjects used magnitude estimation to report their judgments of solution thickness. Prior to testing, subjects were given practice in the use of magnitude estimation by being asked to judge the circumference of a series of circles. They were allowed to use any positive number and no numerical reference was provided for the first sample (modulus-free). Subjects reported their estimates verbally except in the Oral Task, in which they wrote their estimates on individual sheets of paper that were collected after each solution presentation. In the instructions to subjects the terms "viscosity" and "thickness" were intermingled and examples were given of common liquid foods that varied in viscosity. Subjects did not express any difficulty with the textural concept.

In one sensory test, subjects orally judged the viscosity of 10 ml quantities of each solution (Oral Task). To minimize non-oral cues, the solutions were presented to subjects via a plastic syringe fitted with a piece of Tygon tubing that served as a straw. The tubing protruded through a slot in an opaque barrier that separated the experimenter and subject. The experimenter delivered the solution to the subject, attempting to hold the rate of solution delivery constant. Subjects were instructed to swish the solutions in their mouth as they would a mouthwash in order to judge thickness. After expectorating, subjects rinsed their mouths twice with deionized water before receiving the next solution. As another means of minimizing nonoral cues, subjects recorded on paper their numerical judgments of solution thickness before expectorating each solution. The concentrations of sodium alginate used in the study seemed largely tasteless and odorless, although it cannot be said with certainty that flavor cues were entirely absent. Pilot studies (triangle tests) showed that subjects were unable to detect the presence of the small quantity of added food coloring.

For the nonoral tactile task (Rod Task), blindfolded subjects were instructed to determine solution thickness by stirring each test solution with a glass stirring rod. For this task, the test solutions (175 mL) were presented in wide-mouthed jars (9 cm diameter × 9 cm height).

For the Visual Task, subjects were instructed to determine solution thickness by observing the motion of solutions that they tilted. The solutions (20 mL) were presented in 50-ml capacity glass tubes sealed at both ends and mounted with brackets to a heavy frame so that tactile cues from tilting could not be felt.

Experiment 2. The second experiment was similar in design to the first. The same series of thickened solutions was used and subjects participated in a single test session. The differences were that subjects used a different non-oral tactile task to judge solution viscosity and, unlike the previous experiment, the sensory tasks were intermingled rather than blocked during the test session. Instead of using a stirring rod in the Rod Task, blindfolded subjects were required to stir each solution with their index finger and then rub the solutions between their thumb and index finger (Fingers Task).

Statistical analyses

The psychophysical data were first normalized by converting the scores to log values and adding the log difference between the grand mean and the mean for individual subjects from each sensory task (Experiment 1) or from the entire test session (Experiment 2) to each subject's set of log judgments (Engen, 1971). Individual power functions were calculated from log values of the physical and perceptual measures of viscosity. The individual slopes from Experiments 1 and 2 were analyzed with a single factor repeated measures analysis of variance and the sensory data from Experiment 2 were analyzed with a two-factor repeated measures analysis of variance (Sensory method × Viscosity level) with Newman-Keuls tests for post-hoc comparisons (Winer, 1971).

RESULTS

DATA from the first experiment are illustrated in Fig. 1. Mean slopes of the power functions relating physical and perceptual measures of viscosity were not significantly different for the three sensory strategies: 0.34 (Oral), 0.35 (Rod) and 0.39 (Visual). The experimental design did not permit a comparison of apparent differences in the magnitude of perceived viscosity because, although the same subjects participated in all three tasks, their number scales may have changed between tasks.

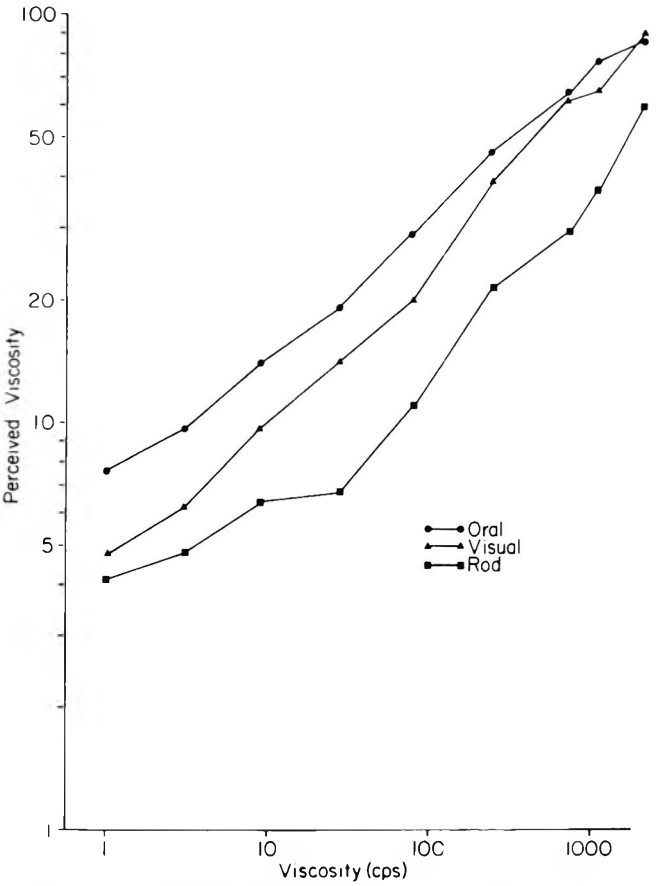

Fig. 1—Relationship between log physical viscosity and log perceived viscosity as a function of the mode of sensory evaluation. Judgments represent the geometric mean of normalized values from 20 subjects.

Figure 2 illustrates results from the second experiment. Slopes of the power functions relating physical and perceptual measures of viscosity were again similar and closely replicated the results from the first experiment: 0.34 (Oral), 0.33 (Fingers) and 0.39 (Visual). Similar to the first experiment, visual judgments yielded a function having a steeper slope than oral and non-oral tactile judgments, but the differences in slope were not statistically significant.

Because subjects used all three modes of sensing within the same test session, differences in the magnitude estimates of thickness for the three methods could be compared. Analysis of variance revealed a significant main effect for sensory task ($F[2,30] = 4.42, p<0.05$) with the interaction term approaching significance. Post-hoc tests demonstrated that this effect was attributable to oral judgments which were significantly higher than visual or non-oral tactile judgments. Although the interaction failed to reach significance, it is evident from Fig. 2 that judgments of solution thickness were converging at higher viscosities. For example, up to 100 cps, oral judgments were approximately 45% higher than visual judgments; for thicker solutions, oral judgments were only 20% higher until the sensory judgments converged.

DISCUSSION

THE MOST STRIKING aspect of the data is the similarity in judgments of solution viscosity despite the use of distinctly different sensory modalities and strategies. This similarity is especially surprising because different sensory modalities are used: vision and touch (somesthesis/kinesthesis). For visual

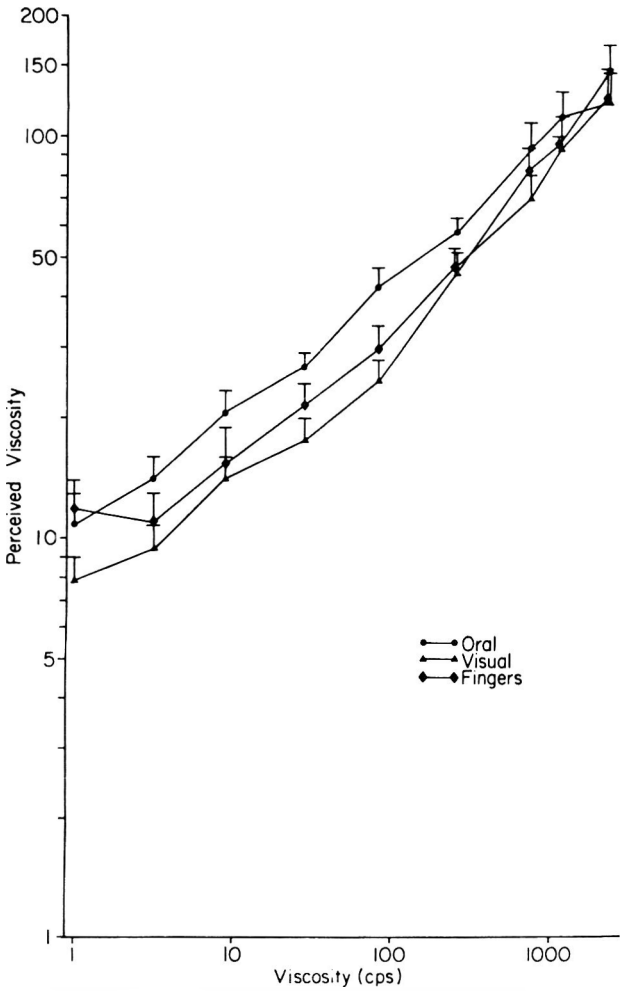

Fig. 2—Relationship between log physical viscosity and log perceived viscosity as a function of the mode of sensory evaluation. Judgments represent the geometric mean and standard error of the mean of normalized values from 16 subjects.

determinations, subjects presumably perceive viscosity by relating the rate of movement of the solution in response to a presumed shear force which is predominantly gravity. For tactile judgments, subjects probably use mechanoreceptors (touch-pressure) to detect the solution's resistance to flow and the rate of flow over the receptor surface, and use intramuscular receptors to detect the force or effort applied to move the solution.

The slopes of the functions relating physical and perceptual measures of viscosity are low. A slope of 1.0 indicates that physical and perceptual measures change at an identical rate; i.e. a 10-fold increase in the physical parameter is also judged as a 10-fold increase. The slopes for viscosity indicate a compression in perceptual judgments; a 10-fold increase in solution viscosity produces little more than a 2-fold increase in perceived viscosity. When compared to the slopes (0.42 - 0.46) found by previous investigators (Stevens and Guirao, 1964), the values in the present study are only slightly lower. This may well be due to the different ranges in physical viscosity used in the two studies.

Despite similarities in the shapes of functions relating physical and perceived measures of viscosity, there were differences in the magnitude of perceived viscosity judgments for oral as compared to visual and non-oral tactile methods of assessment. Contrary to our prediction that oral determinations of viscosity might be lower, solutions were perceived to be more viscous in the mouth, especially for thinner solutions. There is no readily apparent explanation for these differences. It is not likely that saliva increases solution viscosity because saliva has a low viscosity; in normal individuals, it may range from 13 cps at low shear rates to 2 cps at higher shear rates (Roberts, 1977). Other physiological differences between oral and non-oral regions may be responsible; for example, the numbers of tactile or kinesthetic receptors may be different. There is precedent for sensory differences between oral and non-oral regions for other somesthetic stimuli (e.g. temperature, Green, 1984). It is also possible that differences in cognitive or higher-order processing of sensory information account for differences in oral judgments of viscosity as has been suggested for differences in oral and non-oral judgments of size and volume (Anstis and Loizos, 1967; Salthouse et al., 1980).

The results of the present study suggest caution in the design and interpretation of experiments on the sensory evaluation of food texture. It should not be assumed that texture judgments obtained by one sensory modality or strategy will be the same as texture judgments obtained by another sensory means. In practical terms, if the goal is to understand consumer behavior then judgments of food texture should be obtained under conditions most closely mimicking those occurring during food preparation and consumption.

REFERENCES

Anderrson, Y., Drake,; B., Granquist, A., Halldin, L., Johansson, B., Pangborn, R.M. and Akesson, C. 1973. Fracture force, hardness and brittleness in crisp bread. with a generalized regression analysis approach to instrumental sensory comparisons. J. Text. Stud. 4: 119.
Anstis, S.M. and Loizos, C.M. 1967. Cross-modal judgments of small holes. Amer. J. Psychol. 80: 51.
Christensen, C.M. 1979. Oral perception of solution viscosity. J. Text. Stud. 10: 153.
Christensen, C.M. and Vickers, Z.M. 1981. Relationships of chewing sounds to judgments of food crispness. J. Food Sci. 46: 574.
Engen, T. 1971. Psychophysics: II. Scaling methods. Ch. 3. In "Woodworth and Schlosberg's Experimental Psychology," J.W. Kling and L.S. Riggs (Ed.), p. 47. Holt, Rinehart & Winston, New York.
Green, B.G. 1984. Thermal perception on lingual and labial skin. Percep. Psychophys. 36: 209.
Moskowitz, H.R. 1972. Scales of subjective viscosity and fluidity of gum solutions. J. Text. Stud. 3: 89.
Roberts, B.J. 1977. A study of the viscosity of saliva at different shear rates in dentate and edentulous patients. J. Dentistry. 5: 303.
Salthouse, T.A., Kolditz, T.A., Bumberry, J. and Johnston, M. 1980. An illusion of ingestion. Percep. Psychophys. 27: 564.
Shama, F. and Sherman, P. 1973a. Identification of stimuli controlling the sensory evaluation of viscosity. I. Non-oral methods. J. Text. Stud. 4: 102.
Shama, F. and Sherman, P. 1973b. Identification of stimuli controlling the sensory evaluation of viscosity. II. Oral methods. J. Text. Stud. 4: 111.
Stevens, S.S. and Guirao, M. 1964. Scaling of apparent viscosity. Science 144: 1157.
Weinstein, S. 1968. Intensive and extensive aspects of tactile sensitivity as a function of body part, sex, and laterality. Ch. 10. In "The Skin Senses," D.R. Kenshalo (Ed.), p. 195. C.C. Thomas, Springfield, IL.
Winer, B.J. 1971. "Statistical principles in experimental design", McGraw-Hill, New York.
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Examining Methods to Test Factor Patterns for Concordance

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ABSTRACT

To test whether the individual factor patterns of panelists and the panel factor pattern agreed, five grades of tea of the same type, ranging from low to high quality were studied. When a likelihood-ratio (LR) test and cluster analysis (CA) were applied to the 55-term correlation matrices of the seven individual panelists, according to the LR test only two of the matrices were homogeneous, but the CA test grouped all the matrices into the same cluster. The study demonstrated that the correlation matrices of the individual panelists or factor patterns themselves should be examined for agreement among panelists when factor analysis is used.

INTRODUCTION

MOST SENSORY TECHNOLOGISTS accept as a matter of course that data sets should be examined to learn if product-panelist interaction exists. The same has generally not been true as to factor analysis (FA). With increasing frequency, FA is being applied to sensory results (Harries et al., 1972; Wu et al., 1977; Powers et al., 1977; Lyon, 1980; Kwan and Kowalski, 1980; Galt and McLeod, 1983; McLellan et al., 1983, 1984), but only Harries et al. (1972) and Powers et al. (1985) appear to have evaluated concurrence among the panelists as to their individual factor patterns. The matter is important, for a factor pattern derived from panel results pooled might be merely the mean of a group of diverse patterns and thus misleading. Harries et al. (1972) compared by inspection the first three eigenvalues for the responses of six panelists to note how well they agreed with the eigenvalues for the panel results pooled. They agreed quite well. Powers et al. (1985) used the DISCRIM program of the SAS package (1982) to learn whether there was homogenity between the variance-covariance matrices of the individual panelists. All but two of the matrices differed significantly.

Means of examining factor patterns for significance have been described by Bartlett (1950), Burt (1952), Rao (1955), Lawley (1956), Anderson (1958), Lawley and Maxwell (1971) and Arnold (1981), but programs to make the analysis are not generally available. The purpose of this study was to learn whether programs available in common statistical packages would permit sensory technologists to make, as a routine matter, tests to ascertain whether a factor pattern probably represents real underlying structural order or is but the mean of a group of divergent responses.

MATERIALS & METHODS

THE TEA used had been procured for us by McCormick & Co. for a prior study (Godwin, 1984). An expert tea buyer had chosen five grades of tea, ranging from low to high quality, of the same type and fanning grade. Immediately upon receipt, the five lots had been subpackaged in No. 303 cans, flushed with N_2, sealed and stored at $-10°C$. Although the two panels described below evaluated the tea at times 6 months apart, it was assumed that the tea would not be a cause of any major experimental error because of the way it was stored. Later statistical analysis showed that to be true and the panel results were then pooled for further analysis.

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The tea infusion was prepared by adding 400 mL freshly boiled water to 6g tea in a flask, followed by steeping for 4 min. At 30 sec intervals, the flasks were gently swirled to facilitate leaching. At the end of the steeping period, the infusion was filtered through four layers of cheesecloth. To standardize the contact time, the infusions were collected for 30 sec only. Each of the five lots of tea was started 1 min apart. Once the infusion was collected, the flask containing the tea was held in a water bath at 85°C to maintain a constant temperature. Tea infusions were served to the panelists approximately 5 min after preparation.

To ensure that each sample was presented to the panelists at the same temperature, a single sample was dispensed to the panelist upon request. Four cups coded with a 3-digit number and randomized as to order were placed in each booth beforehand and the panelists were instructed to request samples according to the codes on the cups, progressing from left to right.

Matzoh crackers were provided for mouth-clearing if the panelist so wished. The panelists were instructed that if they used the crackers, they had to use them between each sample. Water was also provided. The panelists were required to rinse their mouths with water between samples.

Panelist selection

Six formal training sessions were conducted to train 15 experienced sensory panelists to evaluate tea. Five grades of tea used for the main trial and four other types of tea, somewhat similar, were used so that the panel candidates would have an idea of the range of characteristics to be encountered. Ultimately from among the trainees, five individuals were chosen for the first panel and six for the second. Some terms were provided to the panelists initially; they added two, "oregano-like" and "citrus-like" upon their own volition. The set of terms finally employed included: the desirability of flavor and aroma, and the intensities of briskness, grass-like, oregano-like, bitterness, floral-note, hay-like, metallic, sweetness and citrus-like. A 15 cm unstructured scale was used. During the training period the trainees received five types or grades of tea. At the end of each session, the identities were revealed and various facets of the evaluation were discussed in an effort to bring the panelists as much into agreement with each other as was possible. When the main trials commenced, four samples were provided at each session; no information was given about these samples or the panelists' performance until the conclusion of the study.

Statistical analysis

The panelists' results were first analyzed by one-way analysis of variance (ANOVA) to ascertain the effectiveness of each panelist for each attribute. Panelists were accepted who were significant at $p < 0.50$ for 8 of the 11 descriptors, both trials pooled. After pooling, there were 10 replications for Panel 1, 15 for Panel 2. Two-way ANOVA was used to detect product-panelist interaction. Its application showed that significant product-panelist interaction existed for only two of the terms; nonetheless discriminant analysis and cluster analysis were also applied to the panel results to learn how pervasive product-panelist interaction was.

The simple correlations between each panelist's responses for each combination of the 11 descriptors were examined by the DISCRIM program of SAS (1985) to secure an estimate of the degree of homogeneity between correlation matrices. The output from the DISCRIM program cannot be used directly; a correction in degrees of freedom has to be made to account for each "within" correlation matrix (Powers et al., 1985). Bartlett's sphericity test (Bartlett, 1950) was applied to each matrix to test for randomness (Knapp and Swoyer, 1967; Tobias and Carlson, 1969). The VARCLUS program of SAS (1985) was used to cluster the panelists according to their correlation matrices. The CANDISC program of SAS (1985) was also employed to observe interspatial distances between panelists. Both the SAS and

BMDP (1979) programs for principal-component and for the maximum-likelihood method of FA were utilized inasmuch as there are some differences in the output and for some purposes the factor patterns of one program were more useful than the other. Kendall's tau procedure was used to ascertain the correlation between factors produced by the panel and by different subgroups of the panel.

RESULTS

OUT OF THE 11 PANELISTS chosen for the main trials, the results of three were deleted because they did not attain significant differences for 8 of the 11 attributes examined. The results of one panelist were deleted because he refused to take part in the follow-up trial 6 months later.

The only two terms for which significant product-panelist interaction existed were "floral" and "hay-like." When discriminant analysis was used to classify the panelists, success ranged from 38 to 60% indicating that product-panelist interaction was somewhat more prevalent than significance for two out of 11 attributes would suggest. When Ward's minimum variance cluster analysis was applied to the scale values, panelist 6 broke off immediately, but no further segregation of the panelists occurred until the semi-partial R^2 value had fallen to 0.27. Had panelist 6 been eliminated at this stage, her results would have had to be restored later, for cluster analysis applied to the correlation matrices resulted in an entirely different conclusion being arrived at as to agreement among the panelists.

The correlation matrix of each panelist was subjected to Bartlett's sphericity test to determine whether the correlation matrix was significantly different from an identity matrix. At the two different trial periods and for the two different panels, only once was a panelist's correlation matrix determined to be nonsignificant. When the VARCLUS program was applied to the correlation matrices, only one cluster of seven panelists resulted. The program had to be forced to form two or more clusters for the reason explained below.

Likelihood-ratio test

Except for the correlation matrices of two of the panelists, the DISCRIM program demonstrated that heterogeneity existed between the matrices of all the other panelists. That result agreed exactly with that of Powers et al. (1985) who found the variance-covariance matrices of only two panelists to be homogeneous. Some of the x^2 values associated with the liklihood-ratio test are listed in Table 1.

The two multivariate methods of analysis thus gave conflicting answers. The liklihood-ratio test indicated that the correlation matrices of individual panelists were nearly all heterogeneous. The result is understandable. Only rarely are panel members' results for all attributes free of product-panelist interaction. A few discrepancies can cause matrices to be heterogeneous. In producing 55 simple correlation coefficients from the scale values of 11 descriptors, any discrepancy in scaling is correspondingly magnified. The liklihood ratio test thus said heterogeneity abounded; clustering said the correlation matrices were so much alike that all the panelists belonged in the same cluster. For practical reasons, a course has to be struck somewhere between the very demanding requirement of the liklihood-ratio test that differences be almost nil and the clustering process which is designed to accommodate some variation in location in multidimensional space. The problem was to select panelists whose correlation matrices were similar enough to yield comparable factor patterns even though the liklihood-ratio test would still indicate heterogeneity was significant. The CANDISC program of SAS (1985) was initially utilized. The correlation matrices of the panelists for each tea were examined separately. An indication was thus obtained as to those panelists who tended to be a part of the same group notwithstanding relations among the panelists varied according to the particular tea being examined. A disadvantage to using the CANDISC procedure is that the final decision is subjective. The VARCLUS program was therefore returned to except it was forced to form two and three clusters. Panelists who were grouped together in both clusters were considered to be responding sufficiently alike.

Table 2 shows the results when the Varclus program was forced to generate two and three clusters. The correlation matrices of panelists 2, 5, 6, and 7 resulted in their being included in the dominant cluster upon both occasions with the matrix of panelist 11 included within the dominant cluster when only two clusters were formed. The CANDISC procedure, having generally led to panelists 11 being grouped with 2 and 7, he was therefore also included with panelists 2, 5, 6, and 7. The factor pattern based upon the results of these five panelists was quite similar to that of the panel results pooled (Fig. 1), indicating that the panel results were the consequence of agreement among a majority of the panelists.

It might be argued that when the results of 5 out of 7 panelists are used, the patterns for the subgroup and the panel would generally be similar whether or not there was agreement within the subgroup. To show that two of the panelists likewise yielded a pattern comparable to that of the panel, Table 3 lists the pattern results for the panel and for panelists 5 and 6 combined. There are some differences, but not many. If a corresponding value is not listed, that is because the attribute's correlation with the factor was non-significant. Table 4 lists Kendall's tau correlation values when the corresponding factors were compared. For the three factors and the communality estimates, the correlations were all significant.

Table 1—Liklihood-ratio test for homogeneity of correlation matrices of individual panelists

Panelists	x^2 Observed	x^2 Tabular p = 0.10	df
7 panelists pooled	1064.18	363.30	330
2,7,11	185.86	129.37	110
2,7	38.32	68.78	55
9,10	184.59	68.78	55
5,6	187.83	68.78	55

Table 2—VARCLUS program (oblique principal component) forced to form two and three clusters based upon individual panelist's correlation matrices

Cluster groupings	Panelists[a]
Original cluster	2 5 6 7 11 9 10
Two cluster stage	2 5 6 7 11 9 10
Three cluster stage	2 5 6 7 10 11 9

[a] Panelists underscored by the same line were assigned to the same cluster.

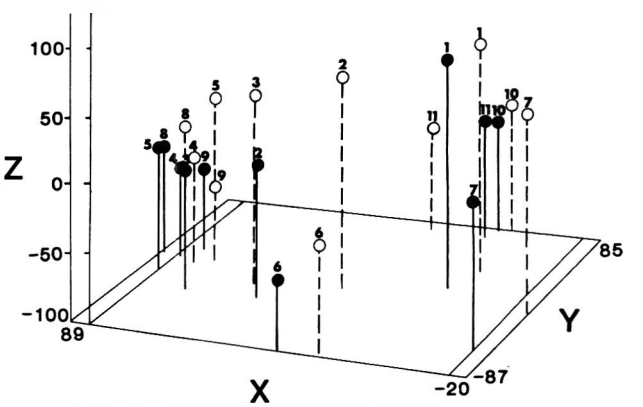

Fig. 1—Factor patterns of panel members pooled (7 members) and of panelists 5 and 6 combined, tea, product 5. ● = pattern for panelists 5 and 6 combined; ○ = pooled pattern; the numbers, in the same order, stand for the terms listed in Table 1.

Table 3—Factor reference structure (semipartial correlations), seven panelists pooled and panelists 5 and 6 combined, tea lot 1

	Factor 1		Factor 2		Factor 3	
Attribute	Pooled panelists	Panelists 5 & 6	Pooled panelists	Panelists 5 & 6	Pooled panelists	Panelists 5 & 6
Flavor		64	59			
Aroma	56	62				
Briskness	68	79				
Oregano-like		72				
Bitter	65	70				
Floral	66	79				
Metallic	43	53				
Grass-like			87	92		
Hay-like			83	84	44	
Sweetness					72	78
Citrus-like					83	83

Table 4—Correlations of factor pattern for panel (7 members) and pattern yielded by panelists 5 and 6, combined, tea lot 1

	Variables			
Variables	R11	R12	R13	R14
R21	0.51 (0.029)			
R22		0.75 (0.001)		
R23			0.49 (0.036)	
R24				0.78 (0.0008)

Three-factor patterns were derived for Fig. 1 because graphic presentation naturally cannot go beyond three dimensions. To facilitate visual inspection, Table 3 likewise lists only three factors. In some instances, four- and five-factor patterns were more appropriate. The forcing of clusters, more and more compact, still applied. If however the number of factors appropriate for the subgroup is different from that of the panel, overall correlation naturally is poor. In fact, numerical analysis is generally not at all necessary in that case. On their faces the patterns are not in accord with each other. In other instances, correlation was poor, but for an entirely different reason. Sometimes all the factors would be well correlated, but the best correlation for a variable would not be with its corresponding factor in the other pattern but with a different factor. When oblique factors were calculated and the Procrustean transformation (SAS, 1985) is a part of the calculation process, some shifting of the factors between position is understandable. The intent of a Procrustean transformation is to harmonize as much as possible the configurations of the different panelists making up a set. In rotating 5 configurations as compared with 7 to effect maximum harmony within a set, a variable may shift from one factor to another. The problems above are pointed out because forcing to form clusters more and more compact does not necessarily lead to factor patterns being more and more alike. Our panel of 11 was initially culled so as to retain only the most consistent and discriminating panelists and those who were not contributing substantially to product-panelist interaction; nonetheless additional culling was sometimes needed to secure a "panel", the factor pattern of which could be demonstrated to result from substantial agreement among the individual members rather than being mere averaging of a mixture of similar and dissimilar patterns.

One observation which clearly came out of the study is that various products or grades should be examined separately. Ideally, the investigator would like to have a factor pattern which is so general it encompasses all variations of the same commodity. Cluster analysis applied to the five grades of tea showed that Products 1 and 5 each formed a class. Products 2, 3 and 4 formed a third class though upon further clustering it ultimately could be split apart. The factor patterns for Products 1 and 5 were different as was that for products 2, 3 and 4 pooled. Before any firm decisions are made as to the factor pattern existing for a given food, the products should be grouped into any classes known in advance or subjected to principal-component analysis or cluster analysis to learn if separate classes possibly exist. That rubric is in line with Cattell's (1965) recommendation. If different classes exist, separate factor analyses should be calculated for each class to demonstrate whether the patterns are alike or different. For any given commodity there is a core pattern, but variations of the pattern should be expected if the characteristics of the product vary as they did here for the different grades of tea. This seems so obvious as not to merit mention. Frequently however factor patterns are presented for a product as if the pattern were a general characteristic for all variations of that product when in fact each grade or species may have its own pattern. While the ultimate objective of FA is to reduce a set of entities to a lesser number of factors, the products and the performance of the panelists should be examined separately since they may be confounded in some fashion.

SUMMARY

THIS STUDY shows that the factor patterns of individual panelists and their patterns for individual products should be examined to learn whether the panelists' responses are sufficiently concordant to suggest that an underlying structural order exists. Patterns may appear to be logical, but before any import is attributed to the pattern, the responses of individual panelists should first be examined to learn if they agree reasonably well. "Reasonably well" is not used as weasel words, but rather in a practical sense. Some heterogeneity is to be expected. The degree of heterogeneity needs to be ascertained so that the investigator has an indication as to whether the factor pattern discerned borders on randomness due to the panelists responding in different ways or there is sufficient agreement among the panelists to indicate that relations among the attributes are being reflected by the panelists' scale values.

The methods examined here, while unquestionably not as rigorous as the tests devised by various statisticians (see citation in the introduction), have the merit of being procedures found in most computer packages and thus available for routine use by sensory technologists.

The study demonstrated that panelists shown to be consistent and discriminating in their responses and exhibiting little product-panelist interaction as judged by analysis of their original scale values may nonetheless be the cause of interaction when their results are subjected to FA. Unless a reasonable percent of the panelists are shown to yield comparable factor patterns, the trustworthiness of the panel's pattern is considerably in doubt.

REFERENCES

Anderson, T.W. 1958. "An Introduction to Multivariate Statistical Analysis," p. 247. Chapman & Hall Ltd., London.
Arnold, S.F. 1981. "The Theory of Linear Models and Multivariate Analysis." John Wiley & Sons, New York.
Bartlett, M.S. 1950. Tests of significance in factor analysis, British J. Stat. Psychol. 111: 77.

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Sensory Quality of Selected Sweeteners: Aqueous and Lipid Model Systems

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ABSTRACT

The sweetness characteristics of sucrose, fructose, aspartame, acesulfame K, sodium saccharin and calcium cyclamate were studied in aqueous and lipid model food systems with and without lemon or vanilla flavoring. Anchored linear scales were used to evaluate sweetened model systems for initial, maximum and residual sweetness intensity and nonsweet aftertaste. Data were analyzed by analyses of variance. Flavor did not influence sweetness, except where residual sweetness was more intense in lemon and vanilla solutions than in plain solutions. No sweetener was perceived exactly like sucrose. Intensity and sweetness profiles varied between systems and among sweeteners. Character of the food system influenced perceptions of sweetness and aftertaste.

INTRODUCTION

NUMEROUS INVESTIGATORS have studied effects of basic tastes on sweetness intensity in aqueous solutions, dispersions and gels. Relative sweetness and intensity and duration of sweetness of sucrose, fructose, glucose, xylitol and other sweeteners have been investigated in this manner (Fabian and Blum, 1943; Gregson and McCowen, 1963; Hyvonen et al., 1977, instead of; 1978; Cardello et al., 1979). However, sweet substances seldom are tasted alone, nor are food systems composed of so few ingredients. Such studies might not depict accurately the character of sweetness in foods, particularly those foods composed of relatively high concentrations of fat or those that undergo thermal processing. Type, texture and character of the dispersion media affect perceived sweetness. Studies on a model food system similar to a baked system were not found in the literature. The most relevant studies have dealt with combinations of carbohydrate sweeteners in simple dispersions.

Changing attitudes about sucrose consumption have heightened the need for evaluating alternative sweeteners. Little information has been published on sweetness perception of aspartame, saccharin and other intense sweeteners in food systems, especially baked products. Total gustatory response to all components and reactions within a food system must be considered if perceived sweetness is to be defined and characterized. The objective of this study was to investigate effects of selected ingredients on sweetness perception of some carbohydrate and nonnutritive sweeteners in various model systems.

MATERIALS & METHODS

Sample preparation

Model food systems evaluated included a solution and a cream, each sweetened with sucrose, fructose, aspartame, acesulfame K, sodium saccharin, or calcium cyclamate. Lemon or vanilla liquid flavoring (#4267 Lemon Natural Oil and #3454 Vanilla, Warner Jenkinson Flavors, St. Louis, MO) were used. Control samples contained no flavoring and were referred to as "plain." Chemically pure sucrose was used for solutions; granulated food-grade sucrose (C&H) was used for model creams. Chemically pure crystalline β-D-fructose (Sigma Chemical Co., St. Louis, MO), unbulked aspartame (G.D. Searle, Skokie, IL), acesulfame K (Hoescht AG, Frankfurt, W. Germany), sodium saccharin (Sherwin Williams, Cincinnati, OH), and calcium cyclamate (Abbott Laboratories, Chicago, IL) were used.

Approximately thirty difference and scaling tests were employed to determine sweetness levels. Solutions of fructose and intense sweeteners were compared to the 5% sucrose reference. Increasingly narrow concentrations were evaluated until the majority of the panelists perceived the composite sweetness intensity approximately equivalent to the sucrose reference. Some researchers (Cloninger and Baldwin, 1974; Larson-Powers and Pangborn, 1978a, b) used a 10% sucrose reference but in this study, where panelists evaluated six samples per session, sweetness equivalent to 10% sucrose was ascertained in preliminary testing to fatigue and impair sensory acuity.

Sweetness equivalent to 5% sucrose was difficult to perceive in the more viscous lipid system. Therefore, equisweet concentrations of all nonsucrose sweeteners were determined for the cream using a 25% sucrose reference cream.

Inherent problems existed in determining the amount of acesulfame K, saccharin and cyclamate equivalent to sucrose in both solutions and creams. The sharp, immediate sweetness of saccharin, the slow onset of acesulfame K sweetness, and the persistent bitterness associated with saccharin and cyclamate allowed panelists to distinguish these sweeteners from sucrose in difference tests. Larson-Powers and Pangborn (1978 a, b) encountered difficulties because cyclamate and saccharin imparted a noticeable bitterness to beverages and gelatins in their study.

Solution

The predetermined amount of sweetener (5.0% sucrose, 4.1% fructose, 0.028% aspartame, 0.026% acesulfame K, 0.014% sodium saccharin or 0.140% calcium cyclamate, all w/v basis) was combined with deionized distilled (d/d) water (approximately 25 ± 1°C) in an Osterizer blender (Model 542-D) until dissolved. Solutions were plain or flavored with 0.11% lemon or vanilla flavoring. Solutions were prepared at least 2.5 hr prior to tasting to allow for equilibration of α- and β-forms (Pangborn and Gee, 1961) and held at room temperature (25° ± 1°C) until evaluation. Ten milliliter samples of solution were presented to panelists in coded, disposable cups.

Cream

The model cream consisted of a concentrated sweetener solution dispersed in 140g bland, hydrogenated shortening (Crisco). The designated amount of sweetener (25.0% sucrose, 20.63% fructose, 0.22% aspartame, 0.22% acesulfame K, 0.22% sodium saccharin or 0.50% calcium cyclamate all w/v basis) was blended with 60 ml d/d water at high speed for 60 sec. The shortening was beaten on high speed for 30 sec, the sweetener solution and liquid flavoring were added, and the mass was whipped at high speed for an additional 45–60 sec. The model cream was prepared 24 ± 1 hr prior to tasting and stored in plastic bags (7 ± 1°C). Three to four gram samples were dispensed into coded, disposable cups. Samples were capped with foil and presented at room temperature, 25 ± 1°C to panelists. Small, blunt edged stainless steel knives were used for tasting because the flat blade facilitated quick release of the sample onto the tongue. Amount of sample tasted was approximately 0.3–0.6 g (1/8–1/4 tsp).

Sensory analysis

Seven panelists from the Dept. of Foods & Nutrition at Kansas State Univ. were chosen on the basis of interest and availability.

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During 15 hr of training, panelist performance was monitored, and panelists worked together to become familiar with sweeteners, to determine common descriptive terminology and to develop specific procedures for evaluating sweetness quality. Each set of six samples presented to panelists during one taste session was prepared with the six sweeteners and one flavor as shown in Table 1.

Food systems were evaluated individually by judges for four parameters of sweetness quality on a 60 point (6 inch scale divided into tenths), computerized scale. All samples were scored for initial, maximum, and residual sweetness intensity and for intensity of non-sweet aftertaste. Initial sweetness was defined as intensity of sweetness perceived when a sample was first taken into the mouth. Maximum sweetness, the most intense sweetness perceived, was assessed during manipulation. Residual sweetness was the sweetness that lingered after swallowing. Intensity and identity of non-sweet tastes and/or feeling factors prior to and after swallowing were recorded. Panelists did not retaste samples and used timers to ensure adequate time for evaluation and recovery. A specific cleansing and rinsing procedure with sliced raw carrots and d/d water was followed to minimize carry-over effect.

Analysis of data

A 3 × 6, flavor by sweetener, treatment combination was used in a split plot design to compare measurements from the 18 treatments. Three replications of the entire sensory experiment were conducted for each system. Data were tested using analysis of variance (ANOV) procedures of the Statistical Analysis System (SAS, 1982). Means were compared and differences, when found, were separated using Least Significant Difference (LSD) procedures.

RESULTS & DISCUSSION

NONE of the flavor by sweetener interactions was significant for any of the sensory properties evaluated in solutions (Table 2). Maximum sweetness was the only factor for which interaction was significant in the creams. Highly significant differences ($p \leq 0.0001$) existed among sweeteners for initial, maximum and residual sweetness intensities and nonsweet aftertaste in both systems.

Of the four sweetness parameters studied, flavor influenced only residual sweetness in solutions (Table 3). Residual sweetness was greater ($p \leq 0.05$) in lemon- and vanilla-flavored solutions than in plain solutions.

Table 1—Treatment combinations used for each food system for each replication

Sweetener	Flavor		
	Lemon	Vanilla	Plain
Sucrose	1	7	13
Fructose	2	8	14
Aspartame	3	9	15
Acesulfame K	4	10	16
Saccharin	5	11	17
Cyclamate	6	12	18

Table 2—F-values and probabilities[a] from ANOV for sweetness quality determined by sensory analysis of solutions and model creams

Source of variation	DF	Sensory parameters			
		Initial sweetness	Maximum sweetness	Residual sweetness	Nonsweet aftertaste
Solutions					
Replications (R)	2	0.91 (NS)	1.45 (NS)	16.23 (0.01)	2.46 (NS)
Flavor (F)	2	1.31 (NS)	0.13 (NS)	6.91 (0.05)	1.85 (NS)
Sweetener (S)	5	16.31 (0.0001)	30.06 (0.0001)	32.18 (0.0001)	5.03 (0.0018)
F × S	10	1.68 (NS)	1.15 (NS)	0.25 (NS)	2.00 (NS)
Creams					
Replication (R)	2	6.61 (0.05)	11.39 (0.02)	7.77 (0.04)	1.30 (NS)
Flavor (F)	2	0.48 (NS)	0.09 (NS)	0.22 (NS)	1.79 (NS)
Sweetener (S)	5	25.69 (0.0001)	40.82 (0.0001)	38.01 (0.0001)	15.99 (0.001)
F × S	10	1.79 (NS)	2.15 (0.05)	0.98 (NS)	0.44 (NS)

[a] Probabilities are given in parentheses; NS = not significant.

Sweetness intensity scores were plotted as profiles to observe the general character of sweeteners in solutions (Fig. 1) and creams (Fig. 2). Generally, order of sweetness in solution was sucrose > aspartame > fructose > saccharin ≥ cyclamate > acesulfame K. In cream systems, order of sweetness differed: saccharin ≥ aspartame > sucrose ≥ acesulfame K ≥ fructose > cyclamate.

From the profiles, one can see that none of the sweeteners was perceived exactly like sucrose. Aspartame sweetness has been described as "sucrose-like" (Inglett, 1981; Homler, 1984), so it was not surprising that aspartame and sucrose sweetness profiles were shaped similarly for the aqueous solutions. The four remaining sweeteners, fructose, acesulfame K, cyclamate, and saccharin, had sweetness profiles similar to each other. Nonsweet aftertaste was much lower for aspartame and sucrose than for any other sweetener in solution, and considerably less than their respective residual sweetnesses. In contrast, acesulfame K had a nonsweet aftertaste much higher than its residual sweetness. Intensities of nonsweet aftertaste for fructose, cyclamate and saccharin were similar to each other and to their residual sweetness level in solutions.

In lipid-based creams, the shapes of the sweetness profiles for all sweeteners were similar. Dissimilarities portrayed in the profiles were evident in the nonsweet aftertastes. The nonsweet aftertaste was not as high as residual sweetness for acesulfame K in the cream; however, the non-sweet aftertaste cyclamate exhibited was high relative to its residual sweetness.

Aspartame and acesulfame K were described as similar in relative sweetness (Arpe, 1978; O'Brien and Gelardi, 1981; Rymon-Lipinski and Huddart, 1983), but individual aspects of sweetness quality were not compared. In this study, differences ($p \leq 0.05$) between the two sweeteners were found in both aqueous and lipid systems. Acesulfame K was reported (Rymon-Lipinski and Huddart, 1983) to have a sweet aftertaste that is lasting but not overpowering. Accordingly, residual sweetness of acesulfame K was lower than initial sweetness in solution but was similar to initial sweetness in creams. On the other hand, the high aspartame sweetness in solutions and creams had a lingering sweetness that was fairly intense. Aftertaste of acesulfame K in solution was the same ($p \leq 0.05$) as saccharin or cyclamate but not significantly different from aspartame in either system. The character of acesulfame K described in the literature (Arpe, 1978; O'Brien and Gelardi, 1981) as "sweetness that is quickly perceptible" and "no aftertaste" appears to be dependent upon the dispersion medium, at least for the levels of sweeteners used in this study.

Cyclamate was low in initial and maximum sweetness in solutions and creams, differing from aspartame, fructose and sucrose in initial sweetness and from all sweeteners in maximum and residual sweetness. Conceivably, the amount of cyclamate might have been too low; its inherent bitterness made achieving equivalent sweetness difficult. Increasing the concentration of cyclamate would have increased that bitter component as well. Even at the level used, bitterness might have impaired or changed the perception of cyclamate sweetness. Aftertaste of cyclamate was especially intense in the cream system and much higher ($p \leq 0.05$) than aftertaste of all other sweeteners. Intensity of non-sweet aftertaste noted by panelists seemed to dispel the claim that cyclamate is "almost free from aftertaste" (O'Brien and Gelardi, 1981). The nonsweet flavor notes associated with saccharin (bitterness, metallic, dryness) reported by Rader et al. (1967) were noted also for cyclamate and acesulfame K.

Aspartame was not completely free of aftertaste either; bitterness was perceived frequently in both solutions and creams. No significant difference in sweetness between aspartame and sucrose solutions was reported by Samundsen (1985) but both solutions had aftertastes of differing quality. Aftertaste of as-

Table 3—Mean[a] sensory values[b] pooled for all sweeteners for lemon, vanilla, and plain flavored solutions and creams

	Sensory Parameters							
	Initial sweetness		Maximum sweetness		Residual sweetness		Nonsweet aftertaste	
Flavor	Solutions	Creams	Solutions	Creams	Solutions	Creams	Solutions	Creams
Lemon	26.26[a]	21.93[a]	31.57[a]	28.04[a]	19.17[a]	20.29[a]	15.71[a]	10.23[a]
Vanilla	27.64[a]	20.89[a]	31.19[a]	27.53[a]	19.00[a]	20.87[a]	16.78[a]	11.40[a]
Plain	27.47[a]	20.68[a]	30.89[a]	28.16[a]	17.13[b]	20.04[a]	13.49[a]	12.98[a]
LSD (0.05)	2.58	3.78	3.73	4.45	1.69	3.61	4.84	4.06

[a,b] Three replications with six sweeteners for a total of 18 observations/mean; means in same column with same letter are not significantly different ($p \leq 0.05$); no comparison intended among columns or between systems. Sixty point computer scale with 60.0, highest and 0.0, lowest; equivalent to 6-inch ODA scale with 1 inch = 10 points.

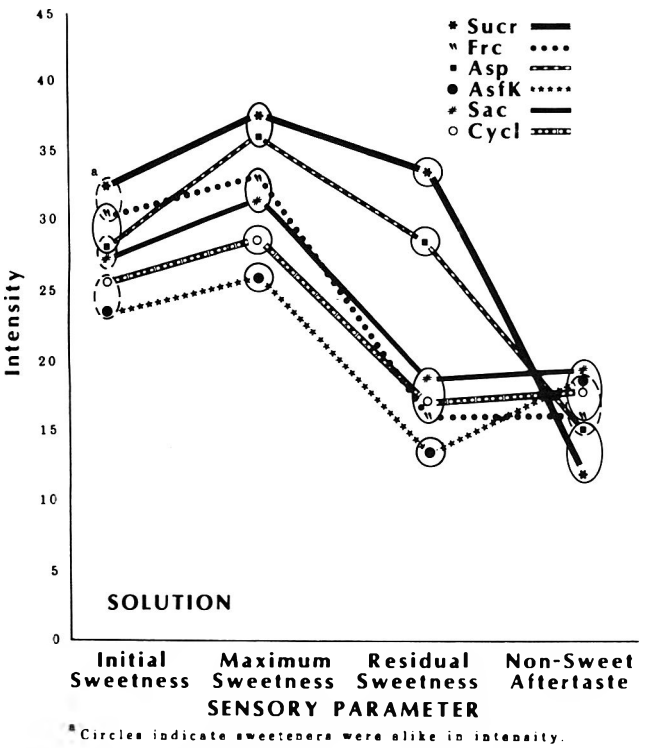

Fig. 1—Sensory intensity profiles for sweeteners in aqueous solutions when flavors were pooled.

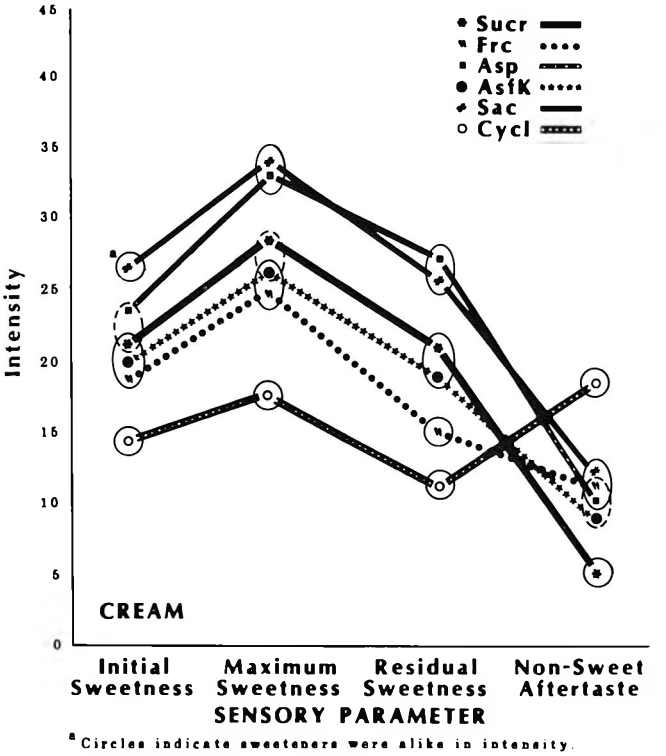

Fig. 2—Sensory intensity profiles for sweeteners in lipid-based creams when flavors were pooled.

partame was described as bitter-sweet with a slightly powdery sensation, whereas sucrose aftertaste was characterized as drying with a slight bitterness. In this study, panelists described aftertaste of sucrose sweetened systems as sour.

Obvious difficulties exist in trying to compare sweetness quality in solutions and lipid media. Comparisons to research conducted previously were not easy to make either. Many researchers (Schutz and Pilgrim, 1957; Stone and Oliver, 1969; Hyvonen et al., 1977; Cardello et al., 1979) have studied relative sweetness or sweetness intensity but few have evaluated sweetness as was done in this study. Yamaguchi et al. (1970) reported on relative sweetness of fructose, sodium cyclamate, sodium saccharin and other sweet substances in solution but gave only the relationship between concentration and sweetness. Harrison and Bernhard (1984) investigated initial sweetness intensity and duration of sweetness in solution relative to concentration but used xylitol, lactose and saccharin. Moskowitz (1970) investigated quality and intensity of sweeteners and reiterated that sweetness quality of artificial sweeteners differs markedly from that produced by sugars. Panelists in this study perceived the carbohydrate and intense sweeteners differently both in solutions and in creams.

Skramlik (1926) demonstrated that sweet substances were perceived differently in water than in oil. The influence of viscosity has been studied (Stone and Oliver, 1969; Vaisey et al., 1969; Arabie and Moskowitz, 1970) but the systems were solutions thickened with hydrocolloids or starch or had a gelatin matrix. Mackey (1958) observed that sensitivity to sucrose was highest in water and less in more viscous systems thickened with carboxymethylcellulose or cornstarch. Sweetener intensity profiles resulting from this study could reflect an effect similar to that reported by Mackey (1958): sucrose sweetness was greater, relative to other sweeteners, in solutions than in creams and, in most instances, initial and maximum sweetnesses of any given sweetener were higher in solutions than in cream systems. Perhaps, the viscous cream left a film on the tongue that inhibited release of sweetener into taste receptors, as Stone and Oliver (1966) theorized. If so, then sweetness intensity of an individual sweetener in solution and cream would differ. Also, sensitivity to saccharin bitterness might be diminished.

Panelists observed that onset of acesulfame K sweeteners was slow in both solution and cream compared to other sweeteners. Inglett (1981) reported that sensory properties of acesulfame K are similar to those of saccharin but in this study, acesulfame K contrasted the sharp, immediate sweetness of saccharin.

CONCLUSIONS

SWEETNESS INTENSITIES of individual sweeteners changed from system to system. Character of the food system seemed

to influence perception, because sweeteners were rated differently in solutions and creams. None of the sweeteners (fructose, aspartame, acesulfame K, sodium saccharin, calcium cyclamate) was perceived exactly like sucrose. Sweetness character and intensity of non-sweet aftertaste varied among sweeteners and between solutions and creams. Sucrose was sweetest of the six sweeteners or like fructose in solution, but saccharin was sweetest and cyclamate was least sweet in the creams. Flavor did not influence sweetness except residual sweetness which was more intense in lemon and vanilla solutions than in plain solutions. Information on the changing character of intense sweeteners in different food systems should be important to manufacturers because food products contain ingredients in addition to water which could influence sweetness.

REFERENCES

Arabie, P. and Moskowitz, H.R. 1971. The effects of viscosity upon perceived sweetness. Percept. Psychophys. 9: 410.

Arpe, H.J. 1978. Acesulfame K, a new noncaloric sweetener. Health and Sugar Substitutes Proc. ERGOB Conf., Geneva, Switzerland.

Cardello, A.V., Hunt, D., and Mann, B. 1979. Relative sweetness of fructose and sucrose in model solutions, lemon beverages and white cake. J. Food Sci. 44: 748.

Cloninger, M.R. and Baldwin, R.E. 1974. L-Aspartyl-L-phenylalanine methyl ester (aspartame) as a sweetener. J. Food Sci. 39: 347.

Fabian, F.W. and Blum, H.B. 1943. Relative taste potency of some basic food constituents and competitive and compensatory action. Food Res. 8: 179.

Gregson, R.A.M. and McCowen, P.J. 1963. The relative perception of weak sucrose-citric acid mixtures. J. Food Sci. 28: 371.

Harrison, S.K. and Bernhard, R.A. 1984. Time-intensity sensory characteristics of saccharin, xylitol and galactose and their effect on the sweetness of lactose. J. Food Sci. 49: 708.

Homler, B. 1984. Properties and stability of aspartame. Food Technol. 38(7): 50.

Hyvonen, L., Kurkela, R., Koivistoinen, P., and Ala-Kulju, M. 1978. The relative sweetness of fructose, glucose, and xylitol in acid solutions at different temperatures. Lebensm-Wiss-Technol. 11: 11.

Hyvonen, L., Kurkela, R., Koivistoinen, P., and Merimaa, P. 1977. Effects of temperature and concentration on the relative sweetness of fructose, glucose and xylitol. Lebensm-Wiss-Technol. 10: 316.

Inglett, G.E. 1981. Sweeteners - a review. Food Technol. 35: 37.

Larson-Powers, N. and Pangborn, R.M. 1978a. Paired comparison and time intensity measurements of the sensory properties of beverages and gelatins containing sucrose or synthetic sweeteners. J. Food Sci. 43: 41.

Larson-Powers, N. and Pangborn, R.M. 1978b. Descriptive analysis of the sensory properties of beverages and gelatins containing sucrose and synthetic sweeteners. J. Food Sci. 43: 47.

Mackey, A. 1958. Discernment of taste substances as affected by solvent medium. Food Res. 23: 580.

Moskowitz, H.R. 1970. Ratio scales of sugar sweetness. Percep. Psychophys. 7(5); 315.

O'Brien, L. and Gelardi, R. 1981. Alternative sweeteners. Chem. Technol. 11: 274.

Pangborn, R.M. and Gee, S.C. 1961. Relative sweetness of alpha- and beta-forms of selected sugars. Nature 191: 810.

Rader, C.P., Tihanyl, S.G., and Zienty, F.B. 1967. A study of the true taste of saccharin. J. Food Sci. 32: 357.

Rymon-Lipinski, G.W.R. and Huddart, B. 1983. Acesulfame K. Chem. Ind. (June 6): 427.

Samundsen, J.A. 1985. Has aspartame an aftertaste? J. Food Sci. 50: 1510.

SAS. 1982. "SAS User's Guide." SAS Institute, Inc. Raleigh, NC.

Schutz, H.G. and Pilgrim, F.J. 1957. Sweetness of various compounds and its measurement. Food Res. 22: 206.

Skramlik, E. 1926. Vondie physiologie des gerushsund geschmacksinnes. In "Handbuch det Physiolgie des nierderen Sinne." In 1. Leipzig: Thieme.

Stone, H. and Oliver, S.M. 1966. Effects of viscosity on the duration of relative sweetness intensity in sucrose solutions. J. Food Sci. 31: 129.

Stone, H. and Oliver, S. 1969. Measurement of the relative sweetness of selected sweeteners and sweetener mixtures. J. Food Sci. 34: 215.

Vaisey, M., Brunon, R., and Cooper, J. 1969. Some sensory effects of hydrocolloid sols on sweetness. J. Food Sci. 34: 397.

Yamaguchi, S., Yoshikawa, T., Ikeda, S., and Ninomiya, T. 1970. 1. Studies on the taste of some sweet substances. II. Interrelationships among them. Agric. Biol. Chem. 34(2): 187.

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 From page 450

BMDP. 1979. "BMDP-79 Biomedical Computer Programs P-Series." University of California Press, Berkeley, CA.

Brach, N.M. 1982. Interrelationships among sensory attributes of tea. Ph.D. dissertation, Univ. of Georgia, Athens, GA.

Burt, C. 1952. Tests of significance in factor analysis. British J. Stat. Psychol. V: 109.

Cattell, Raymond B. 1965. Factor analysis: an introduction to essentials. II. The role of factor analysis in research. Biometrics 21: 405.

Galt, A.M. and MacLeod, G. 1983. A research note: the application of factor analysis to cooked beef aroma descriptors. J. Food Sci. 48: 1354.

Godwin, D.R. 1984. Relationships between sensory response and chemical composition of tea. Ph.D. dissertation, University of Georgia, Athens, GA.

Harries, J.M., Rhodes, D.N. and Chrystall, B.B. 1972. Meat texture. 1. Subjective assessment of the texture of cooked beef. J. Text. Studies 3: 101.

Knapp, T.R. and Swoyer, V.H. 1967. Some empirical results concerning the power of Bartlett's test of the significance of a correlation matrix. Am. Educ. Res. Journ. 4: 13.

Kwan, W., and Kowalski, B.R. 1980. Data analysis of sensory scores. Evaluations of panelists and wine score cards. J. Food Sci. 45: 213.

Lawley, D.N. 1956. Tests of significance for the latent roots of variance and correlation matrices. Biometrika 43: 128.

Lawley, D.N., and Maxwell, A.E. 1971. "Factor Analysis as a Statistical Method." Butterworth & Co., London.

Lyon, B.G. 1980. Sensory profiling of canned boned chicken: sensory evaluation procedures and data analysis. J. Food Sci. 45: 1341.

McLellan, M.R., Cash, J.N., and Gray, J.I. 1983. Characterization of the aroma of raw carrots (Daucus carota L.) with the use of factor analysis. J. Food Sci. 48: 71.

McLellan, M.R., Lind, L.R. and Kime, R.W. 1984. Determination of sensory components accounting for intervarietal in apple sauce and slices using factor analysis. J. Food Sci. 49: 751.

Powers, J.J. 1984. Current practices and applications of descriptive analysis. In "Sensory Analysis of Foods," Piggott, J.R. (Ed.), p. 179. Applied Sci. Publishers, London.

Powers, J.J., Godwin, D.R., and Bargmann, R.E. 1977. Relation between sensory and objective measurements for quality evaluation of green beans. In "Flavor Quality: Objective Measurements," Scanlan, R.A. (Ed.), p. 51. ACS Symposium Series No. 51, Am. Chem. Soc., Washington, DC.

Powers, J.J., Shinholser, K. and Godwin, D.R. 1985. Evaluating assessors' performance and panel homogeneity using univariate and multivariate statistical analysis, in "Progress in Flavour Research 1984", Adda, J. (ed.), Elsevier Sci. Publishers, Amsterdam. p. 202.

Rao, C.R. 1955. Estimation and tests of significance in factor analysis. Psychometrika. 20: 93.

SAS. 1982. SAS User's Guide: Statistics. SAS Institute, Inc., Box 8000, Cary, NC 27511.

SAS. 1985. SAS User's Guide: Statistics. Version 5 Edition. SAS Institute Inc., Cary, NC.

Tobias, Sigmund and Carlson, James E. 1969. Brief Report: Bartlett's test of sphericity and chance findings in factor analysis. Multivariate Behavioral Research 4: 375.

Wu, L.S., Bargmann, R.E. and Powers. J.J. 1977. Factor analysis applied to wine descriptors. J. Food Sci. 42: 944.

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Mathematical Modeling of Microwave Thawing by the Modified Isotherm Migration Method

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ABSTRACT

A mathematical model of microwave thawing of homogeneous food products is developed and solved numerically using the Modified Isotherm Migration Method. The model is used to predict thawing time and temperature profiles for microwave thawed meat cylinders at three frequencies (2450 MHz, 915 MHz, 300 MHz) and different power levels. Model and experimental results for thawing a lean beef cylinder heated at low microwave power using 2450 MHz frequency compare well. The advantage of using 915 or 300 MHz power over 2450 MHz power is shown by calculations. The results show that microwaves significantly accelerate the thawing rate. The mathematical model is explored as a tool for designing optimal microwave/convective heating protocols for rapidly thawing foods in desired temperature ranges.

INTRODUCTION

THAWING OF FROZEN raw materials is often integral to many food manufacturing processes. Optimizing thawing rates in order to avoid microbial problems, chemical deterioration and (excessive) water loss caused by dripping or dehydration, is important. Faster and controlled thawing rates would allow more efficient use of plant space and better production scheduling (Decareau, 1968). Microwave irradiation has the potential to accelerate the thawing process, preferably in a continuous operation. Localized overheating (runaway heating) has limited the application of microwave thawing to food systems which require larger manufacturing conditions. The development of microwave equipment that achieves a more uniform power distribution and a better control of microwave heating by combining lower frequency microwave generation with convection heating (or cooling) allows the exploration of such systems on an industrial scale. Tempering or partial thawing of meat products with microwaves prior to processing is already practiced (Bezanson et al., 1973; Swift and Tuomy, 1978; Edgar, 1981). Low power-low frequency (Bengtsson, 1963) or high power-surface cooling combinations (Bialod et al., 1978) have been proposed for thawing of even large sized products without runaway heating. Microwave thawing techniques are also being proposed for quick thawing of non food systems such as fresh frozen plasma in plastic envelopes (Checcucci et al., 1983).

The phenomenon of thawing or freezing a food is a complex one. It is an unsteady state heat transfer process involving a phase change, which makes the problem a nonlinear one. Furthermore, unlike a pure fluid, the freezing of a food does not take place at a specific freezing point but rather occurs over a range of temperatures. Thermal properties of the food undergoing freezing (or thawing) can vary significantly with temperature, yet little information is available on these properties under variable conditions. Irregular shape and heterogeneity of the food are further complicating factors.

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Numerous approaches to the problem of predicting freezing or thawing rate and time exist in the literature, recently reviewed by Ramaswamy and Tung (1984) and evaluated by Cleland and Earle (1984). These approaches can be categorized into: (1) analytical solutions or semiempirical expressions, and (2) numerical solutions. Most expressions and equations use a single freezing point and constant thermal properties and give the thawing or freezing time for homogeneous and regularly shaped foods. The numerical solutions use finite difference or finite element methods and can approximate more realistically the actual process. Some solutions use mathematical transformations, like the Modified Isotherm Migration Method (Talmon and Davis, 1981; Talmon et al. 1982) which treats the problem as the propagation of a phase front. In addition to the thawing time, numerical methods give the temperature profiles in the food throughout the process, such profiles being relevant to the analysis of possible chemical or microbial reactions incurred by the food during processing.

The purpose of the present paper is to develop the Modified Isotherm Migration Method (MIMM) for predicting the microwave thawing of frozen, simply shaped food samples, and to test MIMM by comparing predictions with experiments carried out on cylinders of lean beef. We also explore the use of MIMM predictions to design optimal microwave/convective heating combinations for rapid, temperature controlled thawing.

THEORY

A FROZEN FOOD SAMPLE of a slab, long cylinder or sphere shape at an initial temperature T_i is exposed at time $t = 0$, to an environment of temperature T_∞ and a uniform microwave field. The sample is assumed to have a single thawing point T_F. During thawing two phases coexist in the sample separated by a propagating thawing front as illustrated in Fig. 1. If the frozen region reaches the thawing point T_F locally, then it can be partially thawed, the local volume fraction ϕ of frozen sample depending on how long the sample has been at the thawing point. Partial thawing inside the propagating thawing front is peculiar to microwave heating, because of volumetric penetration of microwave radiation, and does not occur in convective heating.

The microwave refractive index of foods is large and can be calculated as a function of dielectric properties (von Hippel, 1954). For nonfrozen meat it varies from 7.1 and 8.8 over the range 300 to 2450 MHz in the nonfrozen state and from 2.5 to 2.9 in the frozen state. Therefore the transmitted microwave beam at each point of the surface of the sample is assumed to be transmitted normally to the sample surface. The intensity of the transmitted microwave radiation, expressed as energy flux, I, in W/m^2, at depth x from the point at which the microwave beam hits the sample surface is given by Lambert's law (von Hippel, 1954):

$$I(x) = I_o \exp(-2x/R_i) \quad (1)$$

where I_o is the intensity of the microwave field at the surface.

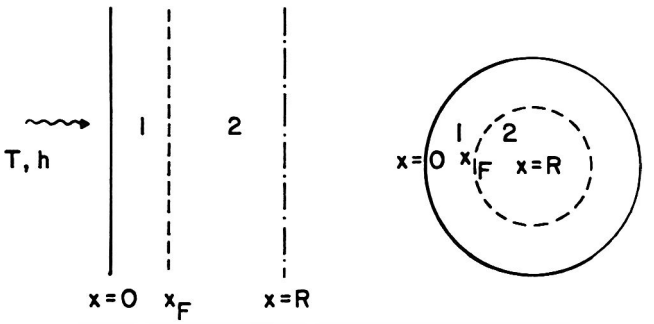


Fig. 1—Cross-sections of thawing slab and cylinder and sphere. For the slab only the left-hand half is shown, x = R being a place of symmetry. For cylinders and sphere, x represents the radial distance from the curved surface to the center. Region 1 is the thawed region, region 2 the frozen region and X_F the position of the propagating thawing front.

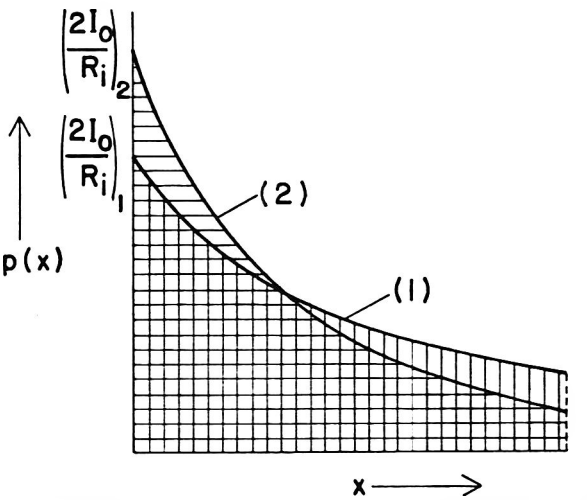


Fig. 2—Qualitative representation of absorbed microwave power per unit volume, p(x), as a function of sample depth, x, in a sample of large penetration depth (1) and one of a smaller one (2).

The power absorbed by a unit volume of the sample at depth x, p(x), is:

$$p(x) = -\frac{dI(x)}{dx} = p_o \exp(-2x/R_i) \qquad (2)$$

p_o ($= 2I_o/R_i$) is the power density absorbed at the surface and R_i the penetration depth, defined as the depth at which the microwave power intensity has decreased to $1/e^2$ of the intensity at the surface, given as

$$R_i = \frac{\lambda}{\pi(2K')^{1/2}} \{[1 + (K''/K')^2]^{1/2} - 1\}^{-1/2} \qquad (3)$$

λ, K', K'' are the radiation wavelength, the dielectric constant and the dielectric loss factor of the sample, respectively. From Eq. (3) it follows that the penetration depth is larger for lower frequencies (longer wavelengths). For the same frequency the penetration depth for the thawed stage is significantly smaller than for the frozen stage because of differences between K' and K'' for the frozen and thawed states. The effect on the absorbed power distribution is shown in Fig. 2.

The assumptions of normal microwave power transmission into the sample, uniform microwave field, and symmetrical conditions reduce the problem to a one-dimensional heat transfer problem with change-of-phase and "effective internal heat source." Heat and microwave transmission through the slab edges or cylinder ends is neglected in the analysis presented here. The following assumptions are also made: Average constant thermal conductivity, k_j, heat capacity, c_j, thermal diffusivity, α_j, and dielectric properties (K_j', K_j'') in each region (j = 1 for the thawed region, j = 2 for the frozen or partially frozen region); negligible shrinkage, i.e., the density, p, is the same for the two phases; and negligible water loss

The total microwave power absorbed by the sample, P, is assumed constant during the thawing procedure. The experiments on which this assumption is based are described in the next section.

For each region the heat conduction equation will be:

$$\frac{1}{\alpha_j}\frac{\partial T_j}{\partial t} = \frac{1-f}{R-x}\frac{\partial T_j}{\partial x} + \frac{\partial^2 T_j}{\partial x^2} + \frac{p_j(x,t)}{k_j} \qquad (4)$$

where f = 1,2,3 are form factors for the slab, cylinder and sphere, respectively, and R is the half width of the slab or the radius of the cylinder or sphere. The power $p_j(x,t)$ absorbed per unit volume is a function of both depth x and time t because the distribution of the power absorbed depends on the position of the propagating front, $x_F(t)$.

The movement of the thawing front is governed by the energy flux balance at $x = x_F$:

$$k_2\left(\frac{\partial T_2}{\partial x}\right)_{x=x_F} - k_1\left(\frac{\partial T_1}{\partial x}\right)_{x=x_F} = \rho\Delta H\frac{dx_F}{dt} \qquad (5)$$

where ΔH is the latent heat of fusion per unit mass of the sample.

The boundary conditions at the change of phase front, the surface, and the center line are:

$$T_1(x_F) = T_2(x_F) = T_F \qquad (6)$$

$$h(T_o - T_\infty) = -k_1\left(\frac{\partial T_1}{\partial x}\right)_{x=0} \qquad (7)$$

$$\left(\frac{\partial T_2}{\partial x}\right)_{x=R} = 0 \qquad (8)$$

h is the average heat transfer coefficient at the surface of the sample, T_o the surface temperature of the sample and T_∞ the temperature far from the sample.

The above equations can be expressed in more convenient form by introduction of the dimensionless variables $\tau \equiv \alpha_1 t/R^2$ and $X \equiv x/R$, the dimensionless parameters $A \equiv \alpha_2/\alpha_1$, $B \equiv k_2/k_1$, and $Bi \equiv hR/k_1$ (a Biot number), and a reduced latent heat, $S \equiv \rho\Delta H\alpha_1/k_1$, having units of temperature. Furthermore, they can be transformed according to the Modified Isotherm Migration Method (MIMM), developed by Talmon et al. (1981) from the Isotherm Migration Method (IMM) initially introduced by Dix and Cizek (1971) and Crank and Phahle (1973). MIMM is preferred to IMM because the former can handle radiation boundary conditions and multiple transition fronts (Talmon et al., 1981, 1983). The key step in IMM and MIMM is the transformation of variables so that position becomes the dependent variable, a function of temperature and time. With the aid of implicit function theory, equations (4) to (8) are transformed to dimensionless partial differential equations of x.

Based on these equations a finite difference numerical scheme is developed and solved:

$$X_j^{n-1} = X_j^n + \Delta\tau^n\left\{4\frac{X_{j-1}^n - 2X_j^n + X_{j-1}^n}{(X_{j-1}^n - X_{j-1}^n)^2} + \frac{f-1}{1-X_j^n} + \frac{R^2}{k_1}p_1(X_j^n,\tau^n)\frac{X_{j-1}^n - X_{j-1}^n}{2\Delta T_1}\right\}, \qquad (9)$$

for $j < F$,

$$X_j^{n+1} = X_j^n + A\Delta\tau^n\left\{4\frac{X_{j+1}^n - 2X_j^n + X_{j-1}^n}{\{X_{j+1}^n - X_{j-1}^n\}^2} + \frac{f-1}{1-X_j^n} + \frac{R^2}{k_2}p_2(X_j^n,\tau^n)\frac{X_{j-1}^n - X_{j-1}^n}{2\Delta T_2}\right\}, \quad (10)$$

for $j > F$, and

$$X_F^{n+1} = X_F^n + \frac{\Delta\tau^n}{S}\left\{\frac{\Delta T_1}{X_F^n - X_{F-1}^n} - B\frac{\Delta T_2}{X_{F-1}^n - X_F^n}\right\}, \quad (11)$$

for $j = F$, the moving thawing front.

The new surface temperature at each time step is calculated based on eq. (7) taking into account an absorbed microwave power term $p_i(0,\tau)R^2/k_1\Delta X$, added to the convection term. This term approaches zero for $\Delta X \rightarrow 0$ but must be included for consistency between the energy flux balance and the convection equation when the finite difference discretization is used. The temperature T_o^{n+1} is thus given as:

$$T_o^{n+1} = \frac{T_o^n + BiT_\infty X_o^{n+1} + \frac{p_1(0,\tau)^n R^2}{k_1}(X_o^{n+1})^2}{1 + BiX_o^{n+1}} \quad (12)$$

where X_o^{n+1} is the position of isotherm T_o^n at the n + 1st time step. A more detailed discussion on how MIMM handles the boundary and symmetry conditions is given by Talmon et al. (1981, 1983).

The "thawing time," t_{TH}, is defined as the time needed for the thawing front to reach the center. At a certain time during thawing, t_o, usually small compared to the thawing time, the temperature profile in region 2 becomes flat and for all practical purposes equals T_F. Thus no heat conduction takes place in region 2 for $t > t_o$. Nevertheless, region 2 continues to absorb microwave energy. This energy uptake causes the formation of "microregions" of thawed phase dispersed in the frozen phase. It appears in our calculations as a change in latent heat for thawing at the propagating front expressed as $\phi(X_F,t)\Delta H$, where ϕ is the local volume fraction of frozen sample at position x and time t. Then Eq. (5) governing the movement of the front is replaced by:

$$-k_1\left(\frac{\partial T_1}{\partial x}\right)_{x=x_F} = \rho\Delta H\phi(x_F,t)\frac{dx_F}{dt} \quad (13)$$

and Eq. (11) in the numerical scheme by:

$$X_F^{n+1} = X_F^n + \frac{\Delta\tau^n}{\phi(X_F^n,\tau^n)S}\left(\frac{\Delta T_1}{X_F^n - X_{F-1}^n}\right) \quad (14)$$

For $t > t_o$, the penetration depth R_i, in region 2 is a function of position and time because it depends on ϕ. $R_i(x,t)$ is estimated as a linear function of R_{i_1} and R_{i_2} as:

$$R_i(x,t) = [1 - \phi(x,t)]R_{i_1} + \phi(x,t)R_{i_2} \quad (15)$$

Eq. (15) agrees very closely with the penetration depth calculated by Fricke's model (1955) for the prediction of the dielectric properties of a dispersed phase based on the dielectric properties of each component (frozen and thawed) and assuming uniform dispersion of spherical or cylindrical particles. Eq. (15) holds also for region 1 ($\phi = 0$ and $R_i(x,\tau) = R_{i_1}$) and region 2 for $\tau < \tau_o$ (when $\phi = 1$ and $R_i(x,\tau) = R_{i_2}$). An energy balance in region 2 (for $\tau > \tau_o$) gives:

$$\frac{\partial\phi}{\partial\tau} = -\frac{p_2(x,\tau)R^2}{k_1}\frac{1}{S} \quad (16)$$

Eq. (16) allows the numerical calculation of $\phi(X,\tau)$ at each time step.

The absorbed microwave power functions in the two regions are given by the following expressions:

$$p_1(X,\tau) = \frac{2I_o}{R_{i_1}}\left[\exp\left(\frac{-2RX}{R_{i_1}}\right) + \exp\left(\frac{-2R(2X_F - X)}{R_{i_1}}\right)\exp\left(-4R\int_{X_F}^{1}\frac{dx'}{R_i(x',\tau)}\right)\right] \quad (17)$$

$$p_2(X,\tau) = \frac{2I_o}{R_i(X,\tau)}\exp\left(\frac{-2RX_F}{R_{i_1}}\right)\left[\exp\left(-2R\int_{X_F}^{X}\frac{dx'}{R_i(x',\tau)}\right) + \exp\left(-2R\int_{X_F}^{1}\frac{dx'}{R_i(x',\tau)} - 2R\int_{X}^{1}\frac{dx'}{R_i(x',\tau)}\right)\right] \quad (18)$$

These expressions are more complex than Eq. 1 because microwave beams are transmitted through two regions (thawed and frozen) with varying penetration depths and the power absorbed at each point is calculated as the sum of two diametrically opposed beams. For more details on the derivation of Eq. (13) to (18) see the Appendix. The microwave intensity at the surface, I_o, can be calculated at each time step from the equation

$$P = \int_{V_1} p_1(X,\tau)dV_1 + \int_{V_2} p_2(X,\tau)dV_2 \quad (19)$$

where V_1 and V_2 are the volumes of the thawed region 1 and the frozen or partially frozen region, respectively.

The MIMM scheme requires the temperature profile at the time when surface temperature reaches the thawing temperature, T_F. This profile can be either calculated numerically or approximated using analytical solutions.

MATERIALS & METHODS

SAMPLES were prepared from commercial bovine semitendinosus (ST) muscles (6.6 to 11.0 kg) obtained from USDA choice carcasses. The muscles were frozen to −24° C and cut by an electric meat saw into rectangular blocks. Then they were cut by a special sample cutter in a hydraulic press into cylindrical samples 6.4 cm diameter and 20 cm length (Godsalve et al., 1977). Holes were drilled axially in the cylinder for the insertion of a teflon string to suspend the cylinder in the oven cavity and for 6 thermocouples (1 at surface, 1 at center, 2 at R/3 and 2 at 2R/3 position). Throughout the preparation procedure the samples were frozen. After preparation they were wrapped with aluminum foil and saran film and stored at −24° C, for thawing experiments.

A specially constructed hybrid (convective/microwave heating) controlled environment oven was used for the thawing experiments (Hung, 1980). With this oven the microwave power, the oven temperature and air flow rate could be controlled. The transmitted microwave power can be continuously set in the range 0–2.5 kW at 2450 MHz. Both transmitted and reflected power were controlled and measured. The oven cavity was large resulting in a relatively uniform microwave field at the center of the cavity (Hung, 1980). The frozen cylinders

with both ends covered with aluminum foil and insulating teflon covers were suspended in the center of the cavity. The suspension system was connected to a balance to monitor water loss. In addition to 6 gauge copper-constantan thermocouples connected to a Honeywell recording potentiometer through the especially constructed door of the oven, a Luxtron fluorometric temperature probe inserted through the oven's top measured the sample's surface temperature. At the low microwave powers used in this study the microwave field-thermocouple interactions were negligible.

We used the calorimetric technique of Nykvist and Decareau (1976) to estimate the energy absorbed by a sample. In this method, the power absorbed by liquids at the same volume and geometry (600 mL nonabsorbent Teflon beakers) at a given transmitted microwave power is measured. Water, water-methanol mixtures, isopropanol, 1-butanol, acetone and acetic acid with dielectric properties giving a range of penetration depths from 1.2 to 12 cm for 2450 MHz were used (dielectric properties from von Hippel, 1954; Bengtsson and Risman, 1971a; Mudgett et al., 1974).

The average heat transfer coefficient, h, used in the calculations reported here for the measured air flow in the oven and for our sample geometry, has been calculated in previous work by Wei et al. (1984) and found to be 20 W/m^2° C.

RESULTS & DISCUSSION

THE EXPERIMENTALLY determined absorbed microwave power was practically the same at a given nominal microwave power level. In a qualitative sense the absorbed power per unit volume as a function of penetration for samples of smaller and larger penetration depths can be found in Fig. 2. The total absorbed power in each case is represented by the area below each curve. In our experiments we used samples of the same geometry and volume, and different dielectric properties. The total absorbed power was not different between 1.2 cm and 12 cm penetration depths. Thus foods, frozen and thawed, whose penetration depth fall between 1.2–12 cm are assumed to absorb constant total power during thawing.

In using the temperature data for the experiments with lean beef, the physical properties of beef at different temperatures are available in literature (Dickerson, 1968; Heldman, 1975; Mudgett et al., 1979; To et al., 1974). In lean beef 76% of the water freezes in the temperature range -5° C to -1° C and another 13% freezes from -24° C to -5° C (Heldman, 1975). We have somewhat arbitrarily chosen -1° C as the melting point in the single melting point model used in this work. The physical properties of frozen meat presented in Table 1 were determined by averaging their literature values over the range -5° C to -1° C where most of the freezing takes place. Values for thawed meat were obtained by averaging meat data over the range 0° C to 30° C. We found that small variation (say 10%) of the physical parameters about their average values has a correspondingly small effect on predicted temperature profiles and thawing times. It is worth mentioning that disagreement among experimental values reported in the literature is often greater than the observed temperature variations of the corresponding quantities. We test our model by comparing experimental results with calculations based on the parameter values given in Table 1.

Thawing time and temperature profiles were predicted at different microwave powers. Results of such computations for cylindrical lean beef samples thawed in a "convection only" environment (Fig. 3(a)) and under a low absorbed microwave power (Fig. 3(b)) are presented for comparison. Initial and ambient temperatures and Biot number are identical for both cases. Temperature profiles at different times during the thawing of the cylindrical samples are shown. The initial temperature profiles were calculated from Heissler charts (Holman, 1981) for case (a) and from a modified version of the analytical solution for microwave heating of semi-infinite slabs derived by Hung (1980) for case (b) and were fed as input data. In both cases the time for the surface to reach the thawing point and the time for the frozen regions temperature profile to become flat, at $T = T_F$, were a small fraction of the total thawing time, about 7% and 10% respectively. The difference in the temperature profiles during thawing with and without microwaves is clear. Notice that in case (b) after the surface exceeded the environment temperature, the profile near the surface become flatter as heat was lost at the surface to the environment. The thawing time, 72 min., was 4.5 times smaller than without microwave power (316 min. for R = 3.2 cm). In Fig. 4 the position of the thawing front and the frozen volume fractions, ϕ, at the front are plotted as a function of time. $\phi(X_F)$ is the value of frozen volume fraction at position X_F, one Δt time-step before the front crosses that position.

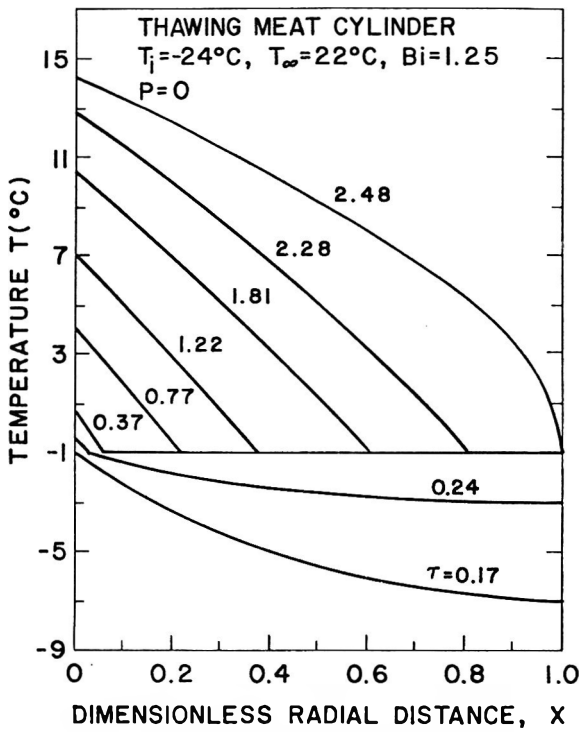
Table 1—Average thermal and dielectric properties of lean beef[a] $T_F = 1$°C, $\Delta H = 245$kJ/kg

		Nonfrozen phase	Frozen phase
Thermal conductivity		0.50 W/m°C	1.30 W/m°C
Density		1057 kg/m^3	961 kg/m^3
Heat capacity		3.52 kJ/kg°C	2.09 kJ/kg°C
Thermal Diffusivity		1.34 × 10^{-7}m^2/sec	6.47 × 10^{-7}m^2/sec
2450 MHz	K′	50	6.0
	K″	17	1.5
	R_i	0.0164 m	0.0641 m
915 MHz	K′	55	6.8
	K″	21	1.5
	R_i	0.0375 m	0.1825 m
300 MHz	K′	65	8.0
	K″	60	2.3
	R_i	0.0465 m	0.3954 m

[a] Table composed from data by Dickerson (1968), Heldman (1975), Bengtsson and Risman (1971b), To et al. (1974) and Mudgett et al. (1979).

Thawing experiments, at the conditions used in the computations of Fig. 3, with meat cylinders of radius R = 3.2 cm were conducted. For P = 0 calculated and experimental temperature profiles agreed within 2° C at the positions measured (Fig. 5). It can be seen that although a flat profile at -1° C was not attained as meat does not actually have a single freezing point, an almost flat profile at a temperature lower but close to -1° C was reached. The experimental thawing time was 323 min, 2.2% higher than 316 min, the value predicted by the model. Experimental results at other Biot numbers, for "convection only" conditions, also agreed closely to model computations.

Microwave thawing experiment results, at the conditions of Fig. 3(b), are presented in Fig. 6. There was some evidence (observed dehydration) of non-uniform heating at the area near the top end of the cylinder across the entrance of the microwave field. Nevertheless, the absorbed power halfway along the cylinder (where temperatures were measured) was fairly uniform. The average temperature histories at the points measured, followed the pattern of the predicted ones. Here too, an almost flat profile at a temperature close to -1° C was reached, indicating that the propagating thawing front is a realistic representation of the actual phenomenon. The average experimental thawing time was 56 min, 22% less than predicted. In addition to the above mentioned nonuniformity of the field this difference could be caused by some microwave focusing at the center of the sample. Another source of discrepancy is the microwave field-thermocouples interactions, which, although minimal at low microwave powers, could result in somewhat higher temperature readings. At higher powers a localized overheating around the thermocouples due to microwave-thermocouple interactions was evident and the use of the thermocouples at these powers was impossible. Another quantity measured in these experiments was drip loss which was 5.5% of the total sample weight compared to about 10% drip loss without microwave power.



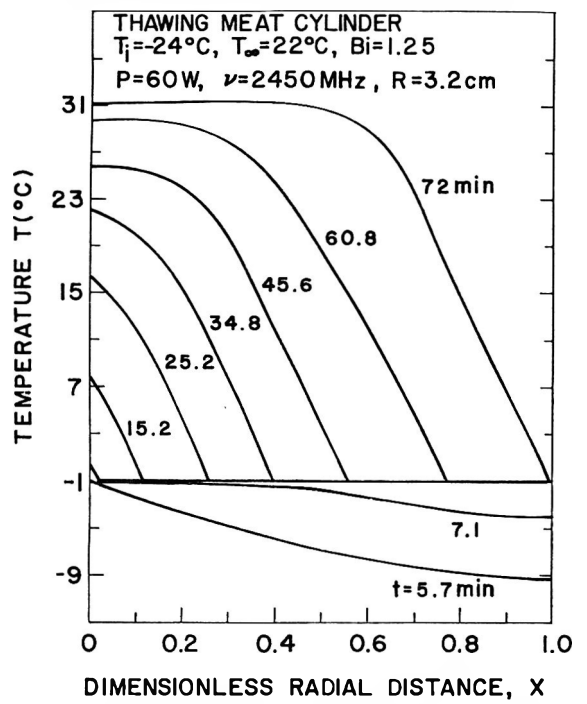


Fig. 3—(a) Temperature profiles in a "convection only" thawing meat cylinder at different reduced times. ($X \equiv x/R$, $\tau \equiv \alpha_r t/R^2$, $Bi \equiv hR/k_r$.) τ multiplied by a factor of 127.4 gives the actual time in min for R = 3.2cm. In this case t_{TH} = 316 min. (b) Temperature profiles in a high frequency-low power microwave thawing meat cylinder at different times (in min).

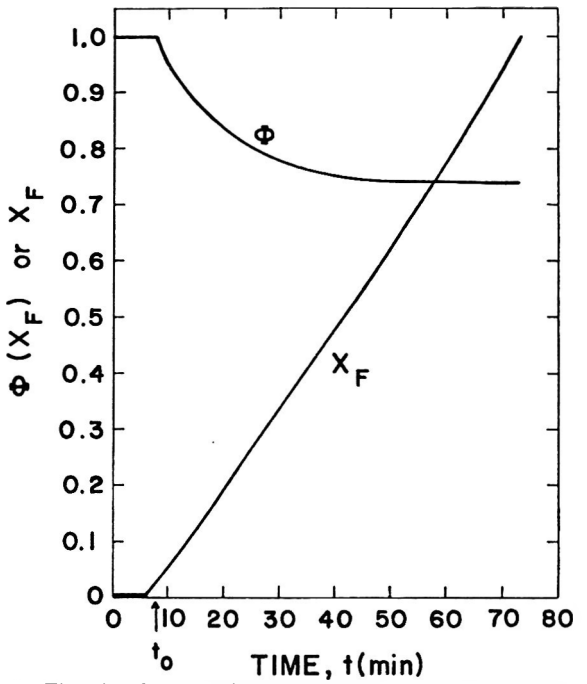


Fig. 4—Thawing front position, X_F, and frozen volume fraction, $\phi(X_F)$, as a function of time during microwave thawing of a meat cylinder. T_i = −24°C, T_∞ = 22°C, Bi = 1.25, P = 60W, ν = 2450MHz, and R = 3.2cm. L = 20cm (Same as Fig. 3b).

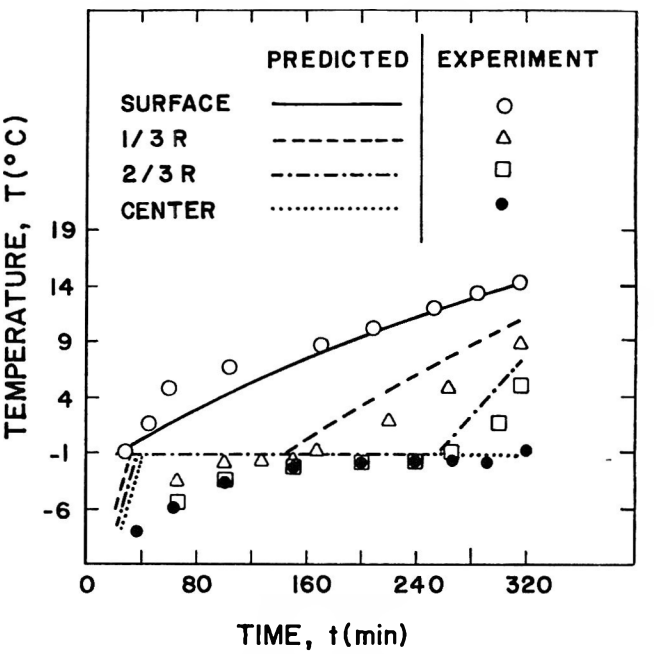


Fig. 5—Predicted and experimental temperature histories at three radial distances during "convection only" thawing of a meat cylinder of radius R = 3.2 cm, Ti = −24°C, T_∞ = 22°C, Bi = 1.25, P = 0.

Thawing time and temperature profile predictions were computed for meat cylinders of the same size and for the same initial temperature and environmental conditions with absorbed microwave power of 0-500W at three different frequencies, 2450 MHz, 915 MHz and 300 MHz. In Fig. 7 the effect of the absorbed microwave power and the microwave frequency on thawing time of the meat cylinder is shown. Thawing times decreased by applying even very low power microwave heating. Above a power of about 200 watts further increase in power produced only a small decrease in thawing time. When the same absorbed microwave power at lower frequency was used thawing time was predicted to be shorter.

In microwave thawing the final temperature profile is important since the temperature in any region should not exceed a certain level in order to maintain product quality. The final temperature profiles for the three frequencies at the same absorbed microwave power (P = 100W) are shown in Fig. 8. Temperatures in the outer regions of the samples were significantly lower at the low frequencies. At the high frequency (2450 MHz), at absorbed powers higher than 100 W the predicted final surface temperature exceeded 45° C. On the other hand the predictions show that the lower frequencies would allow us the use of higher power without excessive surface

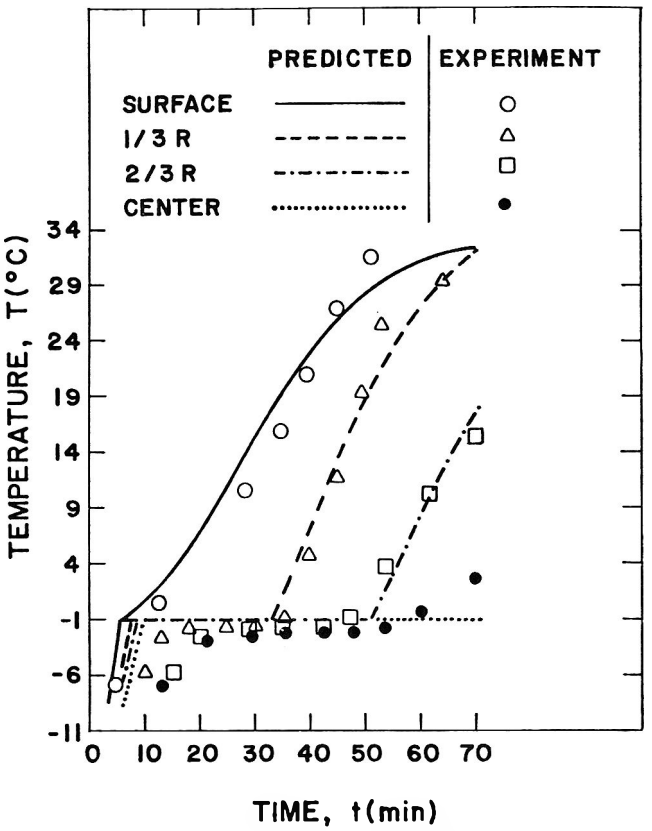


Fig. 6—Predicted and experiment temperature histories at three radial distances during microwave thawing of a meat cylinder. T_i = −24°C, T_∞ = 22°C, Bi = 1.25, P = 60W at ν = 2450 MHz, and R = 3.2cm. L = 20cm.

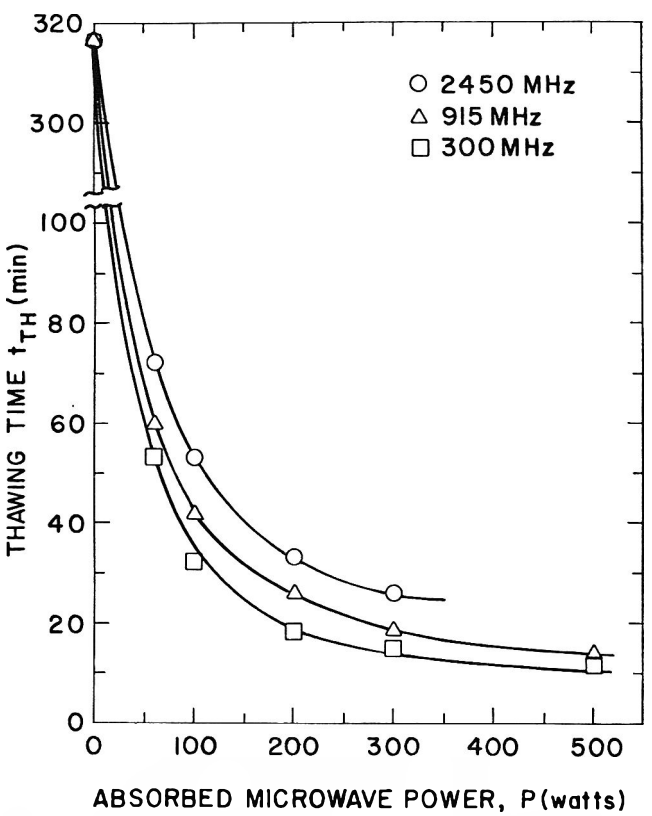


Fig. 7—Predicted thawing times of meat cylinders versus absorbed microwave power for three frequencies. T_i = −24°C, T_∞ = 22°C, Bi = 1.25, R = 3.2 cm, L = 20cm. (Points are thawing time predictions of the model and continuous lines are fitted lines).

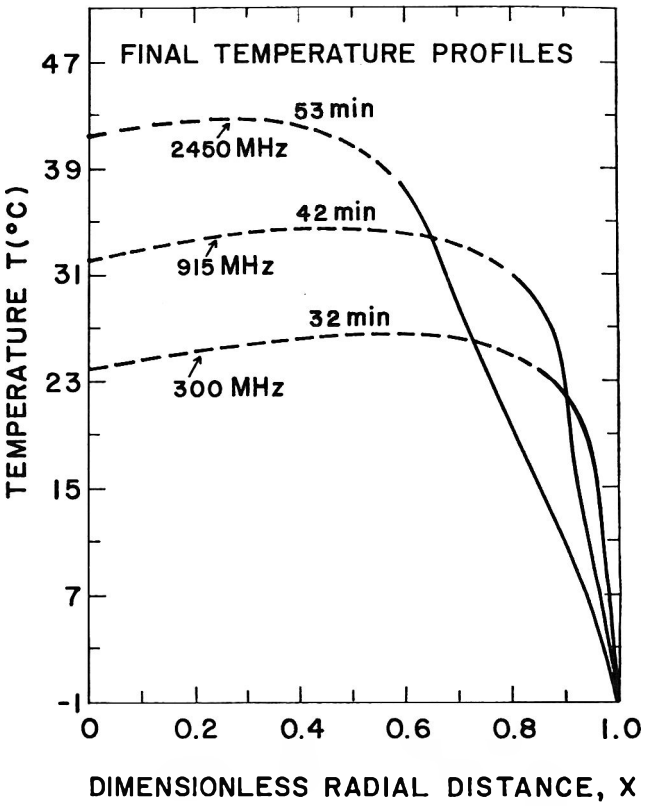


Fig. 8—Final temperature profiles at completion of thawing of meat cylinder at P = 100W for the three frequencies. T_i = −24°C, T_∞ = 22°C, Bi = 1.25, R = 3.2cm, L = 20cm.

heating. As seen in Fig. 8 the model predicts a nearly flat temperature profile through part of the sample at the final stage of thawing. This is due to the inherent limitation of the Isotherm Migration Methods that restrict their use in situations with monotonous temperature profiles. Thus, the method will calculate the position of the isotherm that has slightly lower temperature than the surface temperature (T_o-ΔT_1). Between the surface and that position, the method gives no other information, and so a linear interpolation is used in that region. Actually, in the case of microwave heating at the final stages of thawing, after the surface temperature has exceeded the environment temperature, the temperature will show a maximum of a few degrees higher than T_o due to surface cooling. (Dashed lines in Fig. 8 are assumed temperature profiles, showing the existence of a maximum in the sample at the final stage of thawing.) This does not change much the general picture and the profiles will be close to flat. It can be seen that at the lower frequencies, due to higher penetration depths of the microwaves, the temperature was (almost) uniform through much of the sample.

The lower final temperature profiles at low microwave frequencies are beneficial not only in terms of product quality but in terms of energy requirements, as well. At high frequency the overheating of the thawed region resulted in an excessive microwave energy expenditure. At the low frequencies the microwave energy was distributed more effectively and is consumed more for actual thawing than for heating the already thawed outer region. The differences in energy requirements are shown in Fig. 9.

Complete microwave thawing of larger size samples (diameters over 10 cm) at 2450 MHz resulted in excessive overheating of the outer region even when low powers were used. The use of the lowest possible frequency e.g. 300 MHz, were this frequency to be permitted for industrial use, allows complete thawing of larger samples (up to 20 cm diameter for low powers) with reasonable final outer region temperatures. If

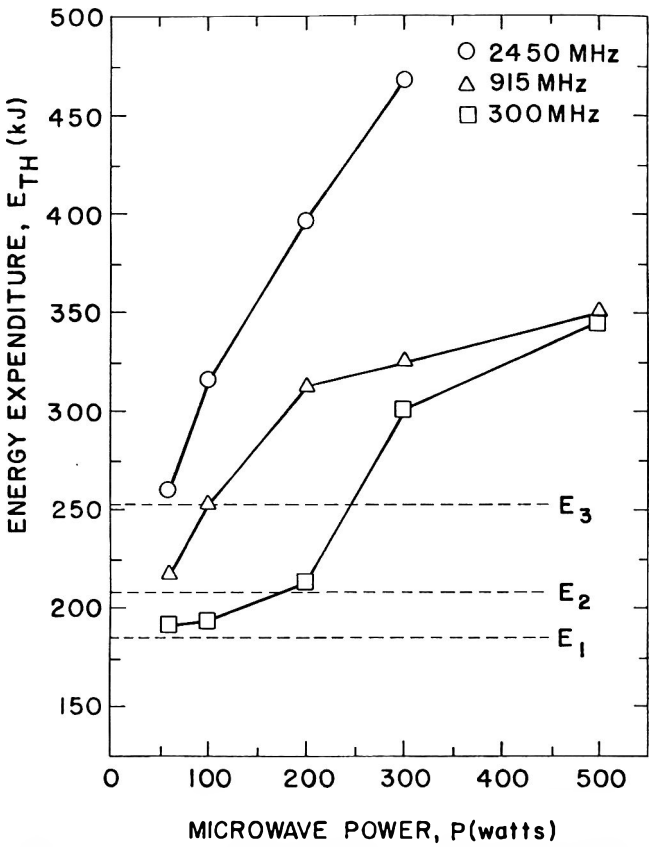


Fig. 9—Microwave energy expenditure to thaw a meat cylinder at different powers at the three frequencies. T_i = −24°C, T_∞ = 22 °C, Bi = 1.25, R = 3.2 cm, L = 20cm. E_1, E_2, E_3 are the theoretical energies required for thawing from −24°C to a uniform temperature of −1°C, 10°C and 20°C, respectively.

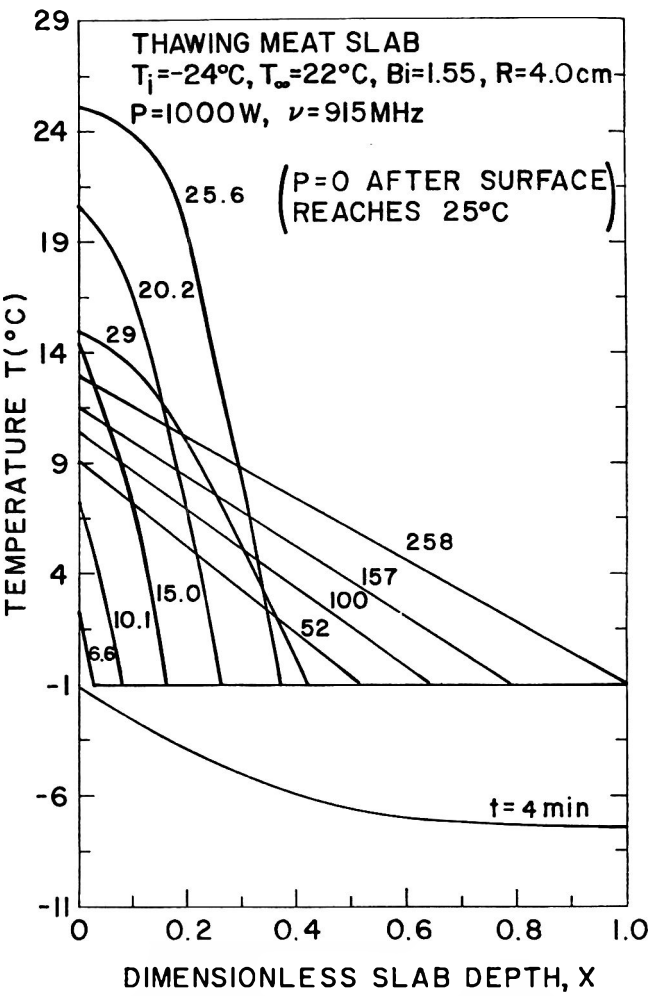


Fig. 10—Temperature profiles in a thawing meat slab. Microwave power of ν = 915 MHz and P = 1000W is applied until surface reaches 25°C. Then "convection only" thawing. (Dimensions: 40 cm × 30 cm × 8 cm).

lower final surface temperatures are required or the products are of larger volumes the following procedure can be used: microwave thawing of the product at the lowest available frequency at a certain absorbed power (e.g. 150–300 W/1000 cm^3) until the surface reaches the maximum allowable temperature; interruption of the microwave heating and completion of the thawing in "convection only" conditions. A similar technique would be to use pulsed microwave heating, to allow for equilibration and surface cooling through convection. Such a procedure nevertheless would be difficult to control in a real operation.

The calculated temperature profiles during the thawing of a meat slab of dimensions 40 cm × 30 cm × 8 cm are shown in Fig. 10. This example could represent an actual situation of a meat product packed in rectangular boxes. The meat slab is heated by microwaves of 915 MHz frequency and 1000 W "absorbed" power. The microwaves are turned off when the surface reaches 25° C and the thawing is completed under "convection only" conditions. Microwave heating is applied for 26 min and the total thawing time is 258 min. Under the same convection conditions with no microwave heating the required thawing time, as predicted by our model, would be 594 min, about 2.3 times longer. It is obvious that an initial stage of microwave heating improves substantially the thawing process. The effect would be even more pronounced if the product was packaged, e.g. in carton boxes that would act as insulation in the case of "convection only" thawing. The initial microwave heating to a certain temperature overcomes this problem.

The model presented is applicable to products with a narrow thawing temperature range. For such products the representation of the phenomenon of thawing with the propagation of a thawing front is very realistic, as the experimental results indicated. The model, although based on simplifying assumptions, can be a useful tool for a preliminary estimation of the conditions and the time needed for the successful microwave or microwave-convection combination thawing of a given product. To our knowledge no other such method of prediction of thawing time and temperatures for microwave thawing exists in the literature.

APPENDIX

If ϕ (x,t) is the local volume fraction of frozen sample at position x in region 2 and time $t>t_o$, the energy balance on a shell of thickness Δx and centered at position $x(x \geq x_F)$ is:

$$\frac{d}{dt}\int_{\Delta x} [\rho_l H_l(1-\phi) + \rho_s H_s \phi] A(x)dx = \int_{\Delta x} p_2(x,t)A(x)dx \quad (A1)$$

where the area A(x) = constant, $2\pi xL$ and $4\pi x^2$ for slab, cylinder and sphere respectively. Since Δx is fixed and x is arbitrary the balance yields:

$$-\rho\Delta H\frac{\partial\phi}{\partial t} = p_2(x,t) \quad (A2)$$

where $\rho\Delta H = \rho_l H_l - \rho_s H_s$ and $\phi(x,t) = 1$ when $t \leq t_o$. Eq. (A2) takes the form of Eq. (16) when the dimensionless variables are introduced. Based on Eq. (16) a numerical scheme

to calculate the value of ϕ from its value at the previous time step will be:

$$\phi(X,\tau^{n+1}) = \phi(X,\tau^n) - \frac{p_2(X,\tau^n)}{k_1}\frac{1}{S} \quad (A3)$$

with $\phi\ (X,\tau_o) = 1$.

The energy balance at the boundary $x = x_F$ (on a shell $\Delta x = x_2 - x_1$ including x_F) gives:

$$\frac{d}{dt}\left\{\int_{x_1}^{x_2}\rho_l H_l A(x)dx + \int_{x_F}^{x_2}[\rho_l H_l(1-\phi) + \rho_s H_s \phi]A(x)dx\right\} = -k_1\left(A(x)\frac{\partial T_1}{\partial x}\right)_{x_F} + \int_{x_1}^{x_F} p_1(x,t)A(x)dx + \int_{x_F}^{x_2} p_2(x,t)A(x)dx \quad (A4)$$

or

$$-\rho\Delta H\phi(x_F,t)\frac{dx_F}{dt} - \int_{x_F}^{x_2}(\rho\Delta H)\frac{\partial\phi}{\partial t}\frac{A(x)}{A(x_F)}dx = -k_1\left(\frac{\partial T_1}{\partial x}\right)_{x_F} + \int_{x_1}^{x_F} p_1(x,t)\frac{A(x)}{A(x_F)}dx + \int_{x_F}^{x_2} p_2(x,t)\frac{A(X)}{A(x_F)}dx \quad (A5)$$

x_1, x_2 are arbitrary, and so in the limit $x_1 = x_F = x_2$ Eq. (A5) leads to Eq. (13).

The prediction formula for dielectric properties of a dispersion as given by Mudget *et al.* (1974), based on Fricke's (1955) complex conductivity model, is:

$$K^* = \frac{K_c^*[K_s^*(1+\omega V_s) + K_c^*(1-V_s)\omega]}{[K_c^*(\omega + V_s) + K_s^*(1-V_s)]} \quad (A6)$$

where K_c^* the complex permittivity of the continuous phase, K_s^* of the suspended phase, V_s the volume fraction of the suspended phase $\omega = 2$ for spherical dispersion and $\omega = 1$ for cylindrical. In our case $K_c^* = K_2^*$, $K_s^* = K_1^*$ and $V_s = 1 - \phi$. If $R_i(x,t)$ is calculated using the dielectric properties predicted from Eq. (A6) the results differ 0-10% from $R_i(x,t)$ estimated from Eq.(15) for $\omega = 2$ and 0-5% for $\omega = 1$. Thus Eq. (21) gives a fairly good approximation of $R_i(x,t)$.

For $t > t_o$, and if $I(x,t)$ the intensity of the microwave beam at position x, then at position $x + dx$:

$$I(x+dx,t) = I(x,t)e^{-2dx/R_i(x,t)} = I(x,t)\left[1 - \frac{-2dx}{R_i(x,t)} + 0(dx^2)\right]$$

or

$$\frac{dI(x,t)}{dx} = \frac{-2I(x,t)}{R_i(x,t)} \quad (A7)$$

For region 2 we integrate Eq. (A7) from x_F to obtain:

$$I(x,t) = I(x_F,t)\exp\left(-2\int_{x_F}^{x}\frac{dx'}{R_i(x',t)}\right) \quad (A8)$$

where

$$I(x_F,t) = I_o(t)\exp\left(\frac{-2x_F}{R_{i_1}}\right) \quad (A9)$$

We have $p_2(x,t) = p_2'(x,t) + p_2''(x,t)$, where $p_2''(x,t)$ is the absorbed power from the diametrically opposed microwave beam and:

$$p_2'(x,t) = -\frac{dI(x,t)}{dx},\ p_2''(x,t) = \frac{dI(2R-x,t)}{dx} \quad (A10)$$

Thus

$$p_2'(x,t) = \frac{2I(x_F,t)}{R_i(x,t)}\exp\left(-2\int_{x_F}^{x}\frac{dx'}{R_i(x',t)}\right) \quad (A11)$$

and

$$p_2''(x,t) = \frac{2I(x_F,t)}{R_i(x,t)}\exp\left(-2\int_{x_F}^{2R-x}\frac{dx'}{R_i(x',t)}\right). \quad (A12)$$

These equations combine to yield Eq. (18).

For $x < x_F$, $p_1(x,t)$ can be analogously derived.

Eq. (18) and Eq. (17) for $t < t_o$ become:

$$p_2(x,t) = \frac{2I_o}{R_{i_2}}\exp\left(\frac{-2x_F}{R_{i_1}}\right)\left\{\exp\left[\frac{-2(x-x_F)}{R_{i_2}}\right] + \exp\left[\frac{-2(2R-x-x_F)}{R_{i_2}}\right]\right\}$$

and

$$p_1(x,t) = \frac{2I_o}{R_{i_1}}\left\{\exp\left(\frac{-2x}{R_{i_1}}\right) + \exp\left[\frac{2(x-2x_F)}{R_{i_1}} - \frac{4(R-x_F)}{R_{i_2}}\right]\right\}$$

NOTATION

A	=	α_2/α_1
B	=	k_2/k_1
Bi	=	Biot number, hR/k_1
c	=	heat capacity (kJ/kg°C)
E_{TH}	=	microwave energy expenditure for thawing (kJ)
f	=	form factor, 1 for slab, 2 for cylinder, 3 for sphere
H	=	enthalpy (J/kg)
h	=	heat transfer coefficient (W/m^2°C)
K′	=	dielectric constant
K″	=	dielectric loss factor
K*	=	complex permitivitty
k	=	thermal conductivity (W/m°C)
L	=	length of cylinder (m)
P	=	total microwave power absorbed by the sample (W)
p	=	power absorbed by a unit volume at the sample (W/m^3)
R	=	half width of slab, radius of cylinder or sphere (m)
R_i	=	penetration depth of the microwaves (m)
S	=	reduced latent heat of change of phase (°C)
T	=	temperature (°C)
T_F	=	thawing temperature (°C)
T_i	=	initial uniform temperature at the sample (°C)
t	=	time (min)
t_o	=	time for region 2 to reach uniform temperature T_F (min)
t_{TH}	=	time for completion of thawing, thawing time (min)
V	=	volume (m^3)
X	=	dimensionless distance, x/R
x	=	distance from surface of the sample (depth) (m)

Greek Letters

α = thermal diffusivity (m^2/s)
ΔH = latent heat of change of phase (J/kg)
ΔT = temp. difference between two adjacent isotherms, $T_j - T_{j+1}$
Δt = time step
λ = microwave radiation wavelength (m)
ν = microwave frequency (MHz)
ρ = density (kg/m^3)
τ = dimensionless time, $\alpha_1 t/R^2$
ϕ = local volume fraction of frozen sample

Subscripts

j = j^{th} isotherm or region j (1:thawed, 2:frozen or partially frozen)
F = moving front or F-th isotherm
l = liquid
o = at the surface
s = solid
∞ = ambient

Superscripts

n = n^{th} time step

REFERENCES

Bengtsson, N. 1963. Electronic defrosting of meat and fish at 35 and 2450 mcs - a laboratory comparison. Food Technol. 17(10): 97.
Bengtsson, N.E. and Risman P.O. 1971a. Dielectric properties of foods at 3GHz as determined by a cavity perturbation technique I. Measuring technique. J. Micr. Power 6(2): 101.
Bengtsson, N.E. and Risman P.O. 1971b. Dielectric properties of foods at 3GHz as determined by a cavity perturbation technique. II. Measurements on food materials, J. Micr. Power 6(2): 107.
Bezanson, A., Learson, R., and Teich, W. 1973. Defrosting shrimps with microwaves. Microwave Energy Appl. Newsletter 6(4): 3.
Bialod, D., Jolion, M., and LeGoff, R. 1978. Microwave thawing of food products using associated surface cooling. J. Microwave Power 13(3): 269.
Checcucci, A., Benelli, G., Duminuco, M., Gaetani, M.L., Paoletti, P., Vannini, S., and Morfini, M. 1983. Reliability of microwave heating for hemoderivative thawing. J. Microwave Power 18(2): 163.
Cleland, A.C. and Earle, R.L. 1984. Assessment of freezing prediction methods. J. Food Sci. 49: 1034.
Crank, T. and Phahle, R.D. 1973. Melting ice by isotherm migration method. Bull. J. Inst. Maths. Applics. 9: 12.
Decareau, R. 1968. High frequency thawing of food. Microwave Energy Appl. Newsletter 1(2): 3.
Dickerson, R.W. 1968. Thermal properties of foods. In "The Freezing Preservation of Foods." 4th ed., Vol. 2, (Ed.) Tressler, D.K., Arsdel, W.B., and Copley, M.J. Avi Publishing Co., Inc., Westport, CT.
Dix, R.C. and Cizek, J. 1971. The isotherm migration method for transient heat conduction analysis. Proc. Fourth Int'l Heat Transfer Conference, Paris, Vol. 1, ASME, New York.
Edgar, R. 1981. Microwave tempering in the food processing industry. Digest Sixteenth Annual Symposium on Microwave Power, Toronto, p. 99.
Fricke, H. 1955. The complex conductivity of a suspension of stratified particles of spherical or cylindrical form. J. Phy. Chem. 56: 168.
Godsalve, E.W., Davis, E.A., Gordon, J., and Davis, H.T. 1977. Water loss rates and temperature profiles of dry cooked bovine muscle. J. Food Sci. fB42: 1038.
Heldman, D.R. 1975. "Food Process Engineering," 2nd ed. AVI Publishing Co., Inc., Westport, CT.
Holman, J.P. 1981. "Heat Transfer," 5th ed. McGraw-Hill, New York.
Hung, C.C. 1980. Water migration and structural transformation of oven cooked meat. Ph.D. thesis, Univ. of Minnesota.
Mudgett, R.E. 1982. Electrical properties of foods in microwave processing. Food Technol. 36(2): 109.
Mudgett, R.E., Wang, D.I.C., and Goldblith, S.A. 1974. Prediction of dielectric properties in oil-water and alcohol-water mixtures at 3,000 MHz, 25°C based on pure component properties. J. Food Sci. 39: 632.
Mudgett, R.E., Mudgett, D.R., Goldblith, S.A., Wang, D.J.C., Westphal, W.B. 1979. Dielectric properties of frozen meats. J. Microwave Power 14(3): 209.
Nykvist, W.E. and Decareau, R.V. 1976. Microwave meat roasting. J. Microwave Power 11(1): 3.
Ramaswamy, H.S. and Tung, M.A. 1984. A review on predicting freezing times of foods. J. Food Proc. Eng. 7: 169.
Swift, J. and Tuomy, J.M. 1979. Evaluation of microwave tempering of meat for use in central food preparation facilities. Microwave Energy Appl. Newsletter 9(1): 3.
Talmon, Y. and Davis, H.T. 1981. Analysis of propagation of freezing and thawing fronts. J. Food Sci. 46: 1478.
Talmon, Y., Davis, H.T., and Scriven, L.E. 1981. Progressive freezing of composites analyzed by isotherm migration methods. AIChE J. 27: 928.
Talmon, Y., Davis, H.T. and Scriven, L.E. 1983. Moving boundary problems in simple shapes solved by isotherm migration. AIChE J. 29: 795.
Talmon, Y., Davis, E.A., Gordon, J., and Davis, H.T. 1982. Temperature profiles in heated frozen foods. In "Food Process Engineering," (Ed.) H.G. Schwartzberg, D. Lund, and J.L. Bomben, AIChE Symposium Series #218, Vol 78: 76.
To, E.C., Mudgett, R.E., Wong, D.I.C., Goldblith, S.A., and Decareau, R.V. 1974. Dielectric properties of food materials. J. Microwave Power 9(4): 303.
von Hippel, A.R. 1954. "Dielectrics and Waves." John Wiley, New York.
Wei, C.K., Davis, H.T., Davis, E.A., and Gordon, J. 1985. Heat and mass transfer in water-laden sandstone: Microwave heating. AIChE J. 31: 842.

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Modification of Physical Properties of Gelatin by Use of An Immobilized Protease in Combination with Molecular Sieves

TOSHIKAZU NISHIO and RIKIMARU HAYASHI

ABSTRACT

The viscosity of gel-strength of industrial gelatin were modified by the use of immobilized subtilisin which was covalently bound by the glutaraldehyde method on glass bead supports with mean pore-sizes of 170, 350, and 500 Å. Depending on the exclusion limit of these molecular sieves and the incubation time, the products of digestion of gelatin by immobilized protease fixed in the matrix had different properties. Thus, gelatins with low viscosity were prepared with minimal changes of gel-strength, and *vice versa*.

INTRODUCTION

GELATIN is principally derived from type I collagen, which is composed of α-, β-, and γ-chains. However, gelatins manufactured in industry contain smaller polypeptides and also polymerized chains larger than the collagen components, depending on the manufacturing process (Johns and Courts, 1977). These contaminants affect the physical properties of the product gelatin (Finch and Jobling, 1977). Therefore, industrial gelatins are prepared and blended for suitable gel-strength and/or viscosity as needed to meet the customer's requirements (Hinterwaldner, 1977). In order to avoid such tedious methods and to provide the required properties, the development of a new method is necessary.

Previously, we showed that a protease immobilized on a support consisting of a matrix of molecular sieve preferentially degraded substrate proteins which were forced into the matrix because the protease and substrate were entrapped inside the matrix (Nishio and Hayashi, 1984). Thus, if such immobilized proteases are used to digest a mixture composed of proteins of various molecular weights, it is possible to hydrolyze selectively a particular range of protein molecules by appropriate choice of the size of the matrix. Based on this idea, we attempted to utilize subtilisin BPN' (*Bacillus* proteinase strain N'; EC 3.4.21.14), immobilized on a series of controlled pore glasses with molecular sieve function, to provide the desired properties to an industrial gelatin.

MATERIALS & METHODS

Materials

Commercial grade alkali-type gelatin (Nitta Gelatine Co., Ltd., Osaka, Japan) was used throughout the experiment. Controlled pore glass (CPG-10) with mean pore diameters of 170, 350, and 500 Å (CPG-170, CPG-350, and CPG-500, respectively) were obtained from Electro Nucleonics Inc. (New Jersey). The following materials were purchased from the sources indicated. Crystalline subtilisin BPN' (Nagarse) was from Nagase Seikagaku Kogyo (Tokyo, Japan); glutaraldehyde (25% aqueous solution), prepared for electron microscopy, was from Nakarai Chemicals (Kyoto, Japan); Cbz-Gly-Leu-NH_2 was from the Protein Research Foundation (Osaka, Japan); Hammersten casein was from E. Merck (Darmstadt, Germany).

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Immobilization of enzyme

Subtilisin BPN' was immobilized on controlled-pore glass (CPG) by the method of Weetall (1976) as modified by us (Nishio and Hayashi, 1984). The actual amounts of bound enzyme were 4.6, 6.8, and 7.2 mg protein per g CPG-170, CPG-350, and CPG-500, respectively, on a dry basis. Hereafter, the amount of enzyme used is expressed as the whole weight including CPG, unless otherwise specified.

Determination of activity

Activity toward Cbz-Gly-Leu-NH_2 was determined as described by Morihara et al. (1970). Activity toward casein was determined according to the method of Kunitz (1947) with a modification (Nishio and Hayashi, 1984).

Activity toward gelatin was determined by the trinitrobenzene-sulfonic acid methods (Fields, 1972): free (0–50 μg) or immobilized (0–4 mg) protease was mixed with 10 mL 5% gelatin dissolved in 0.05M sodium phosphate, pH 7.5. The mixture was incubated at 40°C for 1 hr. After incubation, 1 mL 0.1M sodium borate (0.1 M sodium tetraborate in 0.1 M sodium hydroxide) was mixed with 5 μL of the reaction mixture. Twenty microliters 1.1M trinitrobenzene sulfonate (TNBS) solution was then added and the mixture incubated at 25°C for 2 min, after which it was mixed with 2 mL 0.1M sodium dihydrogen phosphate containing 1.5 mM sodium sulfate. The absorbance at 420 nm was then measured.

Preparation of proteolytic digests of the gelatin

Free (0.5 mg) or immobilized (1.31g) protease was mixed with 1000 mL 5% gelatin dissolved in 0.05M sodium phosphate, pH 7.5, and the mixture was incubated at 40°C with constant stirring. A part of the mixture (about 100 mL) was removed at appropriate time intervals to determine the extent of the digestion and the physical properties (see below). After digestion, free enzyme was inactivated by heating at 100°C for 5 min. The immobilized enzyme was removed by filtration and the filtrate was also heated.

The extent of digestion was estimated from the ninhydrin color value (Moore and Stein, 1954) of 25 μL of the digestion mixture, as previously described (Nishio and Hayashi, 1984).

Determination of physical properties

Gel-strength was determined with a rheometer (Model NRM-2010J-CW, Fudo Kogyo Co., Ltd., Tokyo, Japan) equipped with a flat-bottomed plunger (1.27 cm in diameter). Samples were prepared as follows: 40 mL digestion mixture was heated at 100°C for 5 min and then cooled at 10°C for 16 hr to achieve gelatinization. The plunger depressed the gel surface 4 mm at a speed of 2 cm per min. The measured force was used as an indication of gel strength.

Viscosity was determined with an Ubbelohde viscometer at 40°C. The gelatin used for the gel-strength measurement was remelted and

Table 1—Specific activities of free and immobilized subtilisin BPN' toward a synthetic substrate and proteins

	Specific activity[a] toward		
	Cbz-Gly-Leu-NH_2	Casein	Gelatin
Subtilisin BPN'			
Free	0.39	4.63	2.00
Immobilized on CPG-170	0.41	0.70	0.08
Immobilized on CPG-350	0.41	0.92	0.15
Immobilized on CPG-500	0.39	1.32	0.24

[a] Activities are expressed on the basis of amount of enzyme protein as follows: μmol/mg enz/min for Cbz-Gly-Leu-NH_2, A_{280}/mg enz/min for casein, and A_{570}/mg enz/min for gelatin.

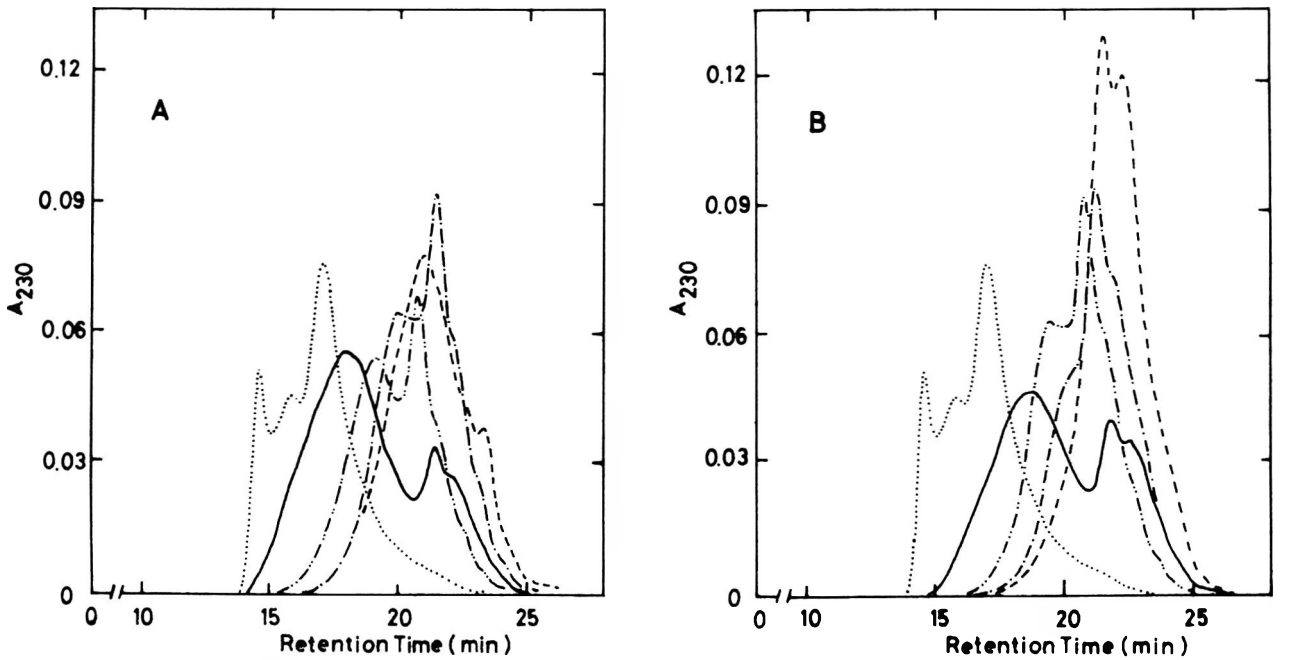

Fig. 1—High performance liquid chromatography of gelatin digests by free and immobilized subtilisin BPN'. A, 10% digestion; B, 20% digestion. Original gelatin (· · · ·), digests by the enzyme immobilized on CPG-170 (——), CPG-350 (– · –), CPG-500 (– · · –), and the digest by the free enzyme (- - - -). The α-and β-chains are eluted at 17 and 15.6 min, respectively.

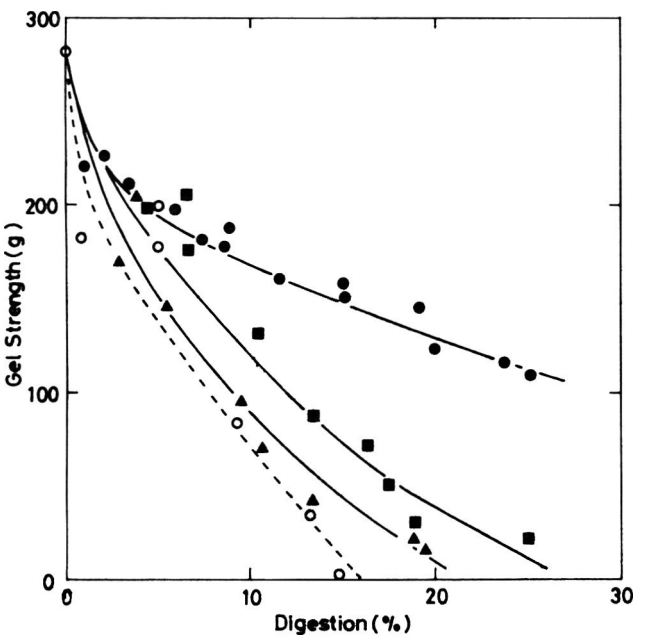

Fig. 2—Change in gel strength accompanying gelatin digestion by free and immobolized subtilisin BPN'. Digest by the free enzyme (○), and digests by the enzyme immobilized on CPG-170 (●), CPG-350 (▲), and CPG-500 (■).

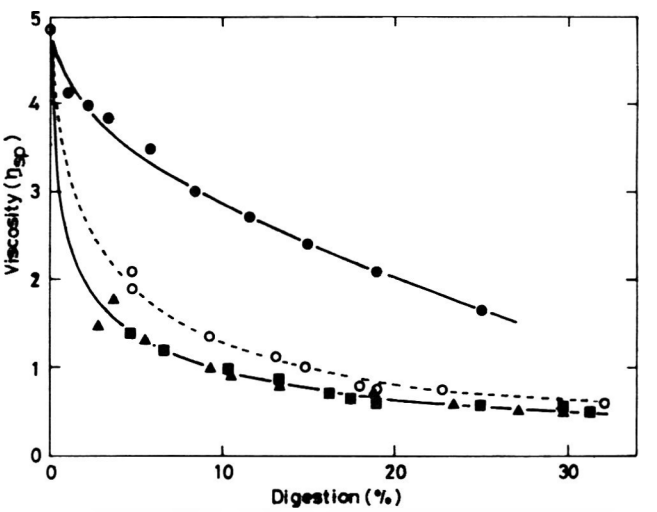

Fig. 3—Change in viscosity accompanying gelatin digestion by free and immobolized subtilisin BPN'. Digest by the free enzyme (○), and digests by the enzyme immobolized on CPG-170 (●), CPG-350 (▲), and CPG-500 (■).

used as the sample. The specific viscosity (η_{sp}) was calculated from the flow time of the solution (t) and the solvent (t_o) according to the equation:

$$\eta_{sp} = dt/d_o t_o - 1$$

where d and d_o denote the specific gravity of the solution and the solvent, respectively.

Other methods

The amounts of enzyme protein bound on CPG were estimated from the amino acid content after the immobilized enzymes had been hydrolyzed with 6N HCl at 110°C for 24 hr in vacuo (Nishio and Hayashi, 1984). The distribution of the molecular size was estimated by high performance liquid chromatography using molecular sieves (TSK G-4000PW gel, Toyo Soda Manufacturing Co., Ltd., Tokyo, Japan); 100 μL gelatin digests were applied to the column and developed with 0.05M sodium phosphate, pH 7.5, at 40°C with a flow rate of 1.0 mL/min (Nishio and Hayashi, 1985).

RESULTS

Properties of immobilized proteases

The specific activity of immobilized subtilisin BPN' toward Cbz-Gly-Leu-NH_2 was the same as that of the free enzyme when it was expressed on the basis of amount of enzyme bound to CPG, and was constant, being independent of the pore size of the CPG used as the enzyme support. Thus, the protease

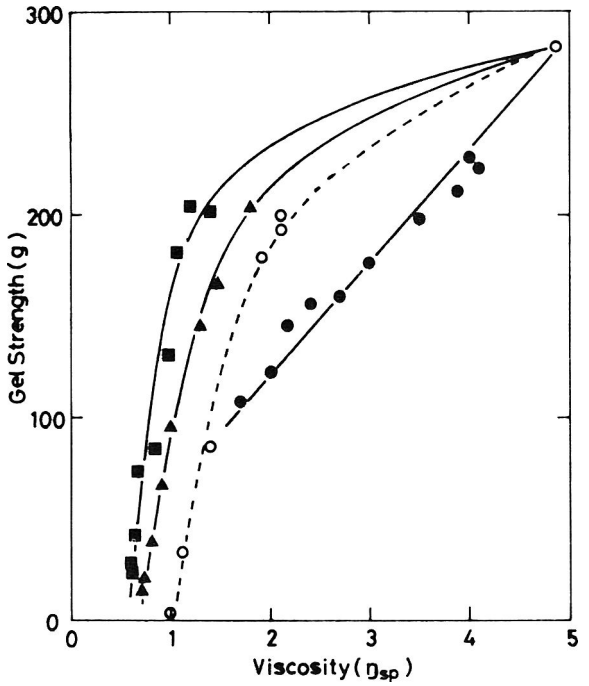

Fig. 4—Relationship between the gel-strength and viscosity of gelatin digests. Digest by the free enzyme (○), and digests by the enzyme immobolized on CPG-170 (●), CPG-350 (▲), and CPG-500 (■).

was covalently bound on the carriers without any inactivation throughout all procedures of the immobilization.

However, the specific activities of the immobilized protease toward casein and gelatin were much smaller than those of the free enzyme, and decreased as the pore size of the CPG decreased (Table 1). This indicates the restricted diffusion of the substrate proteins, reducing contact with the immobilized enzyme (Nishio and Hayashi, 1984).

Distribution of the molecular size of the gelatin digests

Different chromatographic patterns were obtained with the gelatin digests by the free and immobilized proteases in both the 10% (Fig. 1-A) and 20% (Fig. 1-B) digestions. The original gelatin was quickly digested by the free enzyme, resulting in smaller peptides.

The enzyme immobilized on CPG-170 produced the large peptides in the gelatin, slowly producing small peptides. These chromatographic patterns are completely different from those with the free enzyme. The chromatographic patterns of digests by the enzyme immobilized on CPG-350 and CPG-500 were similar to, but intermediate between, those with the free enzyme and the enzyme immobilized on CPG-170.

Changes in gel-strength accompanying proteolytic digestion

Change in gel-strength was measured during the digestion process (Fig. 2). Free subtilisin BPN' rapidly lowered the gel-strength of the gelatin, resulting in complete loss of ability to gel at 15% digestion.

In contrast, the decrease in gel-strength proceeded slowly with digestion by the protease immobilized on CPG-170; one-half the original gel-strength was obtained at 15% digestion, and a gel with gel-strength of about one-third of the original was obtained even at 30% digestion. Digestion by proteases immobilized on CPG-350 and CPG-500 also resulted stronger gels than that produced by the free enzyme.

Changes in viscosity accompanying proteolytic digestion

The viscosity of the gelatin was also reduced in the early stage of digestion by free subtilisin BPN' (Fig. 3). The proteases immobilized on CPG-350 and CPG-500 changed the viscosity in the same way as the free enzyme. The digestion by the protease immobilized on CPG-170, however, resulted in a gradual decrease and gave one-half the original viscosity at 15% digestion.

Relationship between gel-strength and viscosity of the gelatin digests

The gel-strength and viscosity of the gelatin changed with digestion in different ways depending on which CPG was used. The relationship between these two properties (Fig. 4) indicates a way of providing new properties to gelatin. For example, free subtilisin BPN' decreased the specific viscosity of gelatin from 5 to 1 η_{sp} producing gelatin digests which did not gel, whereas the protease immobilized on CPG-500 changed the viscosity to the same extent but with only a small decrease in gel-strength, from 280g to 200g. Digestion by the free enzyme to a gel-strength of 200g resulted in a great decrease in viscosity, whereas the enzyme immobilized on CPG-170 decreased the specific viscosity only from 5 to 3.5. Thus, one can obtain gelatins of desired viscosity and gel-strength by use of the differently immobilized proteases.

DISCUSSION

THE PRESENT EXPERIMENTS were conducted to make practical our general idea that enzymes immobilized on support which also has special function(s) such as molecular sieving, ion-exchange, or affinity to special compounds, can perform new reactions in combination with the enzymatic reaction. For example, protease fixed on a molecular sieve matrix would digest only proteins which can enter the matrix, without affecting larger proteins which are excluded from the matrix. Thus, the new immobilized protease performs two functions, that is, the selection and digestion of protein molecules. This idea would provide a new way for enzyme use, especially in food processing where we frequently meet mixtures of proteins of various molecular sizes.

Permeation of high molecular weight compounds into the matrix of molecular sieves depends on their steric structure (Nozaki et al., 1976). The exclusion limits of CPG-170, CPG-350, and CPG-500 are 4×10^5, 3×10^6, and 1×10^7, respectively, for globular proteins, but are smaller by one order for a chain polymer, polystilene (Electro-Nucleonics Inc. manual). Since commercial gelatins have random structure at 40°C as revealed by circular dichroism spectra in the far-ultraviolet region (Nishio and Hayashi, 1985), CGP-170 should exclude the α-, β-, and γ-chains of collagen (molecular weights 1×10^5, 2×10^5, and 3×10^5, respectively), and CGP-350 should exclude the β- and parts of the γ-chains. However, none of these three chains is excluded by CGP-500. Thus, subtilisin BPN' fixed on CGP-170, CGP-350, and CGP-500 should digest proteins smaller than the three chains, the β-chain and part of the γ-chain, and all three chains and the larger contaminant proteins, respectively.

Although such clear-cut selection of molecules was not seen during the digestion process, the rate of production of the smaller peptides increased with the pore size of the enzyme support (Fig. 1), being clearly different from the patterns with the free enzyme. This selective digestion resulted in production of new gelatins with various gel-strengths and viscosities (Fig. 2 and 3). This explanation is substantiated by similar digestion of single proteins (Nishio and Hayashi, 1984).

The procedure presented here enables one-step adjustment of viscosity and/or gel-strength of commercial gelatin. Use of columns packed with molecular sieves containing immobilized

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Development and Characteristics of Modified Agar Gels

G. PAPPAS, V.N.M. RAO, and C.J.B. SMIT

ABSTRACT

Icing formulations that are being used in bakery and confectionary industries have a lot of post storage problems. The use of stabilizers like agar involves a high cost due to the dissolving process. This study involved the development of freeze-dried and drum-dried agars which are soluble in warm water (55°C). The two modified products were compared with the unmodified one to determine differences in gel formation, gel strength, viscosity and micro structure. The agar modified by drum drying dissolved at 55°C and its gel had characteristics similar to the gel made with unmodified agar heated to 100°C.

INTRODUCTION

ICINGS have been defined as coatings or glazes of sugar and water with various other ingredients present, such as fat, milk solids, egg white, chocolate and other flavors, salt and sometimes coloring substances (Lipman, 1955). The water is essential to make the icing palatable and of proper consistency. All icings must have a desirable texture, attractive appearance and, probably most important, they must exhibit stability towards changes in temperature and humidity for a longer shelf life. Lipman (1955) defined icing stability as the ability of an icing to retain its structure, its smooth, nongranular texture, and to resist liquid separation during storage.

Once the icing has been spread it usually looses moisture by evaporation, causing the icing to become dry, hard, stale and less palatable. Wrapping the products in waxed paper, moisture-proof cellophane or other materials prevents the loss of moisture. However, during distribution and storage there are other changes that also affect the quality of the final product. Glicksman (1969) described these changes in discussing condensation problems within the wrappings. Because moisture is no longer free to escape by evaporation, it condenses as free water on the icing. As a result, the moisture content of the icing changes drastically and additional sugar is dissolved, making the icing soft, sticky, runny and less palatable.

Resolution of this problem may be achieved in two ways: (1) by using a minimum amount of water in the icing (Glicksman, 1969), and (2) by a adding a hydrocolloid to tie up the free water (Glicksman, 1969; Lipman, 1955). The use of hydrocolloids is probably the most effective way to control icing stability. The types and the amounts used have been the subject of several reports (Lipman, 1983; Meer et. al, 1973; Guckenberger; 1981). Although numerous natural substances have been used, the most widely acceptable is the seaweed hydrocolloid agar. Agar is unique among the gelling agents in that gelation occurs at a temperature far below the melting temperature (Glicksman, 1969). Agar is probably the strongest gelling agent known, and will form a gel in concentrations as low as 0.5 percent.

One serious drawback in using agar is that, in order to get it into a condition to gel, it must first be mixed with water and then boiled. Most commercial icing bases are available as "boiling" or "nonboiling" types. The boiling type produces a more stable icing compared with the nonboiling one (Lipman, 1983). Therefore, the development of an icing base that will not require boiling but still can produce stable icing will be of value to the bakery and food industry.

The objectives of this study were to produce modified agar, using freeze drying or drum drying, which would be soluble in warm water (55°C) and form a stable gel upon cooking, and to study the microstructure of each product and relate it to the functional properties of the agar gel.

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MATERIALS & METHODS

THE AGAR USED for the experiments was of Chilean origin, produced in 1984. The base mixture was formulated with 100 parts water and one part agar.

Freeze drying modification

The mixture was boiled, and, with the help of a heating plate, was kept in solution before it was frozen in liquid nitrogen. Although freezing with liquid nitrogen was very fast, there was a possibility of gel formation which would have been undesirable in this experiment. In order to freeze the solution instantaneously, small amounts at a time were frozen in 1 fl oz plastic containers filled to one-third of their capacity. The containers were kept inside the nitrogen tank until the contents were frozen completely. The small containers were then stored in a blast freezer before they were put in a freeze dryer. The final dry product was ground using a micro-mill and vacuum packed in polyester heat sealable, vapor-proof pouches.

Drum drying modification

A steam kettle was used to boil the mixture which was then poured hot into the nips of the drum dryer. The speed of the drums was adjusted to 1 rpm. The final product was ground and packed as described before.

Gel formation

To test the ability of the modified products to form a gel, 1g portions of unmodified agar, freeze-dried agar, and drum-dried agar were mixed with 100 mL water. For the unmodified agar, the mixture (water and agar at 23 ± 2°C) was heated with continuous stirring. After it was brought to a boil the solution was left at room temperature (23 ± 2°C) to cool and form a gel. However, for the two modified products, water at 55°C was used. The two solutions containing the modified agars were then left at room temperature (23 ± 2°C) to cool.

Gel strength evaluation

In order to determine the gel strength two tests were conducted: (1) an empirical test consisting of compressing the gel, using a cylindrical indenter with a flat end, until the gel was fractured, and (2) a fundamental rheological test known as relaxation test (Mohsenin, 1980). For both tests the gels were prepared as before and the mixtures poured into cylinders made of plexiglass rings (15 mm high; 45 mm diam) connected together. The gels were left for 24 hr at room temperature (23 ± 2°C) to equilibrate. Prior to the test, each gel was cut into pieces with a cheese cutter, so that each piece had the dimensions specified above. For each product tested, five replications were made. Both tests were conducted using the INSTRON universal testing machine. For the compression, the crosshead was equipped with a stainless steel cylindrical indenter 11 mm diam. The indenter then was

Table 1—Relaxation and compression test parameters of unmodified and drum dried agar gels

Product	Relaxation test				Puncture test
	E1[c]	E2[c]	T1[c]	T2[c]	F_{max}[d]
Unmodified agar	838.37[a]	324.05[a]	831.54[b]	29.73[a]	8.11[a]
Drum-dried agar	916.33[a]	211.30[b]	927.16[a]	27.26[a]	7.74[b]
F-value	1.89	147.38	8.00	1.37	9.07
Prob > F	0.2064	0.0001	0.0222	0.2755	0.0168

[a,b] Means within the same column having different superscripts are significantly different at 5% level.
[c] E1, E2 are the Young's moduli of spring 1 and 2 respectively, in (Pa). T1, T2 are the relaxation times of element 1 and 2 respectively, in (sec).
[d] F_{max} is the force recorded at the point of rupture, in (Newtons).

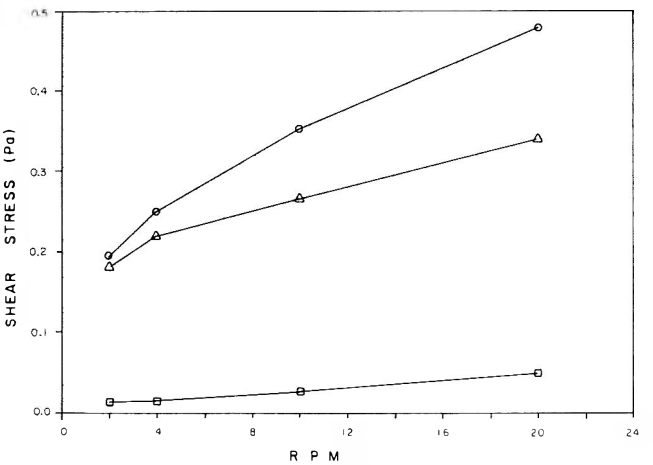

Fig. 1—Flow characteristics of agar solutions at 0.05% concentration using a rotational viscometer. (△ drum-dried, □ freeze-dried, ○ unmodified agar).

moved at 50 mm per min towards the stationary part to compress the gel until it was fractured. The maximum force required to break the gel was recorded for each replication. For the stress relaxation the compression cell was equipped with a 5 cm diam stainless steel disc. The relaxation test was done according to the procedure outlined by Gross et al. (1980). Due to the weakness of the freeze-dried agar gel it was impossible to apply either test. Consequently, only the unmodified and the drum dried agar gels were tested.

The viscosity of the products was measured using the Brookfield narrow gap viscometer at concentrations of 0.075, 0.05 and 0.025 percent. Four speeds were used (2, 4, 10 and 20 rpm) with three replications in each case.

Microstructure evaluation

The microstructure of the unmodified agar and the two modified products was studied using the scanning electron microscope. Samples (0.2 g) from the 3 products were mounted on aluminum plates with double sided adhesive tape. As the samples were dry powders, no further drying was required. The aluminum plates were sputter coated with gold-palladium mixture and allowed to dry for 5 min at room temperature (23 ± 2°C). The aluminum plates were then loaded in the microscope and the scanning cycle started. Four magnifications (100, 500, 2500 and 5000X) were used for each product tested.

Analysis of the results

Preliminary experiments showed that the behavior of the gels during a relaxation test could be represented by two Maxwell models in parallel without the incorporation of a residual spring. The elastic moduli corresponding to spring 1 and 2 and the relaxation times corresponding to elements 1 and 2 were calculated by fitting the points obtained from the chart to the non-linear model. The NLIN procedure, a non-linear regression analysis technique, was used, as described in SAS (1984). Also, one-way analysis of variance was performed on the results of the relaxation and the compression tests.

The results obtained using the Brookfield viscometer were analyzed using a linear regression model. Because the concentrations used were very low, the assumption of Newtonian behavior with a yield stress (commonly referred to as Plastic or Bingham fluid) was made (Van Wazer, et al. 1963). The apparent viscosity at 10 rpm was also calculated as the ratio of shear stress to the shear rate at 10 rpm.

RESULTS & DISCUSSION

Gelling ability

The gelling ability of the drum-dried agar was comparable with the unmodified agar. The modified powder dissolved completely in water at 55°C, resulting in a clear gel, free of suspended material. However, in the case of the freeze-dried powder the gel was very weak and contained aggregations of undissolved particles. Since freeze drying results in an open porous structure it was initially thought that the freeze-dried product would dissolve easily. Instead of absorbing water and dissolving at a faster rate, a suspension was formed and the ability to retain water was very poor. When such a gel was left for a period of 24 hr it lost almost half of the water initially added.

Relaxation and compression test results

There was a significant difference in the maximum force values between unmodified and drum dried agar gels obtained from the compression tests at the 5% significance level (Table 1). Although such a test is considered to be "empirical," it gives an indication of the ultimate force required to rupture the products. Unmodified agar gel was stronger than the drum dried agar gel. The results from the relaxation test agreed with the results obtained from the compression test. The Young's modulus of the first spring element was similar in both gels.

Table 2—Flow parameters of agar solutions at different concentrations

Product		Concentration		
		0.025%	0.050%	0.075%
Agar unmodified	T_o[e]	53.35^{a}_{a}	176.33^{b}_{b}	487.00^{c}_{c}
	Viscosity[f]	10.41^{a}_{a}	36.13^{b}_{b}	99.68^{c}_{c}
Drum-dried agar	T_o	38.88^{a}_{a}	178.67^{b}_{b}	443.73^{c}_{c}
	Viscosity	8.89^{a}_{a}	42.47^{b}_{b}	85.03^{c}_{c}
Freeze-dried agar	T_o	4.17^{a}_{b}	6.87^{a}_{c}	14.28^{b}_{d}
	Viscosity	3.17^{a}_{b}	3.47^{a}_{c}	4.26^{a}_{d}

[a-d] Different superscripts in the same row indicate significant differences by Duncan's multiple range test at 5% significance level. Different subscripts in the same column for the same parameter indicate differences by Duncan's multiple range test at 5% significance level.
[e] T_o is the yield stress in mPa from the linear model [shear stress = yield stress + (plastic viscosity) (shear rate)].
[f] Viscosity is the apparent viscosity in mP1 at shear rate corresponding to 10 rpm.

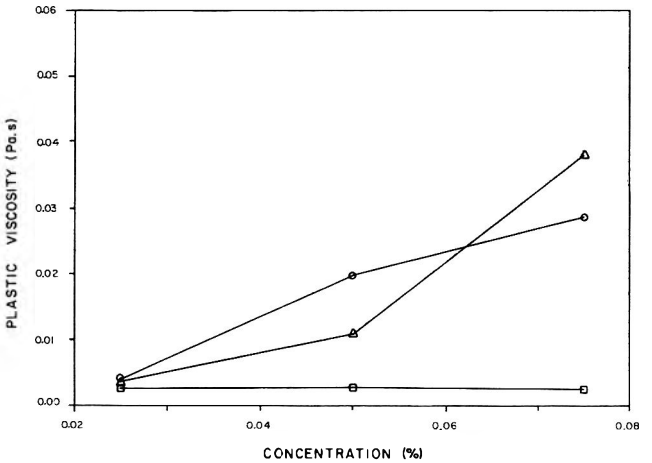

Fig. 2—Plastic viscosity of the three agar sclutions using a rotational viscometer. (△ unmodified agar, ○ drum-dried agar, □ freeze-dried agar).

There was, however, a significant ($P < 0.05$) difference in the modulus of the second spring. In the case of the unmodified agar gel the modulus was higher, explaining the results from the compression test. There was also a significant ($P < 0.05$) difference in the modulus of the second spring. In the case of the unmodified agar gel the modulus was higher, probably explaining the results from the compression tests. There was also a significant ($P < 0.05$) difference in the relaxation time of the first element, but no significant ($P > 0.05$) difference in the relaxation time of the second element. Relaxation times relate to the viscous properties of a particular material; smaller relaxation time indicates a smaller coefficient of viscosity. Consequently, it appears that the drum dried agar gel had a higher coefficient of viscosity than the unmodified agar gel.

Viscosity results

Results of viscosity measurements are shown in Fig. 1. Because the concentrations used were very low, the assumption of Newtonian behavior with a yield stress was made. The shear stress was linearly related to the shear rate as evidenced by higher correlation coefficients for all three products and for every concentration (0.97 to 0.99, significant at 1% level). The apparent viscosity of the drum dried agar solution and the unmodified agar solution was not significantly different ($P < 0.05$) by Duncan's multiple range test. The viscosity of the freeze dried solution was significantly ($P \leq 0.05$) lower. The change in plastic viscosity as a function of the concentrations is shown in Fig. 2. In the case of the unmodified agar, as well as the drum-dried agar, there was a marked increase when higher concentrations were used. However, in the case of freeze-dried agar, there was no change in the magnitude of plastic viscosity. The expected increase in viscosity at higher concentrations was not apparent with this product because of low solubility. There was a visible separation of liquid and solid phase.

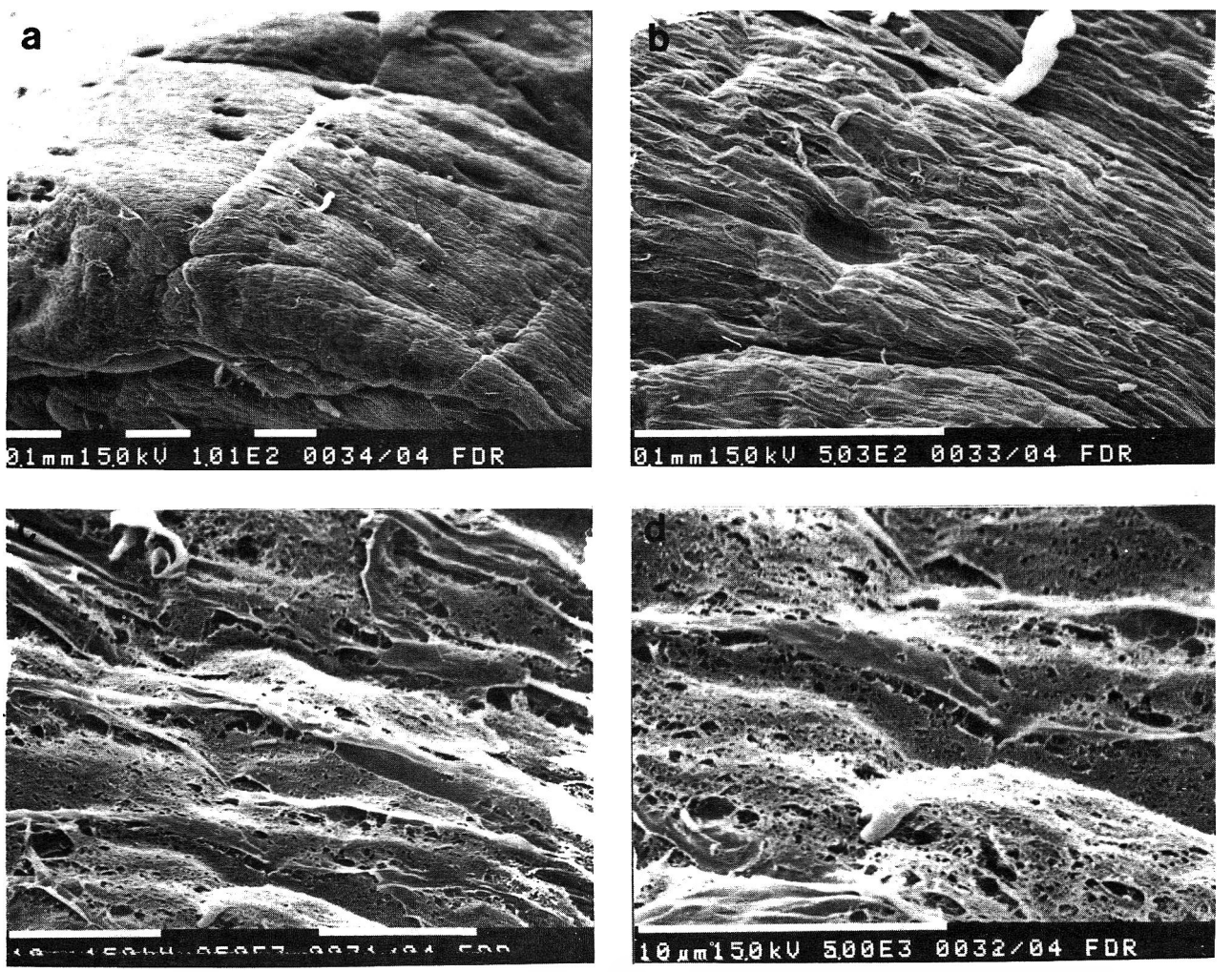

Fig. 3—Scanning electron microscope photographs of the freeze-dried agar (a: 100X; b: 500X; c: 2500X; d: 5000X).

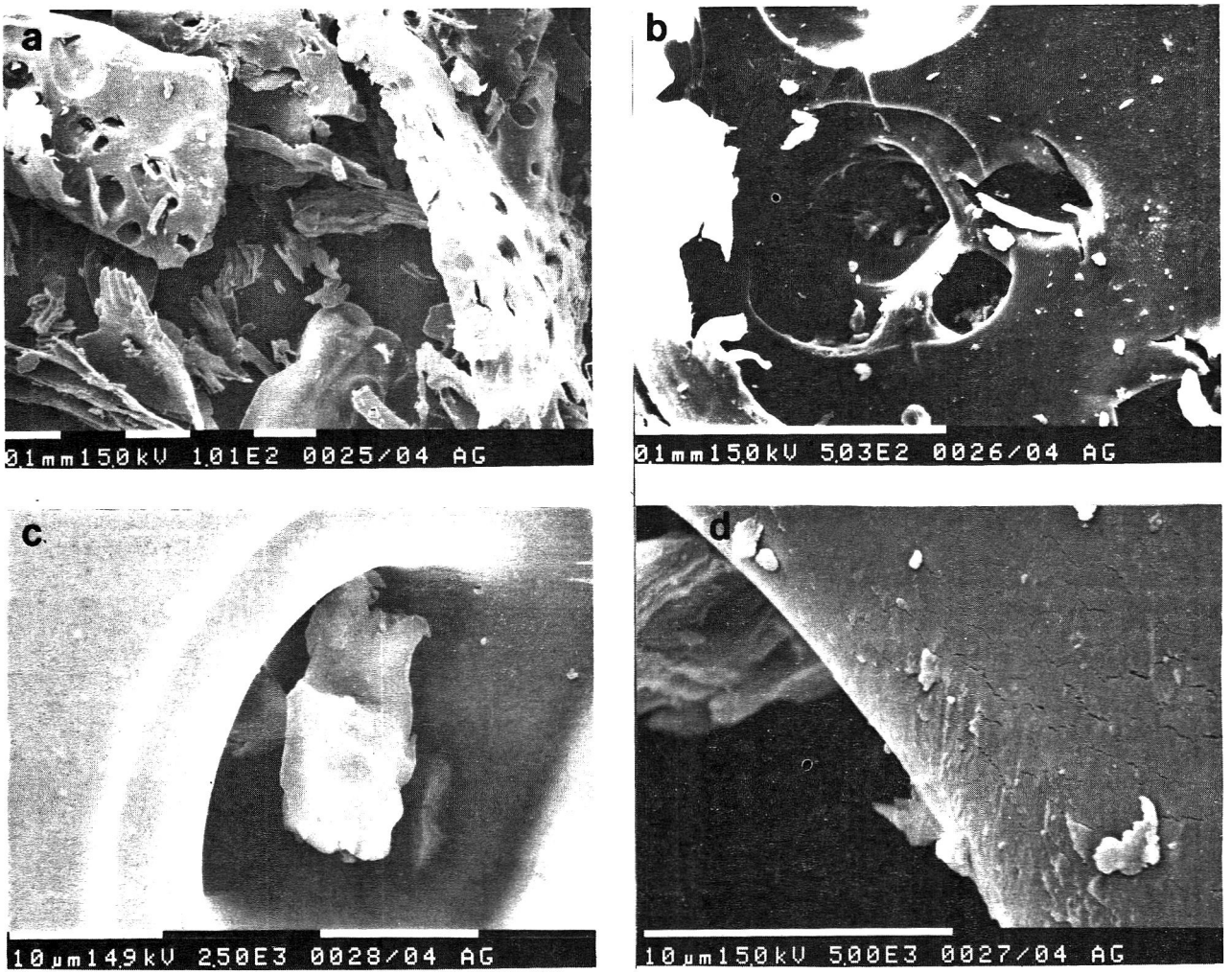

Fig. 4—Scanning electron microscope photographs of the drum-dried agar (a: 100X; b: 500x; c: 2500X; d: 5000X).

Scanning electron microscope (SEM)

The scanning electron microscope revealed differences between the structures of the three powders. The microstructure of the modified products shows that expansion took place, possibly during the heating of the liquid mixture and the drying process. In the case of the freeze-dried powder, the process resulted in a product having many small pores (Fig. 3). However, the microstructure of the drum-dried product was solid with small gaps in the surface (Fig. 4). The unmodified agar powder microstructure appears as a continuous surface without any indication of pores or points of discontinuity (Fig. 5). Consequently, the structural differences between freeze-dried and drum-dried product are related to the solubility and gelling ability of the two modified products. The large expansion that occurred in the case of the freeze-dried product would seem to facilitate the absorption of water. However the resulting gel was weak and unstable. It appears that the large particle size of the freeze-dried product inversely affected the dissolving ability. A reason explaining the large particle size may be associated with the way the freeze-dried agar was produced. During the freezing step with liquid nitrogen, it is possible that some parts of the solution formed a gel before freezing.

The drum-dried agar dissolved completely producing a stable gel, even though it was much less expanded. Although further research is required, it seems that there is an optimum particle size to achieve maximum solubility characteristics.

The drum-dried agar was supplied to a local bakery as an ingredient for icing and tested for the stability of icings on doughnuts. The results of the test showed that the icings were stable at room temperature (23 ± 2°C) for 24 hr.

CONCLUSIONS

THE DRUM-DRIED modified agar developed in this study was able to produce a stable gel at low temperatures with no significant reduction in the rheological characteristics. Two basic advantages of the procedure described in this paper are: (1) use of a gum which is readily available and considered to be inexpensive; (2) the drum drying process is simple, very short and without major technological problems.

Finally, further research is required to investigate the possibility of incorporating certain types of additives prior to the drum drying process.

REFERENCES

Glicksman, M. 1969. Gum technology in the food industry. Academic Press, New York, NY.

Gross, M.O., Rao, V.N.M., and Smit, C.J.B. 1980. Rheological characterization of low-methoxyl pectin gel by normal creep and relaxation. J. Texture Studies. 11(2): 271.

Guckenberger, J.D. 1981. Bakery icings. Baker's Digest. 55(1): 12.

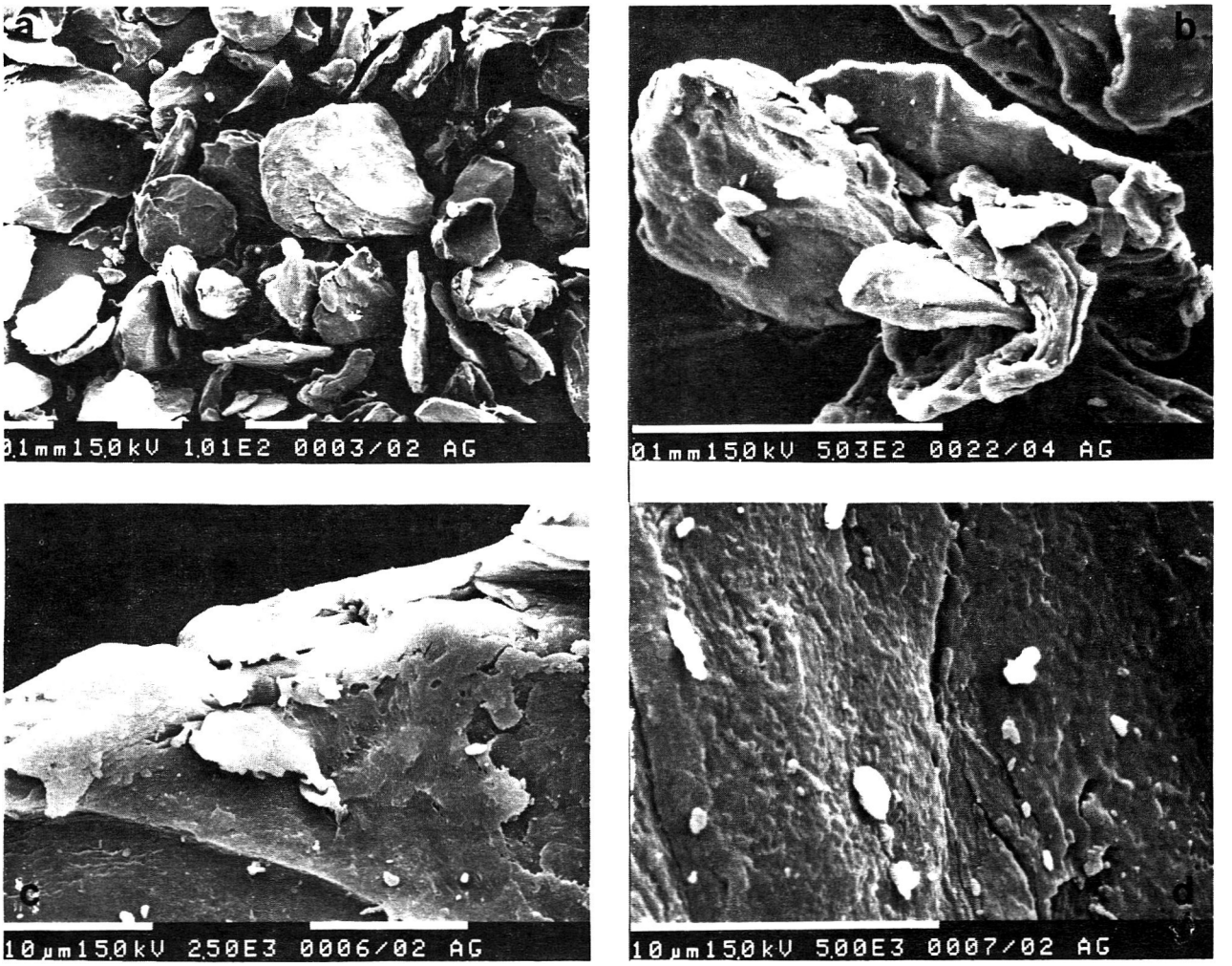

Fig. 5—Scanning electron microscope photographs of the unmodified agar (a: 100X; b: 500X; c: 2500X; d: 5000X).

Lipman, H.J. 1955. Icings. Baker's Digest. 29(5): 111.
Lipman, H.J. 1983. Advances in icing technology. Baker's Digest. 57(4): 32.
Meer, A.W., Meer, G., and Gerard, T. 1973. Natural plant hydrocolloids in bakery applications. Bakery Digest. 47(3): 45.
Mohsenin, N.N. 1980. "Physical Properties of Plant and Animal Materials." Gordon and Breach Science Publishers, New York, Paris, London.
SAS User's Guide. 1984. SAS Institute Inc., Cary, NC.
Van Wazer, J.R., Lyons, J.W., Kim, K.Y., and Colwell, R.E. 1963. Viscosity and flow measurement. Interscience Pub., New York.
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Odor Barrier Properties of Multi-Layer Packaging Films at Different Relative Humidities

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ABSTRACT

A permeation cell method was developed for the determination of transmission rates of organic vapors through flexible packaging materials. The permeation rates at 23°C of some compounds for several composite films at 0% and 75% relative humidity (RH) indicated that the polyethylene vinyl alcohol and nylon combinations exhibited superior barrier properties even at elevated RH, provided that moisture barrier films were present in the laminate construction.

INTRODUCTION

THE EXPANDING USE of plastics in packaging applications competing with glass and metal puts great emphasis on the high barrier properties of the involved materials against moisture and glass (Allison, 1985) to assure an acceptable shelf life of a packaged product.

The permeation of packaging materials to odorous vapors is of significant importance, either to protect the contents against contamination from foreign odors or to retain favorable volatile flavors. Although the permeation rates of permanent gases and water vapor through many plastics have been obtained, there is a deficiency of data for permeation of organic vapors (Zobel. 1985).

The object of this study was to develop a method for quantitative evaluation of the aroma barrier of packaging materials. A series of flexible plastic films of various compositions were then tested for their permeability to some flavoring and malodorous compounds.

MATERIALS & METHODS

THE METHOD is based on the Gilbert-Pegaz permeation cell (Gilbert and Pegaz, 1969). As Fig. 1 indicates, the cell can accomodate simultaneously two films by clamping each between aluminum devices to form a pair of outer chambers and a single inner one (Gilbert et al., 1983). The devices are equipped with Viton O-rings to assure good seal between the films and the surroundings. Chambers on either side of the film have valves for inlet and outlet of permeant supply and septa for sampling.

Nitrogen is bubbled through the liquid permeant and then passed with the permeant vapors through either the middle chamber or the lower and upper compartments. Thus either one cell can be used for duplicates or in the case of very good barriers, the exposed area can be doubled. The nitrogen stream carrying the permeant vapors can be mixed before the cell with either dry or wet nitrogen to adjust the permeant final concentration or the relative humidity of the high concentration chamber. In the case of permeants with very low vapor pressure at ambient temperature, a small amount can be placed in an aluminum dish on the bottom of the cell. If adjustment of the humidity is desired, another dish with an aqueous saturated salt solution can also be placed on the bottom (Fig. 2).

The tested films appear in Table 1 with their respective compositions and thicknesses. Each film was Gelbo flexed (ASTM, 1982) for 20 cycles prior to testing to simulate severe abuse which may be encountered in packaging and distribution. All films to be exposed to a 75% RH environment at 23°C were kept for a period of 2 wk in a desiccator over saturated sodium chloride solution. Then they were tested with the permeant vapor stream combined with controlled humidity nitrogen to provide the elevated humidity level during testing. The permeants used for the tests are also given in Table 1.

The concentrations of the permeating vapors and related humidity were monitored by gas chromatography with removal of small aliquots

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Table 1—Films used for the permeation studies

A. 1.25 Mil HDPE/TIE/NYLON/EVA
B. 1.25 Mil HDPE/TIE/EVOH/EVA
C. 1.40 Mil HDPE/TIE/NYLON/EVOH/NYLON/TIE/HDPE
D. 2.20 Mil HDPE/TIE/MODIFIED/NYLON/TIE/HDPE
E. 1.0 Mil (oriented) PP/TIE/EVOH/TIE/PP
F. 1.0 Mil PP/TIE/PET-G/TIE/PP
G. 1.8 Mil PVDC coated co-ex OPP

Where: HDPE	= High density polyethylene
TIE	= Adhesive layer
NYLON	= Nylon 6
EVA	= Polyethylene vinyl acetate
EVOH	= Polyethylene vinyl alcohol
Modified NYLON	= Mineral filled nylon-6
PP	= Polypropylene
PET-G	= Polyethylene terephthalate-glycol
PVDC	= Polyvinyledene chloride
OPP	= Oriented polypropylene

Permeants used for the test

1. Ethyl acetate	4. Limonene
2. Toluene	5. beta-Pinene
3. Styrene	6. Ethyl phenyl acetate

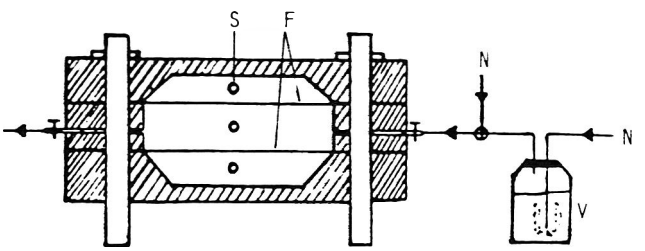

Fig. 1—Permeation cell arrangement for high vapor pressure permeants: (N) Nitrogen inlet; (V) Glass vial containing liquid permeant; (F) Film samples; (S) Septa for sampling.

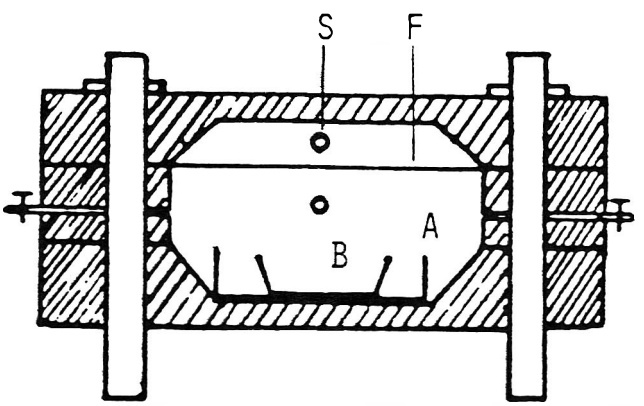

Fig. 2—Permeation cell arrangement for low vapor permeants: (A) Dish with aqueous saturated salt solution; (B) Dish with permeant; (F) Film sample; (S) Septa for sampling.

Table 2—Permeation Rates, 0% RH, 23°C (g/m²·day·100 ppm)

Film	Ethyl acetate	Toluene	Styrene	Limonene	B-Pinene	Ethyl phenyl acetate
A	<0.003	0.001	<0.0003	0.0409	0.0120	0.0085
B	0.30	0.027	0.0610	0.0012	0.0013	<0.0060
C	<0.0004	0.002	0.0054	0.0014	<0.0004	<0.0080
D	<0.0004	0.001	<0.0003	0.0018	<0.0011	<0.0080
E	<0.0004	0.0003	<0.0002	0.0400	<0.0036	<0.0070
F	6.86	1.310	0.0018	0.0315	0.0088	0.234
G	0.52	0.470	0.0046	0.0400	0.0320	0.016

Table 3—Permeation Rates, 75% RH, 23°C (g/m²·day·100 ppm)

Film	Ethyl acetate	Toluene	Styrene	Limonene	B-Pinene	Ethyl phenyl acetate
A	0.0632	0.0199	0.0120	0.0009	0.0013	0.0053
B	0.0034	0.0050	0.0037	0.0037	0.0001	<0.002
C	0.0041	0.0088	0.0060	<0.0003	0.0049	<0.002
D	0.0066	0.0008	0.0046	0.0076	0.0020	0.0076
E	0.0092	0.0034	0.0338	0.0061	0.0031	0.0061
F	0.0040	0.0020	0.0096	0.0071	too fast	0.0071
G	0.0095	0.0007	0.0051	0.0060	0.1419	0.0060

using a gas tight syringe (Gilbert and Pegaz, 1969). The plot of the monitored concentration versus sampling time gives the permeation curve. The slope of the steady state portion was determined and used in combination with the chamber volume, exposed film surface and permeant concentration difference across the film for the calculation of permeation rate in appropriate units.

However, for anomalous diffusion, often found with glassy and semicrystalline polymers (Crank and Park, 1968), when the obtained permeation curves did not show a constant or steady state rate following a lag phase, the linear portion of the permeation curve was used after a testing period of two weeks. With very good barriers no significant permeating amount could be detected for certain permeants. In this case the rate was expressed as a value below the detection limit of the gas chromatograph for the permeant driving force across the film.

While a normalized driving force was used in the calculation of the permeation coefficient, the actual driving force depended on the vapor pressure of the permeant at the test temperature. For a specific penetrant all films were tested at the same vapor driving force concentration for both relative humidities. Linear concentration dependence of permeation rate was assumed, although this might not be the case, especially with organic vapors (Zobel, 1985).

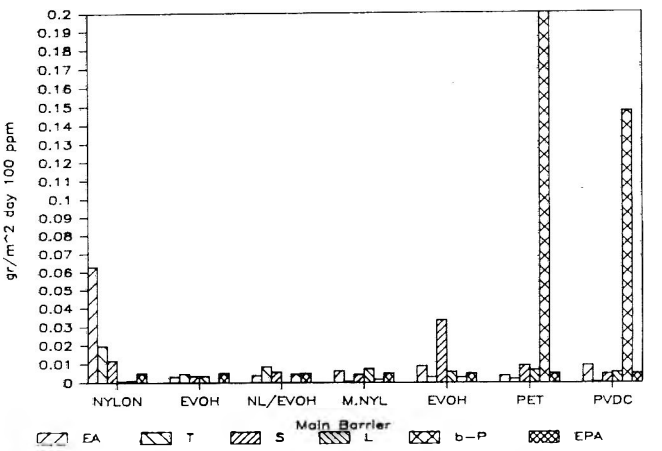

Fig. 3—Permeation rates at 0% RH, 23°C. EA:ethyl acetate, T:toluene, S:styrene, L:limonene, b-B:beta pinene, EPA:ethyl phenyl acetate.

RESULTS & DISCUSSION

THE CALCULATED PERMEATION rates for each film and permeant are given in Table 2 for 0% RH, and Table 3 for 75% RH, respectively. The indicated values were normalized to 100 ppm (g permeant/cc of air) permeant concentration difference across the film. For comparison purposes the values from Tables 2 and 3 are presented in Fig. 3 and 4, respectively. It should be emphasized that normalizing the permeation rate to a unit driving force concentration, allows for a comparison of relative barrier properties of the tested films at only one vapor concentration. However this relationship may change as the vapor concentration is changed.

At 0% RH the nylon-EVOH and modified nylon 6 structures exhibited the best barrier properties for all the permeants (Fig. 3). The nylon-6 and both EVOH films followed next although the HDPE/EVOH/EVA combination was significantly more permeable to ethyl acetate. The PET and PVDC structures had the least barrier performance at 0% RH, especially for ethyl acetate and toluene, with PET much more inferior than PVDC.

For the 75% RH environment all the tested films exhibited almost equivalent barrier properties, with some exceptions for certain permeants. PET and PVDC were very permeable to beta-pinene. Also, the PP/EVOH/PP film appeared sensitive to styrene, and the HDPE/nylon 6/EVA structure had a lower barrier to ethyl acetate and toluene compared to EVOH and modified nylon. Overall at 75% RH the best barrier performance can be attributed to nylon 6/EVOH, modified nylon-6

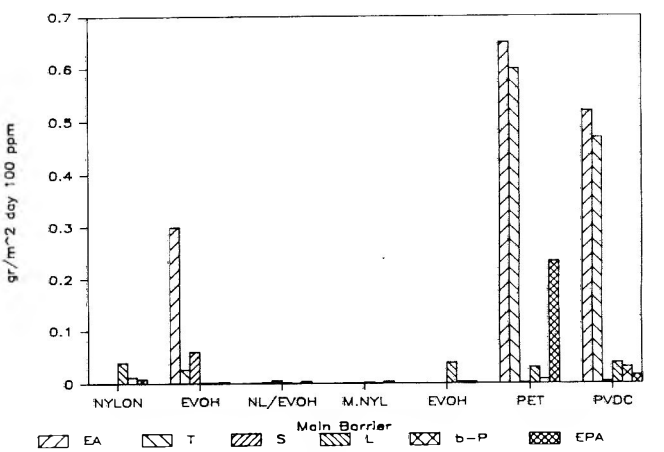

Fig. 4—Permeation rates at 75% RH, 23°C EA:ethyl acetate, T:toluene, S:styrene, L:limonene, b-B:eta pinene, EPA:ethyl phenyl acetate.

Table 4—Ethyle Acetate Permeation Tests, 23°C, 93% RH

Film	Lag time (hr)	Permeation rate (g/m²·day·100 ppm)
A	5.6	0.247
B	24.2	0.021

and both EVOH combinations. The HDPE/nylon 6 EVA film lost its excellent barrier properties at this RH level probably due to moisture sorption from the polyamide.

Although polyethylene vinyl alcohol (EVOH) also sorbed water, the resulting structure change at the 75% RH did not affect the permeation as much as in nylon-6. A similar difference was observed with polyvinyl alcohol and nylon 6 films for carbon dioxide and oxygen permeation (Ito, 1961; Meyer et al., 1957; Toyoshima, 1973). At 75% RH, polyvinyl alcohol was a better barrier than nylon-6 for the above gases. However, the opposite was true at 93% RH. This could be attributed to higher water sorption by polyvinyl alcohol at 93%, possibly because of a more disrupted structure for the polymer compared to nylon 6.

To determine whether EVOH, despite its ethylene content, would exhibit a similar barrier change at 93% RH as polyvinyl alcohol, when compared to nylon-6, the films HDPE/nylon 6/EVA and HDPE/EVOH/EVA were tested at 93% RH for ethyl acetate permeation. The test results are given in Table 4. The permeation rate at the steady state for nylon-6 was about twelve times faster than for EVOH. This did not agree with what was observed for permanent gases and can be attributed to the ethylene content (about 35% w/w) of EVOH. The ethylene modified the polymer structure so it was not disrupted by water sorption as much as in polyvinyl alcohol.

The lag-time, which is related to the diffusivity through the film (Crank and Park, 1968), was about four times slower for nylon-6 versus EVOH (Table 4). This indicated that ethyl acetate could diffuse four times faster in nylon 6 than in EVOH at 90% RH.

CONCLUSIONS

The nylon and polyethylene vinyl alcohol (EVOH) laminations at 0% RH appeared to have the best barrier performance which was superior to the polyethylene terephthalate-glycol (PET-G) and polyvinyledene chloride (PVDC) laminations.

A similar trend was observed even at the 75% RH level, where the water sensitive nylon and EVOH seemed to be generally well protected by the outer hydrophobic laminates. Thus the nylon and EVOH combinations maintained a superior barrier performance compared to PET-G and PVDC.

The determined barrier properties of the tested films could help in designing packaging materials which could offer better aroma protection and consequently contribute to qualitative and quantitative shelf life improvement.

REFERENCES

Allison, H.L. 1985. High-barrier packaging materials-What are the options? Packaging March: 25.
ASTM. 1982. Flex durability of flexible barrier materials. In "Annual Book of ASTM Standards," Vol. 21, p. 414. American Society for Testing and Materials, Philadelphia, PA.
Crank, J. and Park, G.S. 1968. Methods of measurement. Ch. 1. In "Diffusion in Polymers," J. Crank and G.S. Park (Ed.), p. 1. Academic Press, New York.
Gilbert, S.G., Hatzidimitriu, E., Lai, C., and Passy, N. 1983. Study of barrier properties of polymeric films to various organic aromatic vapors. In "Instrumental Analysis of Foods," G. Charalambous and G. Inglet (Ed.), Vol. 1, p. 405. Academic Press, New York.
Gilbert, S.G. and Pegaz D. 1969. Find new way to measure gas permeability. Packaging Jan. 66.
Ito, Y. 1961. Permeability of measuring high-polymer films, Kobunshi Kagaku 18: 158.
Meyer, J.A., Rogers, C.E., Stannett, V., and Scwarc, M. 1957. Studies in the gas and vapor permeability of plastic films and coated papers. Tappi 40: 142.
Toyoshima, K. 1973. Properties of polyvinyl alcohol films, Ch. 14 in "Polyvinyl Alcohol," C.A. Finch (Ed.), p. 339. John Wiley & Sons, New York.
Zobel, M.G.R. 1985. The odor permeability of polypropylene packaging film. Polymer Testing 5: 153.
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proteases of different specificities is now being tested to further improve the present data.

REFERENCES

Fields, R. 1972. The rapid determination of amino groups with TNBS. In "Methods in Enzymology", Vol. 25, p. 464. Academic Press, New York.
Finch, C.A. and Jobling, A. 1977. Physical properties of gelatin. In "The Science and Technology of Gelatin", p. 250. Academic Press, New York.
Hinterwaldner, R. 1977. Technology of gelatin manufacture. In "The Science and Technology of Gelatin", p. 315. Academic Press, New York.
Johns, N.R. 1977. Uses of gelatin in edible products. In "The Science and Technology of Gelatin", p. 366. Academic Press, New York.
Johns, P. and Courts, A. 1977. Relationship between collagen and gelatin. In "The Science and Technology of Gelatin", p. 138. Academic Press, New York.
Kunitz, M. 1947. Crystalline soybean trypsin inhibitor. II. General properties. J. Gen. Physiol. 30: 291.
Moore, S. and Stein, W.H. 1954. A modified ninhydrin reagent for the photometric determination of amino acid and related compounds. J. Biol. Chem. 211: 907.
Morihara, K., Oka, T., and Tsuzuki, H. 1970. Subtilisin BPN': Kinetic study with oligopeptides. Arch. Biochem. Biophys. 138: 515.
Nishio, T. and Hayashi, R. 1984. Digestion of protein substrates by subtilisin: Immobilization changes the pattern of products. Arch. Biochem. Biophys. 229: 304.
Nishio, T. and Hayashi, R. 1985. Regeneration of a collagen-like circular dichroism spectrum from industrial gelatin. Agric. Biol. Chem. 49: 1675.
Nazaki, Y., Schecter, N.M., Reynolds, J.A., and Tanford, C. 1976. Use of gel chromatography for the determination of the Stokes radii of proteins in the presence and absence of detergents. A reexamination. Biochemistry 15: 3884.
Weetall, H.H. 1976. Covalent coupling methods for inorganic support materials. In "Methods in Enzymology", Vol. 44, p. 134. Academic Press, New York.
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Autoxidizing Process Interaction of Linoleic Acid with Casein

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ABSTRACT

Autoxidized linoleic acid was classified to linoleic acid (LA), its hydroperoxide (LAHPO) and secondary autoxidation products (SP), and interactions of these products with casein were investigated. Casein was antioxidative to the autoxidation of LA. The products were readily bound to casein at 37°C within 1 hr in order of SP > LAHPO > LA. SP and LAHPO facilitated the access of fluorescent probes to casein and made casein insoluble. Casein was subsequently became undigestible during 1 day incubation with SP or after 2 days with LAHPO. Incubation with SP for 4 days or more caused casein to lose its amino acids and to polymerize.

INTRODUCTION

POLYUNSATURATED FATS react easily with atmospheric oxygen to form their peroxides (Labuza and Ragnarsson, 1985). The peroxides are further autoxidized to a complex mixture of various oxidized products (Kanazawa et al., 1973), generally termed secondary autoxidation products (SP). Oxidative deterioration and off-flavor in foods are caused by these products. These autoxidation products readily reacted with proteins and decreased bioavailability (Andrews et al. 1965; Chiba et al., 1976; Chang et al., 1985). When the proteins were incubated with peroxidizing lipids for 5 days or more, their amino acid residues were lost (Roubal and Tappel, 1966; Kanazawa et al., 1975a) and the proteins were polymerized (Shimada and Matsushita, 1978; Nielsen, 1979). Interaction of peroxidizing lipids with protein has been investigated using linoleate and lysozyme in a model system (Schaich and Karel, 1975; Kanner and Karel, 1976; Funes and Karel, 1981). However, processes of interaction of proteins with the autoxidizing lipids prior to undergoing the drastic damage, are not well understood.

In this study, the effects of linoleic acid (LA) its hydroperoxide (LAHPO) and its SP on the hydrophobicity, solubility and digestibility of casein were investigated.

MATERIALS & METHODS

Materials

Casein according to Hammarsten was purchased from Merck & Co., Inc. [U-^{14}C]LA, obtained from New England Nuclear, USA, was diluted with cold LA (Tokyo Kasei Kogyo Co., Ltd.) to a specific activity of 15.6 kBq/mmol. The LA was autoxidized at 37°C for 7 days. LA, LAHPO and SP fractions were obtained from the autoxidized LA by silica gel column and thin layer chromatography (Kanazawa et al., 1975b). The purity of LA and LAHPO were > 95% and > 89%, respectively. The SP fraction consisted of a 36% mixture of polymers, 26% epoxyhydroperoxides or endoperoxides, 4.8% 9-oxononanoic acid, 3.7% hexanal, 2.5% nonanedioic acid, 2.4% short-chain carboxylic acids, 0.75% 8-oxooctanoic acid, 0.34% 12-oxododecadienoic acid and the remainder (Kanazawa et al., 1983).

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Methods

Incubation of casein with the autoxidation products. The following experiments were performed in triplicate. Casein was preswollen in water, dissolved in 0.05N NaOH, and then diluted to a 0.5% solution of casein with 0.1M Sörensen buffer (pH 6.5). LA, LAHPO or SP in methanol were added to aliquots of the casein solution at concentrations of 5.0mM (1.4 mg/ml of the casein solution), 5.0mM (1.56 mg/ml) or 1.9 mg/ml, respectively, and incubated at 37°C. A control solution of casein was prepared in the same volume of methanol.

Autoxidation of LA and decomposition of LAHPO in the solution were measured by peroxide value (PV) analysis (Sully, 1954) and thiobarbituric acid (TBA) test (Asakawa and Matsushita, 1980). The TBA data were expressed as milligram equivalent units of malonaldehyde (MA).

Radioassay. The amounts of the radioactive products bound to casein were periodically determined with 1 mL of each of the incubation mixtures, which was washed with 1 mL diethyl ether 5 times and then mixed with 3 mL ethanol for precipitation of casein. The casein precipitate was re-washed with 1 mL diethyl ether: ethanol (1:1) 3 times and re-suspended in 2 mL buffer. One milliliter of the suspension was absorbed on a cellulose sheet. The sheet was dried and burned in a sample oxidizer (Packard Model 305 Tri-Carb) as described previously (Kanazawa et al., 1985). The radioactivity was measured with a scintillation counter using a toluene-based scintillation cocktail. Recovery of radioactivity with the sample oxidizer was 97.2 ± 1.2% (n = 10).

Measurement of fluorescence. Fluorescent intensity of hydrophobic pockets in casein was determined with a Hitachi Fluorescence Spectrophotometer (Model 203, equipped with a mercury lamp) using fluorescent probes, 8-anilino-1-naphthalenesulfonic acid ammonium salt (ANSA), Tokyo Kasei Kogyo Co., Ltd. and 2-p-toluidinylnaphthalene-6-sulfonic acid potassium salt (TNS), Nakarai Chemicals Ltd. The above incubation mixtures of casein were diluted five times with water and a pre-determined amount of ANSA or TNS solution added. Their fluorescent intensities at the excitation wavelength of 367 nm were measured at the emission wavelength of 463 nm when ANSA was added or 430 nm with TNS added. Fluorescent intensity of a quinine sulfate (1 μg/mL) standard was set as 100. When dissociation constants of these probes with casein were determined, ANSA (15.4 to 45.2μ M) or TNS 43 to 147 μM) was added to the casein solution after the incubation with the autoxidation products, and the fluorescent intensities were determined. These intensities versus the concentrations of probes were plotted reciprocally and analyzed by the Lineweaver-Burk method (Lineweaver and Burk, 1934).

Measurements of solubility and digestibility. Five milliliter aliquots of the incubation mixtures were withdrawn, and the solubility and digestibility of the casein was determined. After washing with diethyl ether, a small amount of 0.1N HCl was added to precipitate the casein. The precipitates were collected by centrifugation, 1500 × *g* for 10 min, and dissolved in 3.5 mL 0.05N NaOH. After standing for 2 hr, the solutions were centrifuged at 1500 × g for 10 min and the supernatants were analyzed by the ninhydrin method (Yemm and Cocking, 1955). The color intensity was compared with that of casein incubated without the autoxidation products.

The above casein precipitates were also suspended in 2.5 mL 0.1M phosphate buffer (pH 7.6) and mixed with 2.5 mL 0.02% trypsin or chymotrypsin (Boehringer Mannheim GmbH) solution. The mixtures were incubated for 20 min at 37°C. In the case of pepsin (Sigma Chemical Co.), the enzyme was reacted in 0.1M acetate buffer (pH 2.0). The reactions were stopped by addition of 5 ml 5% trichloroa-

cetic acid and then the reaction mixture was filtered through filter paper. Reproducibility of recovery of the digested products was better with the filtration than centrifugation. The filtrates were treated with ninhydrin and the color intensities compared spectrophotometrically at 570nm with that of casein incubated without the autoxidation products and digested by the enzymes.

RESULTS

Autoxidation of LA in casein solution

The first product in the autoxidation of LA is LAHPO, which was quantified by the PV method. LAHPO decomposes mainly to aldehyde species and the aldehydes are sensitive to the TBA test (Kanazawa et al., 1983). Autoxidation of LA and decomposition of LAHPO in the casein solution were measured (Fig. 1). A peak of PV appeared on the third day after the incubation in the LA solution without casein, while the peak appeared on the seventh day in the LA solution containing casein (Fig. 1A). The maximum value of PV was lower for the casein-containing solution than for the solution without casein. A peak of the TBA value also appeared earlier in the LA solution without casein than in the LA solution containing casein (Fig. 1B). The PV of the LAHPO solution with casein decreased more slowly than the PV of the solution without casein, and the TBA values of LAHPO were lower for the solution with casein than that without casein. Thus, casein acted antioxidatively for both the autoxidation of LA and the decomposition of LAHPO.

On the other hand, casein was shown to undergo drastic damage in the autoxidation of LA, including losses of amino acid residues (Yanagita et al., 1973). Kanazawa et al. (1975) reported that the reactivity of the autoxidation products to protein was in order of SP > LAHPO > LA. When 1.9 mg/ml SP was added to 0.5% casein solution and incubated at 37°C, part of the casein was polymerized on the fourth day, by analysis with SDS polyacrylamide gel electrophoresis, and 30% or more losses of basic amino acids and methionine were observed on the 7th day (data not shown). When incubated for 2 days, however, casein was not polymerized and the following losses of amino acids were observed: 35% methionine, 7% lysine, 5% histidine, aspartic acid and alanine, and no losses of the other amino acids. Eighty percent of the lost methionine was recovered as methionine sulfoxide. Casein was unlikely to undergo drastic damage during 2 days of incubation except for the oxidation of methionine. Therefore, in the following experiments, interactions of LA, LAHPO and SP with casein during the short period of incubation were evaluated.

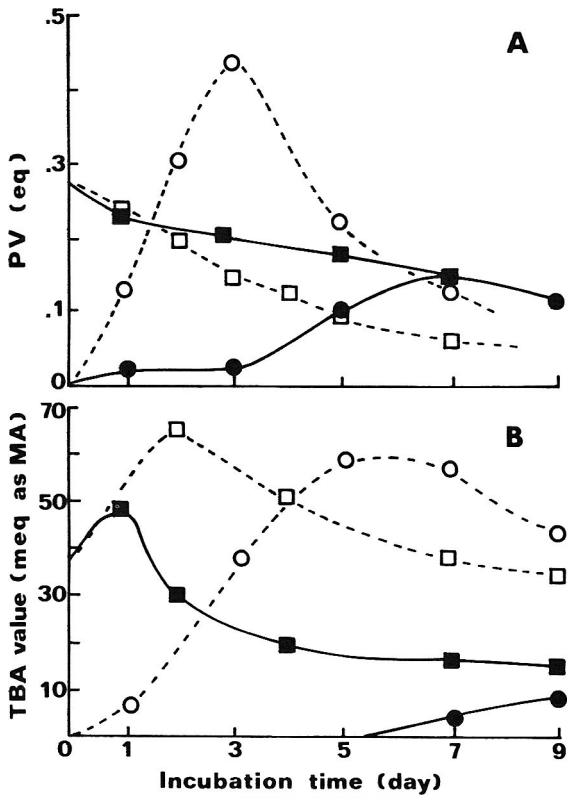

Fig. 1—Changes in peroxide value (A) and thiobarbituric acid (B) values of linoleic and linoleic acid hydroperoxide during the autoxidation. Linoleic acid with (—●—) and without (—○—) casein, linoleic acid hydroperoxide with (—■—) and without (—□—) casein.

Binding of the autoxidation products to casein

The rates of binding of radioactive autoxidation products to casein are shown in Fig. 2. When 5mM of LA or LAHPO were added to the casein solution, 15% LA or 30% LAHPO was bound to casein within 5 min after addition, and the amounts

Table 1—Amounts of radioactive linoleic acid and linoleic acid hydroperoxide and secondary autoxidation products bound to casein after 24 hr incubation

	Amounts (mg[a]/5mg of casein)	
	Added to casein	Bound to casein
Linoleic acid	0.8	0.2
	1.4	0.4
	4.5	1.0
	9.0	1.5
Linoleic acid hydroperoxide	0.9	0.5
	1.7	0.9
	3.7	1.5
	7.8	2.9
	9.9	3.6
Secondary autoxidation products	1.0	0.7
	2.1	1.5
	3.8	3.1
	7.1	5.7
	10.0	8.2
	38.0	25.5

[a] The amounts of autoxidation products were calculated from their radioactivities.

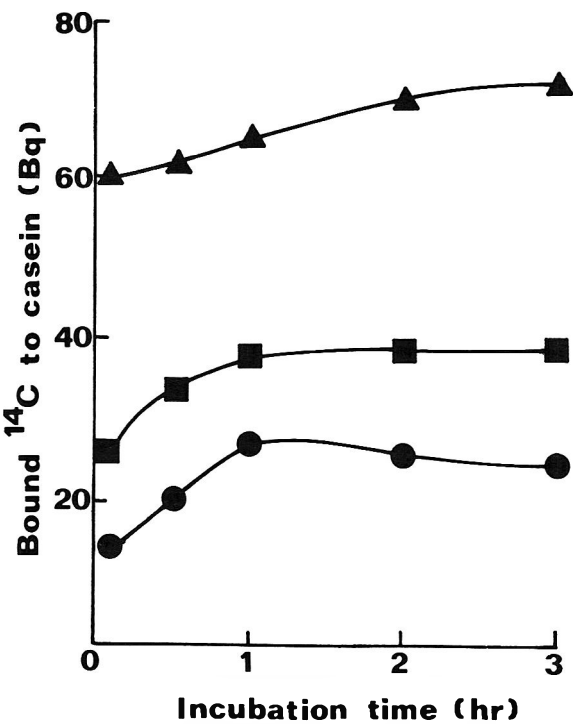

Fig. 2—Binding of radioactive linoleic acid (LA) (●), linoleic acid hydroperoxide (LAHPO) (■) and secondary autoxidation products (SP) (▲) to casein.

bound reached a maximum at 1 hr of incubation. SP was bound more readily than LA and LAHPO, and 75% of SP in the solution was bound within 5 min. Table 1 shows the amounts of these bound products after 24 hr incubation. While 25% of the added amount of LA was bound to 5 mg casein when 0.8~4.5 mg LA were added, 17% of the added LA (1.5 mg/5 mg casein) was bound when 9 mg LA (about 2-fold more than casein) was mixed with casein. Nielsen et al. (1985) reported that the amount of lipid bound to whey protein was 0.33 g/g protein. Fifty five percent of the added amount of LAHPO was bound to casein when 0.9 mg of LAHPO was added and 36% of LAHPO was bound when 9.9 mg of LAHPO was added. On the contrary, the amount of SP bound to casein increased linearly as the amount of added SP increased. When 38 mg SP (8-fold to casein) were added, 25 mg SP (5-fold) were bound to 5 mg casein. SP was composed of both hydrophobic (high molecular-weight polymers) and hyrophilic (low molecular-weight aldehydes or acids) components (Kanazawa et al., 1983). These components seemed to have easy access to both hydrophobic and hydrophilic positions in casein.

Changes in hydrophobicity of casein

Changes in hydrophobicity of casein by the autoxidation products were measured (Fig. 3). The incubation of casein with methanol as a control did not affect the fluorescence of casein. The incubation with LA slightly attenuated the fluorescent intensity of casein by ANSA (Fig. 3A) and enhanced that by TNS (Fig. 3B). LAHPO and SP apparently increased the fluorescent intensities by both probes, ANSA and TNS. Dissociation constants of casein with the probes were determined after the incubations of casein with the autoxidation products for 72 hr (Table 2). The dissociation constant of casein incubated with LA was the same as the constants of nonincubated and control casein with ANSA or TNS. LAHPO decreased the constant 10-fold with ANSA and 5-fold with TNS. SP decreased the constant 3-fold with ANSA or TNS. Thus, LAHPO and SP made easy the access of these probes to casein. LAHPO increased the hydrophobicity of casein more markedly than SP, contrary to the results of the radiochemical experiment. The molecular weight of LAHPO is lower than that of SP. When LAHPO was added LAHPO bound to the hydrophobic pockets, leaving more space for ANSA to approach in comparison to when SP was added.

Table 2—Dissociation constants of 8-anilino-naphthalenesulfonic acid ammonium salt (ANSA) or 2-p-toluidinylnaphthalene-6-sulfonic acid potassium salt (TNS) with casein incubated with the autoxidation products for 72 hr

	Dissociation constant ($\times 10^{-5}$M)	
	with ANSA	with TNS
Incubation with		
Linoleic acid	8.40	13.9
Linoleic acid hydroperoxide	0.73	2.46
Secondary autoxidation products	2.98	4.41
Methanol	12.9	9.80
Nonincubation	8.55	12.0

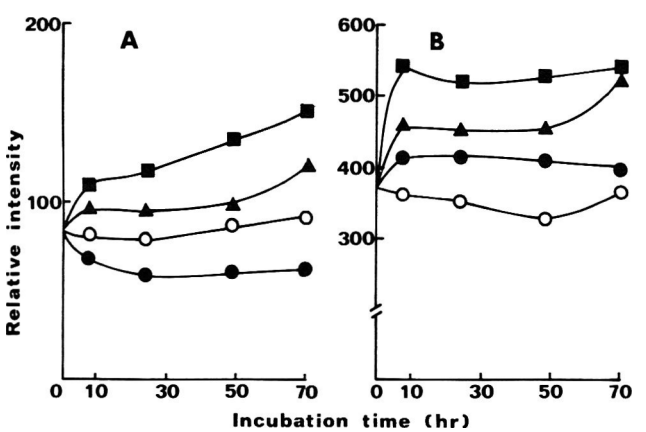

Fig. 3—Changes in fluorescent intensities of casein with ANSA (A) and TNS (B). Casein was incubated with linoleic acid (LA) (●), linoleic acid hydroperoxide (LAHPO) (■), secondary autoxidation products (SP) (▲) or methanol (○). The casein solutions were withdrawn at the indicated time, diluted 5 times and assayed.

Changes in solubility and digestibility

LAHPO and SP made casein insoluble during the incubation, but LA did not (Fig. 4). Incubation with LAHPO for 4 days decreased the solubility of casein by 60% and incubation with SP for 2 days decreased the solubility by 90%. These changes in the solubility of casein coincided with the decreases in digestibility of casein by digestive enzymes (Fig. 5). While the digestibility of casein by trypsin increased after incubation with LA, the digestibility readily decreased after incubation with LAHPO. SP acted more drastically on the digestibility than LAHPO. Similar results of digestibility were obtained with chymotrypsin and pepsin.

DISCUSSION

DAMAGE TO PROTEINS caused by peroxidizing lipids is well known, but the interactions between proteins and lipids prior to the damage are not understood. In a model system of LA and casein, interaction of LA with casein during the autoxidation process is suggested as follows.

LA was readily bound to casein. Small amounts of LAHPO were formed during the incubation, although the autoxidation of LA was suppressed by casein. LAHPO was easily bound to the hydrophobic pockets of casein. The decomposition of LAHPO was also delayed by casein, but SP was soon produced. SP was bound more easily to both the hydrophobic and hydrophilic positions on the surface of casein than LA and

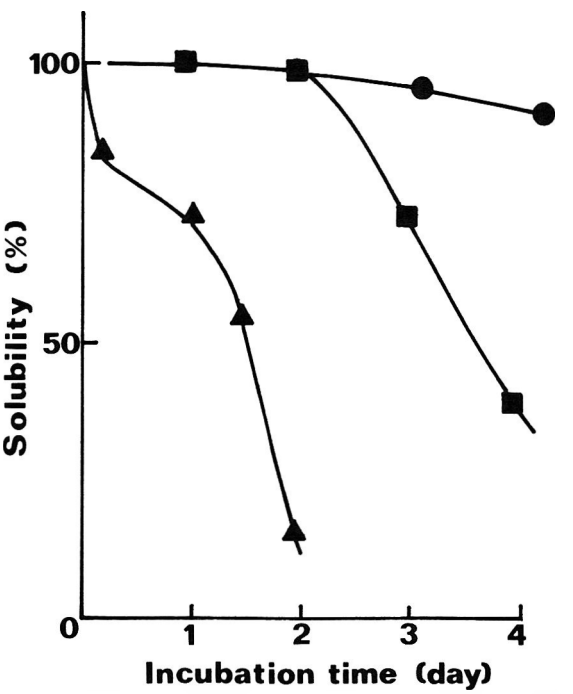

Fig. 4—Changes in solubility of casein during the incubations with linoleic acid (LA) (●), linoleic acid hydroperoxide (LAHPO) (■) and secondary autoxidation products (SP) (▲).

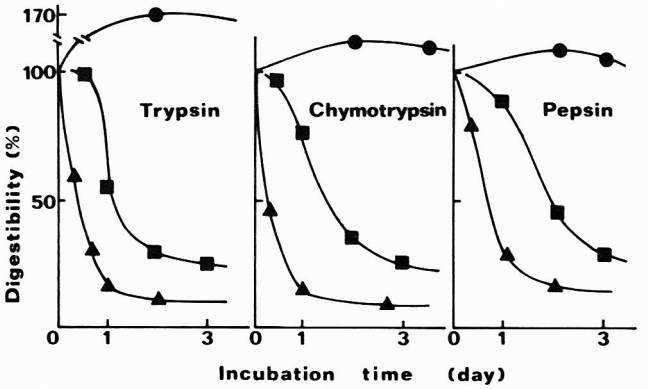

Fig. 5—Changes in the digestibility of casein by enzymes, during incubation with linoleic acid (LA) (●), linoleic acid hydroperoxide (LAHPO) (■) and secondary autoxidation products (SP) (▲).

LAHPO. SP might have produced rigid casein. Casein became insoluble, drastically decreasing its digestibility by enzymes. A part of casein was soon polymerized by SP and the methionine residue of casein was oxidized. Further, the basic amino acid residues were likely to be lost by formation of Schiff bases with aldehyde components of SP as shown by Malshet and Tappel (1973).

Thus, the severe deterioration of casein was caused by SP. One of the deleterious components contained in SP may be 9-oxononanoic acid (Kanazawa and Natake, 1986), which was a major aldehyde in the autoxidative degradation of LA. Another may be 4-hydroxynonenal (Esterbauer et al., 1982). Toxicities of these aldehydes have been elucidated in other biological systems (Minamoto et al., 1985).

REFERENCES

Andrews, F., Bjorksten, J., Trenk, F.B., Henick, A.S., and Koch, R.B. 1965. The reaction of an autoxidized lipid with proteins. J. Am. Oil Chem. Soc. 42: 779.

Asakawa, T. and Matsushita, S. 1980. Coloring conditions of thiobarbituric acid test for detecting lipid hydroperoxides. Lipids. 15:137.

Chang, K.C., Kendrick, J.G., Marshall, H.F., and Satterlee, L.D. 1985. Effect of partial methionine oxidation on the nutritional quality of soy isolate and casein. J. Food Sci. 50: 849.

Chiba, H., Doi, H., Yoshikawa, M., Sugimoto, E. 1976. Deterioration of casein components by malonaldehyde. Agric. Biol. Chem. 40: 1001.

Esterbauer, H., Cheeseman, K.H., Dianzani, M.U., Poli, G., and Slater, T.F. 1982. Separation and characterization of the aldehydic products of lipid peroxidation stimulated by ADP-Fe^{2+} in rat liver microsomes. Biochem. J. 208: 129.

Funes, J. and Karel, M. 1981. Free radical polymerization and lipid binding of lysozyme reacted with peroxidizing linoleic acid. Lipids. 16:347.

Kanazawa, K., Mori, T., and Matsushita, S. 1973. Oxygen absorption at the process of the degradation of linoleic acid hydroperoxides. J. Nutr. Sci. Vitaminol. 19: 263.

Kanazawa, K., Danno, G., and Natake, M. 1975a. Lysozyme damage caused by secondary degradation products during the autoxidation process of linoleic acid. J. Nutr. Sci. Vitaminol. 21: 373.

Kanazawa, K., Danno, G., and Natake, M. 1975b. Stabilization of linoleic acid at the process of autoxidation by potassium iodide. Agric. Biol. Chem. 39: 1177.

Kanazawa, K., Danno, G., and Natake, M. 1983. Some analytical observations of autoxidation products of linoleic acid and their thiobarbituric acid reactive substances. Agric. Biol. Chem. 47: 2035.

Kanazawa, K., Kanazawa, E., and Natake, M. 1985. Uptake of secondary autoxidation rpoducts of linoleic acid by the rat. Lipids. 20: 412.

Kanazawa, K. and Natake, M. 1986. Identifications of 9-oxononanoic acid and hexanal in liver of rat orally administered with secondary autoxidation products of linoleic acid. Agric. Biol. Chem. 50: 115.

Kanner, J. and Karel, M. 1976. Changes in lysozyme due to reactions with peroxidizing methyl linoleate in a dehydrated model system. J. Agric. Food Chem. 24: 468.

Labuza, T.P. and Ragnarsson, J.O. 1985. Kinetic history effect on lipid oxidation of methyl linoleate in a model system. J. Food Sci. 50: 145.

Lineweaver, H. and Burk, D. 1934. The determination of enzyme dissociation contents. J. Am. Chem. Soc. 56: 658.

Malshet, V.G. and Tappel, A.L. 1973. Fluorescent products of lipid peroxidation: 1. Structural requirement for fluorescence in conjugated Schiff bases. Lipids. 8: 194.

Minamoto, S., Kanazawa, K., Ashida, H., Danno, G., and Natake, M. 1985. The induction of lipid peroxidation in rat liver by oral intake of 9-oxononanoic acid contained in autoxidized linoleic acid. Agric. Biol. Chem. 49: 2747.

Nielsen, H. 1979. Reaction between peroxidized phospholipid and protein: II. Molecular weight and phosphorous content of albumin after reaction with peroxidized cardiolipin. Lipids. 14: 900.

Nielsen, H.K., Löliger, J., and Hurrell, R.F. 1985. Reactions of proteins with oxidizing lipids. 1. Analytical measurements of lipid oxidation and of amino acid losses in a whey protein-methyl linolenate model system. Brit. J. Nutr. 53: 61.

Roubal, W.T. and Tappel, A.L. 1966. Damage to proteins, enzymes, and amino acids by peroxidizing lipids. Arch. Biochem. Biophys. 113: 5.

Schaich, K.M. and Karel, M. 1975. Free radicals in lysozyme reacted with peroxidizing methyl linoleate. J. Food Sci. 40: 456.

Shimada, K. and Matsushita, S. 1978. Polymerization of soybean 11S globulin due to reactions with peroxidizing linoleic acid. Agric. Biol. Chem. 42: 781.

Sully, B.D. 1954. A modified iodimetric determination of organic peroxides. Analyst. 79: 86.

Yanagita, T., Sugano, M., Cho, S., and Wada, M. 1973. Changes in available lysine and in vitro digestibility of casein accompanied with oxidation of ethyl linoleate. Nogei Kagaku Kaishi. 47: 73.

Yemm, E.W. and Cocking, E.C. 1955. Determination of amino acids with ninhydrin. Analyst. 80: 209.

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A Research Note

Presence and Stability of Patulin in Pasteurized Apple Cider

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ABSTRACT

Pasteurized apple cider produced in Georgia was surveyed for patulin. Levels from 244–3993 μg patulin/L cider were found. Eight high temperature-short time (HTST) treatments (60°, 70°, 80°, and 90°C for 10 sec; 90°C for 20, 40, 80, and 160 sec) and one batch treatment (90°C for 10 min) were used to determine the stability of patulin in pasteurized cider. The 60°, 80°, and 90°C HTST treatments and the batch pasteurization significantly reduced the patulin level, but did not completely destroy the toxin. Storage of the cider had no effect on the patulin level.

INTRODUCTION

SEVERAL DIFFERENT SPECIES of *Penicillium, Aspergillus* and *Byssochlamys*, common spoilage microorganisms of fruits, are capable of producing patulin (Stott and Bullerman, 1975). Patulin, a highly reactive-unsaturated 5 membered ring lactone (Busby and Wogan, 1981), is overtly toxic to animals such as rats, cats, rabbits, and mice. Along with the toxic responses demonstrated, carcinogenic activity has also been observed with sublethal doses (Stott and Bullerman, 1975).

Patulin contamination of apple cider can occur if apples supporting patulin producing molds are included in the starting material during cider production (Scott, 1984). This toxin has been found in apples and apple products available to consumers in several investigations. Apple cider samples surveyed both in Wisconsin (Brackett and Marth, 1979) and in the Washington, DC area (Ware et al., 1974) were found to contain patulin. Wilson and Nuovo (1973) reported patulin contamination of "organic apple cider" up to 45,000 μg patulin/L. In "sweet apple cider" (unfermented apple juice), Scott et al. (1972) found 1 μg patulin/L.

In chemical stability studies, Scott and Somers (1968) reported a 15% reduction of patulin in fresh apple juice stored at 22°C for 1 wk, and a 55% reduction in canned apple juice stored at 22°C for 5 wk. Lovett and Peeler (1973) demonstrated patulin to be resistant to thermal destruction at a pH range of 3.5–5.5 when heated up to 125°C. Destruction times were observed to increase at lower pH levels, thus establishing that patulin is more stable in acidic solutions.

Apple products (e.g. apple cider) are produced and sold within Georgia where an extensive screening of patulin contamination of these products is lacking. The purpose of this study was to screen pasteurized apple cider samples from Middle and North Georgia for patulin; to quantitate patulin in contaminated batches; and to determine the effects of thermal treatments on patulin stability.

MATERIALS & METHODS

Survey of pasteurized apple cider

Samples of pasteurized apple cider were obtained from roadside stands or directly from producers in Middle and North Georgia. Fifty milliliters apple cider were extractd with three 50 mL portions ethyl acetate. The combined extracts were filtered through anhydrous sodium sulfate (Sigma, St. Louis, MO) and evaporated to dryness with a Buchi rotovapor R110 (Brinkman, Westburg, NJ). The residue was dissovled in 100 μL ethyl acetate. Thin-layer chromatography was carried out on 20 × 20 cm, 0.25 mm layers of Silica Gel 60 (EM Science, Cincinatti, OH) which were developed in toluene-ethyl acetate-88% formic acid (5:4:1, v/v/v). Plates were sprayed with 4% aqueous phenyl-hydrazine hydrochloride (Sigma, St. Louis, MO) and heated at 105°C for 2–3 min. Patulin levels in the cider samples were estimated visually by comparing intensities of the yellow spots with standards at the same R_f-value (0.44). The lowest detectable level of patulin by this method was determined to be 200 μg. All solvents were obtained from Fisher (Springfield, NJ).

Percent recovery of patulin from apple cider was determined by adding a solution of patulin in ethyl acetate (2.5 μg/uL) to the cider in concentrations ranging from 250–750 μg/50 mL. Apple cider used in this analysis was previously determined to contain no patulin. Duplicate samples of spiked juices were extracted and chromatographed as previously described. The levels of patulin in the cider samples reported were corrected for the average percent recovery of 88.9%.

Pasteurization treatment

Approximately 150–250 mL portions of patulin-containing apple cider (approx. 20 μg patulin/mL cider) were treated as described. Four different temperature treatments (60°, 70°, 80°, and 90°C were used in conjunction with a high temperature-short time pasteurization unit (Morgan, 1985). A 163.8 cm holding tube with an inner diameter of 0.13 cm was used. The flow rate was adjusted to 12.42 mL/min to obtain a holding time of 10 sec. Additional samples were pasteurized utilizing the HTST pasteurization unit at 90°C with holding times of 20, 40, 80 and 160 sec. The holding tube described above, with adjustments of the flow rates, was used for the 20, 40 and 80 sec holding times, while a 77.5 cm tube with an inner diameter of 0.13 cm was used for the 160 sec holding time. Two 250 mL portions of the patulin-containing cider were given a batch pasteurization treatment by maintaining each portion at 90°C for 10 min. One of these portions was analyzed for patulin immediately after treatment while the second was stored at 20–22°C for one month before analysis. Two portions received no heat treatment with one analyzed for patulin on the day of preparation, while the second portion was analyzed after storage at 20–22°C for one month. Three replications of each treatment were done.

Two 100 mL portions from each treatment were extracted and chromatographed by TLC as described above. Patulin appeared as yellow spots with a R_f value of 0.44. Spots were scraped from the plates and reconstituted in 5 mL of 95% ethyl alcohol. The sample was mixed for 1.5 min and centrifuged at 3000 × *g*. The supernatant fluid was removed and was reconstituted into a total of 6 mL. A Hewlett Packard 8451A diode array spectrophotometer (Fort Collins, CO) was used to measure the absorbance of patulin at 420 nm. Standard analysis of variance and Duncan's multiple range test were performed using SAS (1985).

RESULTS & DISCUSSION

ALL CIDER SAMPLES obtained from Middle and North Georgia producers were found to contain patulin (Table 1). Previously, Brackett and Marth (1979) reported 58% of the apple juice samples in Wisconsin contained patulin with an average of 50.7 μg/L. In 1974, Ware et al. determined that 61% of the apple cider samples analyzed in the Washington D.C. area contained 44-309 μg patulin/L. However, our re-

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Table 1—Patulin levels in pasteurized apple cider[a]

Sample	μg Patulin/L apple cider
A	3993
B	244
C	662
D	3112
E	1500
Average	1902

[a] Values corrected for percent recovery.

Table 2—Patulin concentration in apple cider processed by various thermal treatments

Pasteurization			
Temp (°C)	Time	μg Patulin/mL apple cider[a]	% Reduction[b]
—	0 sec	11.5[c]	0
70	10 sec	10.6[c,d]	7
60	10 sec	10.0[d,e]	12
90	10 min	9.8[d,e]	15
80	10 sec	9.7[d,e]	15
90	10 sec	9.3[e]	19

[a] Means of six determinations on each of three samples.
[b] % reduced compared to the unheated samples.
[c,d,e] Means with the same letter are not significantly different ($p > 0.05$).

sults differed in that a higher patulin range of 244–3990 μg/L with an average of 1902 μg/L was observed.

The excessive level at which patulin was found in pasteurized apple cider in this study warranted additional studies into the parameters of its destruction during normal processing treatments. A batch-style pasturization treatment similar to that used by local processors and various HTST treatments were used to evaluate the thermal stability of patulin.

The 60°, 80°, and 90°C HTST (10 sec) and the 90°C batch pasteurization (10 min) treatments resulted in a significant reduction in the patulin concentration (Table 2). Increasing the holding time of the 90°C HTST had no significant effect on decreasing the patulin concentration; although the 90°C HTST (10 sec) pasteurization treatments resulted in an 18.8% reduction in patulin concentration, the thermal processing was not sufficient to insure a product free of the toxin. In a prior study, Lovett and Peeler (1973) reported a 90% reduction of patulin in an aqueous solution (pH 3.5) heated to 125°C and held for 268 min. Locally produced Georgia apple cider is not subjected to thermal processing parameters as severe as those described by these researchers.

Storage of the cider samples for one month at 22°C had no effect on the patulin concentration. These results conflict with the findings of Scott and Somers (1968) who reported an 11% reduction in the patulin level of canned apple juice stored at 32°C for three weeks.

Our results show a high incidence and varying range of patulin contamination in pasteurized apple cider from Middle and North Georgia. In Georgia, this appears to be a variable situation not only between processors but also between batches from one processor. Our results also indicate that thermal processing can reduce the level of patulin in cider, but processes which may be used are insufficient to totally eliminate the toxin from the product. Storage of patulin-contaminated cider did not result in a decrease in toxin levels.

REFERENCES

Brackett, R.E. and Marth, E.H. 1979. Patulin in apple juice from roadside stands in Wisconsin. J. Food Prot. 42: 863.
Busby, Jr., W.F. and Wogan, G.N. 1981. Patulin and penicillic acid. In "Mycotoxins and N-Nitroso Compounds: Environmental Risks," Vol. 2. CRC Press, Inc., Boca Raton, FL.
Lovett, J. and Peeler, J.T. 1973. Effect of pH on the thermal destruction kinetics of patulin in aqueous solution. J. Food Sci. 38: 1094.
Morgan, J.N. 1985. Optimization of human milk treatment. Ph.D. dissertation, Univ. of Georgia, Athens, GA.
SAS Institute Inc. 1985. SAS User's Guide: Statistics, Version 5 ed. SAS Institute Inc., Cary, NC.
Scott, P.M. 1984. Effects of food processing on mycotoxins. J. Food Prot. 47: 489.
Scott, P.M., Miles, W.F., Toft, P., and Dube, J.G. 1972. Occurrence of patulin in apple juice. J. Agric. Food Chem. 20: 450.
Scott, P.M. and Somers, E. 1968. Stability of patulin and penicillic acid in fruit juices and flour. J. Agric. Food Chem. 16: 483.
Stott, W.T. and Bullerman, L.B. 1975. Patulin: a mycotoxin of potential concern in foods. J. Milk Food Tech. 38(11): 695.
Ware, G.M., Thorpe, C.W., and Pohland, A.E. 1974. Liquid chromatographic method for determination of patulin in apple juice. J. Assoc. Offic. Anal. Chem. 57(5): 1111.
Wilson, P.M. and Nuovo, G.J. 1973. Patulin production in apples decayed by *Penicillium expansum*. Appl. Microbiol. 26: 124.
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A Research Note

Debittering of Citrus Juices Using Supercritical Carbon Dioxide

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ABSTRACT

Supercritical carbon dioxide was found to be effective in removing limonin, a primary bitter component in citrus juices, from reconstituted early season California Washington navel concentrate. Using pressures between 3000–6000 psi and temperatures between 30–60°C an average reduction of 25% in limonin contents was found in 91 sixty-minute runs. Four-hour runs debittered the juice reducing the limonin content from approximately 17.5 ppm to approximately 7.0 ppm with negligible effect on the ascorbic acid, pulp, titratable acidity as citric acid, and total amino acids. Oil levels dropped slightly but can be easily replenished in commercial operation without violation of the federal standards of identity.

INTRODUCTION

ONE OF THE MAJOR problems in the citrus industry is the occurrence of natural bitter triterpenoids, primarily in the form of limonin, that generally diminish the quality of citrus products. There have been several methods investigated in order to reduce this bitter effect (Anonymous, 1973; Guadagni et al., 1976; Hasegawa and Maier, 1983; Shaw and Wilson, 1983; Orme and Hasegawa, 1986; Chandler and Johnson, 1979; Maeda et al., 1984) but every method poses problems for industrial use in the United States. Even though a knowledge of supercritical fluids has been around for over 100 years, application on a commercial scale has been achieved only recently with the decaffeination of coffee in Europe (Zosel, 1978) and the extraction of hops in Australia (Hubert and Vitzthim, 1978). The fact that some compounds dramatically increase their solubilizing ability under supercritical conditions renders supercritical extraction as an attractive means of selectively removing undesireable compounds from complex mixtures that would be difficult to remove using more conventional methods. In food processing, the ideal supercritical fluid is carbon dioxide which is nontoxic, nonflammable, nonexplosive, readily and economically available, and has a low critical temperature and pressure which is important in industrial scale up and to the heat sensitivity of foods compared to petroleum and mineral applications.

The purpose of this study was to investigate the use of supercritical carbon dioxide in removing limonin from citrus juices.

MATERIALS & METHODS

Supercritical extraction

Supercritical extraction was performed using a SCE screening unit (Autoclave Engineers, Erie, PA) incorporating a 300 mL liquid extraction vessel and a syphon CO_2 cylinder. The feed line from the CO_2 cylinder to the pump was cooled using a Forma Scientific 2095 bath/circulator attached by flex tubing to a 1-inch diameter glass tube stopped at both ends and surrounding the line. This allowed the refrigerant from the bath/circulator to circulate through the glass tubing and around the line in order to keep the infeed CO_2 in a liquid state to facilitate pumping. In addition to this refrigeration jacket on the infeed line, the pump head was cooled with ice. The unit provided for the measurement and control of pressure, temperature, flow rate of CO_2 as well as the total cubic feet of atmospheric CO_2 used. Approximately 150 mls of reconstituted Washington navel concentrate was used for each run. Since our labs have shown that only 20–80% of the limonin develops during processing, the juice samples were heated until no further limonin development occurred for purposes of the test. Without this induced limonin development, decreases in limonin from extraction were difficult to distinguish from increases due to limonin development from its natural precurser after each 60-minute run. In order to assimilate industrial conditions, four-hour runs were performed using reconstituted navel concentrate that had not been heated.

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Sample analyses

Limonin was determined as previously reported (Kimball, 1984). Brix (% soluble solids), % acid, % oil, % pulp, ascorbic acid, and total amino acids (formol test) were performed using standard methods (Praschan, 1976).

RESULTS & DISCUSSION

NINETY-ONE 60-min runs were performed using temperatures between 30–60°C and pressures between 3000–6000 psi. Limonin was reduced an average of 25% from an initial level of 17.5 ppm. An analysis of variance revealed that temperature had no significant effect on the limonin reduction at the 5% level but pressure was highly significant at the 1% level. A dramatic increase in the limonin extraction occurred between 40°C and 45°C at 3000 psi with the analysis of variance also indicating a significantly higher limonin reduction at higher temperatures and pressures at the 5% level.

In order to estimate the rate of limonin extraction, the time of the runs was varied and the reduction in limonin versus the time of the run is plotted in Fig. 1.

In an attempt to assimilate industrial conditions in totally debittering the juice down to the taste threshold of approximately 7 ppm, four-hour runs were performed at three representative temperatures and pressures. The samples used were tested before and after each run for limonin, ascorbic acid, citrus oil, pulp, total acid as citric acid, and amino acids with the results illustrated in Table 1. As can be seen, the limonin level was reduced to its taste threshold while the levels of ascorbic acid, pulp, total acid as citric acid, and amino acids remained essentially unchanged. Oil levels decreased slightly but the oil content in commercial juices can be replenished without violating the standards of identity for pure citrus juices.

When the CO_2 is depressurized after passing through the juice, its solubilizing ability is lost and the solute or solutes are deposited in a collection vessel in the SCE unit. Inspection of this deposit revealed traces of a dark brownish orange liquid that contained small amounts of d-limonene, the main component of citrus oils. This deposit represented a negligible amount of mass compared to the juice sample and it took several runs before enough was collected to even see. This extractant had an intensely bitter taste and washings of the collection vessel revealed high levels of limonin. The limonin-rich deposit may show promise as an antifeedant for certain agricultural pests,

Table 1—Juice characteristics before and after 4 hr of supercritical CO_2 treatment[a]

PRESSURE (psi)		3500	4000	5500
TEMPERATURE (°C)		30.2 ± 0.4	40.2 ± 0.3	40.0 ± 0.0
CO_2 used (ft^3)		29.329 ± 1.187	28.027 ± 2.128	24.910 ± 0.830
Limonin (ppm)	Before	17.6 ± 0.8	17.4 ± 0.4	17.5 ± 0.4
	After	7.6 ± 0.2	6.9 ± 0.5	7.0 ± 0.6
Vitamin C[b] (mg/100 mL)	Before	59.61 ± 2.68	60.89 ± 6.75	58.31 ± 5.51
	After	59.57 ± 1.44	60.40 ± 6.49	57.28 ± 4.71
Oil (% v/v)	Before	0.009 ± 0.001	0.010 ± 0.002	0.009 ± 0.001
	After	0.007 ± 0.002	0.007 ± 0.002	0.006 ± 0.001
Pulp[b] (% v/v)	Before	8.7 ± 0.4	8.9 ± 0.0	8.9 ± 0.0
	After	8.6 ± 0.4	9.0 ± 0.9	8.6 ± 0.5
Acid (% w/w)	Before	0.95 ± 0.03	0.96 ± 0.02	0.95 ± 0.01
	After	0.98 ± 0.03	0.96 ± 0.04	0.98 ± 0.05
Amino acids (meq/100 mL)	Before	1.65 ± 0.07	1.66 ± 0.05	1.70 ± 0.05
	After	1.64 ± 0.05	1.59 ± 0.10	1.69 ± 0.08

[a] Data represent averages of 6 replicates with standard deviations.
[b] Data represent averages of 3 replicates with standard deviations.

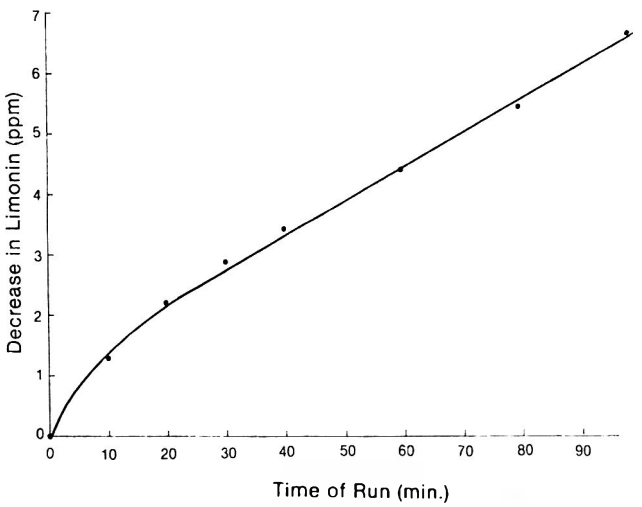

Fig. 1—Reduction of limonin from 15.3 ppm versus time of run at a pressure of 4500 psi and a temperature of 50°C.

including cotton worms, and may become an environmentally safe alternative to pesticides (Klocke and Kubo, 1982).

The cost of industrial scale-up of the process described in this work is largely unknown since no commercial supercritical plants involving extraction from a liquid exist in the world. It is estimated, however, that the cost for the high pressure equipment and controls would be a major disadvantage of the method. As the price of equipment comes down and greater extraction efficiences emerge, this process may become more competitive with other debittering methods presently under investigation.

REFERENCES

Anonymous 1973. Debittering citrus juices. Citrograph 58: 403.
Chandler, B.V. and Johnson, R.L. 1979. New sorbent gel forms of cellulose esters for debittering citrus juices. J. Sci Food Agric. 30: 825.
Guadagni, D.G., Maier, V.P., and Turnbaugh, J.G. 1976. Effect of neodiosmium on threshold and bitterness of limonin in water and orange juice. J. Food Sci. 41: 681.
Hasegawa, S. and Maier, V.P. 1983. Solutions to the limonin bitterness problem of citrus juices. Food Technol. 37: 73.
Hubert, P. and Vitzthim, O.G. 1978. Angew. Chem. Int. Ed. 17: 710.
Kimball, D.A. 1984. Factors affecting the rate of maturation of citrus fruits. Proc. Fla. State Hort. Soc. 97: 40.
Klocke, J.A. and Kubo, I. 1982. Citrus limonoid by-products as insect control agents. Ent. Exp. and Appl. 32: 299.
Maeda, H., Takahashi, Y., Miyake, M., and Ifuku, Y. 1984. Removal of bitterness and reduction of acidity in Hassaku (*Citrus hassaku* hort. ex Tanaka) juice with ion exchange resins and adsorbents. Nippon Shokuhin Kogyo Gakkaishi 31: 413.
Orme, E.D. and Hasegawa, S. 1986. Inhibition of limonoin accumulation in Citrus limon fruit by naphthaleneacetic acid. J. Agric. Food Chem. (In press).
Praschan, V.C. 1976. "Quality Control Manual for Citrus Processing Plants." Intercit, Inc., Safety Harbor, FL.
Shaw, P.E. and Wilson, C.W. 1983. Debittering citrus juices with β-cyclodextrin polymer. J. Food Sci. 48: 646.
Zosel, K. 1978. Agnew. Chem. Int. Ed. 17: 702.
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A Research Note

The Proteins of Muscadine Grapes

OLUSOLA LAMIKANRA

ABSTRACT

The concentrations, molecular weights and isoelectric points of proteins present in muscadine grape cultivars were determined. The total protein contents of these grapes, are consistent with values reported in literature for *V. vinifera* grape proteins. Molecular weights of most of the protein fractions were, however, relatively high when compared with those of other grape cultivars. Isoelectric points of muscadine proteins ranged between pH 5.6 and pH 7.6.

INTRODUCTION

GRAPE JUICE and wine turbidity can result from the presence of several types of suspended materials. Of these, proteins and polypeptides appear to be most critical, in that they greatly affect the stability of these beverages. In white wines, they are second only to bitartrate instabilities as the most common non-microbial defect (Boulton, 1980).

The ability of proteins to cause clouding in wines and juices depends on the properties of their molecules, and cannot be correlated with the total amount present in solution. An important property that influences clarity and stability of wines is the protein isoelectric point, since this represents the pH value at which the molecule is least soluble (Boulton, 1980). Isoelectric properties of proteins affect not only their natural tendency to precipitate, but also the affinity of such molecules to various adsorbents with some ion exchange capacity, which are commonly referred to as fining or settling agents.

Several reports have been published on the nature of must and wine proteins for grape cultivars (Bayly and Berg, 1967; Anelli, 1977; Somers and Ziemelis, 1973). Wine proteins are essentially a mixture of grape proteins and proteins from autolyzed yeast. Bayly and Berg (1967) were, however, able to demonstrate that the proteins that are responsible for wine clouding originate from grapes.

While a number of studies have been carried out on the total nitrogen and free amino acid concentrations for muscadine (*Vitis rotundifolia* Michx.) grapes (Marcy et al., 1981; Saunders and Takeda, 1981), the nature of their proteins has never been reported. The objective of this study was therefore to separate and characterize muscadine grape proteins based on their molecular weights and isoelectric points.

MATERIALS & METHODS

Juice extraction

Freshly picked Carlos, Welder and Higgins muscadine grapes were washed, and separately blended in a laboratory blender. Juice from these were separated from their pomace by centrifugation at 2,000 × *g* for 10 min. Analyses on the extracted juice for pH, titratable acidity as tartaric acid by titration with sodium hydroxide (0.1M), and soluble solids with the aid of a refractometer were then carried out. The protein contents of the juices were also determined by phosphomolybdic acid precipitation and the biuret test (Amerine and Ough, 1980).

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Protein extraction

Grape proteins were precipitated by dissolving ammonium sulfate (80%) in the juice (100 mL). The solution obtained was left for 15 h at 8 °C, after which it was centrifuged at 20,000 × *g* for 20 min at 4 °C. Tris buffer (pH 8.2; 0.1M; 3ml) containing dissolved sodium chloride (5%) was added to the precipitate, and thoroughly mixed to form a fine suspension. Undissolved materials were removed by centrifugation at 20,000 × *g*. The solution obtained was then dialyzed on a membrane with molecular weight cutoff range between 6,000 and 8,000 daltons for 24 hr.

Separation and analysis of proteins

Two dimensional gel electrophoresis, using isoelectric focussing, followed by SDS-polyacrylamide gel electrophoresis was carried out on replicated protein extracts as described by Garrels (1979, 1983). The first dimension isoelectric focusing gels contained pH 3-10 ampholytes and 12.5% acrylamide slab gels were employed in the second dimension. Gels were stained with silver nitrate solutions. Calibration for isoelectric points and molecular weights was carried out by running a blend of standards with known molecular weights and isoionic points (REF-52) with the grape protein extracts.

RESULTS & DISCUSSION

THE CHEMICAL CHARACTERISTICS of grapes used are shown in Table 1. Welder grape juice had a protein concentration that was higher than that of the Carlos and Higgins juices. The amount of protein obtained for these grape cultivars (13.34, 7.95 and 3.27 mg/100ml, respectively) are consistent with those found in many *V. vinifera* grape cultivars (Bayly and Berg, 1967), but lower than those reported for *V. labrusca* grape varieties by Kluba et al., (1978). Variations in the protein concentrations, as observed in these muscadine cultivars are, however, common. Bayly and Berg (1967) reported a range between 2.0 to 26.0 mg/100 mL for *V. vinifera* grapes while Kluba et al., (1978) determined protein concentrations between 97.8 and 170 mg/100 mL on *V. labrusca* grape cultivars. Two dimensional gel electrophoresis showed about 20 protein fractions in Welder juice, 10 for the Carlos and 7 for Higgins (Table 2). In all cases, protein bands with molecular weights of 32,000 and 24,000 daltons were observed, with the more intense fractions having molecular weights of 32,000 daltons. Proteins with higher molecular weights (49,000, 58,000 and 100,000 daltons) were also detected in the Welder juice (Table 2). The Higgins grapes showed one fraction with a molecular weight of 14,000 daltons. The isoelectric points for the grape proteins ranged from 5.6 to 7.25 in the Carlos, 5.6 to 6.3 in Higgins and 5.6 to 7.6 in the Welder grape cultivar. No protein band was observed when electrophoresis runs, using acidic ampholytes (pH 2.5–4.0) was carried out.

Table 1—Characteristics of muscadine cultivars used for analyses

Cultivar	pH	°Brix	Total acidity, as tartaric (%)	Total protein (mg/100mL)
Welder	3.32	18.0	0.75	13.34
Carlos	3.14	14.0	0.70	7.95
Higgins	3.19	10.2	0.67	3.27

Table 2—Characteristics of protein fractions separated from Welder, Higgins and Carlos muscadine grapes

Fraction no.	pH	Mol wt (daltons)	Fraction no.	pH	Mol wt (daltons)
		WELDER			
1	6.30	100,000	11	5.60	32,000
2	6.35	100,000	12	5.70	32,000
3	6.40	100,000	13	5.80	32,000
4	6.90	100,000	14	6.30	32,000
5	7.00	58,000	15	6.35	32,000
6	7.50	58,000	16	6.40	32,000
7	7.60	58,000	17	6.90	32,000
8	7.00	49,000	18	6.40	24,000
9	7.50	49,000	19	6.60	24,000
10	7.60	49,000	20	6.90	24,000
		CARLOS			
1	5.60	32,000	6	7.25	32,000
2	5.70	32,000	7	6.40	24,000
3	5.80	32,000	8	6.60	24,000
4	6.40	32,000	9	6.90	24,000
5	6.90	32,000	10	7.25	24,000
		HIGGINS			
1	5.60	32,000	5	6.60	32,000
2	5.70	32,000	6	6.67	24,000
3	5.80	32,000	7	5.70	14,000
4	6.47	32,000			

The occurrence of grape proteins with molecular weights over 30,000 daltons, as was observed in these muscadine grape cultivars, are not common. The values reported for non-muscadine grape cultivars is between 10,000–28,000 daltons. (Moreti and Berg, 1965; Bayly and Berg, 1967; Yokotsuka et al., 1977; Boulton, 1980). A minimum of four Welder fractions had molecular weights around 100,000 daltons. Differences also exist between the values of isoelectric points for muscadine grapes and the non muscadine cultivars. Most grape cultivars have proteins with isoelectric points of pH 2.5 to 8.7 (Moreti and Berg, 1965; Bayly and Berg, 1967; Anelli, 1977; Ough and Anelli, 1979; Boulton, 1980). The lowest isoionic point value that was observed for the two muscadine cultivars was 5.6. Some of the differences observed between the muscadine grape proteins and those of other cultivars that were earlier reported may be due to differences in the techniques used for their separation and staining of gels. Two dimensional gel electrophoresis is expected to result in a better resolution of the proteins, and the use of silver nitrate for protein detection is usually a more sensitive stain for detecting protein fractions that are present in small quantities. The isoionic properties of the protein fractions indicate that muscadine grape protein pH is usually much higher than that of its corresponding must and wine pH values, which commonly falls between 2.75 to 3.25 (Carroll et al., 1978; Carroll and Marcy, 1982). It, therefore, suggests that muscadine proteins will be more readily removed from such solutions by negatively charged fining agents such as bentonite and (−) kieselsol, since these protein fractions will carry considerable positive charges in such low pH media. However, since proteins are not the only colloids that affect clarity and stability of musts and wines, these fining agents may not necessarily be suitable for removing all suspended materials in muscadine grape products. More work is required to determine the extent of protein involvement in muscadine wine and juice stability, and to identify the less stable fractions that might be responsible for this lack of stability, if contributions from proteins are found to be significant.

REFERENCES

Anelli, G. 1977. The proteins of musts. Am. J. Enol. Vitic. 28: 200.

Amerine, M.A. and Ough, C.S. 1980. "Methods for Analysis of Musts and Wines". p. 169. Wiley-Interscience, New York.

Bayly, F.C. and Berg, H.W. 1967. Grape and wine proteins of white wine varietals. Am. J. Enol. Vitic. 24: 18.

Boulton, R. 1980. The nature of wine proteins. Proc. Wine Indust. Tech. Seminar 6: 67.

Carroll, D.E., Ballinger, W.E., McClure, W.F., and Nesbitt, W.B., 1978. Wine quality versus ripeness of light sorted Carlos muscadine grapes. Am. J. Enol. Vitic. 29: 169.

Carroll, D.E. and Marcy, J.E. 1982. Chemical and physical changes during maturation of muscadine grapes (*Vitis rotundifolia*). Am. J. Enol. Vitic. 33: 168.

Garrels, J.I. 1979. Two-dimensional gel electrophoresis and computer analysis of proteins synthesized by clonal cell lines. J. Biol. Chem. 254: 7961.

Garrels, J.I. 1983. Quantitative two dimensional gel electrophoresis of proteins. Meth. Enzym. 100: 411.

Kluba, R.M., Mattick, L.R., and Hackler, L.R. 1978. Changes in the free and total amino acid composition of several *Vitis labruscana* grape varieties during maturation. Am. J. Enol. Vitic. 29: 102.

Marcy, J.E., Carroll, D.E., and Young, C.T. 1981. Changes in free amino acid and total nitrogen concentrations during maturation of muscadine grapes (*V. rotundifolia*). J. Food Sci. 46: 543.

Moreti, R.H., Berg, H.W. 1965. Variability among wines to protein clouding. Am. J. Enol. Vitic. 16: 69.

Ough, C.S. and Anelli, G. 1979. Zinfandel grape juice protein fractions and their amino acid makeup as affected by crop level. Am. J. Enol. Vitic. 30: 8.

Saunders, M.S. and Takeda, F. 1981. Chemical analysis of pomace from muscadines for potential by-product useage. Hortscience 16: 281.

Somers, T.C. and Ziemelis, G. 1973. Direct determination of wine proteins. Am. J. Enol. Vitic. 24: 47.

Yokotsuka, K., Yoshii, M., Aihara, T., and Kushida, T. 1977. Isolation and characterization of proteins from juices, musts and wines from Japanese grapes. J. Ferment. Technol. 55: 510.

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A Research Note

Thermal Degradation Kinetics of Prickly-Pear-Fruit Red Pigment

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ABSTRACT

The color stability of betacyanine from prickly pear was determined in the fruit juice at temperatures up to 90 °C. The degradation rates were dependent on pigment concentration, being slower for higher concentrations. The lack of a significant oxygen effect or inhibitory action of ascorbic acid did not support autooxidation as the exclusive chemical process responsible for decolorization.

INTRODUCTION

THE UNCERTAIN FUTURE of synthetic red pigments for food has intensified the interest in natural alternatives. The plants belonging to the order of Centrospermae contain the attractive red-violet betacyanins in addition to the yellow betaxanthins (Mabry, 1966; Mabry and Dreiding, 1968; Piatteli, 1976). Among them, colorants containing pigments of the red beet are manufactured commercially and their properties have been investigated extensively (von Elbe et al., 1974a, b; Savolainen and Kunsi, 1978; Saguy et al., 1978; Driver and Francis, 1979; Pasch and von Elbe, 1979; Saguy, 1979).

The need for varieties suitable for rocky and arid lands prompted us to evaluate the agroeconomical aspects of the prickly pear (Rosenthal et al., 1985). The prickly pear is the fruit of the genus *Opuntia* belonging to the Cactaceae family. The fruit-yielding *Opuntia* genus is classified into several species. One of them, *O. ficus-indica* Mill (*O. gymnocarpa* Web.), grows wild over large areas in Israel. The Cactaceae family belongs to the order Centrospermae (Mabry et al., 1963), a group of families whose color is attributed to the presence of betalain pigments.

Botanically, the highly gastronomically-appreciated prickly pear is a many seeded berry, consisting of a thick, violet pericarp with a number of clefts of small prickles enclosing a red-violet or orange-yellow, sweet, luscious pulp, intermixed with a number of small, black, shiny seeds. The pear-shaped fruit is 3–4 cm in diameter and 5–7 cm in length and weighs approximately 30–70g. The intense red-violet color of this fruit suggests its possible use as a source of natural food pigment. In contradistinction to the red beet pigments, this color has been much less investigated, and there are no reports of its components. The structural analysis of indicaxanthin, the yellow betaxanthin from a species of *O. ficus-indica*, has been reported (Piatteli et al., 1964).

The objective of this study was to evaluate the kinetics and stability of a red pigment, obtained from prickly pear, for its potential use as a natural food colorant.

MATERIALS & METHODS

PRICKLY PEAR JUICE was obtained by homogenizing whole fruits in a Waring Blendor, straining, pasteurizing and precipitating the gum mucilage with six volumes of ethanol. The ethanolic solution of the dye was filtered and evaporated under reduced pressure at 40°C. The solid residue was freeze-dried and kept in an air tight container at −18°C.

The visible spectra were recorded with a Cary 210 spectrophotometer. Ten milliliters aqueous stock solution of dye were heated in sealed vials in a thermostatically controlled bath to the experiment temperature (50°, 70°, 90 °C) as measured by a thermometer immersed in a vial with the same dye solution. After the thermometer reading reached the appropriate temperature, the vials (in duplicate) were held at the given temperature for the given time and then were removed periodically and cooled immediately in an ice bath. Subsequently, the absorption spectra of the solution were recorded, 1 hr after the last vial was removed from the water bath. The data for thermal deterioration of the pigment in aqueous solution were collected for two concentrations which differed by a factor of 10, as established from records of the absorption at λ_{max}. The absorption spectra of these two sets of experiments were recorded in 1 cm and 0.1 cm optical path-cells to enable a direct comparison of the spectra obtained for the two concentrations.

Linear regression analyses of the kinetic data were performed with a Macintosh computer using Stat-works program.

RESULTS & DISCUSSION

WHEN SOLUTIONS of prickly pear pigment were heated, the color (λ_{max} = 535 nm) gradually disappeared, yielding a new compound (λ_{max} 415 nm) through an isosbestic point at 456 nm (Fig. 1a). The kinetic analysis of the data obtained indicated that the discoloration for both concentrations obeyed a pseudo-first-order reaction pattern (Fig. 1b). Regression analysis and correlation cooficients were calculated and the regression lines are plotted on Fig. 1b. Calculated r(°C) and standard deviation (SD °C) for the 1 cm cell are: r_{50} = 0.953; SD_{50} = 3.04 × 10^{-4}; r_{70} = 0.994, SD_{70} = 1.63 × 10^{-4} and r_{90} = 0.993, SD_{90} = 1.17 × 10^{-3}; and for the 0.1 cm cell r_{50} = 0.999, SD_{50} = 1.02 × 10^{-5}; r_{70} = 0.979, SD_{70} = 2.83 × 10^{-4} and r_{90} = 0 .987, SD_{90} = 8.81 × 10^{-4}. Table I sum-

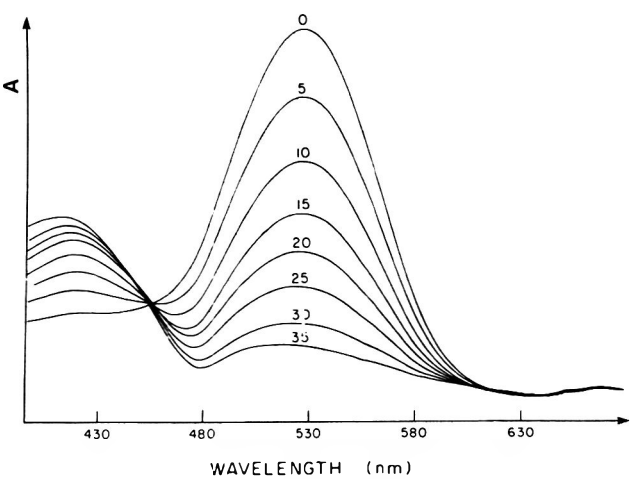

Fig. 1a—Visible absorption set of spectra for the thermodegraded prickly pear pigment at 90°C. The exposure times as given for each curve are in minutes (A - absorbance).

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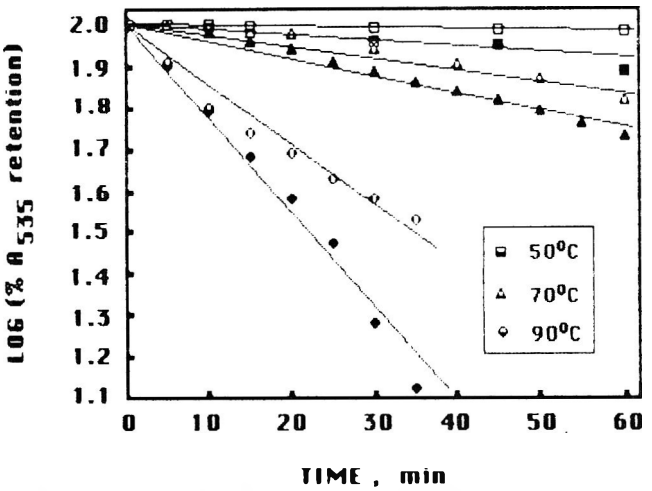

Fig. 1b—Extent of pigment loss $\frac{A_{535} \text{ at time } t}{A_{535} \text{ at time } 0} \times 100$ *as measured in 1 cm cell for a diluted color solution (■, ▲, ♦) and in 0.1 cm cell for concentrated color solution (□, △, ◇) as a function of time.*

Table 1—Rate constants and half-life times of thermodegraded prickly pear pigments

	Diluted[a]		Concentrated	
Temp °C	$k_1 \times 10^3$ $(min)^{-1}$	$T_{1/2}$ (min)	$k_1 \times 10^3$ $(min)^{-1}$	$T_{1/2}$ (min)
50	3.80	182.4	0.7	990.0
70	10.76	64.4	6.9	100.4
90	57.11	12.1	29.9	23.2

[a] Diluted solution was ten times more dilute than the concentrated solution as estimated from absorbance measurements. The spectra measurements were obtained in 1.0 cm and 0.1 cm optical-path cells, respectively.

marizes the temperature-related rate constants and half-life values (Labuza and Kamman, 1983) for chromophore degradation. The results indicated that the color was more stable in the more concentrated solution. A concentration effect was also observed in the deterioration of crude anthocyanins (Mishkin and Saguy, 1982).

The presence of an isosbestic point on the discoloration curves (Fig. 1a) indicates that the colorless product is formed directly from the pigment as a result of only one chemical reaction. Activation energies were computed using Arrhenius equation. The values obtained for the two different pigment concentrations are very close: E_a = 7.72 kcal/mole (diluted solution) and E_a = 10.73 kcal/mole (concentrated solution). These values are substantially lower than the 19.2 kcal/mole obtained for red beet betanine degradation by Saguy (1979), but similar to the value of 10 kcal/mole as reported by von Elbe et al. (1974b) for beet juice. These differences might be due to incomplete pigment regeneration after the heat treatment.

The presence of oxygen has only a marginal effect on the thermostability of the dye. Thus, the chromophore loss after 1 hr heating at 70°C was 60.6% and 53.2% in air- and nitrogen-saturated solutions, respectively. Similarly, only a slight inhibition of the discoloration reaction was observed when the thermal stability test at 70°C of the diluted solution of the pigment was performed in the presence of 0.1% ascorbic acid. Thus, the rate constant in this case was 9.9×10^{-3} min^{-1} as compared with 10.76×10^{-3} min^{-1} in the absence of ascorbic acid. It should be noted that before the test, the pH of the pigment solution with ascorbic acid was adjusted with NaOH to the value of the ascorbic acid-free solution, that is, 4.35. These observations suggested that autooxidation was not the major chemical mechanism responsible for degradation. The dye was most stable in solution at around pH 4, where about 50% of its absorbance was lost after 4 months of storage at 4°C.

Finally, the color in solution was unchanged within ± 10%, over a pH range of 2.35 to 7.9, in regard with λ_{max} = 535 nm, and absorbance even after 4 months of cold storage. A bathochromic shift to λ_{max} = 545 nm was observed at pH = 10.

REFERENCES

Driver, M.G. and Francis, F.J. 1979. Stability of phytolaccanin, betanine and FD&C red #2 in dessert gels. J. Food Sci. 44: 518.
Labuza, T.P. and Kamman, J.F. 1983. Reaction kinetics and accelerated tests simulation as a function of temperature. In "Computer-Aided Techniques in Food Technology," (Ed.) I. Saguy, p. 71. Marcel Dekker, Inc., New York.
Mabry, T.J. 1966. The betacyanins and betaxanthins. In "Comparative Phytochemistry," (Ed.) T. Swain, p. 231. Academic Press, New York.
Mabry, T. J. and Dreiding, A.S. 1968. The betalains. In "Recent Advances in Phytochemistry," (Ed). R.E. Alston and V.C. Runeckles, Vol. 1, p. 145. Appleton, New York.
Mabry, T.J., Taylor, A., and Turner, B.L. 1963. The betacyanines and their distribution. Phytochemistry 2: 61.
Mishkin, M. and Saguy, I. 1982. Thermal stability of pomegranate juice. Z. Lebensm. Untersuch. Forsch. 175: 410.
Pasch, J.H. and von Elbe, J.H. 1979. Betanine stability in buffered solutions containing organic acids, metal cations, antioxidants, or sequestrants. J. Food Sci. 44: 72.
Piatelli, M. 1976. Betalains. In "Chemistry and Biochemistry of Plant Pigments," (Ed.) T.W. Goodwin, Vol. 1, p. 560. Academic Press, New York.
Piatelli, M., Minale, L., and Prota, G. 1964. Isolation, structure and absolute configuration of indicaxanthin. Tetrahedron 20: 2325.
Rosenthal, I., Merin, U., Bernstein, S., and Popel, G. 1985. Technological evaluation of the prickly pear cultivar "Ofer". Hassadeh 65: 1416.
Saguy, I. 1979. Thermostability of red beet pigments (betanine and vulgaxanthin-I): influence of pH and temperature. J. Food Sci. 44: 1554.
Saguy, I., Kopelman, I.J., and Mizrahi, S. 1978. Thermal kinetics degradation of betanine and betalamic acid. J. Agric. Food Chem. 26: 360.
Savolainen, K. and Kunsi, T., 1978. The stability properties of golden beet and red beet pigments: influence of pH, temperature, and some stabilizers. Z. Lebensm. Untersuch. Forsch. 166: 19.
von Elbe, J.H., Klement, J.T., Amundson, C.H., Cassens, R.G., and Lindsay, R.C. 1974a. Evaluation of betanine pigments as sausage colorants. J. Food Sci. 39: 128.
von Elbe, J.H., Maing, I.Y., and Amundson, C.H. 1974b. Color stability of betanine. J. Food Sci. 39: 334.
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A Research Note

Fermentation Characteristics of Lactobacilli in Okra (*Hibiscus esculentus*) Juice

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ABSTRACT

Okra juice was fermented with nine strains of homofermentative and ten strains of heterofermentative lactobacilli as well as one commercial culture to study the metabolic activities of these cultures in an effort to select organisms for use in okra fermentation. During a 24 hr fermentation one *Lactobacillus cellobiosus* strain converted 29.7% of the mucilage, utilized 85.7% of the reducing sugars and 90.3% of the sucrose decreasing the pH value to 3.65. During the same period seven other strains converted from 20.2–26.8% of the mucilage, 78–98% of the reducing sugars and 75–80% of the sucrose giving a pH value between 3.45 and 4.23. All strains showed considerable variation in the ability to produce lactic acid, acetic acid and ethanol, and they all possessed low levels of lactate dehydrogenase activity.

INTRODUCTION

FERMENTATION of okra (*Hibiscus esculentus*) before canning is used for product acidification (pH < 4.5) to minimize thermal processing thus maintaining the natural color and removing a substantial amount of mucilage, which is undesirable to the consumer (Roukas and Kotzekidou, 1986).

The use of pure cultures of homo- and heterofermentative lactic acid bacteria in vegetable fermentations has been thoroughly reviewed by Fleming and McFeeters (1981). Homofermentative organisms catabolize glucose by the glucolytic pathway almost completely to lactic acid (Thornhill and Cogan, 1984) while heterofermenters participate in mixed fermentations yielding lactic acid, ethanol, acetic acid, glycerol, mannitol and CO_2 (Stamer, 1979). While acid production by pure cultures has been extensively studied (Etchells et al., 1975), there is little information available on the quantitative substrate and product changes caused by lactic acid bacteria in complex media like foods. Chen et al. (1983) reported on the fermentation characteristics of heterolactic acid bacteria in green bean juice. This is nearly the only systematic attempt to study the fermentation characteristics of some heterofermenters on a vegetable product.

The objective of this study was to determine the fermentation characteristics of homo- and heterofermentative lactobacilli in okra juice.

MATERIALS & METHODS

Preparation of okra juice

Fresh okra (cultivar Pylea No 1) was used in this study. After removing the seeds, okra was chopped and blended with five times its weight of water. The blend was centrifuged at 4,000 × *g* for 15 min and dry salt was added to the viscous supernatant solution at a proportion of 2.5% (w/v). Then, 50 mL aliquots were dispensed into large test tubes (25 × 200 mm). The tubes were intermittently sterilized as described by Seeley and VanDemark (1972). The effectiveness of sterilization was checked by examining (noninoculated) tubes after incubation at 30°C for 48 hr. None of these tubes supported growth.

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Strains studied

The majority of lactobacilli tested were isolated from okra (Kotzekidou and Roukas, 1986). These cultures were maintained at 3°C in MRS broth (de Man et al., 1960; E. Merck, Darmstadt, W. Germany) and were subcultured every two months.

Homo- and heterofermentative organisms were distinguished by measuring gas production from glucose as suggested by Gibson and Abd-el-Malek (1945) using modified MRS agar (Hitchener et al., 1982).

Each of the strains was inoculated in MRS broth and incubated at 30°C for 12 hr. The cultures were centrifuged at 3,000 × *g* for 10 min and the pellet of cells was suspended in sterile saline solution 5% (w/v) to obtain an absorbance equal to point 2 of the McFarland Scale (Paule, 1971). These culture solutions were inoculated in okra juice in proportion 1% (v/v). The commercially available starter culture "Vege Start" (Chr. Hansen's Laboratorium A/S, Copenhagen, Denmark) was handled according to the instructions of the manufacturer.

The inoculated tubes were incubated for 24 hr and 48 hr at 30°C under anaerobic conditions (BBL Gas Pak, H_2 + CO_2 anaerobic system, Becton Dickinson and Co., Cockeysville, MD). Each strain was inoculated into duplicate tubes with okra juice. After fermentation, these tubes were analyzed separately.

Determination of mucilage, reducing sugars, sucrose, organic acids, ethanol and pH

The amount of mucilage contained in okra juice was determined by precipitation with ethanol as described by Woolfe et al. (1977). Reducing sugars were measured using the method suggested by Nelson (1944) with glucose as colorimetric standard. Sucrose was determined after inversion using the method suggested by Nelson (1944). L- and D-Lactic acids were determined enzymatically by reduction of NAD to NADH, using LDH and D-LDH, respectively (Gutmann and Wahlefeld, 1974; Gawehn and Bergmeyer, 1974). Acetic acid was determined also enzymatically with acetate kinase and hydroxylamine (Holz and Bergmeyer, 1974). Ethanol was measured using the method described by van Gent and Kerrich (1965). Special chemicals and enzymes for these assays were obtained from Sigma (Sigma Chemie GmbH, Taufkirchen, W. Germany). All other chemicals were reagent grade (Merck, Darmstadt, W. Germany). pH measurements were made by a Knick 646 pH meter equipped with a glass electrode.

LDH activity

LDH activity was measured in cultures grown on MRS broth at 30°C for 18 hr using the method suggested by Bergmeyer and Bernt (1974). Before measurements the cultures were sonicated by 22 amplitudes for 25 min in a Soniprep 150 Ultrasonic Disintegrator (MSE, England, U.K.).

RESULTS & DISCUSSION

THE PRODUCTS of fermentation are presented in Table 1. Some of the *Lactobacillus cellobiosus* strains and the *Lactobacillus brevis* strain produced no D-lactic acid after 24 hr, but they did produce acid after 48 hr fermentation. Two *Lactobacillus plantarum* strains produced acetic acid in addition to lactic acid, while one strain produced lactic acid and ethanol.

Table 1—pH changes and lactic acid, acetic acid and ethanol production by lactobacilli during fermentation of okra juice

Strain		24 hr L-Lactic acid (mM)	24 hr D-Lactic acid (mM)	24 hr Acetic acid (mM)	24 hr Ethanol (mM)	24 hr pH[d]	48 hr L-Lactic acid (mM)	48 hr D-Lactic acid (mM)	48 hr Acetic acid (mM)	48 hr Ethanol (mM)	48 hr pH[d]
L.cellobiosus	2421	3.76	0	0	1.20	4.18	10.43	0.30	0.50	2.90	4.00
L.cellobiosus	2427	3.89	1.85	0.5	0.80	4.21	6.85	4.74	3.00	1.90	4.19
L.cellobiosus	2414	4.20	4.38	0.5	1.26	4.23	7.78	5.70	1.45	3.20	4.10
L.cellobiosus	0001[a]	5.83	1.70	0.3	0.75	4.12	11.71	3.02	0.58	1.85	4.00
L.cellobiosus	4811	4.51	0	0.8	1.10	4.28	7.56	0.10	1.72	3.00	4.20
L.cellobiosus	4822	14.86	0	0.7	3.29	3.56	32.97	0.34	1.35	5.60	3.40
L.cellobiosus	2417	16.72	3.90	0.9	0.55	3.60	31.65	9.50	2.20	1.60	3.52
L.cellobiosus	3624	12.20	4.80	0.46	0.35	3.50	29.26	10.38	2.70	1.28	3.40
L.cellobiosus	2420	14.51	4.49	0.60	0.25	3.65	28.73	11.49	3.90	1.30	3.52
L.brevis × 20[b]		5.90	0	0.55	0	4.23	13.75	2.90	2.95	0.10	4.10
L.plantarum	3621	16.28	4.65	0	0	3.45	33.94	10.62	0	0	3.40
L.plantarum	4812	24.31	4.58	0.46	0	3.45	34.21	15.58	7.2	0	3.35
L.plantarum	4818	22.76	15.10	0	0.10	3.48	28.99	10.23	0	0.50	3.35
L.plantarum	4819	23.65	8.47	0	0	3.58	33.59	11.03	0	0	3.46
L.plantarum	4826	12.96	2.92	0	0	3.60	35.05	12.92	0.3	0	3.48
L.plantarum	4827	11.23	2.50	0	0	3.56	27.85	7.89	0	0	3.49
L.plantarum	1227	23.65	5.16	0	0	3.58	34.03	7.75	0	0	3.47
"Vege Start"[c]		19.85	8.23	0	0	3.46	35.09	37.21	0	0	3.20
L.salivarius subsp. salicinius	0018[a]	16.05	0	0	0	3.60	30.98	0	0	0	3.50
L.salivarius subsp. salicinius	2407	16.94	0	0	0	3.56	30.10	0	0	0	3.44

[a] Cultures isolated from okra fruits. All other cultures were isolated from fermented okra.
[b] Culture obtained from National Institute for Research in Dairying, Reading, UK.
[c] Commercial culture, obtained from Ch. Hansens Laboratorium A/S, Copenhagen, Denmark.
[d] Okra juice had an initial pH value of 5.80.

Table 2—Reducing sugar, sucrose and mucilage utilization by lactobacilli in okra juice[a]. Lactate dehydrogenase (LDH) activity in cell extracts of lactobacilli grown on MRS broth for 18 hr

Strain		Reducing sugar utilization (%) 24 hr	Reducing sugar utilization (%) 48 hr	Sucrose utilization (%) 24 hr	Sucrose utilization (%) 48 hr	Mucilage conversion (%) 24 hr	Mucilage conversion (%) 48 hr	LDH volume activity (U/L)
L.cellobiosus	2421	63.5	75.6	80.1	95.3	15.5	20.7	30.88
L.cellobiosus	2427	60.2	70.7	84.3	96.5	18.7	21.4	6.18
L.cellobiosus	2414	56.9	77.0	81.6	96.7	22.0	24.7	77.21
L.cellobiosus	0001	65.8	79.2	82.4	98.3	4.6	6.7	123.53
L.cellobiosus	4811	57.5	69.6	70.5	89.7	24.1	28.3	67.94
L.cellobiosus	4822	33.1	93.5	70.1	95.4	16.7	28.0	12.35
L.cellobiosus	2417	72.4	80.2	85.6	90.6	10.3	17.4	6.18
L.cellobiosus	3624	48.0	91.2	86.7	93.6	24.0	31.4	15.44
L.cellobiosus	2420	85.7	90.7	90.3	100.0	29.7	35.4	12.35
L.brevis × 20		97.9	99.7	80.1	88.8	23.5	29.5	98.83
L.plantarum	3621	90.2	95.7	80.3	87.8	22.0	35.6	142.06
L.plantarum	4812	84.6	91.7	75.8	89.3	20.9	25.2	15.44
L.plantarum	4818	81.3	91.5	71.3	80.4	10.1	13.3	15.44
L.plantarum	4819	78.0	91.2	78.6	86.7	26.8	33.5	21.62
L.plantarum	4826	82.4	92.3	75.8	87.6	22.8	24.8	18.53
L.plantarum	4827	52.4	76.9	60.5	69.5	22.0	33.5	18.53
L.plantarum	1227	59.1	95.8	68.4	73.6	17.9	19.5	15.44
"Vege Start"		78.0	99.6	75.6	85.3	20.2	30.4	—[b]
L.salivarius subsp. salicinius	0018	85.7	92.4	60.6	74.5	18.3	29.1	21.62
L.salivarius subsp. salicinius	2407	89.0	95.5	75.8	89.8	20.4	35.8	37.06

[a] Okra juice initially contained 410 mg/100 mL reducing sugars, 736.4 mg/100 mL sucrose and 923.3 mg/100 mL mucilage.
[b] Not determined.

Table 1 indicates that there are differences in the amount of L- and D-lactic acid produced by the different strains. These variations may be due to strain differences in L-LDH, D-LDH and racemase content (Nordal and Slinde, 1980).

As presented in Table 1 for any given strain the pH did not change substantially in the period between 24 hr and 48 hr of fermentation. In all fermentations the final pH was lower than 4.28.

L. cellobiosus strains converted more than 70% of the sucrose present in okra juice (Table 2). *L. cellobiosus* 2420 after 48 hr of fermentation converted 91% of the reducing sugars and 100% of the sucrose. Seven of the homofermentative strains after 24 hr of fermentation converted 78 to 90% of the reducing sugars and 71 to 80% of the sucrose in the substrate. Complete removal of fermentable carbohydrates precludes a possible secondary fermentation by lactic acid bacteria and yeasts according to the observations made by Fleming et al. (1983).

Both homo- and heterofermentative cultures fermented okra mucilage, a polysaccharide composed of galacturonic acid, galactose, rhamnose and glucose in a mole ratio 1.3:1.0:0.1:0.1 (Woolfe et al., 1977). *L. cellobiosus* 2420 after 24 hr of fermentation converted approximately 30% of the mucilage. After 48 hr fermentation with the strains *L. cellobiosus* 2420, *L. plantarum* 3621 and *Lactobacillus salivarius* subsp. *salicinius* 2407, the converted mucilage in the fermentation juice reached approximately 35% of the initial content. During fermentation with *L. brevis* × 20 the great decrease in sugars was not followed by a similar decrease in mucilage. The decrease in both mucilage and sugars of okra juice during fermentation varied with the strain and with the age of the culture.

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A Research Note

Effect of Temperature and Period of Fermentation on Protein and Starch Digestibility (*In vitro*) of *Rabadi*— A Pearl Millet Fermented Food

NEERJA DHANKHER and B.M. CHAUHAN

ABSTRACT

Rabadi was prepared by allowing pearl millet flour-country buttermilk mixture to ferment for three different periods (3, 6 and 9 hr) at four different temperatures (35°, 40°, 45°, and 50°C). As fermentation time increased, protein as well as starch digestibility (*in vitro*) of *rabadi* increased significantly at all the temperatures. Maximum increase in the digestibility of both protein (51%) and starch (58%) occurred after 9 hr fermentation at 45°C.

INTRODUCTION

PEARL MILLET (*Pennisetum typhoides* Rich.) constitutes a major source of protein and calories in the diet of millions of the Indian population. Protein digestibility of pearl millet like that of other plant foods is low (Chauhan et al., 1986). Starch, a major component of carbohydrates in pearl millet, is relatively more resistant to attack by pancreatic amylase (Sullins and Rooney, 1977). Fermentation has been known to improve protein digestibility (Kao and Robinson, 1978; Boralkar and Reddy, 1985) as well as starch digestibility (Boralkar and Reddy, 1985) of food grains.

Rabadi is a traditional fermented food consumed regularly by people in the northwestern pearl millet producing region of India. It is prepared by mixing pearl millet whole flour with country buttermilk and then fermenting the mixutre for 4–6 hr at 35–45°C (Dhankher, 1985). The present study reports the effect of different periods (3, 6 and 9 hr) and temperatures (35°, 40°, 45°, and 50°C) of fermentation on protein and starch digestibility (*in vitro*) of *rabadi*.

MATERIALS & METHODS

PEARL MILLET whole grains, procured from the local market, were ground to fine powder (0.5 mm particle size) with an electric grinder (SEW, New Delhi, India). For preparing *rabadi*, the flour (150g) was mixed with buttermilk 600 mL) and made to a homogenous mixture by stirring. The mixture, contained in a conical flask, was allowed to ferment for three time periods (3, 6 and 9 hr) at four different temperatures (35°, 40°, 45°, and 50°C) in a BOD incubator (Scientronic Instruments, New Delhi, India). The fermented product was oven dried at 70°C to a constant weight, milled to pass through 0.5 mm sieve in a Cyclotec sample mill (Tecator, Höganäs, Sweden) and stored until required for further analysis. The flour-buttermilk mixture, dried without carrying out any fermentation, formed the control group.

Country buttermilk, the source of inoculum for lactic acid fermentation, was procured from a nearby rural household. It was bought fresh early in the morning on the day of *rabadi* preparation and was used immediately. At the time of mixing the buttermilk had a pH of 4.0.

Protein digestibility (*in vitro*) of dried sample of *rabadi* was determined by employing pepsin and pancreatin (Akeson and Stahman, 1964). Nitrogen content of the sample and of the undigestible residue was determined by the micro-Kjeldahl method (AOAC, 1980).

$$\text{Protein digestibility (\%)} = \frac{\text{Digestible protein}}{\text{Total protein}} \times 100$$

Pancreatic amylase was employed for assessing *in vitro* starch digestibility (Singh et al., 1982). The maltose so liberated was measured colorimetrically by using dinitrosalicylic acid reagent.

The data were processed for analysis of variance (Panse and Sukhatme, 1961) to test the significant differences among treatments.

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RESULTS & DISCUSSION

Protein digestibility

A gradual increase in protein digestibility of pearl millet flour-buttermilk mixture was observed at all the temperatures as the duration of fermentation increased (Table 1). During 3, 6 and 9 hr of fermentation the increase in the digestibility ranged from 9–23%, 25–33%, and 40–51%, respectively. Protein digestibility was highest when *rabadi* was fermented for 9 hr at 45°C. Microflora may produce some proteolytic enzymes during fermentation (Hesseltine, 1983) which may be responsibile for increased protein digestibility of *rabadi*. In addition, antinutrients like phytate and polyphenols present in cereals that affect protein digestibility adversely (Tan et al., 1984; Knuckles et al., 1985) may also decrease during fermentation (Lopez et al., 1983; Dhankher, 1985). Boralkar and

Table 1—Effect of temperature and period of fermentation on in vitro *digestibility of protein (%) and starch (mg maltose release/100 mg* rabadi*) of* rabadi *(on dry matter basis)*[a]

Period of fermentation (hr)	35°C		40°C		45°C		50°C	
	Protein digestibility	Starch digestibility	Protein digestibility	Starch digestibility	Protein digestibility	Starch digestibility	Protein digestibility	Starch digestibility
0	55.6 ± 0.6	14.6 ± 0.2	55.0 ± 0.8	14.4 ± 0.4	55.1 ± 0.6	14.4 ± 0.1	56.3 ± 0.8	14.6 ± 0.2
3	68.1 ± 0.6 (14)	16.5 ± 0.2 (13)	65.6 ± 0.8 (18)	17.0 ± 0.1 (18)	60.9 ± 1.9 (9)	15.6 ± 0.1 (8)	69.1 ± 0.9 (23)	18.4 ± 0.2 (26)
6	70.1 ± 0.5 (27)	18.0 ± 0.1 (23)	73.5 ± 1.0 (33)	19.4 ± 0.3 (35)	69.4 ± 0.3 (25)	19.2 ± 0.0 (33)	72.3 ± 0.6 (29)	19.4 ± 0.2 (33)
9	81.8 ± 0.3 (47)	21.1 ± 0.4 (44)	77.4 ± 0.1 (40)	21.6 ± 0.2 (50)	83.4 ± 0.7 (51)	22.8 ± 0.7 (58)	80.8 ± 0.9 (43)	21.9 ± 0.1 (50)
CD[b](p<0.05)	2.6	0.2						

[a] Values are means ± S.D. of four independent determinations. The figures in parentheses indicate increase in the digestibility expressed as per cent of control values.

[b] Critical difference at 5% level. Differences of two means within/between the temperature treatment exceeding this value are significant.

Reddy (1985) reported that fermentation of soybean batter for 8, 12, and 16 hr increased protein digestibility (*in vitro*) from 58% to 80%, 84%, and 86%, respectively.

Starch digestibility

Rabadi had better starch digestibility (*in vitro*) than the initial fermentation mixture. Digestibility increased gradually at all the temperatures as the time of fermentation was raised. Nine hour fermentation gave the highest starch digestibility; at 35°, 40°, 45°, and 50°C, it increased by 44%, 50%, 58%, and 50%, respectively. Possible breakdown of starch to oligosaccharides may be partly responsible for the improvement in starch digestibility during fermentation (Cronk et al., 1977). Levels of phytate and polyphenols in cereal foods, which inhibit amylase (Thompson and Yoon, 1984), are decreased during fermentation (Lopez et al., 1983, Dhankher, 1985). This may also contribute to better starch digestibility of fermented products. Boralkar and Reddy (1985) reported a significant increase in starch digestibility of soybean batter during fermentation.

Overall, *rabadi* had higher protein and starch digestibility than the starting pearl millet flour-buttermilk mixture. Improvement was most pronounced when fermentation occurred at 45°C for 9 hr. The traditinal method of preparation of *rabadi* offers unique nutritional advantages of not only improving the amino acid profile of pearl millet but also of making the starch and the resultant protein more digestible.

REFERENCES

Akeson, W.F. and Stahman, M.A. 1964. A pepsin-pancreatin digest index of protein quality evaluation. J. Nutr. 83: 257.

AOAC. 1980. "Official Methods of Analysis," 13th ed. Association of Official Analytical Chemists, Washington, DC.

Boralkar, M. and Reddy, N.S. 1985. Effect of roasting, germination and fermentation on the digestibility of starch and protein present in soybean. Nutr. Rep. Int. 31: 833.

Chauhan, B.M. Suneja, N., and Bhat, C.M. 1986. Nutritional value and fatty acid composition of some high yielding varieties of bajra. Bull. Grain Technol. 24: 44.

Cronk, T.C., Steinkraus, K.H., Hackler, L.R. and Mattick, L.R. 1977. Indonesian tape ketan fermentation. Appl. Environ. Microbiol. 33: 1067.

Dhankher, N. 1985. Studies on the nutritional value of *rabadi*-a fermented bajra product. M.Sc thesis, Haryana Agricultural Univ., Hisar, India.

Hesseltine, C.W. 1983. The future of fermented foods. Nutr. Rev. 41: 293.

Kao, C. and Robinson, R.J. 1978. Nutritional aspects of fermented foods from chickpea, horsebean and soybean. Cereal Chem. 55: 512.

Knuckles, B.E., Kuzmicky, D.D., and Betschart, A.A. 1985. Effect of phytate and partially hydrolysed phytate on *in vitro* protein digestibility. J. Food Sci. 50: 1080.

Lopez, Y., Gordon, D.T. and Fields, M.L. 1983. Release of P from phytate by natural fermentation. J. Food Sci. 48: 9553.

Panse, V.G. and Sukhatme, P.V. 1961. "Statistical Methods for Agricultural Workers", 2nd ed. Indian Council of Agricultural Res., New Delhi.

Singh, U., Kherdekar, M.S., and Jambunathan, R. 1982. Studies on Desi and Kabuli chickpea cultivars. The levels of amylase inhibitors, level of oligosaccharides and *in vitro* starch digestibility. J. Food Sci. 47: 510.

Sullins, L.W. and Rooney, R.D. 1977. In "Proc. Symp. on Sorghum and Millets for Human Food." D.A.V. Dendy (Ed), p. 91. Tropical Products Institute, London.

Tan, N. Wong, K., and de Lumen, B.O. 1984. Relationship of tannin levels and trypsin inhibitor activity with *in vitro* protein digestibility of raw and heat-treated winged bean. J. Agric. Food Chem. 32: 819.

Thompson, L.U. and Yoon, J.H. 1984. Starch digestibility as affected by polyphenol and phytic acid. J. Food Sci. 49: 1228.

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All strains possessed low levels of LDH activity. Despite the fact that some strains showed the same LDH activity (Table 2), they produced different amounts of lactic acid.

The desirable okra fermentation characteristics include: rapid pH decrease (to maintain natural color), mucilage conversion (to improve consumer acceptance) and reducing sugar depletion (to preclude secondary fermentation). Based on the above desirable characteristics among the strains used in this study, *L. cellobiosus* 2420 gave the best fermentation characteristics decreasing the pH to 3.65 and converting 30% of the mucilage, 86% of the reducing sugars and 90% of the sucrose.

REFERENCES

Bergmeyer, H.U. and Bernt, E. 1974. UV-assay with pyruvate and NADH. In "Methods of Enzymatic Analysis," H.U. Bergmeyer (Ed.), p. 574. Academic Press, New York.

Chen, K.H., McFeeters, R.F., and Fleming, H.P. 1983. Fermentation characteristics of heterolactic acid bacteria in green bean juice. J. Food Sci. 48: 962.

de Man, J.C., Rogosa, M., and Sharpe, M.E. 1960. A medium for the cultivation of lactobacilli. J. Appl. Bacteriol. 23: 130.

Etchells, J.L., Fleming, H.P., and Bell, T.A. 1975. Factors influencing the growth of lactic acid bacteria during the fermentation of brined cucumbers. In "Lactic Acid Bacteria in Beverages and Food," J.G. Carr, C.V. Cutting, and C.G. Whiting (Ed.), p. 281. Academic Press Inc., London.

Fleming, H.P. and McFeeters, R.F. 1981. Use of microbial cultures: vegetable products. Food Technol. 35(1): 84.

Fleming, H.P., McFeeters, R.F., Thompson, R.L., and Sanders, D.C. 1983. Storage stability of vegetables fermented with pH control. J. Food Sci. 48: 975.

Gawehn, K. and Bergmeyer, H.U. 1974. D(−)-lactate. In "Methods of Enzymatic Analysis," H.U. Bergmeyer (Ed.), p. 1492. Academic Press, New York.

Gibson, T. and Abd-el-Malek, Y. 1945. The formation of carbon dioxide by lactic acid bacteria and Bacillus licheniformis and a culture method for detecting the process. J. Dairy Res. 14: 35.

Gutmann, I. and Wahlefeld, A.W. 1974. L(+)-lactate:determination with lactate dehydrogenase and NAD. In "Methods of Enzymatic Analysis," H.U. Bergmeyer (Ed.), p. 1464. Academic Press, New York.

Hitchener, B.J., Egan, A.F., and Rogers, P.J. 1982. Characteristics of lactic acid bacteria isolated from vacuum-packaged beef. J. Appl. Bacteriol. 52: 31.

Holz, G. and Bergmeyer, H.U. 1974. Acetate:determination with acetate kinase and hydroxylamine. In "Methods of Enzymatic Analysis," H.U. Bergmeyer (Ed.), p. 1528. Academic Press, New York.

Kotzekidou, P. and Roukas, T. 1986. Characterization and distribution of lactobacilli during lactic fermentation of okra (*Hibiscus esculentus*). J. Food Sci. 51: 623.

Nelson, N. 1944. Determination of reducing sugars. J. Biol. Chem. 153: 375.

Nordal, J. and Slinde, E. 1980. Characteristics of some lactic acid bacteria used as starter cultures in dry sausage production. Appl. Environ. Microbiol. 40: 472.

Paule, R. 1971. Contribution à l'étude biochimique du genre Lactobacillus par une méthode normalisée. Thèse doctorat en pharmacie, Lyon.

Roukas, T. and Kotzekidou, P. 1986. Lactic fermentation of okra (Hibiscus esculentus) with starter cultures for the improvement of quality of canned okra. In "The Shelf Life of Foods and Beverages," G. Charalambous (Ed.), p. 629. Elsevier Science Publishers B.V., Amsterdam.

Seeley, H.W. and Van Demark, P.J. 1972. "Microbes in Action: A Laboratory Manual of Microbiology," 2nd ed. W.H. Freeman & Co., San Francisco, CA.

Stamer, J.R. 1979. The lactic acid bacteria: microbes of diversity. Food Technol. 33(1): 60.

Thornhill, P.J. and Cogan, T.M. 1984. Use of gas-liquid chromatography to determine the end products of growth of lactic acid bacteria. Appl. Environ. Microbiol. 47: 1250.

van Gent, P.K. and Kerrich, J.E. 1965. The colorimetric determination of ethanol in blood with vanadium oxinate. Analyst 90: 335.

Woolfe, W.L., Chaplin, M.F., and Otchere, G. 1977. Studies on the mucilages extracted from okra fruits (Hibiscus esculentus L.) and baobab leaves (Adansonia digitata L.). J. Sci. Food Agric. 28: 519.

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A Research Note

Effect of Polyelectrolyte Treatments on Waste Strength of Snap and Dry Bean Wastewaters

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ABSTRACT

Processing waters from snap beans (SBW) and dry beans (DBW) were treated with 16 different cationic and anionic polyelectrolytes, singly and in combinations. None of the anionic and only two of the cationic polymers tested worked well for turbidity reduction. Treatments of SBW with Floculite 250 at 10–30 mg/L and of DBW with chitosan at 60–80 mg/L reduced turbidity (NTU) by 81 and 90%, chemical oxygen demand (COD) by 21 and 23%, total solids (TS) by 25 and 8%, suspended solids (SS) by 54 and 44%, dissolved solids (DS) by 22 and 0%, nitrate-nitrogen by 0 and 39%, and total phosphorus by 74 and 18%, respectively. Total aerobic bacteria, coliform bacteria, and mesophilic bacterial spores were reduced by 68 and 20%, 81 and 90%, and 94 and 96% for SBW and DBW treatments, respectively.

INTRODUCTION

FOOD PROCESSING PLANTS generally discharge large volumes of wastewaters which are high in organic content. Polyelectrolyte treatments have been shown to reduce the strength of processing wastewaters from leafy green vegetables, eggs, pimiento (Bough, 1974; 1975a,b), poultry (Bough et al., 1975), and potatoes (Karim and Sistrunk, 1985). The objective of this study was to test whether such organic polymers used without floc-forming inorganic salts for treatment of snap bean (SBW) and dry bean (DBW) wastewaters at their natural pH levels had the ability to reduce turbidity (NTU), chemical oxygen demand (COD), total (TS), suspended (SS), and dissolved (DS) solids, nitrate-nitrogen, total phosphorus, and counts of total aerobic, coliform, and mesophilic spore-forming bacteria.

MATERIALS & METHODS

Samples

Composite effluent samples of SBW and DBW from Allen Canning Co. (Springdale, AR) were collected fresh. Samples for determinations of nitrate-nitrogen, total phosphorus, and bacterial spores were placed in C-enamel cans, sealed, and frozen at −20°C and analyzed within 2 months. The other parameters (COD, TS, SS, DS, and total aerobic and coliform bacteria) were determined on fresh samples held at 2°C no longer than 24 hr before analysis.

Polymer treatments

A modified model stirred jar test system (Green and Kramer, 1979) was used. SBW or DBW samples of 100 mL were placed in 150 mL beakers and magnetically stirred at 200 rpm. For treatment involving only cationic or anionic polymers, the polymer was added to the sample and stirring continued for 3 min. For treatment involving both cationic and anionic polymers in combination, the cationic polymer was added first and the sample stirred for 3 min; then the anionic polymer was added and the sample stirred for an additional 2 min at 50 rpm. After treatment, all samples were allowed to settle for 30 min before being filtered through a 200-mesh screen. Assays were then made of the filtrates. While 16 different polymers (8 cationic and 8 anionic) were tested singly, only two cationic polymers, Floculite 250 (F-250, DuBois Chemical Co., Sharonville, OH) and chitosan (Food, Chemical, and Research Laboratories, Inc., Seattle, WA), were effective in reducing NTU of SBW and DBW, respectively. Thus, these two polymers were selected for further detailed study.

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Assays

Turbidity values expressed as nephelometric turbidity units (NTU) were determined using a formazin standard and a Monitek Model 21 Laboratory nephelometer (Monitek, Inc., Hayward, CA). Values for TS, SS, DS, nitrate-nitrogen, and total phosphorus were determined by standard methods (APHA, 1975); values for COD were determined by the method of Mercer and Rose (1956). Determinations for total aerobic bacteria, coliform bacteria, and mesophilic spore-forming bacteria were according to Speck (1984). The data were analyzed statistically by Duncan's multiple range tests and Least Significant Difference (LSD) tests (Steel and Torrie, 1960).

RESULTS & DISCUSSION

Snap bean wastewater

For SBW a 30 min settling period prior to polymer treatments reduced turbidity by 29–40%. At F-250 concentrations above 30 mg/L, the NTU values of treated SBW increased rapidly. Control tests of 0–100 mg/L of F-250 in deionized water gave no appreciable NTU readings (Fig. 1, open circles), indicating that concentrations of F-250 above 30 mg/L appar-

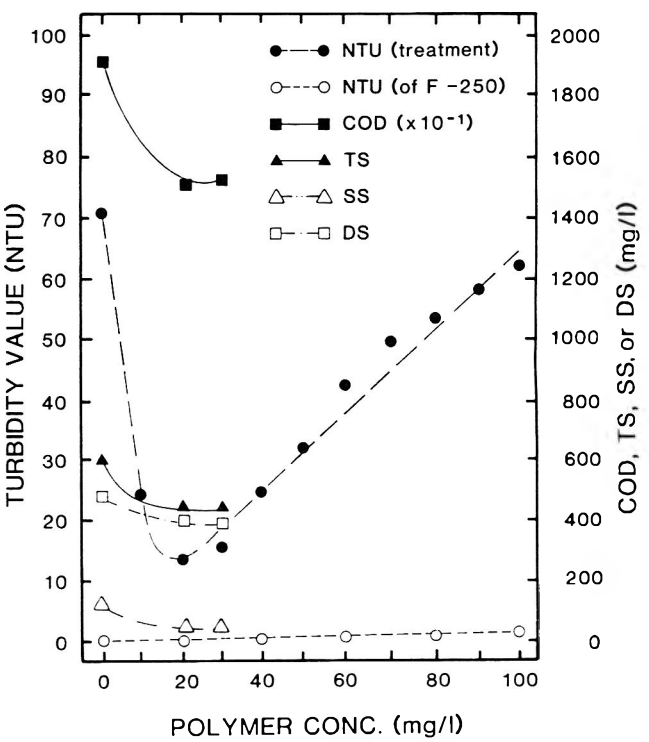

Fig. 1—Effect of F-250 on NTU, COD, TS, SS, and DS in composite effluent wastewater from snap bean processing.

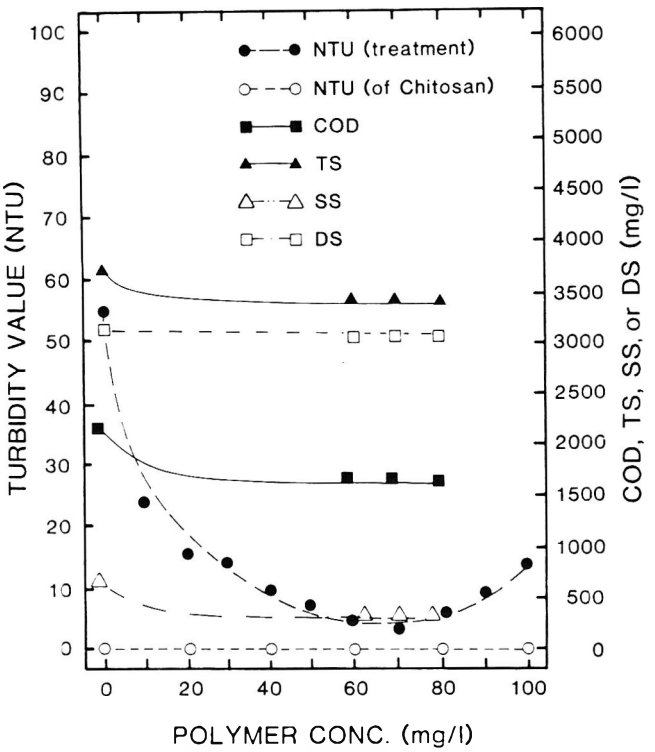

Fig. 2—Effect of chitosan on NTU, COD, TS, SS, and DS in composite effluent wastewater from dry bean processing.

ently caused a re-suspension of SBW solids similar to that reported by O'Melia (1972). Treatment combinations of anionic polymers with F-250 gave no additional reductions in NTU values.

At 20 mg/L, F-250 reduced the values for NTU, COD, TS, SS, and DS by 81, 21, 25, 54, and 22%, respectively (Fig. 1). Total phosphorus was reduced by 74% from 24.9 to 6.5 mg/L, a value still above the desired maximum guideline value of 1-2 mg/L (Ochsner and Blackwood, 1979). Nitrate-nitrogen was not reduced from the initial level of 10 mg/L. Counts of total aerobic bacteria, coliform bacteria, and mesophilic bacterial spores were reduced by 68, 81, and 94% to log counts of 7.1, 5.4, and 1.9, respectively.

Dry bean wastewater

For DBW a 30 min settling period prior to polymer treatments reduced turbidity by 38–43%. Chitosan treatments above 80 mg/L caused an apparent increase in NTU values. Again, control tests of 0–100 mg/L of chitosan in deionized water gave no appreciable NTU readings (Fig. 2, open circles), indicating that re-suspension of DBW solids occurred at chitosan concentrations above 80 mg/L (O'Melia, 1972). Treatment combinations of anionic polymers with chitosan gave no additional reductions in NTU values.

At 60 mg/L, chitosan reduced the values for NTU, COD, TS, SS, and DS by 90, 23, 8, 44, and 0%, respectively (Fig. 2). Total phosphorus was reduced by 17% from 59.1 to 48.8 mg/L, nitrate-nitrogen was reduced by 36% from 11.5 to 7.4 mg/L. Counts of total aerobic bacteria, coliform bacteria, and mesophilic bacterial spores were reduced by 20, 90 and 96% to log counts of 7.5, 4.3, and 0.8, respectively.

SUMMARY & CONCLUSIONS

TO MINIMIZE total water usage, re-use of some wash waters in-plant for initial food product washing is a goal of some food processors. The treatment of wastewaters with organic polymers to reduce turbidity and solids to permit a recycle of such waters is one approach. The initial pH values of untreated SBW and DBW were 5.6–6.5 and 5.7–6.0, respectively. The F-250 treatments did not affect the pH of SBW while the chitosan treatments reduced the pH of DBW by about 0.4 pH units. For treatment of wastewaters from leafy green vegetables, a reduction of pH to 4.0 and addition of inorganic salts (iron, alum) caused greater reductions of COD and solids values than without the inorganic salts (Bough, 1974). However, in the present study it was decided to keep the pH of SBW and DBW near neutrality and omit the use of inorganic salts as floc-formers to obviate the need for pH re-adjustment prior to recycle and the concern about possible carry-over of these metals into these food products when washed with recycled water.

Little has been published about the ability of organic polymers to remove or reduce bacteria from food processing wastewaters. Results of this study indicated that total aerobic bacteria and coliform counts remained relatively high after polymer treatment at log 7.1 and 5.4 for SBW and at log 7.5 and 4.3 for DBW. However, these organisms at these levels are of less concern than is a possible build-up of spore levels in such low acid foods that are to be thermally processed. The ability of the F-250 and chitosan treatments to significantly reduce the mesophilic bacterial spore counts of SBW from log 3.1 to 1.9 and of DBW from log 2.2 to 0.8, for reductions of 94 and 96%, respectively, is of interest. These findings suggest that spore build-up in the food products as a result of wash water recycle after polymer treatments would likely not occur.

REFERENCES

APHA. 1975. "Standard Methods for the Examination of Water and Wastewater," 14th ed. American Public Health Association, Washington, DC.
Bough, W.A. 1974. Physical-chemical treatment of waste effluents from canning of leafy green vegetables. J. Milk Food Technol. 37: 205.
Bough, W.A. 1975a. Coagulation with chitosan—An aid to recovery of by-products from egg breaking wastes. Poultry Sci. 54: 1904.
Bough, W.A. 1975b. Reduction of suspended solids in vegetable canning waste effluents by coagulation with chitosan. J. Food Sci. 40: 297.
Bough, W.A., Shewfelt, A.L., and Salter, W.L. 1975. Use of chitosan for the reduction and recovery of solids in poultry processing waste effluents. Poultry Sci. 54: 992.
Green, J.H. and Kramer, A. 1979. "Food Processing Waste Management." AVI Publishing Co., Westport, CT.
Karim, M.I.A. and Sistrunk, W.A. 1985. Treatment of potato processing wastewater with coagulating and polymeric flocculating agents. J. Food Sci. 50: 1657.
Mercer, W.A. and Rose, W.W. 1956. Chemical oxygen demand as a test of strength of cannery waste water. National Canners Association-Western Research Laboratory Res. Lab. Report #816-56-B, Berkeley, CA.
Ochsner, J.C. and Blackwood, T.R. 1979. Status assessment of toxic chemicals: Phosphates. EPA-600/2-79-210j, U.S. Environmental Protection Agency, Washington, DC.
O'Melia, C.R. 1972. Coagulation and flocculation. Ch. 2. In "Physico-chemical Processes for Water Quality Control," W.J. Weber, Jr. (Ed.), p. 61. Wiley Interscience, New York.
Speck, M.L. 1984. "Compendium of Methods for the Microbiological Examination of Foods." American Public Health Association, Inc., Washington, DC.
Steel, R.G.D. and Torrie, J.H. 1960. "Principles and Procedures of Statistics." McGraw-Hill Book Co., New York.
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A Research Note

Vitamin B_{12} Activity in Miso and Tempeh

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ABSTRACT

The USP microbiological assay with *L. leichmannii*, ATCC 7830, was used to determine vitamin B_{12} activity in light rice miso, dark rice miso, barley miso, tempeh and tempeh burger. Unpasteurized misos were found to have the highest B_{12} content, averaging 0.21 μg/100g. Vitamin B_{12} activity in miso ranged from a high of 0.25 μg/100g in barley miso to a low of 0.15 μg/100g in light rice miso. Pasteurized tempeh contained 0.12 μg vitamin B_{12} per 100g food. Tempeh burger contained 0.06 to 0.11 μg vitamin B_{12} per 100g food. The variation in vitamin B_{12} activity found in these products may be due to different conditions used or produced during fermentation. Collaborative studies are needed and assessment of vitamin B_{12} pseudoform activity before these foods can be considered a source of vitamin B_{12}.

INTRODUCTION

THE DETERMINATION of vitamin B_{12} in miso and tempeh is of considerable interest to the food industry and to the many vegetarians who consume these foods. Beuchat (1984) reported increased consumption of these fermented soybean foods in the United States in the last decade. Vitamin B_{12} is considered to be the nutrient least available in the diet of people who eat foods solely derived from plants (Abdulla et al., 1981).

All vitamin B_{12} found in nature is synthesized by microorganisms. Shurtleff (1983), Steinkraus (1985), Lee et al. (1958), and Liem et al. (1977) suggested that two fermented soybean products, tempeh and miso, contain microorganisms capable of producing B_{12}. Wang (1984) states that microorganisms used in fermentation of these products are not known to synthesize vitamin B_{12}. However, vitamin B_{12} activity is produced by *Klebsiella pneumoniae*, which Steinkraus (1983) suggests is a desirable if not essential microorganism in the natural fermentation process.

The purpose of this study was to determine whether commercially produced, locally available tempeh and miso contained vitamin B_{12}. Vitamin B_{12} activity was measured by the presence or absence of a growth response with Lactobacillus leichmannii (*L. leichmannii*) which responds to both pseudo and true vitamin B_{12} activity.

MATERIALS & METHODS

FIVE LOCALLY PURCHASED fermented foods: pasteurized tempeh, pasteurized tempeh burger, unpasteurized dark rice miso, unpasteurized light rice miso and unpasteurized barley miso were analyzed for vitamin B_{12} activity. Vitamin B_{12} activity was measured in one brand of miso and two brands of tempeh. *L. leichmannii*, USP culture medium, inoculum broth and maintenance agar were obtained from Difco Laboratories (Detroit, MI). Miso was refrigerated at 4° C and tempeh was frozen at −17° C.

Research design

Vitamin B_{12} activity was estimated using the United States Pharmacopia microbiological assay with *L. leichmannii* (ATCC 7830) (USP, 1985). Each assay was repeated three times, with duplicate tubes at three or more concentrations. Pasteurized process cheese food was used as a positive control. A recovery study was conducted to determine the possibility of an inhibitor (Beck, 1983).

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Inhibitor study

The presence of inhibitors was determined as follows: 21.7 pg cyanocobalamin was added to duplicate tubes in the second replicate analysis and the vitamin B_{12} activity determined as described for the USP (1985) assay. If recovery was less than 80%, the presence of an inhibitor was indicated (Beck, 1983).

Extraction and analysis

As recommended by Liem et al. (1977) and Ro et al. (1979) a cyanide-buffer solution was used for extraction of vitamin B_{12}. The cyanide buffer solution converts less stable noncyanocobalamins to more stable cyanocobalamins (Skeggs, 1966). In preliminary assays, extraction without cyanide resulted in no identifiable vitamin B_{12} activity in any food except cheese.

The cyanide buffer, pH 6.0, used in this study contained 3.0g citric acid. H_2O, 6.5g sodium diphosphate (Na_2HPO_4) and 20 mg sodium cyanide (NaCn) and was diluted with deinoized water to a 500 mL total volume. Two grams of each food were homogenized with 50 mL extracting solution. The vitamin B_{12} was extracted by autoclaving for 10 min at 121° C. The samples were cooled to room temperature, then filtered using a water vacuum filtration and Schleicher and Schuell Analytical filter papers No. 589. Cheese was filtered through Whatman No. 42 filter paper and centrifuged at 1,868 g in a Sorvall RC-5B Superspeed refrigerated centrifuge with an SS 34 rotor head, at 5–10° C for 10 min. After a clear solution was obtained, each sample was diluted to desired concentrations and pH adjusted to 6.0.

RESULTS & DISCUSSION

DATA on vitamin B_{12} activity and recovery of vitamin B_{12} are reported in Table 1. Percent moisture is provided to allow comparison of the vitamin B_{12} obtained in this study with other values as they become available. All of the foods investigated contained detectable amounts of vitamin B_{12} activity. Pasteurized processed cheese food contained 1.17 ± 0.048 (mean ± standard error) μg/100g food which compares favorably with 1.1 ± 0.109 (mean ± standard error) μg/100g reported in Handbook 8-1 (Posati and Orr, 1976). No inhibitor to *L. leich-*

Table 1—Moisture, vitamin B_{12} activity and vitamin B_{12} recovery in fermented soybean products

Food	Moisture[a] (%)	Vitamin B_{12} activity[b] (μg/100g)	Recovery[c] (%)
Barley miso	52.1 ± 0.3	0.256 ± 0.011d	112
Dark rice miso	52.0 ± 0.4	0.232 ± 0.005d	111
Light rice miso	48.7 ± 0.7	0.147 ± 0.005	96
Tempeh	59.7 ± 0.2	0.118 ± 0.003	129
Tempeh burger, new	70.5 ± 0.2	0.106 ± 0.001e	107
Tempeh burger, old	68.2 ± 0.1	0.055 ± 0.002	NDf
Cheese food	44.6 ± 0.1	1.170 ± 0.048	91

[a] Means ± standard errors are derived from one determination with duplicate samples.

[b] Means ± standard errors are derived using USP microbiological assay with *L. leichmannii* in triplicate assays with at least four values per assay.

[c] Mean of two measurements from one determination.

[d] Significantly different from light rice miso, $p < 0.05$ with t test.

[e] Significantly different from old tempeh burger, stored over 1 wk, $p < 0.01$ with t test.

[f] ND = not determined.

mannii was found in this study; mean recovery of vitamin B_{12} was 108%.

When the misos are considered together, total vitamin B_{12} activity averaged 0.21 μg/100g. No United States data were available with which to compare the vitamin B_{12} activity of miso. However, as reported by Shurtleff (1983), a Japanese analysis indicated that "light yellow miso" contained 0.17 μg B_{12}/100g food which is in close agreement with 0.15 μg B_{12}/100g of light rice miso found in this study.

Tempeh contained 0.12 μg B_{12}/100g and tempeh burger contained 0.06–0.11 μg B_{12}/100g. These values are much lower than the 0.4 to 6.2 μg B_{12}/100g reported by Liem et al. (1977). Steinkraus et al. (1961), using the microorganism *Ochromonus malhemensis* for measuring B_{12} activity, found tempeh contained 0.5 μg B_{12}/100g. The higher B_{12} activity suggested by Liem et al. (1977) and Steinkraus et al. (1961) could be the result of differences in extraction technique, pasteurization, assay microorganism or in the sources of tempeh.

When tempeh burger was stored for one week or greater, a 52% decrease in vitamin B_{12} activity was found. The mechanism responsible for a decrease in vitamin B_{12} activity is not known.

The results of this study direct attention to the presence of vitamin B_{12} activity in some fermented nonmeat foods previously thought to be devoid of vitamin B_{12} activity. However, the source of vitamin B_{12} in tempeh and miso was not investigated. Foods recommended as containing a nutrient should contain that nutrient by design not chance.

A limitation of this study is that vitamin B_{12} activity, as assessed microbiologically with *L. leichmannii* may be influenced by the absence or presence of cyanide (Ro et al., 1979; Skegga, 1966), the presence of pseudovitamin B_{12} (Ford and Porter, 1953), analogs of vitamin B_{12} (Ro et al., 1979) and thymidine and other deoxyribonucleosides (Kitay et al., 1950). Therefore, collaborative studies are needed and the presence or absence of vitamin B_{12} pseudoform activity should be assessed before these values are added to standard food tables. Chemical assays using vitamin B_{12} radioisotopes and intrinsic factor or microbiological assays with the protozoan, *Ochromonus malhamensis* (Ford, 1953) may be helpful in further quantifying the true vitamin B_{12} activity in miso and tempeh.

REFERENCES

Abdulla, M., Anderson, I., Asp, Nils-Georg, Berthelsen, K., Birkhed, D., and Dencker, I. 1981. Nutrient intake and health status of vegans. Chemical analyses of diets using the duplicate portion sampling technique. Am. J. Clin. Nutr. 34: 2464.

Beck, W.S. 1983. The assay of serum cobalamin by *Lactobacillus leichmannii* and the interpretations of serum cobalamin levels. In "The cobalamins," p. 31. C.A. Hall (Ed.). Churchill Livingstone: New York.

Beuchat, L.R. 1984. Fermented soybean foods. Food Technol. 38: 64.

Ford, J.E. 1953. The microbiological assay of vitamin B_{12}. The specificity of the requirement of *O. malhamensis* for cyanocobalamin. Br. J. Nutr. 7:: 299.

Ford, J.E. and Porter, J.W.G. 1953. Vitamin B_{12} like compounds. 2. Some properties and compounds isolated from bovine gut contents and faeces. Brit. J. Nutr. 7: 326.

Kitay, E., McNutt, W.S., and Snell, E.E. 1950. Deoxyribosides and vitamin B_{12} as growth factors for lactic acid bacteria. J. Bacteriol. 59: 727.

Lee, I.J., Kim, S.I., and Haw, K. 1958. Biochemical studies on Korean fermented foods. VIII. Studies on vitamin B_{12} contents of the fermented food in Korea. Abstract. Report of National Chemistry Laboratories 7: 14 (Korean).

Liem, I.T.H., Steinkraus, K.H., and Cronk, T.C. 1977. Production of vitamin B_{12} in tempeh, a fermented soybean food. Appl. Environ. Microbiol. 34: 773.

Posati, L.P. and Orr, M.L. 1976. Composition of foods: dairy and egg products—raw, processed, prepared. USDA Agriculture Handbook No. 8.1, Supt of Documents, U.S. Govt Printing Office, Washington, DC.

Ro, S.L., Woodburn, M., and Sandine, W.E. 1979. Vitamin B_{12} and ascorbic acid in kimchi inoculated with *Propionibacterium freudenreichii SS. shermanii*. J. Food Sci. 44: 873.

Shurtleff, W. 1983. Vegetarian sources of vitamin B_{12}. Vegetarian Times, February, p 61.

Skeggs, H.R. 1966. Microbiological assay of vitamin B_{12}. Met. Biochem. Anal. 14: 53.

Steinkraus, K.H. 1983. Indonesian tempe and related fermentations. In Microbiology Series "Handbook of Indigenous Fermented Foods," Vol. 9., Section 1, p. 29. Marcel Dekker, Inc., New York.

Steinkraus, K.H. 1985. Bio-enrichment:production of vitamins in fermented foods. In "Microbiology of Fermented Foods," p. 322. Vol. I., B.J.B. Wood. (Ed.). Elsevier, New York.

Steinkraus, K.H., Hand, D.B., Van Buren, J.P., and Hackler, L.R. 1961. Pilot plant studies on tempe. "Proc. Conference on Soybean Products for Protein in Human Foods," p. 83. USDA, Peoria, IL.

USP. 1985. Vitamin B_{12} activity assay. In "The United States Pharmacopia." 21st ed. U.S. Pharmacopeial Convention, Inc., Rockville, MD.

Wang, H.L. 1984. Private communication. Culture Collection Research, Fermentation Laboratory. USDA, Peoria, IL.

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A Research Note

Effects of Cooking Method upon Flavor of Carrots and Peas

S. ISSANCHOU and F. SAUVAGEOT

ABSTRACT

Two modalities of pressure (atmospheric pressure and high-pressure of $5.5.10^4$ Pa) and two modalities of immersion (boiling and cooking in steam) were combined factorially to produce four ways of cooking. These different cooking methods were studied on two vegetables: carrots and peas. Sensory attributes of samples were assessed on an unstructured scale. Steam-cooked vegetables have higher sensory attributes (odor and flavor intensities, typical odor and flavor notes, sweetness). Larger losses of soluble solids and volatiles are believed to account for these differences between vegetables cooked in water and cooked in steam.

INTRODUCTION

CHANGES in the composition of vegetables occur during processing and cooking. The impact of these treatments have been extensively studied on nutrient composition (Lund, 1979). Other studies have reported the effects of cooking on the volatile components of cauliflowers (MacLeod and MacLeod, 1970), carrots (Heatherbell and Wrolstad, 1971) and potatoes (Nursten and Sheen, 1974). But less emphasis has been placed on the impact of processing and cooking on the sensory attributes of vegetables. Although the effects have been studied by Simon et al. (1982) who have established correlations between chemical changes and the results of sensory evaluations on raw, fresh-cooked, canned-cooked and frozen-cooked products. Unfortunately, no authors have attempted to compare the effects of different household cooking practices on the flavor of vegetables. The purpose of this work was to investigate the effects of everyday cooking methods on the sensory characteristics of carrots and peas.

MATERIALS & METHODS

TWO CARROT CULTIVARS were studied (Hybrid F1 × Bastin, grown in Italy, and Clause 50, grown in France). Three green pea cultivars were studied (Lincoln and Frisky, grown in the south of France and GV Thezin, grown locally in France). Peas were hand-picked to avoid damage to the tegument and thus oxydation. All vegetables were stored at 4°C and were processed and evaluated within 10 days of harvest.

Carrots were washed, peeled and sliced (1 cm thick). Peas were shelled and size graded (peas with a diameter <7.5 mm were eliminated).

Four different cooking methods were used: (1) boiling (water cooking) under atmospheric pressure i.e. the traditional way; (2) steam cooking under atmospheric pressure; (3) pressure ($5.5.10^4$Pa) water cooking; (4) pressure ($5.5.10^4$Pa) steam cooking. Thus two factors (pressure and immersion) were studied, each with two modalities. For each method, cooking was performed in a stainless steel household pressure cooker SEB. Salt was added to water to be representative of the usual cooking methods. The amounts of salt used for each method were chosen after preliminary experiments were run to obtain a similar low intensity of saltiness for all the samples. For atmospheric pressure treatments, the water was brought to a boil and vegetables added; timing of the cooking was then started. For high-pressure, vegetables were placed in the cooker with water; timing of the cooking was started when the pressure of $5.5.10^4$Pa was reached. When cooked, the vegetables were rapidly drained. The temperature of samples when they were evaluated was about 60°C. Our aim was to obtain the same texture as evaluated sensorially for each method and within each replicate. Preliminary experiments have shown this to be very difficult. Thus, cooking times after each replicate were readjusted in order to obtain the same texture for each method over the whole experiment.

All panelists had previous experience in sensory evaluation and were familiarized with products and test procedures. Fifteen persons out of a panel of 31 were used at each session.

For each vegetable, the four samples were evaluated in the same session but in a randomized order. Twelve replicates (one per session) were performed on each vegetable (for carrots: 8 with F1 × Bastin and 4 with Clause 50; for peas: 5 with Lincoln, 3 with Frisky and 4 with GV Thezin). Most of the subjects participated in six sessions, the others in five or seven sessions.

Evaluations were conducted before lunch in individual air conditioned (20°C) booths. The lights were dimmed but not colored as there was no noticeable differences in color between the four samples. Drinking water was available for rinsing.

Seven sensory variables were examined: odor intensity, typical odor note, texture, flavor intensity, typical flavor note, sweetness, saltiness. These attributes were rated using an unstructured scale. The scale consisted of a 10 cm horizontal line with verbal anchor points placed at 0.5 cm from each end (left anchor = low value and right anchor = high value). Responses were digitized for statistical analysis by measuring the segment length from the left of the scale to the judge's mark.

A three way analysis of variance (pressure, immersion and cultivar) was conducted on the arithmetic means of the judge's responses obtained on each replicate for each vegetable and each attribute. The four cooking treatments were compared using the t-Bonferroni method (Coursol, 1980).

RESULTS & DISCUSSION

THE MEANS as well as the F-values from the analysis of variance (for the carrots and peas, respectively) are shown in Tables 1 and 2. In spite of readjustments of cooking time a similar texture was not obtained for all cases; steam-cooked vegetables were harder than boiled vegetables. However, as the data showed independence of the texture to the other sensory attributes in the investigated cooking range (Sauvageot and Issanchou, 1987), it was concluded that the differences noticed in odor, flavor or taste between cooking procedures were not bound to texture differences.

Analysis of variance confirmed the importance of sample influence on sensory attributes, particularly on sweetness. These differences between samples may be due to genotype, soil and climate effects. Influences of these factors, particularly genotype were previously noted on fresh and cooked samples (Simon and Lindsay, 1983).

Much larger F-values were found for all odor and flavor attributes with respect to the pressure factor.

Boiled carrots were judged less sweet than steam cooked

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Table 1—Mean responses to sensory attributes of carrots cooked by four different methods and F-values for each source of variation

Sensory attributes	Cooking method: Atmospheric pressure Water	Atmospheric pressure Steam	Over-pressure Water	Over-pressure Steam	F[a] Values Sign. Level Pressure	Immersion	Cultivar	Pressure × Immersion	Pressure × Cultivar
Texture[e]	5.49[cd3]	6.21[c]	4.96[d]	6.02[cd]	1.46 N.S.[b]	8.68 1%	13.78 0.1%	0.33 N.S.	0.05 N.S.
Odor intensity[f]	3.49[d]	4.45[c]	3.94[cd]	4.46[c]	2.31 N.S.	22.35 0.1%	1.92 N.S.	2.10 N.S.	0.50 N.S.
Typical odor note[f]	3.97[c]	4.57[c]	4.22[c]	4.60[c]	0.67 N.S.	8.19 1%	0.06 N.S.	0.39 N.S.	0.45 N.S.
Flavor intensity[f]	5.38[d]	6.07[c]	5.09[d]	6.01[c]	1.26 N.S.	26.50 0.1%	2.24 N.S.	0.46 N.S.	0.04 N.S.
Typical flavor note[f]	5.43[d]	6.06[c]	5.42[d]	5.82[cd]	0.38 N.S.	6.42 2.5%	4.82 5%	0.35 N.S.	0.10 N.S.
Sweetness[f]	5.20[d]	6.00[d]	5.80[cd]	6.15[c]	4.11 5%	9.70 1%	57.88 0.1%	1.45 N.S.	0.01 N.S.
Saltness[f]	2.63[c]	2.79[c]	2.65[c]	2.52[c]	1.04 N.S.	0.02 N.S.	18.07 0.1%	1.26 N.S.	0.77 N.S.

[a] Interaction immersion × cultivar was never significant, it was included in error.
[b] N.S. = Not Significant
[c,d] Means within the same line followed by different letters are significantly ($p<0.05$) different.
[e] On the scale: 0 = very soft, 10 = very firm
[f] On the scale: 0 = low value, 10 = high value

Table 2—Mean responses to sensory attributes of peas cooked by four different methods and F-values for each source of variation

Sensory attributes	Cooking method: Atmospheric pressure Water	Atmospheric pressure Steam	Over-pressure Water	Over-pressure Steam	F[a] Values Sign. Level Pressure	Immersion	Cultivar	Pressure × Immersion	Pressure × Cultivar
Texture[e]	4.84[cd]	5.39[c]	4.58[d]	5.07[cd]	1.28 N.S.[b]	4.11 5%	14.43 0.1%	0.00 N.S.	5.16 5%
Odor intensity[f]	4.75[d]	5.87[c]	5.29[cd]	5.82[c]	2.19 N.S.	24.01 0.1%	2.48 N.S.	3.16 N.S.	0.41 N.S.
Typical odor note[f]	4.78[d]	5.61[c]	5.24[cd]	5.65[c]	2.61 N.S.	16.73 0.1%	1.22 N.S.	2.00 N.S.	0.75 N.S.
Flavor intensity[f]	4.07[d]	5.02[c]	4.62[cd]	4.90[c]	2.12 N.S.	15.60 0.1%	2.47 N.S.	4.80 5%	0.29 N.S.
Typical flavor note[f]	4.16[c]	4.73[c]	4.85[c]	4.86[c]	4.96 5%	2.25 N.S.	1.68 N.S.	2.32 N.S.	1.10 N.S.
Sweetness[f]	3.19[c]	3.52[c]	3.46[c]	3.51[c]	0.95 N.S.	0.86 N.S.	19.58 0.1%	0.44 N.S.	0.16 N.S.
Saltness[f]	2.61[c]	2.75[c]	2.87[c]	2.57[c]	0.14 N.S.	0.45 N.S.	1.52 N.S.	2.26 N.S.	0.13 N.S.

[a] Interaction immersion × cultivar was never significant, it was included in error.
[b] N.S. = Not Significant
[c,d] Means within the same line followed by different letters are significantly ($p<0.05$) different.
[e] On the scale: 0 = very soft, 10 = very firm
[f] On the scale: 0 = low value, 10 = high value

carrots. It may be concluded that boiling leads to larger losses in soluble solids such as sugars which contribute to sweetness of carrots than cooking in steam. On the contrary, there was no difference in sweetness between boiled peas and steam cooked peas. This may be due to the fact that in this experiment the peas were hand-picked and were protected by their tegument, so diffusion was limited. This assumption is supported by results obtained by Varoquaux and Dupuy (1971) who demonstrated that losses of soluble solids during water blanching were more important when peas were mechanically harvested.

Loss of volatiles rather than formation of off-flavor is believed to account for the differences between the flavor of products cooked in water and cooked in steam as the differences in perceived odor intensities were larger than the differences in the typical notes and as no off-odor or off-flavor were noted by judges.

In conclusion, it appears that the immersion factor has more effect upon sensory characteristics than the pressure and that cooking in steam better preserves the original qualities of vegetables. Since high-pressure had no adverse effect on sensory quality, cooking in steam at high-pressure might be recommended because it shortens cooking time and thus allows a gain of energy.

REFERENCES

Coursol, J. 1980. "Technique statistique des modèles linéaires. 1. Aspects théoriques." Les cours du C.I.M.P.A., Nice.
Heatherbell, D.A. and Wrolstad, R.E. 1971. Carrots volatiles. 1. Characterization and effects of canning and freeze-drying. J. Food Sci. 36:219.
Lund, D.B. 1979. Effect of commercial processing on nutrients. Food Technol. 33(2):28.
MacLeod, A.J. and MacLeod, G. 1970. Flavor volatiles of some cooked vegetables. J. Food Sci. 35: 734.
Nursten, H.E. and Sheen, H.R. 1974. Volatile flavour components of cooked potato. J. Sci. Food Agric. 25:643.
Sauvageot, F. and Issanchou, S. 1987. Interactions texture-flaveur après cuisson de choux fleurs, de carottes et de petits pois. In preparation.
Simon, P.W., Peterson, C.E., and Lindsay, R.C. 1982. Genotype, soil and climate effects on sensory and objective components of carrot flavor. J. Amer. Soc. Hort. Sci. 107(4):644.
Simon, P.W. and Lindsay, R.C. 1983. Effects of processing upon objective and sensory variables of carrots. J. Amer. Soc. Hort. Sci. 108(6):928.
Varoquaux, P. and Dupuy, P. 1971. Les pertes en matières solubles au cours du blanchiment des pois. C.R. Acad. Agric. France, 57(1):949.
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A Research Note

A New Method for Aflatoxin-Free Storage of Agricultural Commodities

ARUN SHARMA, S. R. PADWAL-DESAI, and G. B. NADKARNI

ABSTRACT

Aflatoxin production was observed in the 2 kg lots of peanuts and corn that were stored for 90 days at ambient temperature (28–30°C) and a relative humidity of 100% after infecting with the spores of aflatoxin producing strain *Aspergillus parasiticus* NRRL 3145. Treatment of the samples with an aqueous solution of 2-chloroethylphosphonic acid prevented aflatoxin formation in both the commodities, whereas, the untreated lots supported aflatoxin formation.

INTRODUCTION

PRESENCE OF AFLATOXIN in foods and feeds is a potential health hazard (Shank, 1981). Aflatoxin producing strains of *Aspergillus flavus* and *Aspergillus parasiticus* are commonly associated as storage fungi with a number of agricultural commodities, and these organisms elaborate the toxins when conditions are conducive for their growth (Goldblatt, 1968). 2-Chloroethylphosphonic acid (CEPA, ethrel, ethephon), an ethylene generating compound, was found to inhibit aflatoxin biosynthesis in the cultures (Sharma et al., 1985). A study was therefore undertaken to assess the efficacy of CEPA for aflatoxin-free storage of peanuts (*Arachis hypogea*) and corn (*Zea mays*).

MATERIALS & METHODS

Organism and culture conditions

Aflatoxin producing strain *A. parasiticus* NRRL 3145 was used in these studies. The recultivation of the stock culture (maintained on potato-dextrose agar) was carried out by streaking the slant material on potato dextrose agar (Difco Laboratories, Detroit, MI) plates. Single colonies were picked up from the streaked plates and transferred to fresh slants. To maintain the culture, regular transfers to fresh slants were carried out at 1-month intervals.

The spore suspension of the above culture was prepared by transferring slant material to Roux bottles containing potato dextrose agar (pH 5.6, 200 mL), which were subsequently incubated for 10 days at ambient temperature (28–30°C). The harvesting of spores from Roux bottles and the preparation of spore suspension were carried out as described earlier (Sharma et al., 1980).

Treatment and storage studies

Peanuts and corn were procured from a local market and divided into lots of 2 kg each. A 40% aqueous solution of 2-chloroethylphosphonic acid (Amchem Products Inc., Pa) was used. A 15 mL aliquot of spore suspension of *Aspergillus parasiticus* (ca 10^6 spores/mL) was used as inoculum. CEPA solution, the spore suspension (inoculum) and water were blended with the 2 kg lots of peanuts and corn in proportions given in Table 1. Water was added to increase kernel moisture and to facilitate blending of the inoculum and CEPA with the commodity. The blending was carried out in large flexible polyethylene pouches by vigorous manual shaking. The blended lots were stored in polyethylene pouches at ambient temperature (28–30°C) and a relative humidity of 100% for 90 days.

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Table 1—Biogenesis of aflatoxin in CEPA treated and untreated peanuts and corn during storage[a]

		Total aflatoxin (mg)	
		Peanuts	Corn
Control before storage		nd[c]	nd
After storage (90 days, 28–30°C, RH 100%)			
Control uninfected	(0, 0, 70)[b]	traces	nd
Control infected	(0, 15, 55)[b]	5.1	0.8
Treated infected I	(15, 15, 40)[b]	nd	nd
Treated infected II	(20, 15, 35)[b]	nd	nd
Treated infected III	(25, 15, 30)[b]	nd	nd

[a] The methods are outlined in the text. Results are averages of at least three replicates.
[b] Figures in parenthesis are the volumes (mL) of CEPA solution (40%), inoculum (10^6 spores/mL) and water, respectively.
[c] nd = not detected.

The lots of the two commodities under each treatment as well as their controls were stored in triplicate. Estimation of aflatoxin was carried out accordingly in triplicate.

Detection and estimation of aflatoxin

Extraction of aflatoxin was carried out using 70% aqueous acetone, precipitating the extract with lead acetate, centrifuging and extraction of the supernatant with chloroform (Liem and Beljaars, 1970). Detection and estimation of aflatoxin was carried out as described earlier (Sharma et al., 1985).

RESULTS & DISCUSSION

AFTER THEY WERE PROCURED from the market, the two commodities were tested for the presence of aflatoxin contamination, and both were free from aflatoxin (Table 1). Upon storage of the control samples for 90 days at ambient temperature (28–30°C) and a relative humidity of 100% only traces of aflatoxin could be detected in peanuts, and no aflatoxin formation was observed in corn samples. However, both commodities infected with spores of *A. parasiticus* showed aflatoxin formation upon storage. It is worth noting that the commodities which were infected prior to storage after treatment with CEPA did not develop any aflatoxin.

Visual differences in CEPA-treated and untreated peanut kernels were also noticed after the storage of these samples. The untreated kernels showed white mycelial growth which was not present on kernels in the treated lots. It is apparent that besides inhibiting aflatoxin biogenesis, CEPA could also control the growth of molds. This would retain the original appearance of the peanuts. In case of corn the untreated grains also showed discoloration, whereas the grains in the treated lots remained unaffected.

These studies thus demonstrate the efficacy of CEPA for aflatoxin-free storage of agricultural commodities.

REFERENCES

Goldblatt, L.A. 1968. "Aflatoxin: Scientific Background, Control and Implications". (Ed.) L.A. Goldblatt. Academic Press, New York.

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A Research Note

Copper Activity in Sucrose Solutions

Y. P. HSIEH and N. D. HARRIS

ABSTRACT

Copper activity was measured by a cupric electrode in 0.1M sodium acetate-acetic acid buffered (pH 3.2, 4.5, 6.0) and unbuffered sucrose solutions (10% w/v) using the standard addition technique (1–10 ppm of Cu^{++}). In water, the measured copper concentration (activity) was linearly related to the added copper concentration with a slope of unity. The slope decreased in acetate buffer solutions as pH increased indicating that Cu^{++} was complexed with acetate and to a greater extent at higher pH. In the presence of sucrose, copper activity increased approximately 16% in all solutions. Results suggest that the bulking effect of sucrose and the decreased water activity in solutions produced increased copper activity in sucrose solutions.

INTRODUCTION

COPPER is a powerful catalyst for many chemical reactions in solution. The catalytic power of copper is related to its activity rather than to its concentration in solution. The activity of copper can be regarded as the "effective concentration" of copper in solution. Cupric ions can form complexes with many organic ligands (Briggs et al. 1981, Bourne et al. (1971). The degree of complex formation varies from near 100% to nil depending on the concentrations of the ligands and cupric ions and the nature of the ligand. Therefore, measurement of copper activity in solution is critically important when one addresses the effect of copper's catalytic effect. Use of a selective electrode is a convenient means of assessing the activity of copper in solution. The potential measured in the selective electrode method directly corresponds to the activity rather than to the concentration of copper in solution.

Sucrose as well as copper is a common ingredient in many food products. Many sugars have proved to have copper complexing properties (Cross et al. 1985). However, there is no study reported on the effect of sucrose on copper activity in solution. Khamov (1938) conducted an experiment on the effect of sugar on activity of various metal ions including Zn, Cd, and Hg, and concluded that sucrose does increase the activity of electrolytes mainly due to the bulking effect of the sugar.

Copper-catalyzed reactions are important to the chemistry of foods. The modification of copper activity in solutions with and without sucrose, therefore, has implications for the quality, processing and formulation of foods and beverages. The objectives of this experiment were to study the effect of sucrose on copper activity in water and acetate buffered solutions by means of cupric ion selective electrode and to study the copper complexing power of acetate buffer at different pH levels of the solution.

MATERIALS & METHODS

ALL CHEMICALS used in this study were analytical grade. Deionized distilled water was used to prepare all of the solutions.

Sucrose solutions were prepared by dissolving 10g sucrose in water or in 0.1M sodium acetate-acetic acid buffer at pH 3.2, 4.5,and 6.0 and made up to the volume of 100 mL. A standard copper solution was prepared by diluting a stock 5M cupric nitrate solution.

A solid-state cupric ion selective electrode (Orion, Model 94-29) in conjunction with a single-junction reference electrode (Orion, Model 90-01) was used to monitor the chemical potential (activity) of copper in solution. The cupric ion activity is related to free ion concentration by the activity coefficient. Ionic activity coefficients are variable and largely depend on total ionic strength. If the background ionic strength is high and constant relative to the sensed ion concentration, the activity coefficient is constant and activity is directly proportional to concentration. One milliliter of 5M $NaNO_3$ was added to each 50-mL sample or standard solution for a background ionic strength of 0.1M. The potential measurements were made with an ion analyzer (Orion, Model EA 940). A small volume (50 μL) of a concentrated standard copper solution was added to the sample and the copper activity for each sample was measured by using the standard addition technique (Smith and Manahan, 1973). The experiment was done in triplicate. Linear regression analysis on measured Cu^{++} concentration (dependent variable) vs added Cu^{++} concentration (independent variable) was done by a commercial computer statistical program, Statistics with DAISY, on an Apple II computer.

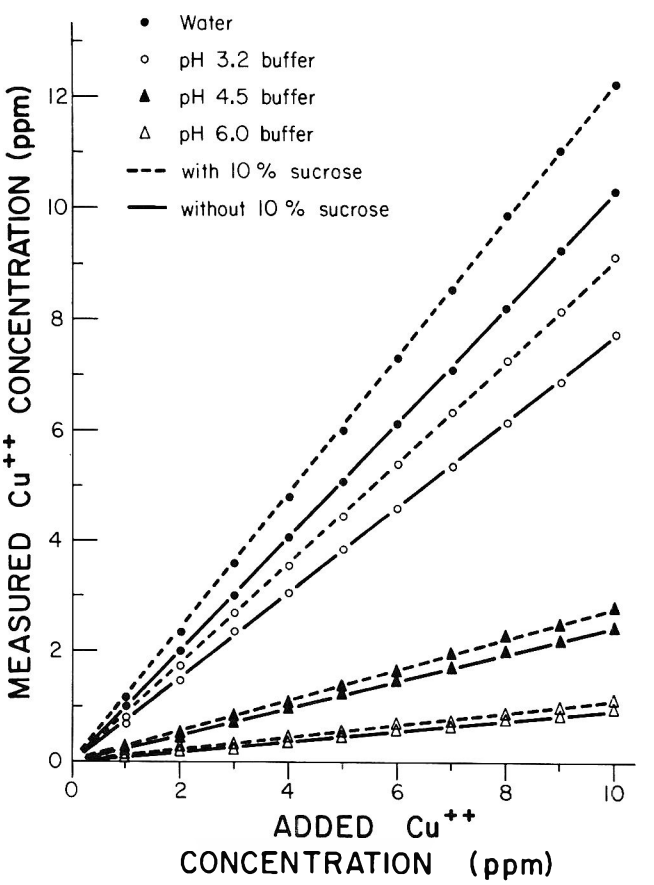

Fig. 1—Relationships between measured Cu^{++} concentration (activity) and added Cu^{++} concentration in water and 0.1M acetate buffer solution in the absence (-) and presence (---) of 10% sucrose.

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RESULTS & DISCUSSION

THE RELATIONSHIP between the measured Cu^{++} concentration by electrode method (activity) and the actual Cu^{++} concentration added is presented in Fig. 1. In water the relationship was linear with a slope of unity as expected since the ionic strength of solutions was the same for all copper concentrations. In buffer solutions, the relationships were still linear but slopes decreased as the pH of the buffer solution increased. The result illustrates that acetic buffer has the power to complex copper ions in solution and thus decrease the "effective concentration" of copper ion. As the pH of the buffer solution increased, the complexing power of the buffer salt increased. This is due to the fact that unprotonated acetate species have more complexing power than protonated species. As the pH increased and approached the pK value of acetic acid, the fraction of the unprotonated species increased sharply. The pK value of acetic acid at infinite dilution is 4.7; however, when corrected for the salt effect (ionic strength) of the buffer and the background, it becomes 4.5 (Stumm and Morgan, 1970). This explains why there was a marked difference in copper complexing power between buffers of pH 3.2 and pH 4.5. At pH 3.2 only a very small fraction of acetate was unprotonated, while at pH 4.5 half of the acetate was unprotonated. In the presence of sucrose, the measured Cu^{++} concentration (activity) is significantly and consistently higher than its nonsucrose counterparts both in water and in buffer solutions. The increase in Cu^{++} activity ranges from 14.5 to 17.3% higher than that of their nonsucrose counterparts as inferred from the slope increase in Table 1. At least two factors can account for this increase in copper activity: First, the bulking effect of the sucrose in solution and second, the reduction of water activity due to sucrose addition. There are 93.8g (not 100g) of water in 100 mL 10% sucrose solutions. Therefore, the bulking effect of 10% sucrose solution accounts for approximately 6.6% of the total increase in Cu^{++} concentration. The increase in Cu^{++} activity due to the reduction of water activity in a 10% sucrose solution is about 1.1% because the water activity is 0.99 in the 10% sucrose solutions (Troller, 1983). The combination of the two effects is 7.7% (1.066*1.011 = 1.077). More than half of the Cu^{++} activity increase observed is still unaccounted for. Khamov (1938) in his investigation on effect of sugar on electrolyte activity attributed almost all of the increase in electrolytic activity to the bulking effect of sugar. This experiment does not totally support his conclusion. In a separate experiment, Hsieh and Harris (unpublished data) measured the rate of ascorbic acid oxidation by a copper-catalyzed reaction in water and sucrose solution. Their results indicated that under the copper-catalzyed condition (1 and 2.5 ppm copper), addition of 10% sucrose reduced the ascorbic acid retention in solutions by 11.1% in comparisons to the nonsucrose control. This 11.1% reduction in ascorbic acid retention corresponded to the effect of approximately 16% increase in Cu^{++} activity. This agrees with the result of this experiment in that the addition of 10% sucrose in solutions increases Cu^{++} activity an average of 16.3% over their nonsucrose counterparts. Therefore, the unaccounted for increase in Cu^{++} activity is not likely due to modification of the electrode behavior or by error alone. Further investigations on this phenomenon are needed.

In summary, sucrose causes an increase of copper activity both in acetate buffered and unbuffered solutions in comparison to its nonsucrose counterpats in the range 1–10 ppm Cu^{++}. The bulking effect of sucrose and the reduced water activity in solution can account for approximately half of the effect. The overall copper activity has been decreased in acetate buffered solutions due to the cupric-acetate complex formation. The copper complexing power of acetate increased as the pH of the solution increased.

Table 1—Results of the regression analysis on measured Cu^{++} concentration (activity) vs added Cu^{++} concentrations

	Solutions			
	Water	pH 3.2 buffer	pH 4.5 buffer	pH 6.0 buffer
Regression slope, w/ sucrose	1.1829 (0.9946)[a]	0.9026 (0.9998)	0.2793 (0.9996)	0.1086 (0.9996)
Regression slope, w/o sucrose	1.0130 (0.9988)	0.7693 (0.9996)	0.2440 (0.9994)	0.0933 (0.9992)
Fraction[b] of free Cu^{++}	1.00	0.76	0.24	0.09
Slope ratio[c]	1.168	1.173	1.145	1.164

[a] R-square value of the regression.
[b] Based on the fraction of free Cu^{++} in water w/o sucrose = 1.00.
[c] Slope ratio = slope with sucrose/slope without sucrose.

REFERENCES

Bourne, E.J., Searle F., and Weigel, H. 1971. Complexes between polyhydroxy compounds and copper (II) ions. Carbohyd. Res. 16: 185.
Briggs, J., Finch, P., Matulewicz, M.C., and Weigel, H. 1981. Complexes of copper (II), calcium, and other metal ions with carbohydrates: thin-layer ligand-exchange chromatography and determination of relative stabilities of complexes. Carbohyd. Res. 97: 181.
Cross, H., Pepper, T. Kearsley, M.W., and Birch, G.G. 1985. Mineral complexing properties of food carbohydrates. Starch/Starke 37: 132.
Hsieh, Y.P. and Harris, N.D. 1986. Unpublished data. Florida State Univ.
Khamov, I.N. 1938. Influence of sugar on the electrode potentials. Chem. Abstr. 33: 7669.
Smith, M.J. and Manahan, S.E. 1973. Copper determination in water by standard addition potentiometry. Analytical Chem. 45: 836.
Stumm, W. and Morgan, J.J. 1970. Acids and bases. In "Aquatic Chemistry," p. 85. Wiley-Interscience, New York.
Troller, J. 1983. Methods to measure water activity. J. Food Protection 46: 129.

Ms received 9/15/86; accepted 11/3/86.

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Shank, R.C. 1981. "Mycotoxins and N-Nitroso compounds, Environmental Risks". Vol. 1, (Ed.) R.C. Shank. CRC Press, Boca Raton, FL.
Sharma, A., Behere, A.G., Padwal-Desai, S.R., and Nadkarni, G.B. 1980. Influence of inoculum size of *Aspergillus parasiticus* spores on aflatoxin production. Appl. Environ. Microbiol. 40: 989.
Sharma, A., Padwal-Desai, S.R., and Nadkarni, G.B. 1985. Possible implications of reciprocity between ethylene and aflatoxin biogenesis in *Aspergillus flavus* and *Aspergillus parasiticus*. Appl. Environ. Microbiol. 49: 79.
Liem, D.H. and Beljaars, P.R. 1970. Note on rapid determination of aflatoxin in peanuts and peanut products. J. Assoc. Off. Anal. Chem. 53: 1064.

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A Research Note

A Rapid Method for the Determination of Thiobarbituric Acid Reactive Substances

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ABSTRACT

A method which significantly reduced the time required for thiobarbituric acid reactive substances determination by using an autoclave for a short period of time instead of using the conventional heating methods was developed. This procedure allowed small samples to be analyzed in a very short length of time. Ground beef and standards were compared using the conventional and new methods with no statistical differences.

INTRODUCTION

THE USE OF 2-thiobarbituric acid (TBA) as an indicator of the degree of rancidity in fat-containing foods was first proposed by Sinnhuber and Yu (1958); however, the most widely used procedure is that of Tarladgis et al. (1960). This procedure may be divided into three distinct sections: the distillation, the TBA reaction, and optical densitometry. The first two steps require approximately an hour to complete in a laboratory set up to perform thiobarbituric acid reactive substances (TBARS) determination on a regular basis. The method of Tarladgis et al. (1960) is obviously tedious in its set-up and execution. Fox (1981) found several simplifications to the classical method which permit a more "bench-top" approach to the Tarladgis method.

The purpose of this research was to decrease the amount of time required in the second (TBA reaction) step. However, a way was found to decrease the time required for the first step, the distillation, as well as cutting the sample size by 90%. These procedural modifications are based on earlier work of Spillman (1981).

MATERIALS & METHODS

Materials

Ground beef. The ground beef was weighed (approximately 5g each) and formed into (2.5 cm) round balls, placed in aluminum foil and cooked for five min at 180°C. Distillate of 24 hr old, cooked ground beef was prepared by the method of Tarladgis et al. (1960) on the original apparatus used in that classical experiment.

1,1,3,3,-Tetraethoxypropane (TEP) standard was diluted in warm deionized water to yield concentrations ranging from 4×10^{-6} M to 2×10^{-5} M. 2-Thiobarbituric acid was dissolved in 90% acetic acid to give a 0.02M solution. Complete dispersion of the TBA required moderate heating on a magnetic stirrer/warming stage.

Thiobarbituric acid reactive substances determination

One milliliter sample or standard was placed in each test tube, 1 mL TBA reagent was added and the tube closed with glass stoppers and vortexed. Solutions of deionized water and TBA were run as standards. The test tubes were then placed in either: (1) a boiling water bath (100°C) for 60 min; or (2) in an autoclave at 8.6 to 9.1 kg pressure and 120°C for 15 min with timing beginning when the temperature reached 120°C.

After heating and allowing the tubes to return to room temperature (20°C), 12 mL 1-butanol were immediately added to each tube. The tubes were again stoppered and vortexed. The percent transmittance (%T) was read at 532 nm (Sinnhuber et al., 1958) using a Bausch and Lomb Spectrophotometer 20 calibrated against a water blank. The results were converted to absorbance (A) using the formula:

$$A = 2 - \log(\%T)$$

Statistical analysis. Results were read as %T rather than as A directly due to the greater accuracy afforded by the %T scale compared to the A scale. A z-score was run on the data and a least square line was fitted to the data.

Table 1—Average absorbance of 1,1,3,3-tetraethoxypropane standards[a]

	Autoclave	Water bath
Conc TEP	Mean A ± s.d.	Mean A ± s.d.
0M	0.029 ± 0.003	0.026 ± 0.016
4.0×10^{-6}M	0.372 ± 0.036	0.348 ± 0.017
8.0×10^{-6}M	0.647 ± 0.013	0.625 ± 0.011
1.2×10^{-5}M	0.934 ± 0.028	0.887 ± 0.033
1.6×10^{-5}M	1.126 ± 0.929	1.099 ± 0.046
2.0×10^{-5}M	1.301 ± 0.0	1.261 ± 0.039

[a] 50 replications

Table 2—Average absorbance of ground beef distillate[a]

	Mean A ± s.d.	Range
Water Bath	0.0799 ± 0.024	0.0969 ± 0.0629
Autoclave	0.0605 ± 0.0	0.0605 ± 0.0

[a] 50 replications

RESULTS & DISCUSSION

TABLE 1 shows the absorbance for each concentration of TEP. The autoclave treatment of the TBA/TEP solution provided the same results as those obtained utilizing the longer water bath method. Examination of the means indicated that the autoclave treatment yielded a more complete reaction; that is, the means of the absorbance for the autoclaved samples were slightly greater than those of the water bath method samples.

Examination of Table 2 (the absorbance of the ground beef distillate) indicates similar results. These data further supported the positive correlation of results obtained by water bath treatment and those obtained by autoclave treatment.

Caution should be exercised in comparing curves or TBA numbers obtained by one preparation to evaluate the results obtained by our method since distillation may cause some concentration of malonaldehyde in the sample and the standard. When both are treated in the same fashion, any error would drop out of the calculations. However, if yields are in fact different, substantial errors could result from crossing procedures to calculate TBA numbers.

REFERENCES

Fox, J.D. 1981. Personal communication. Univ. of Kentucky, Lexington, Ky.

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A Research Note

Determination of Mass Diffusivity of Simple Sugars in Water by the Rotating Disk Method

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ABSTRACT

The rotating disk method is shown to be an accurate method for determining the mass diffusivity of solid food components in liquid solutions. The diffusivities of sucrose and glucose in water were determined to be 0.50 and 0.66 (m^2/sec × 10^9) at 25°C with activation energies of 36.3 and 31.6 kJ/g-mol, respectively.

INTRODUCTION

THE DESIGN and analysis of mass transfer operations requires knowledge of the diffusion coefficients of the components under consideration. These diffusion coefficients are often estimated from correlations, e.g. the Wilke-Chang for small molecules or Stokes-Einstein form for macromolecules. Application of these types of correlations to food systems may result in very crude estimates of diffusivity, since they do not account for the effects of important parameters such as pH and ionic strength on the interactions between the solute and solvent. Experimental methods, especially those that allow the determination of diffusivities of a solute in the particular solvent of interest and at the appropriate temperature, are preferable to empirical correlations. One such method is the rotating disk, which has been used to measure the diffusivity of 1-naphthol in water (Bourne and Tovstiga, 1985).

Theory

If a food component is (a) solid at the temperature of interest, and (b) can be formed into the shape of a disk, then its integral diffusivity in any solvent may be determined by rotating that disk in the solvent and following its rate of weight loss (or concentration gain in solution).

The rotating disk method involves following the dissolution of a partially soluble substance from a disk of area A rotating at an angular velocity ω in a mass M of solution. If x′ is the mass fraction of solute in solution at any time t′ and x* is the same quantity at saturation, the mass transfer coefficient k of a solute dissolving into the liquid may be macroscopically measured by following the mass fraction of solute in the solution as a function of time (Bourne and Tovstiga, 1985).

$$M \frac{dx'}{dt'} = kA(x^* - x') \qquad (1)$$

which when integrated from $x' = 0$ to x and $t' = 0$ to t yields,

$$k = \frac{-M}{At} \ln\left(1 - \frac{x}{x^*}\right) \qquad (2)$$

If molecular diffusion controls the dissolution rate, and if the Schmidt number (Sc = μ/ρD where μ is the viscosity, ρ is the density, and D the diffusion coefficient) is greater than 100, then the mass transfer coefficient from a rotating disk follows the relation (Levich, 1962; Newman, 1973),

$$k = \frac{0.6205\ \rho D^{2/3} \omega^{1/2}}{\upsilon^{1/6}\left(1 + 0.298\ Sc^{-1/3} + 0.014515\ Sc^{-2/3}\right)} \qquad (3)$$

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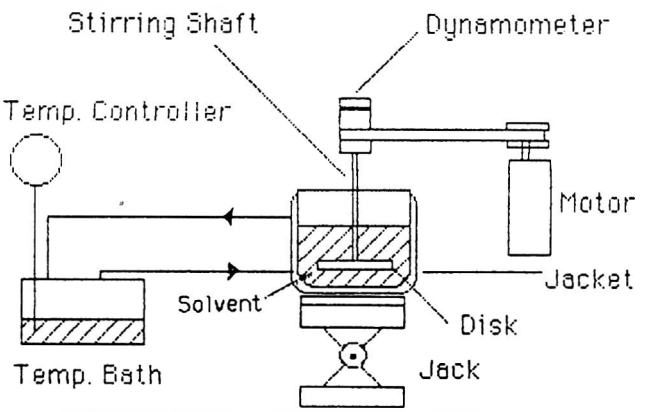

Fig. 1—Schematic of the rotating disk apparatus.

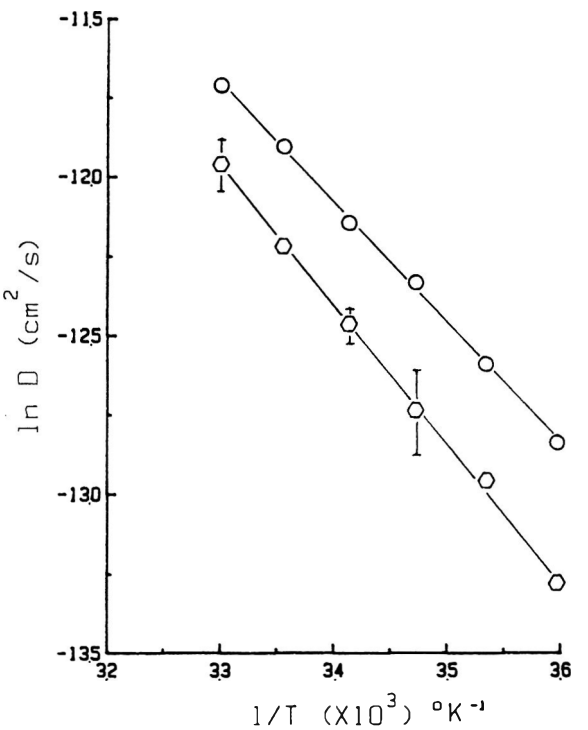

Fig. 2—Arrhenius plot showing the temperature dependence of the mass diffusivity of sucrose (○) and glucose (○) in water for the temperature range 5–30°C.

where $\upsilon = \mu/\rho$. Eq. (3) can be solved for D as,

$$D = \left[\frac{-0.2980k\upsilon^{-1/6} - (0.0888k^2\upsilon^{-1/3} + 2.482\rho\omega^{1/2}k\upsilon^{1/6} - 0.05804k^2\upsilon^{-1/3})^{1/2}}{0.02902k\upsilon^{-1/2} - 1.241\rho\omega^{1/2}}\right]^3 \quad (4)$$

Thus if the viscosity and density of the solution are known or measured, and if the mass transfer coefficient is measured, the diffusivity of the component of interest in a solvent can be computed. The purpose of this paper is to describe the rotating disk method for determining mass diffusivity, and present a simplified equation for the calculation of diffusivity from mass transfer data using sucrose/water and glucose/water as model food systems.

MATERIALS & METHODS

Disk preparation

Glucose disks were prepared by melting pure anhydrous glucose and pouring the molten mass into a 66 m diameter × 8 mm high mold. After pouring, the molds were hardened in a −18°C freezer and stored in a dessicator until used.

Sucrose disks were prepared by dissolving 50g of a mixture of 95% sucrose and 5% 42 DE corn syrup solids into approximately 25g water. The solution obtained was then boiled (sucrose decomposes if heated directly) until the temperature reached 160°C, poured into the mold, hardened in the freezer and stored as were the glucose disks. Corn syrup solids were added to the sucrose disks to prevent crystal growth, maintaining the sucrose in an amorphous form. This was necessary because for highly crystalline sucrose dissolution occurred at crystal boundaries and entire crystals would then separate from the disk.

Measurement procedure

The experimental apparatus is shown schematically in Fig. 1. Two holes, equally spaced from the center, were drilled in the disks which were then bolted onto a specially modified steel nut and screwed onto the end of the stirring shaft. The shaft was rotated at 500 rpm (measured by an electronic tachometer) using a MagneDrive II driven by a belt attached to a variable speed motor (Autoclave Engineers Erie, PA).

A jacketed stainless steel vessel was filled with a pre-weighed amount (approximately 600g) of water and maintained at the desired temperature by means of a circulating ethylene glycol/water bath (Haake Model A81). The vessel was raised using a lab jack, and a timer started when the rotating disk was completely covered with fluid. The test duration was 2 minutes, after which the vessel was lowered and a 20 mL sample of fluid was removed. This sample was placed in a tared beaker, weighed and concentrated by evaporating a portion of the water in a microwave oven until less than 2 mL remained. Sugar concentration in the concentrated sample was measured using a Bausch and Lomb temperature compensated refractometer. Sugar concentration in the original sample was then determined by back calculation. Experiments were conducted at 5°, 10°, 15°, 20°, 25°, and 30°C (2 replicates at each temperature) as measured directly in the solvent using a mercury thermometer. Physical properties of the solution were taken from the literature (Reid et al., 1977) as the physical properties of pure water since sugar concentration never exceeded 4%. Equilibrium concentrations were obtained from the International Critical Tables of Numerical Data (Bates et al., 1927). Mass transfer areas were calculated as the arithmetic mean of the surface area of the disk before and after each experiment. From these data, all of the parameters in Eq. (4) are known and the diffusion coefficients could be calculated.

RESULTS & DISCUSSION

VALUES of diffusivity at 25°C as determined by the rotating disk method were 0.50 and 0.66 ($m^2/sec \times 10^9$) for sucrose and glucose in water, respectively, which compares favorably to reported values of 0.54 and 0.69 ($m^2/sec \times 10^9$) for the diffusivities of sucrose and glucose at infinite dilution in water at 25°C (Chandrasekaran and King, 1972). Arrhenius plots showing the temperature dependence of the mass diffusivities are presented in Fig. 2. Activation energies calculated from the slopes in Fig. 2 are 36.3 and 31.6 kJ/g-mol for sucrose and glucose, respectively.

SUMMARY

THE ROTATING DISK METHOD has been shown to be a relatively simple and reliable technique for determining the mass diffusivity of food components in liquids. The major disadvantage of the methodology results from the requirement that the compounds of interest must be formed into disks of sufficient rigidity to physically endure the test procedure which is not possible for all food components.

REFERENCES

Bates, F., Phelps, F.P., and Synder, C.F. 1927. Saccharimetry, the properties of commercial sugars and their solutions. In "International Critical Tables of Numerical Data Physics, Chemistry and Technology," Vol. II, 1st ed, p 334. McGraw Hill, New York.

Bourne, J.R. and Tovstiga, G. 1985. Measurement of the diffusivity of 1-Naphthol in water with a rotating disk. Chem. Eng. Comm. 36: 67.

Chandrasekeran, S.K. and King, C.J. 1972. Multicomponent diffusion and vapor-liquid equilibria of dilute organic components in aqueous and sugar solutions. AIChE J. 18(3): 513.

Levich, V.G. 1962. "Physico-Chemical Hydrodynamics," ch. 2–3. Prentice-Hall, Englewood Cliffs, NJ.

Newman, J.S. 1973. "Electrochemical Systems," p. 309. Prentice-Hall, Englewood Cliffs, NJ.

Reid, R.C., Prausnitz, J.M., and Sherwood, T.P. 1977. "The Properties of Gases and Liquids." McGraw Hill, New York.

Ms received 10/26/86; accepted 12/17/86.

DETM OF TBA REACTIVE SUBSTANCES. . .From page 500

Sinnhuber, R.O. and Yu, T.C., 1958, 2-Thiobarbituric acid method for the measurement of rancidity in fishery products. 2. The quantitative determination of malonaldehyde. Food Technol. 12: 9.

Sinnhuber, R.O. Yu, T.C., and Yu, T.C. 1958. Characterization of the red pigment formed in the 2-thiobarbituric acid determination of oxidative rancidity. Food Res 23: 626.

Spillman, D.M. 1981. The effects of feeding regime, vacuum packaging and aging on the organoleptic quality, physical characteristics and thiamin, riboflavin and niacin content of beef steaks. Ph.D. dissertation, Univ. of Kentucky, Lexington, KY.

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

Ms received 6/27/86; revised 12/16/86; accepted 12/16/86.

A Research Note

Extraction of Carotenoprotein from Shrimp Process Wastes with the Aid of Trypsin from Atlantic Cod

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ABSTRACT

Atlantic cod trypsin or bovine trypsin were used to aid the extraction of carotenoprotein from shrimp wastes at 4°C. When 25 mg% cod trypsin was added to extraction medium containing 0.5N ethylene diaminetetraacetic acid (EDTA) 64% of the astaxanthin and 81% of the protein of shrimp waste was recovered as carotenoprotein in 24 hr. With 25 mg% bovine trypsin, under otherwise identical conditions, the carotenoprotein recovered represented 49% of the astaxanthin and 65% of the protein of the waste. Semi-purified cod trypsin was not as effective as pure trypsin in facilitating recovery of carotenoprotein from shrimp waste. The recovery of carotenoprotein from shrimp waste, during extraction at 4°C with or without trypsin, was facilitated by EDTA.

INTRODUCTION

CAROTENOPROTEIN, a by-product from crustacean process offal, has potential for use as a feed supplement in rations of farmed fish or as a colorant and flavorant for use in food products (Simpson and Haard, 1985a; Simpson and Haard, 1985b). When the extraction medium includes 25 mg% bovine trypsin, 0.5N ethylene diamine tetra acetic acid (EDTA), pH 7.7 at 4°C as much as 80% of the protein and astaxanthin of shrimp waste can be recovered as a stable carotenoprotein complex. At higher temperatures the speed of carotenoprotein extraction is increased and EDTA is not required for high yield; however the maximum yields are somewhat lower and the odor and taste of the product is adversely affected.

Given the low habitat temperature of Atlantic cod, its trypsin is expected to be a more efficient catalyst than bovine trypsin at low reaction temperatures (Simpson and Haard, 1984a). The present study was undertaken to compare the efficacy of trypsin from Atlantic cod pyloric ceca with that from bovine pancreas in releasing carotenoprotein from shrimp waste at low reaction temperatures. The utility of semi-purified cod trypsin isolates to aid recovery of carotenoprotein from shrimp wastes was also examined.

MATERIALS & METHODS

Materials

Atlantic cod (*Gadus morhua*), obtained live from holding tanks at the Marine Sciences Research Laboratory, were stunned with a blow to the head and the pyloric ceca was removed and used to obtain trypsin. Raw, frozen shrimp (*Pandulus borealis*, Kroyer) were donated by Fishery Products International, St. John's, Nfld. Bovine trypsin, N-benzoylarginine-p-nitroanilide (BAPA), soybean trypsin inhibitor, EDTA, tris(hydroxymethyl)aminomethane(Tris), cyanogen bromide-activated Sepharose 4B were obtained from Sigma Chemical Co., St. Louis, MO. Polyethylene lauryl ether (Brij 35) was obtained from BDH Chemicals, Dartmouth, N.S. All other reagents were of reagent grade and were obtained from Fisher Scientific, Halifax, N.S.

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Isolation of trypsin

Trypsin, from the pyloric ceca of Atlantic cod, was isolated and purified by a procedure used by us earlier with Greenland cod (Simpson and Haard, 1984a). The purified trypsin exhibited one band after electrophoresis in the presence or absence of SDS as described by Simpson and Haard (1984a). Trypsin activity was standardized by the amidase reaction as described by Simpson and Haard (1984a). One BAPA unit is defined as an absorbancy change (410 nm) of 2.933/min at 25°C, pH 8.2 in a 3 mL reaction mixture.

Extraction of carotenoprotein

Carotenoprotein was extracted and isolated from cooked shrimp waste as described by Simpson and Haard (1985a) using 25 mg% trypsin. In certain experiments, when EDTA was not added to the extraction medium, the pH was maintained at 7.7 by titration with 0.5N HCl using a metrohm autotitration unit. In separate experiments, the "Brij fraction" or the "ammonium sulfate fraction" of semi—pure trypsin, equivalent to 25 mg% pure trypsin on the basis of it's amidase activity (after activation) with BAPA substrate at 25°C, was used instead of purified cod trypsin.

Total astaxanthin and protein content of carotenoprotein

The lyophilized carotenoprotein was analyzed for total astaxanthin and protein as described by Simpson and Haard (1985a). The protein and astaxanthin content of carotenoprotein was expressed as % recovered, i.e. the total astaxanthin (or protein) recovered in the carotenoprotein fraction as a per cent of that which was present in the raw shrimp waste prior to extraction.

RESULTS & DISCUSSION

Amidase activity of Atlantic cod trypsin

At 25°C, the specific activity of purified cod trypsin was 1.14 BAPA units/mg protein, and was similar to that of bovine trypsin, 1.13 BAPA units/mg protein. The temperature coefficients for the amidase reaction catalyzed by cod and bovine trypsin were 1.76 and 2.08 respectively. Thus at 5°c, the specific activity of cod trypsin (0.37) was almost 50% higher than that of bovine trypsin (0.26). The relatively low temperature coefficient for Atlantic cod trypsin is similar to that reported earlier by us for Greenland cod trypsin, i.e. 1.62 (Simpson and Haard, 1984a). The specific activities of the "Brij" and "ammonium sulfate" fractions, employed as extraction aids, were 0.4 and 0.41 BAPA units/mg protein at 25°C respectively.

Comparison of bovine and cod trypsin as extraction aids

The recovery of astaxanthin and protein from shrimp waste when extraction was carried out for various times up to 48 hr at 4°C in 0.5M EDTA, pH 7.7 containing either 25 mg% bovine trypsin, 25 mg% pure cod trypsin or no enzyme is shown in Fig. 1. Both sources of trypsin were effective in

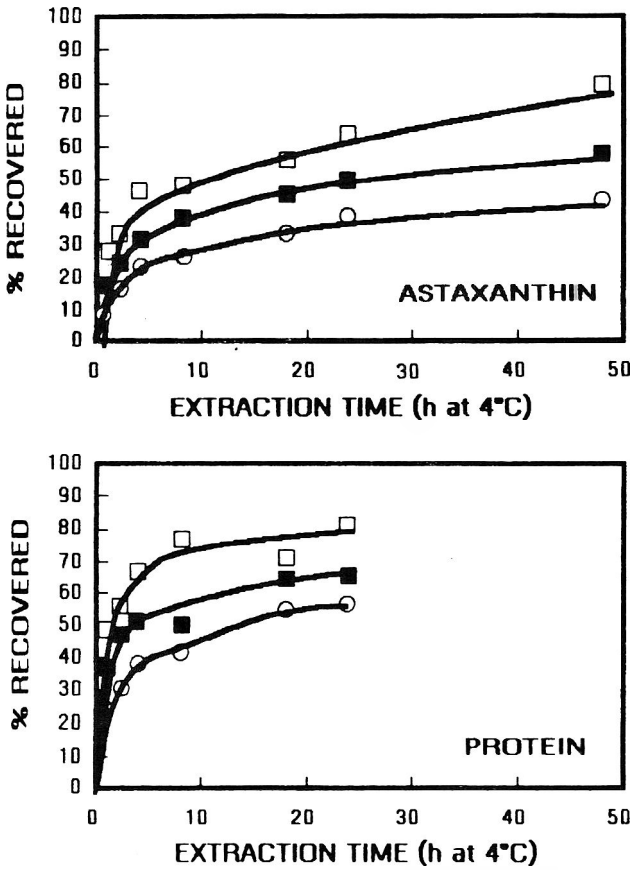

Fig. 1—Recovery of astaxanthin and protein from shrimp wastes as carotenoprotein. Extraction was at 4°C with 0.5M EDTA, pH 7.7 containing no added enzyme (○), purified Atlantic cod trypsin (□), or purified bovine pancreatic trypsin (■). The astaxanthin and protein contents of the shrimp waste employed were 128 mg% and 2.91%, respectively, prior to extraction. Data are the average of duplicate results for one experiment and are representative of results obtained in two other experiments.

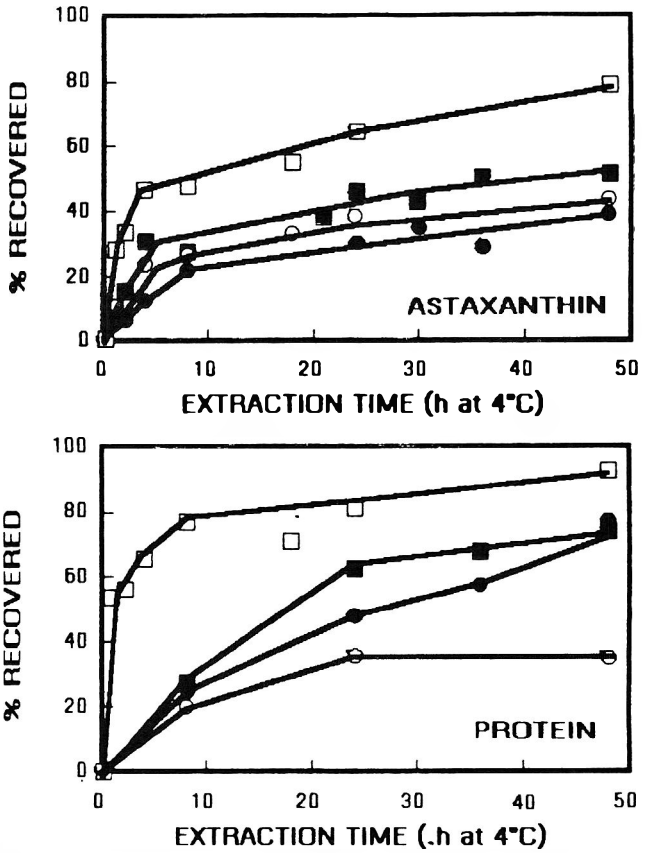

Fig. 2—Recovery of astaxanthin and protein from shrimp wastes as carotenoprotein. Extraction was at 4°C with 0.5 M EDTA, pH 7.7 containing no added enzyme (○), pure cod trypsin (□), "Brij" fraction of cod trypsin (●) or "ammonium sulfate" fraction of cod trypsin (■). Data are the average of duplicate results for one experiment.

aiding the extraction of carotenoprotein compared to the control which did not contain added enzyme. Cod trypsin was more effective than bovine trypsin in aiding pigment and protein recovery. On the basis of the temperature coefficients of BAPA hydrolysis for the two enzymes, the cod trypsin would be expected to be almost 1.5 times as active as bovine trypsin at 4°C. Thus Atlantic cod is an advantageous industrial enzyme for use at low temperature as already shown for Greenland cod trypsin (Simpson and Haard, 1986b).

Extraction of carotenoprotein at 4°C without EDTA

Both cod and bovine trypsin were less effective, and did not appreciably differ, in aiding extraction of carotenoprotein from shrimp waste when EDTA was omitted from the extraction medium. The astaxanthin recoveries after 48 hr extraction with cod trypsin, bovine trypsin and control were only 36%, 35% and 18% respectively. The protein recoveries for these samples were 56%, 54% and 37% respectively. Trypsin aided extraction at 4°C differs from that carried out at 50°C, since at the higher temperature EDTA does not have a marked effect on carotenoprotein extraction (Simpson and Haard, 1985b).

Semi-purified cod trypsin as extraction aids

The "AS" and "Brij" fractions of cod trypsin appeared to be very effective in releasing astaxanthin from shrimp waste since the chitinous residue obtained after extraction was rendered colorless. However, the pigment released during extraction with semi-purified cod trypsin was not recovered, as completely, in the carotenoprotein fraction compared to pure cod trypsin (Fig. 2). After making the extracts containing semi-purified trypsin to 45% saturation with ammonium sulfate and centrifugation, much of the pigment remained soluble or separated as an oil. These observations indicate that impurities, presumably other proteolytic enzymes or lipolytic enzymes which were not separated from pyloric ceca extracts by the initial purification steps, act to degrade the carotenoprotein. Previously we found that technical grade porcine trypsin or Enzeco protease AP-1 were less effective than pure bovine trypsin in facilitating release of intact carotenoprotein from shrimp wastes (Simpson and Haard, 1985a).

CONCLUSIONS

THE PRESENT STUDY indicates Atlantic cod trypsin is a more effective extraction aid than is bovine trypsin for recovering carotenoprotein from shrimp process wastes at 4°C when EDTA is present in the extraction medium. Unfortunately, the semi-purified cod trypsin fractions tested were not very effective in recovering intact carotenoprotein under the conditions employed in this study. It is likely that use of pure trypsin as an extraction aid would be prohibitive for commercial preparation of carotenoprotein because of the expense of purified enzymes. For this reason, additional study should be directed toward finding a practical way of obtaining cod trypsin which does not have residual activity which is autolytic to carotenoprotein.

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A Research Note

Observations on the Functional Properties of U.S. Land-Processed Surimi

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ABSTRACT

Several methods were used to evaluate the quality of ship-processed and U.S. land-processed surimi. The fold test is useful to distinguish between high and low quality surimi but lacks the sensitivity to discriminate between surimi samples having similar yet different functional properties as measured by the punch test and torsional shear test. Moisture content of U.S. land-processed surimi was linearly correlated with several functional properties. Much work is still required to determine the relationship of the results of the various tests to the quality and sensory properties of finished products.

INTRODUCTION

IMPORTS of surimi, a refined protein product obtained by washing mechanically deboned fish flesh, and products prepared from surimi such as seafood analogs have increased rapidly in the last few years reaching over 88 million pounds in 1985 (Parker, 1986). In 1986, Alaska Pacific Seafoods Inc. (APS) in Kodiak, AK, produced over 1.8 million pounds of surimi, successfully completing the first commercial U.S. production of surimi funded by a National Marine Fisheries Service Saltonstall-Kennedy grant through the Alaska Fisheries Development Foundation. As U.S. manufacturers began to use the APS produced surimi, it was not clear as to what criteria should be used to evaluate its quality.

In Japan, several grades of surimi are produced and criteria for grading are well established (Suzuki, 1981), but these criteria may or may not be useful to manufacturers who are producing items other than traditional seafood analogs. Lanier et al. (1985) proposed the use of a torsional shear test in place of the punch test used in Japan to define the functional properties of surimi. In addition, Webb (1985) studied the water-holding capacity of Japanese ship-processed and U.S. land-processed surimi. The purpose of this study was to evaluate the functional properties of U.S. land-processed surimi using a variety of tests and to investigate the interrelationship of the tests.

MATERIALS & METHODS

Raw materials

Blocks of surimi were produced during February and March, 1986 from Alaska pollock (*Theragra chalcogramma*) at APS, Kodiak, Alaska. The process was similar to that used in Japan (Lee, 1984) except a Baader 182 filleting machine and an in-line washing system described by Kelly (1986) were used to prepare and wash the minced pollock flesh. From the surimi produced by APS during this period, 21 blocks (10kg each) were selected, with care given to obtain a wide range of moisture contents. Thus, the samples should only be used to reflect the range rather than define the typical functional properties of surimi produced at APS. These blocks were held at −20°C for no longer than 3 months until tested. Two blocks of high quality Japanese factory ship-processed surimi were also tested.

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Preparation of gels

Frozen surimi quality testing standards (Suzuki, 1981) were used to prepare and evaluate the surimi blocks. Samples were tempered for 16-18 hrs at 4°C and chopped in a silent cutter until the temperature reached 0°C, then fine salt equal to 3% of the weight of the surimi was gradually added. The mixture was chopped for up to 12 min or until the temperature of the batter reached 9°C. The batter was stuffed in 50mm diameter Saran casings for the punch test or metal tubes for the torsion test. The samples were cooked by immersion in a water bath for 20 min at 40°C followed by 20 min at 90°C. The cooked samples were immediately cooled with cold water for 20 min and held at 4°C for 16-18 hrs.

Punch and torsion test

To determine gel strength, cooked samples were equilibrated to 20°C by holding at ambient temperature for 2 hr, cut into 30mm long sections and placed on a Model 302-B Food Checker rheometer (Sun Kagaku Co., Ltd) equipped with a 5mm spherical probe. The probe speed was set at 1mm/sec and force and deflection at failure was recorded. Ten sections were measured for each sample. Torsional stress and strain were determined using the method of Kim et al. (1986). Five replicate determinations were made.

Moisture, fold test and water-holding capacity

Moisture content was determined by drying a weighed sample at 105°C for 16–18 hr. A 3 mm thick slice of the punch sample was used in the fold test following the scoring procedure of Kudo et al. (1973). Water holding capacity (WHC) was determined using a procedure similar to that of Webb (1985) except the slurry (4:1 ratio of 2% NaCl: surimi) was centrifuged 10 min at 10,000g. A low WHC number indicates that most of the water was retained by the surimi. Correlation coefficients and other statistical data were determined using SPSS computer programs (Nie, 1975).

RESULTS & DISCUSSION

FOR THE U.S. land-processed surimi, moisture content was linearly correlated with punch force, punch deflection, shear stress and gel strength ($r = -0.456, -0.752, -0.443, -0.601$ respectively, $P<0.05$). The linear relationship between moisture content and gel strength may allow for a correction factor so that gel strength values could be adjusted to a constant moisture basis making comparisons of different surimi samples easier. Correction factors may be influenced by other properties of surimi and may not be the same if different processing methods are used however.

For example, the factory-ship and U.S. land-processed surimi had similar moisture contents but different functionality (Table 1). Although total protein content, which can be assumed to be inversely related to the moisture content, influences functionality, the type and purity of the protein is also important. The functionality of surimi is dependent on the myofibrillar protein fraction and washing of minced fish during the preparation of surimi increases the concentration of these proteins (Babbitt, 1986). Differences in washing technique and

Table 1—Functional properties of factory-ship and U.S. land-processed surimi[a]

	Land-processed	Factory-ship
Moisture (%)	76.7 ± 1.7	76.1 ± 0.2
WHC (%)	67.5 ± 16.3	69.3
Fold Test	5	5
Punch force (PF)[d] (g)	440 ± 87	930 ± 19
Punch deflection[d] (PD) (mm)	13.9 ± 1.4	16.9 ± 0.5
Gel strength[b,d] (gxcm)	617 ± 153	1564 ± 83
Gel stiffness[c,d] (g/cm)	317 ± 56	542 ± 57
Shear stress[d] (kPa)	49.4 ± 11.5	76.5 ± 6.8
True strain	2.19 ± 0.31	2.30 ± 0.49
Rigidity[d] (stress/strain)	22.9 ± 5.6	33.8 ± 4.2

[a] Mean ± std. dev. of 21 lots of U.S. land-processed and 2 lots of factory-ship processed surimi.

[b] Gel strength (gxcm) = PF × (PD × 0.1).

[c] Gel stiffness (g/cm) = PF/(PD × 0.1).

[d] Properties were significantly different ($P<0.05$, Least significant difference test).

other manufacturing procedures, such as refining as well as freshness of fish at the time of processing, could easily account for the factory-ship samples produced at sea having different functional properties.

For the land-processed surimi, gel strength was significantly correlated to rigidity ($r=0.440$) which may indicate they are influenced by the same factors. WHC was not significantly correlated to other results however. Evidently, the ability to retain water in an uncooked state depended on different properties than the ability to form a strong cooked gel.

Interestingly, all of the surimi samples received the highest score (5, "extremely elastic") in the fold test. Thus, while the traditional fold test is used in Japan to distinguish between high and low grade surimi, it lacks the sensitivity of the punch or torsion tests. For the manufacturer of surimi based products, tests in addition to the fold test may be necessary to assure product consistency. Additional work is needed to determine what properties in fish flesh are responsible for the functional properties of surimi and if differences in functional properties as measured by the punch and torsional shear tests affect the quality of finished products.

REFERENCES

Babbitt, J.K. 1986. Suitability of seafood species as raw materials. Food Technol. 40(3): 97.

Kelly, B. 1986. The 1985 Alaska Pacific Seafoods surimi production experience. In "Proceedings of the International Symposium on Engineered Seafood Including Surimi," p. 247, (Ed.) R.E. Martin and R.L. Collette. National Fisheries Institute, Washington, DC.

Kim, B.Y., Hamann, D.D., Lanier, T.C., and Wu, M.C. 1986. Effects of freeze-thaw abuse on the viscosity and gel-forming properties of surimi from two species. J. Food Sci. 51: 951.

Kudo, G., Okada, M., and Miyanchi, D. 1973. Gel-forming capacity of washed and unwashed flesh of some Pacific coast species of fish. Mar. Fish. Rev. 35(12): 10.

Lanier, T.C., Hamann, D.D., and Wu, M.C. 1985. Development of methods for quality and functionality assessment of surimi and minced fish to be used in gel-type food products. Final Report. Alaska Fisheries Development Foundation, Anchorage, AK.

Lee, C.M. 1984. Surimi process technology. Food Technol. 38(11): 69.

Nie, N.H. 1975. "SPSS, Statistical Package for the Social Sciences," 2nd ed. McGraw-Hill, Inc. New York.

Parker, P. 1986. The surimi market comes of age. Seafood Business. 5(3): 40.

Suzuki, T. 1981. "Fish and krill protein: Processing Technology." Appl. Sci. Publ., Ltd., London.

Webb, N. 1985. Surimi applications to the food industry. Final Report. Alaska Fisheries Development Foundation. Anchorage, AK.

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CAROTENOPROTEIN EXTRACTION FROM SHRIMP WASTES. . .

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REFERENCES

Saito, A. and Regier, L.W. 1971. Pigmentation of brook trout (*Salvelinus fontinalis*) by feeding dried crustacean waste. J. Fish. Res. Board Canada 28: 509.

Simpson, B.K. and Haard, N.F. 1984a. Purification and characterization of trypsin from the Greenland cod (*Gadus ogac*). 1. Kinetic and thermodynamic characteristics. Can. J. Biochem Cell Biol. 62(9): 894.

Simpson, B.K. and Haard, N.F. 1984b. Trypsin from Greenland cod as a food-processing aid. J. Appl. Biochem. 6: 135.

Simpson, B.K. and Haard, N.F. 1985a. The use of proteolytic enzymes to extract carotenoproteins from shrimp wastes. J. Appl. Biochem. 7: 212.

Simpson, B.K. and Haard, N.F. 1985b. Extraction of carotenoprotein from crustacean wastes. Canadian Patent application, file #265-8192-1, filed July 24, 1985.

Ms received 8/11/86; accepted 12/23/86.

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A Research Note

Storage Stability of Nonpackaged Irradiated Indian Mackerel (*Rastrelliger kanagurta*) in Ice

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ABSTRACT

The storage stability of eviscerated Indian mackerel (*Rastrelliger kanagurta*) irradiated at a dose of 1.5 kGy and held under melting ice was examined. Packaging in polyethylene pouches prior to irradiation gave a shelf-life of 25 days, while, nonpackaged, irradiated fish remained in acceptable condition up to 20 days. The nonpackaged irradiated fish has less irradiation odor and better appearance as compared with packaged irradiated fish during the course of ice storage. Uniradiated and nonpackaged mackerel kept in ice were acceptable only for 14 days. The results suggest that pre-irradiation packaging was not essential for commercially adequate extension in shelf-life of the iced fish.

INTRODUCTION

PACKAGING of fresh fishery products prior to radurization treatment is considered essential in order to prevent microbial contamination during post-irradiation refrigerated storage (Agarwal and Sreenivasan, 1973). However, apart from the cost factor, keeping radurized fish in pouches has some disadvantages. These include the possibility of the pouches favoring growth of anaerobic organisms like *Clostridium botulinum* during inadvertent increase in storage temperature, unattractive appearance of certain types of eviscerated fish species due to drip and oozing of blood, and chances of interaction of radiolytic products of packaging material with fish constituents leading to loss of natural flavor.

Radurization treatment reduces the initial load of spoilage microflora present in the fish (Alur et al., 1971, Silliker et al., 1980). If the treatment is given in nonpackaged condition, post-irradiation contamination occurs mainly through the ice in which the fish is held. However, during storage, melting ice has been shown to wash off the microorganisms and enhance acceptability of Indian mackerel (Surendran and Iyer, 1976), prawn (Angel et al, 1985) and fresh water fish (Poulter and Nicolaides, 1985). Therefore, as compared with unirradiated fish, the treated fish held at 0°C is likely to take a longer time to attain the threshold microbial spoilage potential to make it unacceptable. These considerations prompted us to investigate the shelf-life of non-packaged radurized Indian mackerel.

MATERIALS & METHODS

Fish

Fresh mackerel was washed repeatedly in potable water both prior to and after evisceration. The fish were divided into four lots of at least ten each. Two lots were kept without packaging in perforated (hole diam 1.6 cm) aluminum boxes, 30 × 13 × 20 cms. The remaining two lots were sealed in polyethylene (400 gauge) bags, each containing two fish, and the bags were placed in perforated aluminum boxes. Crushed ice was put around the fish. The boxes were exposed to gamma radiation at a dose of 1.5 kGy (Venugopal et al., 1973) in a ^{60}Co package irradiator (AEC Ltd., Canada, dose rate, 3.6 kGy per hr.). After irradiation, the boxes were stored in a cold room maintained at 0°C along with unirradiated packaged and nonpackaged controls. Crushed ice was replenished daily and the water from melting ice was removed periodically.

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Sensory evaluation

Fish were wrapped in aluminum foil and steamed (100°C) for 10 min. The steamed fish was immediately served to five trained and experienced panelists for odor evaluation. The 10-point scoring system (Bilinski et al., 1983) was as follows: 10—fresh odor, characteristic of steamed fresh Indian mackerel, 9—marginal loss of fresh odor, 8—slight loss of fresh odor, 7—definite loss of fresh odor, 6—almost no odor, 5—just detectable ammoniacal and rancid odor, 4 to 2—increasing strong off odor, 1—strong putrid odor. Fish that scored 10 to 5 were considered acceptable and those scored below 5 were spoiled. Untreated fish from the initial lot stored at −10°C, thawed prior to sensory evaluation and steamed with the experimental samples was included as a reference.

Chemical analyses

Total volatile basic nitrogen (TVBN) was estimated by the method of Farber and Ferro (1956). Total volatile acids (TVA) content was estimated as described earlier (Venugopal et al., 1981).

Bacterial counts

A 10-g sample from the belly portion of each fish was aseptically homogenized for 1 min with 90 mL sterile saline in a Sorval ommimixer. Appropriate serial dilutions of the homogenate were placed into petri dishes in duplicate. The colony forming units were determined using plate count agar (Difco). Plates were incubated at 25°C for 48 hr. An average of two replicates was taken.

RESULTS & DISCUSSION

DURING THE COURSE of ice storage accumulation of about 10 mL of drip, including blood, was observed in the packages, which imparted a brownish appearance to the fish. In the case of fish kept without polyethylene packaging, the melting ice removed the drip and, therefore, the fish retained their natural appearance. Although the use of whole mackerel would have prevented the drip formation, eviscerated mackerel was used in the present studies because evisceration is known to enhance the shelf-life (Bilinsky et al., 1983). After 2 wk of storage the nonpackaged irradiated and unirradiated fish were slightly slimy. Yellowing of the skin was also observed which was more pro-

Table 1—Sensory evaluation score of Indian mackerel stored in ice[a]

Storage period (days)	Unirradiated		Irradiated (1.5 kGy)	
	Packaged	Nonpackaged	Packaged	Nonpackaged
0	10	10	10	10
7	5.5	7	8	8
14	3	5	7	6
21	1	4	6	5
28	—	3.2	5	4

[a] The sensory evaluation was done as described in Materials & Methods. Average of three experiments are given. 10 = fresh odor, characteristic of steamed fresh fish; 5 = just detectable ammoniacal and rancid odor; 1 = strong putrid odor.

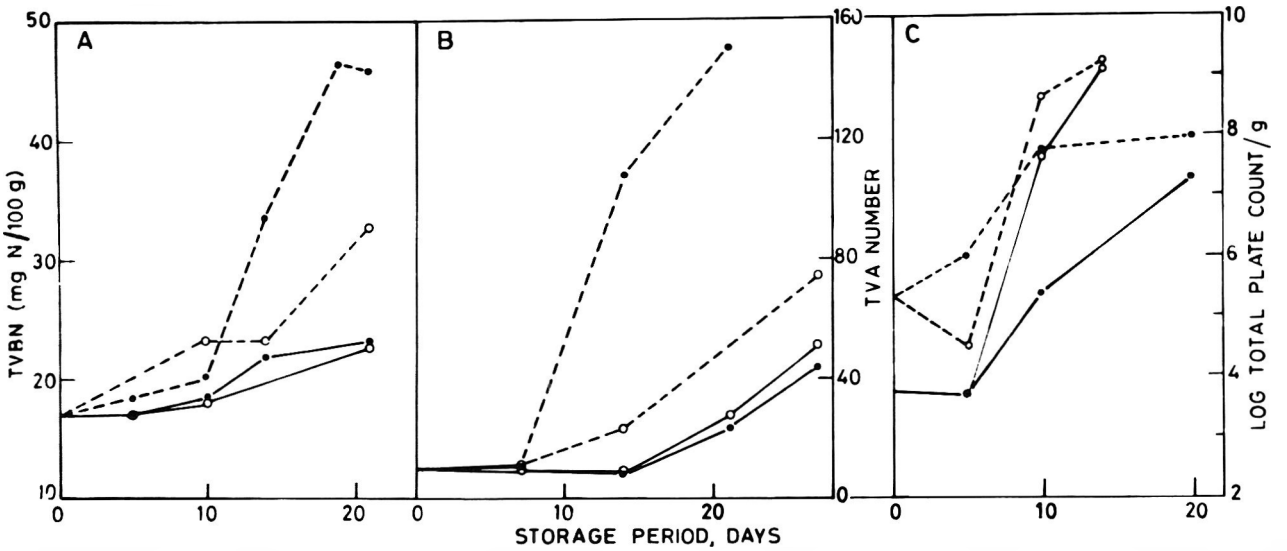

Fig. 1—Values of total volatile basic nitrogen (TVBN) (A), total volatile acids (TVA) number (B), and total plate count (C) of Indian mackerel during ice storage. TVBN values are expressed as mg per 100g fish. TVA values are expressed as ml of N/100 alkali required to neutralize volatile acids from 100g fish. - - - -, unirradiated; _____irradiated (1.5 kGy) ●, packaged; ○, nonpackaged.

nounced in the case of unirradiated nonpackaged fish, whereas the irradiated nonpackaged fish showed little yellowing. The irradiated fish regained its natural silvery look after thorough washing in tap water, whereas, the unirradiated fish remained yellowish in appearance. Appreciable irradiation odor was observed in packaged irradiated fish throughout the period of storage.

The sensory score of the steamed fish during the course of ice storage indicates the acceptability of the fish varied during the course of storage depending upon the treatment (Table 1). Packaging resulted in rapid spoilage of unirradiated fish, while, the same (unirradiated) fish kept without packaging in melting ice was acceptable for longer periods of time. Based on a sensory score of '5' denoting border line of acceptability, the unirradiated packaged and non-packaged fish had a shelf life of eight and fourteen days, respectively. Irradiation at 1.5 kGy enhanced the shelf life of both packaged and non-packaged fish to 25 and 20 days, respectively.

The TVBN and TVA values of Indian mackerel during ice storage are shown in Fig. 1 A and B, respectively. Both TVBN and TVA values increased rapidly in the case of packaged unirradiated fish while for the non-packaged unirradiated fish the rate of increase in these values was significantly slower, indicating a lower rate of spoilage. Irradiation at 1.5 kGy reduced both the TVBN and TVA values in packaged and non-packaged fish.

The bacterial load of the fish during the course of ice storage is depicted in Fig. 1C. Except in the case of packaged irradiated fish, in all samples, the total plate counts increased to a value of about 10^8 per g within a period of 10 days. However, the nonpackaged irradiated fish was still acceptable up to a storage period of 20 days as judged by sensory evaluation. We have shown that radurization treatment significantly reduced the proportion of spoilage organisms leading to predominance of nonspoilers belonging, mainly, to the genus Bacillus and Micrococcus (Alur and Lewis, 1980). During storage under ice although contamination does occur and total plate count increases almost at the same rate, because of compositional difference in the microbial load at a value of 10^8 per g, the irradiated fish was still acceptable while the unirradiated fish was spoiled, as shown by sensory evaluation studies. Similar enhanced acceptabilities of certain other radurized fish items having comparable microbial load with that of unirradiated control samples have been reported (Hanneson and Dadbjartsson, 1973).

The present results suggest the beneficial effect of melting ice in enhancing the keeping quality of irradiated Indian mackerel under nonpackaged condition. Under commecial conditions, which demand handling of bulk quantities of fish with speed, feasibility of packaging is limited. The usual practice is icing of eviscerated fish in 10 to 20 kg lots in perforated boxes which are stored or transported under refrigeration to consumer centers. Proper icing in conjunction with gamma irradiation at a dose of 1.5 kGy can further enhance the keeping quality of Indian mackerel useful for commercial application.

REFERENCES

Agarwal, S.R. and Sreenivasan, A. 1973. Packaging aspects of irradiated flesh foods. A review. J. Food Technol. 3: 27.
Alur, M.D. and Lewis, N.F. 1980. Influence of storage temperature on microflora of Indian mackerel Fleischwirtschaft 60: 453.
Alur, M.D., Lewis, N.F., and Kumta, U.S. 1971. Radiation sensitivity of fish microflora. Ind. J. Exp. Biol. 9: 45.
Angel, S., Weinberg, Z.G., Juven, B.J., and Lindner, P. 1985. Quality changes in the fresh water prawn, *Macrobachium rosenberge* during storage on ice. J. Food Technol. 20: 553.
Bilinski, E., Jonas, R.E.E., and Peters, M.D. 1983. Factors controlling the deterioration of spiny dogfish (*Squalus acanthias*) during ice storage. J. Food. Sci. 48: 808.
Farber, L. and Ferro, M. 1956. Volatile reducing substances and volatile nitrogen compounds in relation to spoilage of canned fish. Food Technol. 10: 303.
Hanneson, G. and Dadbjartsson, B. 1973. Radurization of Norway lobster in tail. In "Radurization of Scampi, Shrimp and Cod." Technical Report No. 124, p. 32. International Atomic Energy Agency, Vienna, 1971.
Poulter, N.H. and Nicolaides, N. 1985. Studies on the ice storage characteristics and composition of a variety of Bolivian fresh water fish. 1. Altiplano fish. J. Food Technol. 20: 437.
Silliker, J.H., Elliot, R.P., Baird-Parker, A.C., Bryan, F.L., Christian, J.H.B., Clark, D.S., Olson, J.C., and Roberts, T.A. 1980. Microbial Ecology of Foods. Vol. 1. Factors affecting life and death of microorganisms. 3. Ionizing irradiation. International Commission of Microbiological Specification of Foods. p. 46–69. Academic Press, New York.
Surendran, P.K. and Mahadeva Iyer, K. 1976. Bacterial flora of fresh and iced Indian mackerel (*Rastrelliger kanagurta*) and its response to chlortetracycline (CTC) treatment. Fishery Technol. (India) 13: 139.
Venugopal, V., Lewis, N.F., and Nadkarni, G.B. 1981. Volatile acids content as a quality index for Indian mackerel (*Rastrelliger kanagurta*). Lebensm. Wiss Technol. 14: 39.
Venugopal, V., Savagaon, K.A., Kumta, U.S., and Sreenivasan, A. 1973. Extension of shelf-life of Indian mackerel (*Rastrelliger kanagurta*) by gamma radiation. Journal of Fish Res. Board of Can. 30: 305.
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A Research Note

SDS-PAGE Conditions for Detection of Titin and Nebulin in Tender and Tough Bovine Muscles

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ABSTRACT

Purified myofibrils were prepared from *infraspinatus* (tender) and *rhomboideus* (tough) muscles at 7 days postmortem and examined for myofibrillar/cytoskeletal protein degradation by using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Four acrylamide/bisacrylamide ratios (37:1, 50:1, 75:1 and 100:1) and two SDS-PAGE gel buffers (Tris-HCl, pH 8.0 and 8.9) were used to determine the optimum conditions for detection of titin and nebulin. Titin was degraded to a greater extent in myofibrils from the *infraspinatus* than in myofibrils from the *rhomboideus*. Very little nebulin was detected in either muscle. Use of acrylamide/bisacrylamide ratio of 37:1 and a gel buffer of pH 8.0 provided the most optimum conditions for detecting differences in the resolution of titin, nebulin and their apparent degradation products.

INTRODUCTION

SODIUM DODECYL SULFATE polyacrylamide gel electrophoresis (SDS-PAGE) has been very useful in examining the change in myofibrillar/cytoskeletal proteins during postmortem aging because of the unique properties of both SDS and polyacrylamide gels. SDS has the ability to bind to these proteins and make them negatively charged molecules and dissociating them to monomeric chains (Shapiro et al., 1967). Consequently, the electrophoretic mobility of the monomeric protein chains is representative of their respective molecular weights (Reynolds and Tanford, 1970). Polyacrylamide gels can be made to contain pore radii of about 0.5 to 4 nm by adjusting the total concentration of acrylamide (the lower the acrylamide concentration, the greater is the pore dimension). Hence, polyacrylamide gels can be used to study myofibrillar/cytoskeletal proteins having a wide range of molecular weights.

Wang et al. (1979), using SDS-PAGE, discovered two large proteins in striated muscle and named them titin and nebulin. Titin (MW, 1×10^6 daltons) migrated as a closely spaced doublet (designated T_1 and T_2), and nebulin (MW, 5×10^5 daltons) migrated as a single band. Lusby et al. (1983) reported that titin and nebulin were very susceptible to postmortem proteolytic degradation and could possibly play a role in meat tenderization. In support of that hypothesis, Paterson and Parrish (1986) reported that T_1 degrades to T_2 in tender bovine muscle, but the T_1T_2 doublet remains intact in less tender bovine muscle.

Porzio and Pearson (1977), using SDS-PAGE, studied methods for improving the resolution of myofibrillar proteins located below myosin heavy chain. Little research, however has been reported on techniques to improve SDS-PAGE for detecting changes in titin and nebulin in postmortem bovine tender and tough muscles.

The purpose of this study was to evaluate the effects of different SDS-PAGE techniques on the resolution of titin and nebulin in myofibrils from the *infraspinatus* (tender) and *rhomboideus* (tough) muscles.

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MATERIALS & METHODS

SAMPLES were obtaine from the infraspinatus (IF) and rhomboideus (RB) muscles of chucks from USDA Choice steer carcasses postmortem stored at 2°C for 7 days; then, the IF and RB muscles were removed, vacuum-packaged and frozen (−30°C). IF and RB muscle samples were thawed for 48 hr at 4°C; then, highly purified myofibrils from the IF and RB were prepared by the method of Goll et al. (1974) for SDS-PAGE. SDS-PAGE was performed by using a modification of Lusby et al. (1983) for 3.2% acrylamide [acrylamide/bisacrylamide, 37:1 (w/v); 50:1 (w/v); 75:1 (w/v); and 100:1 (w/v)] slab gels (16 cm × 14 cm × 0.15 cm). Two SDS-PAGE gel buffers were utilized, Tris-HCl, pH 8.0, and Tris-HCl, pH 8.9. Myofibrils were heated at 50°C for 20 min in 7.5% (v/v) 2-mercaptoethanol, 1.5% (w/v) SDS, 15% (w/v) sucrose, 0.03% (w/v) bromphenol blue and 15mM 2-N-morpholinoethonesulfonic acid (pH 6.5). Sixty micrograms of protein were applied to each gel lane for acrylamide/bisacrylamide ratios of 37:1 and 50:1. One hundred and twenty micrograms of protein were applied to each gel lane for acrylamide/bisacrylamide ratios of 75:1 and 100:1 because 60 μg did not result in visible protein bands at these two acrylamide/bisacrylamide ratios. GELBOND* PAG (Polyacrylamide Gel Support Medium, FMC) was used to support SDS-PAGE slab gels with acrylamide/bisacrylamide ratios of 75:1 and 100:1. Gels were stained overnight in Coomassie brilliant blue [0.2% (w/v) Coomassie brilliant blue, 7% (v/v) acetic acid, 40% (v/v) ethanol]. Gels were destained by using a solution containing 7% (v/v) acetic acid and 40% (v/v) methanol.

RESULTS & DISCUSSION

The SDS-polyacrylamide gel electrophoretogram (Fig. 1, lanes a,b) displayed differences in titin from the infraspinatus (tender = Td) and rhomboideus (tough = Tg) muscles. Titin has

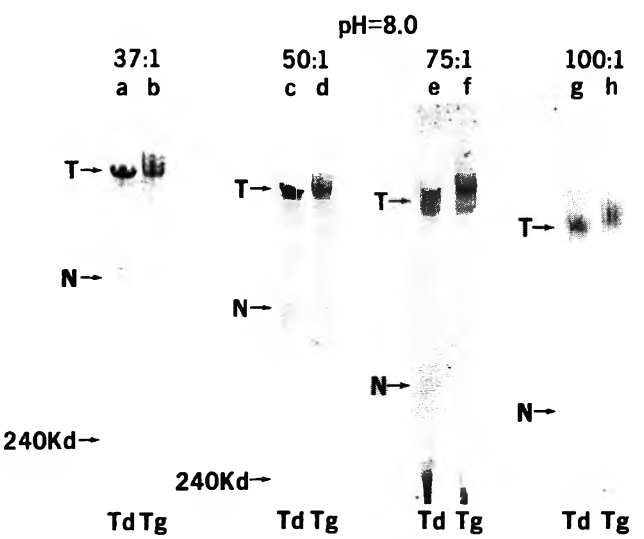

Fig. 1—SDS 3.2%, pH 8.0, polyacrylamide gels of myofibrils prepared from bovine infraspinatus (Td, tender) and rhomboideus (Tg, tough) muscles. Proteins include (T) titin, (N) nebulin and (240 kd) 240-kilodalton protein.

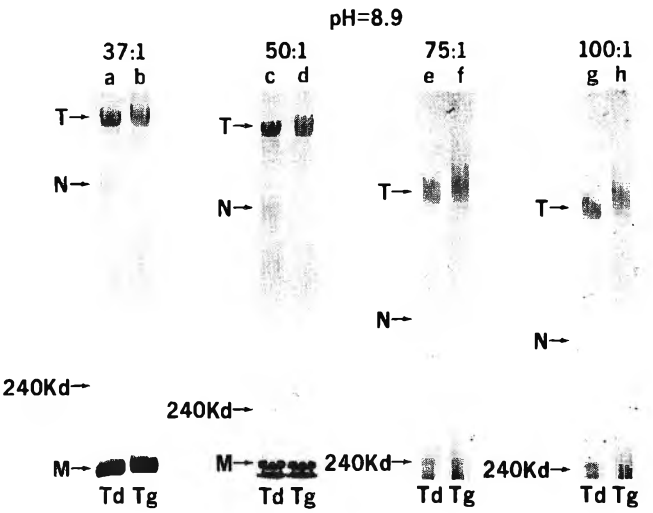

Fig. 2—SDS 3.2%, pH 8.9, polyacrylamide gels of myofibrils prepared from bovine infraspinatus (Td, tender) and rhomboideus (Tg, tough) muscles. Proteins include (T) titin, (N) nebulin, (240 kd) 240-kilodalton protein and (M) myosin.

undergone more complete proteolytic degradation in the Td muscle than in the Tg muscle as evidenced by the presence of a single protein band for titin in the Td muscle and several constituent bands for titin in the Tg muscle. Lusby et al. (1983) have shown that titin, in bovine longissimus muscle, was degraded during portmortem storage and that the initial existing titin doublet became a single band during increased postmortem storage. Wang et al. (1979) characterized the upper titin band as titin-1 (T_1) and the lower titin band as titin-2 (T_2) and have observed that proteolytic degradation of titin is always accompanied by the conversion of T_1 to T_2. In the present study, however, titin (Fig. 1b) appeared as a triplet rather than a doublet as reported previously (Lusby et al., 1983). This difference may be due to different muscles possessing different types of titin. Lusby et al. (1983) utilized bovine longissimus, whereas the infraspinatus and rhomboideus were examined in this study. Yoshidomi et al. (1985) have shown with SDS-PAGE that several types of titin (referred to as connection in their study) were detected in adult skeletal muscles of chicken.

Nebulin (Fig. 1a,b) was present in a very limited amount, and a regular banding pattern was not detectable. Lusby et al. (1983) reported that nebulin is degraded quickly during postmortem storage, which may explain the lack of nebulin in the SDS-polyacrylamide gels in this study. An unidentified protein band, with an estimated molecular weight of 240-kd, was detected in the Td sample but was absent in gels from the Tg sample (Fig. 1a,b). This 240-kd protein may be the cytoskeletal protein spectrin, which has a molecular weight of 240 kd and functions in binding to and crosslinking actin filaments (Pearl et al., 1984). Because the 240-kd protein is more prominent in the Td sample, it is also possible that the 240-kd protein band is a degradation product of some higher-molecular-weight myofibrillar protein.

At pH 8.0, an increase in the acrylamide/bisacrylamide ratio decreased the resolution of titin (Fig. 1,a-h). Titin exhibited very distinct bands at the 37:1 ratio, but became a broad smear at the 100:1 ratio. Likewise at pH 8.9 (Fig. 2a-h), the resolution of titin decreased at the higher acrylamide/bisacrylamide ratios. At pH 8.9, however, the 50:1 ratio, rather than the 37:1 ratio, appeared to produce the most distinct titin bands. Porzio and Pearson (1977) reported that lower ratios of acrylamide to bisacrylamide improved the resolution of low-molecular-weight proteins such as tropomyosin, but high-molecular-weight proteins, such as titin, were not examined in their study. The 240-kd protein appeared as a single band at pH 8.0 (Fig. 1a) but appears as a faint doublet at pH 8.9 (Fig. 2a,c). In addition, the 240-kd protein is present as a faint band in the rhomboideus (Tg) muscle at pH 8.9 (Fig. 2b,d), whereas the 240-kd protein was not present in the Tg muscle at pH 8.0 (Fig. 1b,d).

In summary, the results of this study show that the choice of gel buffer pH and acrylamide/bisacrylamide ratio are very important considerations in studying high-molecular-weight myofibrillar/cytoskeletal proteins by using SDS-PAGE. A pH of 8.0 and an acrylamide to bisacrylamide ratio of 37:1 provided the optimum condition for resolving titin. Higher ratios of acrylamide/bisacrylamide and a pH of 8.9 resulted in reduced resolution of titin, greater smearing of protein bands, and made handling of the gels more difficult.

REFERENCES

Goll, D.E., Young, R.B., and Stromer, M.H. 1974. Separation of subcellular organelles by differential and density gradient centrifugation. In "Proc. 27th Annu. Recipr. Meat Conf." p. 250. National Live Stock & Meat Board, Chicago, IL.

Lusby, M.L., Ridpath, J.F., Parrish, F.C., Jr. and Robson, R.M. 1983. Effect of postmortem storage on degradation of the myofibrillar protein titin in bovine longissimus muscle. J. Food Sci. 48: 1787.

Paterson, B.C. and Parrish, F.C., Jr. 1986. A sensory panel and chemical analysis of certain beef chuck muscles. J. Food Sci. 51: 876.

Pearl, M., Fishleind, D., Mooseker, M., Keene, D., and Keller, T, III. 1984. Studies on the spectrin-like protein from the intestinal brush border, TW 260/240, and characterization of its interaction with the cytoskeleton and actin. J. Cell Biol. 98(1): 63.

Porzio, M.A. and Pearson, A.M. 1977. Improved resolution of myofibrillar proteins with sodium dodecyl sulfate polyacrylamide gel electrophoresis. Biochim. Biophys. Acta 490: 27.

Reynolds, J.A. and Tanford, C. 1970. The gross conformation of protein sodium dodecyl sulfate complexes. J. Biol. Chem. 245: 5161.

Shapiro, A.L., Vinuela, E. and Maizel, J.V. 1967. Molecular weight estimation of polypeptide chains by electrophoresis in SDS-polyacrylamide gels. Biochem. Biophys. Res. Commun. 28: 815.

Wang, K., McClure, J., and Tu, A. 1979 Titin: major myofibrillar components of striated muscle. Proc. Natl. Acad. Sci. (USA) 76: 3698.

Yoshidomi, H., Ohgshi, K., and Maruyama, K. 1985. Changes in the molecular size of connectin, an elastic protein, in chicken skeletal muscle during embryonic and neonatal development. Biomed. Res. 6(4): 207.

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A Research Note

Enzyme Profile of Raw and Heat-Processed Beef, Pork and Turkey Using the "Apizym" System

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ABSTRACT

A semi-quantitative micromethod enzyme system (APIZYM) designed for the detection of 19 individual enzymes was used to determine the presence and level of activity of these enzymes in raw and heat processed (60 and 71.1°C) beef, pork and turkey muscle tissue. Filtrates were obtained from 0.9% saline extracts of the raw and heat processed samples. Heat processing the product to 60°C greatly decreased the number of enzymes that could be detected. Samples heated to 71.1°C exhibited little enzymatic action, except for minimal activity of leucine aminopeptidase. Thus, indicating the possibility of using the APIZYM system as a new and/or improved method for determining the end-point temperature to which meat and poultry products have been heat processed.

INTRODUCTION

THE APIZYM SYSTEM (Analytab Products, Plainview, NY) is a semi-quantitative micromethod designed for the detection of 19 individual enzyme activities in a variety of specimens, e.g. tissues, cells, biological fluids, microorganisms.

The APIZYM system has been predominantly used for detecting the enzyme activities of a variety of bacteria (Tharagonnet et al., 1977; Humble et al., 1977; Lee and Simard, 1984), thus aiding the microbiologist in identifying bacteria. Buiting (1983), on the other hand, used the APIZYM system for determining the adequacy of heat treatment to kill *Enterobacteriaceae* in sludge from flocculation-flotation equipment in poultry and swine slaughter plants.

No information is available concerning the use of the APIZYM system for detecting the enzymatic activity of raw and heat processed muscle tissue. Therefore, the purpose of this study was to: (a) determine which of the 19 enzymes that can be detected with the APIZYM system are present in filtrates obtained from 0.9% saline extracts of raw and heat processed beef, pork and turkey; and (b) which enzymes have low, medium and high enzymatic activities.

MATERIALS & METHODS

FIVE by 7.5 by 7.5 cm pieces of muscle tissue were cut from the semimembranosus muscle of beef and pork and from the pectoralis major muscle of turkey. Heat processed samples were prepared by inserting a Sabrecouple insertion thermocouple (Honeywell, Fort Washington, PA) into the geometric center of each piece, placing the piece of meat in individual cooking bags, evacuating the air from the bag and heat processing the sample in a circulating water bath to end-point temperatures of 60°C (140°F) and 71.1°C (160°F), respectively. Internal temperature was monitored by a recording potentiometer (Electronik 16, Honeywell, Fort Washington, PA).

Raw and heat processed samples were cut into strips and ground through a 3-mm grinder plate. Filtrates were prepared by adding 0.9% saline solution to the ground sample at a ratio of 2:1 (saline:meat) and filtering the extract through #934-AH Whatman filter paper. Sixty microliters of the filtrate were added to each of the 20 microcupules in the APIZYM strip (Analytab Products, Plainview, NY). Detailed procedures for preparing the incubation chamber, incubation conditions and reading of results followed the manufacturer's instructions. Enzymes detected by the system are shown in Table 1.

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RESULTS & DISCUSSION

COLOR INTENSITY VALUES and enzymes detected in raw and heat processed beef, pork and turkey are shown in Table 2. Enzymes in raw beef, pork and turkey having high activity (based on color intensity values of 4 to 5) were leucine aminopeptidase (F), phosphoamidase (L) and acid phosphatase (K). Beef had slightly less leucine aminopeptidase activity than either pork or turkey. All three species of muscle exhibited the same amount of phosphoamidase activity. Acid phosphatase was about equal for beef and pork but considerably less for turkey.

Enzymes having medium activity in beef, pork and turkey (based on color intensity values of 2 to 4) were valine aminopeptidase (G), N-acetyl-β-glucosaminidase (R), alkaline phosphatase (B) and α-glucosidase (P). Both pork and turkey had more valine aminopeptidase activity than beef. N-acetyl-β-glucosaminidase activity was highest in turkey, lowest in beef with pork being intermediate. Chymotrypsin (J) was present only in turkey. Alpha-glucosidase activity was higher in turkey than pork with no activity in beef.

Enzymes having low activity in beef, pork and turkey (based on color intensity values of 2 and lower) were esterase lipase (D), cystine aminopeptidase (H), β-galactosidase (N), β-glucuronidase (O) and trypsin (I). Esterase lipase and β-galactosidase activity was detected in pork and turkey but not in beef. Cystine aminopeptidase activity was highest in pork, lowest in beef and intermediate in turkey. Equal amounts of β-glucuronidase (O) activity were found in all three species of meat.

Table 1—Enzymes detected by the APIZYM system

Cupule	Enzyme assayed
A	Control
B	Alkaline phosphatase
C	Esterase (C4)
D	Esterase lipase (C8)
E	Lipase (C_{14})
F	Leucine aminopeptidase
G	Valine aminopeptidase
H	Cystine aminopeptidase
I	Trypsin
J	Chymotrypsin
K	Acid phosphatase
L	Phosphoamidase
M	α-Galactosidase
N	β-Galactosidase
O	β-Glucuronidase
P	α-Glucosidase
Q	β-Glucosidase
R	N-Acetyl-β-Glucosaminidase
S	α-Mannosidase
T	α-Fucosidase

Table 2—Color intensity values and enzymes detected in filtrates from saline extracts of raw and heat processed (60° and 71.1°C) beef, pork and turkey muscle tissue

Product	Enzymes detected[a]																			
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T
Beef-raw	0	0	0	0	0	4.5	0.5	0.2	0.2	0	4.0	4.0	0	0	1.0	0	0	2.0	0	0
Pork-raw	0	1.5[b]	0	2.0	0	5.0	3.0	2.0	0	0	4.5	4.0	0	2.0	1.0	1.0	0	2.5	0	0
Turkey-raw	0	3.5	0	1.5	0	5.0	3.0	1.0	0	3.0	2.5	4.0	0	1.0	1.0	2.5	0	3.5	0	0
Beef-60°C	0	0	0	0	0	1.0	0	0	0	0	1.0	1.0	0	0	0	0	0	2.0	0	0
Pork-60°C	0	2.0	0	1.5	0	1.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Turkey-60°C	0	1.0	0	0.5	0	3.5	0	0.5	0	0	1.0	4.0	0	1.0	3.5	0	0	3.0	0	0
Beef-71.1°C	0	0	0	0	0	0.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pork-71.1°C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Turkey-71.1°C	0	0	0	0	0	1.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

[a] A (control, B (alkaline phosphatase), C (Esterase C_4), D (Esterase-lipase C_8) E (lipase C_{14}), F (leucine aminopeptidase), G (valine aminopeptidase), H (cystine aminopeptidase), I (trypsin), J. (chymotrypsin), K (acid phosphatase), L (phosphoamidase), M (α-galactosidase, N (β-galactosidase),) (β-glucurondiase), P (α-glucosidase), Q (β-glucosidase), R (N-acetyl-β-glucosaminidase), S (α-mannosidase), T (α-fucosidase).

[b] Color intensity values: < 1 (trace), 1-2 (low, 5-lOnM), 2-4 (medium, 10-30nM) and 4-5 (maximum intensity, 30-40 nM).

A trace of trypsin activity was detected in raw beef but not in raw pork or turkey. No esterase (C), lipase (E), α-galactosidase (M), β-glucosidase (Q), α-mannosidase (S) and α-fucosidase (T) activity was detected in any of the samples.

Although Buiting (1983) used the APIZYM system to determine the adequacy of heat treatment to kill *Enterobacteriaceae* in sludge from flocculation-flotation equipment in poultry and swine slaughter plants, there are some general similarities between his results and ours. For example, Buiting reported chymotrypsin activity in poultry sludge but not in swine sludge which agrees with our findings for turkey meat compared to pork. This might be attributed to the fact that the sludge probably contained tissue particles and/or sarcoplasm from poultry. In contrast to our findings, Buiting detected high levels of alkaline phosphatase (B), esterase (C) and α-glucosidase (P) activity and medium high levels of β-glucuronidase (O) activity in both poultry and swine unheated sludge. These and other differences may have been due to other substances and/or microorganisms in the sludge.

Results of our study showed that of the 19 enzymes that can be detected with the APIZYM system, 8 to 12 of these enzymes were detected in the filtrates obtained from raw beef, pork and turkey muscle tissue. Chymotrypsin was detected only in raw turkey and could possibly be used for identifying turkey in a raw meat formulation containing turkey, beef or pork. However, this requires further investigation.

When the three species of samples were heat processed to an internal temperature of 60°C (140°F): 4 of the 19 enzymes were detected in the filtrates from beef; 3 of the 19 enzymes were detected in the filtrates from pork, and 9 of the 19 enzymes were detected in filtrates from turkey. In addition, there was approximately a 75 to 80% loss in leucine aminopeptidase, acid phosphatase and phosphoamidase activity in beef and pork muscle and minimal loss in activity for turkey. Samples heat processed to an internal temperature of 71.1°C (160°F) exhibited no enzyme activity, except for a minimal amount of leucine aminopeptidase activity, thus, indicating the possibility of using the APIZYM system for development of new and/or improved methods for determining the end-point temperature to which meat and poultry products have been heat processed.

REFERENCES

Buiting, M.H.B.M. 1983. A fast and simple test for determinatin of carbesterase activity in flotation sludge. IPS Research Report No. 134. Spelderholt Institute for Poultry Research. The Netherlands.

Humble, M.W., King, A., and Phillips, I. 1977. APIZYM: A simple rapid system for the detection of bacterial enzymes. J. Clinical Path. 30: 275.

Lee, B.H. and Simard, R.E. 1984. Three systems for biochemical characterization of Lactobaccilli associated with meat spoilage. J. Food Protection. 47: 937.

Tharagonnet, D., Sission, P.R., Roxby, C.M., Ingham, H.R., and Selkon, J.B. 1977. The APIZYM system in the identification of Gram-negative anaerobes. J. Clinical Path. 30: 505.

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Use of a company or product name by the U.S. Department of Agriculture does not imply approval or recommendation of the product to the exclusion of others which may also be suitable.

A Research Note

Extension of the Shelf-Life of Fresh Ground Pork with Polyphosphates

R.A. MOLINS, A.A. KRAFT, and J.A. MARCY

ABSTRACT

The antimicrobial effects of 1.0% sodium acid pyrophosphate (SAPP) and sodium orthophosphate monobasic (ORTHO), alone or combined, were studied in fresh ground pork held at 2–4°C for 6 days. Addition of 1.0% SAPP to the meat inhibited psychrotrophic bacterial growth ($P<0.01$) and resulted in 50% longer meat shelf-life (equivalent to 2 days) in relation to control samples or to meat treated with 0.5% or 1.0% ORTHO. Addition of a 0.5% SAPP/0.5% ORTHO combination was less inhibitory to meat bacteria than 0.5% SAPP alone. Bacterial inhibition did not correlate with soluble orthophosphate content in ground pork.

INTRODUCTION

THE LEGAL LIMIT of 0.5% at which some phosphates may be added to cooked meats and meat products (USDA, 1982) originated from numerous studies that showed such compounds to increase the water-holding capacity of meats and to reduce cooking losses, as reviewed by Knipe (1982). That limit, however, has no microbiological basis.

Several phosphates among those approved for use in cooked and processed meats have been shown to possess antimicrobial properties (Sofos, 1986; Wagner, 1986; Tompkin, 1983). Sodium acid pyrophosphate (SAPP), in particular, has been found to inhibit bacterial growth, including that of inoculated *Clostridium sporogenes* PA3679, in cooked, vacuum-packaged bratwurst held at 24–25°C for 24 and 48 hr (Molins et al., 1985c). Sapp also inhibited *Clostridium botulinum* growth and toxin production in beef/pork frankfurter emulsions (Wagner and Busta, 1983). However, previous work (Molins et al., 1985a) could not demonstrate significant microbiological effects by SAPP added to uncooked bratwurst at a level of 0.5% (w/w), although total mesophilic and psychrotrophic bacterial counts were consistently lower in bratwurst treated with SAPP than in untreated controls. That study theorized that possible hydrolysis of SAPP, brought about by naturally occurring meat phosphatases, might be involved in the loss of that phosphate's bacteriostatic or bactericidal properties in fresh pork meat. In a later study (Molins et al., 1986), increased concentration of soluble orthophosphates in cooked bratwurst containing SAPP correlated with bacterial inhibition, but the apparent relationship between those parameters could not be ascertained.

The purpose of this study was to determine possible microbiological advantages of adding SAPP to fresh ground pork, at levels as high as 1.0% meat weight and to examine the effects of legal and above-legal levels of SAPP/ORTHO combinations on the natural bacterial flora of fresh, ground pork. The latter purpose was directed at studying possible antimicrobial interactions between SAPP and the product of its enzymatic hydrolysis, monosodium orthophosphate (ORTHO).

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MATERIALS AND METHODS

FRESH PORK BUTTS (20% fat), ground through a 1/8-inch (0.32-cm) diameter plate were obtained from the Iowa State University Meat Science Laboratory. Portions of meat weighing 500g were treated with the necessary volumes of a 10% (w/v) aqueous solution of sodium acid pyrophosphate (SAPP) (Stauffer Chemical Co., Washington, PA) and/or of sodium orthophosphate monobasic (ORTHO) (Fisher Scientific, Fair lawn, NJ), to give the following concentrations: 0.5 and 1.0% SAPP, 0.5 and 1.0% ORTHO, 0.5 SAPP/0.5% ORTHO. A portion of ground pork meat received no phosphate and constituted the control. The phosphate solutions were filter-sterilized through 22-μ membrane filters (Millipore Corp., Bedford, MA) before being added to the meat. The volume of added water was equalized in all batches, including controls, with sterile distilled water. After blending for 1 min at low and high speed in a Kitchen Aid Model 4 mixer (The Hobart Mfg. Co., Troy, OH), the batches of meat were aseptically divided into 30-g portions and placed into sterile Stomacher 400 bags (Tekmar Co., Cincinnati, OH). The bags were folded to avoid contamination and stored in a walk-in cooler at 2–4°C. Samples for microbiological examination were taken on the day of preparation (day 0) and after 2, 4 and 6 days of refrigerated storage. On sampling days, 270 mL sterile 0.1% peptone water were added to one sample from each treatment, and the contents were blended for 2 min in a Stomacher Lab Blender 400 (Tekmar Company, Cincinnati, OH). Serial dilutions were prepared according to standard methods, pour-plated with trypticase soy agar (TSA, BBL) and incubated to enumerate mesophilic (30°C/48 hr) and psychrotrophic (5°C/7–10 days) bacterial populations. Changes in soluble orthophosphate content were determined by the method of Dick and Tabatabai (1977), modified for use in meats by Molins et al. (1985b). A Radiometer 28 meter (Radiometer, Copenhagen, Denmark) equipped with an Orion 9163 probe (Orion Research, Cambridge, MA) was used to determine the pH of the samples on the day of preparation (day 0). Experiments were replicated three times. Microbiological data were transformed into logarithms and analyzed by using Statistical Analysis System (SAS, 1986) general linear model procedure.

RESULTS & DISCUSSION

THE ADDITION of 1.0% SAPP to refrigerated fresh ground pork caused highly significant ($P<0.01$) inhibition of mesophilic (data not presented) and psychrotrophic bacterial growth (Fig. 1). After 6 days at 2–4°C, samples containing 1.0% SAPP had not reached 10^7 psychrotrophic colony-forming units (CFU) per gram, indicating that the meat was not spoiled. On the basis of psychrotrophic bacterial populations (Fig 1), meat containing 1.0% SAPP had a 30–50% longer shelf-life than that treated with 0.5% SAPP or with the 0.5% SAPP/0.5% ORTHO combination and a 90% longer shelf-life than samples that received only orthophosphate or no phosphate at all (6 days vs 3.5 days, respectively). Mean pH values on the day of preparation (day 0) were: control, 5.49; 1.0% ORTHO, 5.52; 0.5% ORTHO, 5.49; 1.0% SAPP, 5.33; 0.5% SAPP, 5.44; and 0.5% SAPP/0.5% ORTHO, 5.40. Therefore, the mean pH decrease in meat treated with 1.0% SAPP seemed too small (0.16 pH unit) to account for the bacterial inhibition observed.

Table 1—Mean soluble orthophosphate content of fresh ground pork μg/g of meat

Days at 2-4°C	TREATMENT					
	Control	0.5% SAPP	1.0% SAPP	0.5% ORTHO	1.0% ORTHO	0.5% SAPP 0.5% ORTHO
0	1081	1478	1688	2066	3262	2555
2	958	1796	2164	1942	2975	2743
4	1013	2107	2953	1911	2917	2845
6	984	2284	2763	1951	3034	3070

Standard error: 65

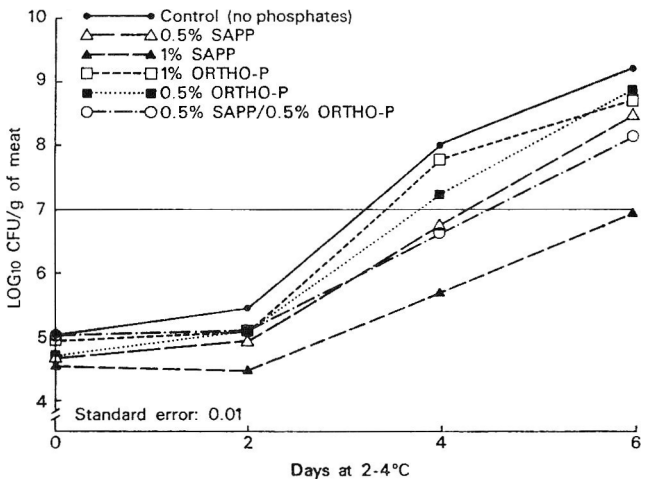

Fig. 1—Total psychrotrophic bacterial numbers in phosphate-treated ground pork.

No correlation was found between the microbiological data and the soluble orthophosphate content of the meat (Table 1). This suggested that increasing the concentration of soluble orthophosphates within the limits of this study did not result in lower numbers of microorganisms in refrigerated ground pork. Therefore, orthophosphate formation alone did not account for the observed antimicrobial activity of SAPP. Future research concerning phosphate levels greater than 0.5% in meats and meat products should include physico-chemical, sensory and nutritional aspects.

REFERENCES

Dick, W.A. and Tabatabai, M.A. 1977. Determination of orthophosphates in aqueous solutions containing labile organic and inorganic phosphorous compounds. J. Environ. Qual. 6(1): 82.

Knipe, C.L. 1982. Effects of inorganic polyphosphates on reduced sodium and conventional meat emulsion characteristics. Ph.D. dissertation, Iowa State University, Ames, IA.

Molins, R.A., Kraft, A.A., and Olson, D.G. 1985a. Effect of phosphates on bacterial growth in refrigerated uncooked bratwurst. J. Food Sci. 50: 531.

Molins, R.A., Kraft, A.A., and Olson, D.G. 1985b. Adaptation of a method for the determination of soluble orthophosphates in cooked and uncooked pork containing acid-labile poly- and pyrophosphates. J. Food Sci. 50: 1482.

Molins, R.A., Kraft, A.A., Olson, D.G., Walker, H.W., and Hotchkiss, D.K. 1986. Inhibition of *Clostridium sporogenes* PA3679 and natural bacterial flora of cooked vacuum-packaged bratwurst by sodium acid pyrophosphate and sodium tripolyphosphate with or without added sodium nitrite. J. Food Sci. 51: 726.

Molins, R.A., Kraft, A.A., Walker, H.W., and Olson, D.G. 1985c. Effect of poly- and pyrophosphates on the natural bacterial flora and inoculated *Clostridium sporogenes* PA3679 in cooked vacuum packaged bratwurst. J. Food Sci. 50: 876.

SAS. 1986. "SAS User's Guide." SAS Institute, Inc., Cary, NC.

Sofos, J.N. 1986. Use of phosphates in low-sodium meat products. Food Technol. 40: 52.

Tompkin, R.B. 1983. Indirect antimicrobial effects in foods: phosphates. J. Food Safety 6: 13.

USDA. 1982. Meat and poultry products: phosphates and sodium hydroxyde. Fed. Register 47: 10779.

Wagner, M.K. 1986. Phosphates as antibotulinal agents in cured meats: a review. J. Food Prot. 49: 482.

Wagner, M.K. and Busta, F.F. 1983. Effect of sodium acid pyrophosphate in combination with sodium nitrite or sodium nitrite/potassium sorbate on *Clostridium botulinum* growth and toxin production in beef/pork frankfurter emulsions. J. Food Sci. 48: 990.

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A Research Note

Neutral Lipids Traced Through the Beverage Alcohol Production Process

JACQUELYNN O'PALKA, IRENE EIDET, and LARRY L. JACKSON

ABSTRACT

Changes in lipids were traced from the malt through the beverage alcohol (bourbon) production process to the dried distiller's grain with solubles (DDGS). Changes in lipid composition previously observed during the fuel alcohol production process also occurred during beverage alcohol production but to a lesser extent. The levels of triacylglycerols fell while free fatty acid levels rose chiefly at the stage of mashing and distillation. Little or no free fatty acid degradation occurred during drying. Minor quantities of fatty acid ethyl esters (4–5%) were formed during fermentation.

INTRODUCTION

ATTEMPTS to utilize dried distiller's grains (DDG), a high protein, high fiber by-product of alcohol distillation, in bakery products have been plagued with flavor, color and texture problems. Defatting DDG has resulted in reduced flavor damage to oatmeal cookies containing 15% barley fuel-alcohol derived DDG (Dawson et al., 1984) and to a corn based nutritional supplement containing 10% corn DDG (Bookwalter et al., 1984).

A comparison of the lipids extracted from barley DDG and the parent barley indicated that the fuel alcohol production process resulted in considerable alteration of the neutral lipid fraction. The fermentation step resulted in nonspecific acid hydrolysis of the triacylglycerol (TAG) fraction to free fatty acids (FFA) while distillation and drying resulted in decomposition of the unsaturated FFA (Dawson et al., 1987).

The purpose of this study was to monitor the changes in the lipid fraction from the original grain to the DDGS in a bourbon distillery to determine if similar changes in lipid occurred during the production of a human food grade product.

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MATERIALS & METHODS

THE JAMES B. BEAM Distilling Co. (Clermont, KY) was selected because of its well-controlled processing conditions. All processing equipment from mashing through drying was stainless steel. Two sets of samples were collected. The consecutive (c) samples traced the same batch of grain through all processing stages. The random (r) samples were collected from a variety of batches of grain but at the same processing stages as the consecutive samples.

Samples were taken of: (1) ground corn (70%), rye, barley malt mixtures; (2) cooked mash (2 hr at 100°C); (3) after ca. 96 hr of fermentation; (4) after distillation and centrifugation to thin stillage (supernatant); (5) thick stillage (solids); (6) concentration of thin stillage to condensed distillers solids (CDS); (7) blending of thick stillage and CDS, drying at 288–315°C to yield DDGS. All samples were transported to the Beam Laboratory and immediately covered with reagent grade hexane.

The hexane extracts of the samples were concentrated and the lipid was placed in individual 4 dram vials, purged with nitrogen, frozen, packed in dry ice and shipped to Montana State University. Using thin layer chromatography, the lipid extracts were separated and quantified into the following five fractions: mono-diacylglycerols (MAG/DAG), free fatty acids (FFA), triacylglycerols (TAG), fatty acid esters, and sterol/wax esters. The composition of all five fractions was determined by gas chromatography (Dawson et al, 1987).

Chemical analysis of the DDGS and original grain samples was as reported previously (Dawson et al., 1984).

RESULTS & DISCUSSION

THE CHEMICAL COMPOSITION of the c and r samples of the original ground malt mixture and DDGS were very similar. Chemical composition of ground malt mixtures on a dry weight basis was as follows: protein 10.3 ± 0.4%, lipid 3.8 ± 0.1%,

Table 1—Composition of neutral lipids throughout processing for consecutive (c) and random (r) samples

Sample	Lipid composition (% by weight) MAG[a] DAG	FFA	TAG	Ethyl esters	Sterol/Wax esters
Ground Malt Mixture(c)[b]	8 ± 1	7 ± 5	80 ± 2	ND	5 ± 2
Ground Malt Mixture(r)	7	3	86	ND	4
Cooked Mash(c)[b]	10 ± 4	14 ± 4	66 ± 5	ND	9 ± 1
Cooked Mash(r)	7	14	73	ND	7
End of Fermentation(c)[b]	10 ± 3	13 ± 3	67 ± 8	5 ± 1	4 ± 1
End of Fermentation(r)	10	19	58	5	8
Thin Stillage(c)	10	19	57	6	8
Thin Stillage(r)	12	19	54	7	8
Condensed Distillers Solubles (c)			(Insufficient Sample)		
Condensed Distillers Solubles (r)	15	21	56	5	6
Thick Stillage (c)	13	12	64	5	6
Thick Stillage (r)	10	14	69	2	5
DDGS(c)[c]	9	11	70	4	5
DDGS(r)	5	12	73	4	7

[a] MAG/DAG = mono- and diacylglycerols; FFA = free fatty acids; TAG = triacylglycerols.

[b] Mean and standard deviation of three samples. ND = not detected.

[c] DDGS = dried distillers grains with solubles.

Table 2—Fatty acid composition of the lipid fractions from the bourbon fermentation process

Lipid fraction	Fatty Acids (% by weight)				
	16:0	18:0	18:1	18:2	18:3
TAG[a]	12 ± 1[b]	1 ± 1	25 ± 1	60 ± 1	2 ± 1
MAG/DAG	18 ± 1	2 ± 1	22 ± 1	55 ± 2	3 ± 1
FFA	23 ± 9	3 ± 1	14 ± 5	45 ± 11	4 ± 2
Ethyl esters	23 ± 5	2 ± 1	14 ± 1	57 ± 2	3 ± 1
Sterol/wax esters	20 ± 7	3 ± 2	18 ± 6	47 ± 6	6 ± 2

[a] TAG = triacylglycerols; MAG/DAG = mono- and diglycerols; FFA = free fatty acids
[b] Mean and standard deviation of three samples

neutral detergent fiber 15.0±0% and ash 1.6±0.1%. Chemical composition of the DDGS was in agreement with values previously reported by Ranhotra et al. (1982) and was as follows: protein 29.1±0.7%, lipid 8.1±0.1%, neutral detergent fiber 39.1±0.5% and ash 4.7±0.3% on a dry weight basis.

The types of changes in lipid composition with processing (Table 1) were similar to those previously observed in the production of fuel alcohol (Dawson et al., 1987). The magnitude of the changes, however, was much less. The greatest reduction in TAG levels occurred during mashing followed by a smaller reduction during distillation (Table 1). The fatty acid composition of the TAG and MAG/DAG fractions was nearly constant throughout processing (Table 2).

The James B. Beam distillery utilized the sour-mash process which involves recycling a portion of the acidic thin stillage in the mashing step which would favor nonspecific acid hydrolysis of TAG. Levels of MAG/DAG and FFA (Table 1) increased during mashing and to a lesser extent during distillation. In contrast to the finding of Dawson et al. (1987), drying resulted in little loss of FFA (Table 1) and had little if any effect on unsaturated fatty acid composition (Table 2). This was probably a reflection of the multistaged, temperature-controlled drying process developed by this distillery.

As in fuel alcohol production, small quantities of ethyl esters were produced during fermentation and persisted through all subsequent processing stages. The composition of the ethyl esters (Table 2) and the composition of the sterol/wax esters were similar (Table 2). Small quantities of sterol/wax esters were present throughout the process (Table 1) and were largely unaffected by processing.

This study indicates that while some changes in lipid composition such as the hydrolysis of TAG during mashing occurred, the magnitude of the changes in lipids were much less than in the fuel alcohol process. Good sanitation, stainless steel equipment and the use of lower processing temperatures seemed to reduce the level of lipid degradation. The control of batch variability affected not only the quality of the bourbon, but also the DDGS as indicated by the similarity between c and r samples of DDGS. In contrast, a higher level of lipid degradation, and considerable variation between the two batches of fuel alcohol/DDG was observed (Dawson et al., 1984; 1987) when mild steel tanks, less stringent sanitation standards and less concern for processing controls were used. A number of process modification may be necessary in the fuel alcohol process to provide a DDG acceptable as a food supplement.

REFERENCES

Bookwalter, G.N., Warner, K., Wall, J.S., and Wu, U.V. 1984. Corn distillers' grains and other by-products of alcohol production in blended foods. II. Sensory, stability and processing studies. Cereal Chemistry 61: 509.

Dawson, K.R., O'Palka, J., Hether, N.W., Jackson, L., and Gras, P.W. 1984. Taste panel preference correlated with lipid composition of barley dried distiller's grains. J. Food Sci. 49: 787.

Dawson, K.R., Eidet, I., O'Palka, J., and Jackson, L. 1987. Barley neutral lipid changes during the fuel ethanol production process and product acceptability from dried distillers grains. J. Food Sci. (submitted).

Ranhotra, G.S., Gelroth, J.A., Torrence, F.A., Bock, M.A., Winterringer, G.L., and Bates, L.S. 1982. Nutritional characteristics of distiller's spent grains. J. Food Sci. 47: 1184.

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ERRATA

J. Food Sci. 51(5):1352–1357 (1986). Nutrient composition of some fresh and cooked retail cuts of veal by K. Ono, B.W. Berry, and L.W. Douglass. Please make the following corrections: p. 1355, Table 2, Riboflavin for rib roast should read 0.35x and 0.28y for B and SFV, respectively.

J. Food Sci. 49(2):468–477 (1984). Parametric analysis for predicting freezing time of infinitely slab-shaped food by J. Succar and K. Hayakawa. Please make the following corrections: p. 472, Eq. (34) and (35):

$$Y = 2.5949 + 0.0222 \cdot X_1 - 0.2117 \cdot X_2 - 0.2181 \cdot X_3 + 0.0561 \cdot X_4 + 0.249 \cdot X_5 + 0.770 \cdot X_6 + 0.2411 \cdot X_7 - 0.3756 \cdot X_4^2 + 0.0507 \cdot X_6^2 + 0.083 \cdot X_6 \cdot X_7 - 0.0092 \cdot X_3^3 - 0.0107 \cdot X_6^3 - 0.0044 \cdot X_3^4 + 0.0334 \cdot X_4^4 - 0.0028 \cdot X_2^4 - 0.0026 \cdot X_5^4 - 0.0010 \cdot X_7^4 \quad (34)$$

$$F_o = e^Y \quad (35)$$

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