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EDITORIAL

With this final issue of 1991, I want to take this opportunity to thank the Editorial Board, the authors of papers, and the reviewers for making the *Journal of Food Processing and Preservation* a worthy refereed publication vehicle for advancing the science of Food Processing and Technology. I especially want to thank Bill Breene, Jerry Cash, Barry Swanson, and Joe von Elbe who have completed a three-year term on the Editorial Board. Fortunately each will continue for another three-year term on the Editorial Board.

We successfully made the transition to a six-issue Journal. However, we are continually looking for quality manuscripts in the area of Food Processing and Preservation. Two areas which have not been overwhelmed are the databank papers and the computer code papers. We continue to seek those papers since they can make a valuable contribution to the literature.

As we move into 1992, undoubtedly a year of profound change, we will also look for continued improvement in the *Journal of Food Processing and Preservation*. With a commitment from the Editorial Board, the authors and the reviewers I am confident that we will grow in quantity and quality.

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DARYL LUND
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CONTENTS

CHANGES IN PEAS DUE TO FREEZING AND STORAGE

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ABSTRACT

The evaluation of the suitability of 9 new lines and 1 cultivar of peas for IQF (Individual Quick Freezing) is reported. Dry matter, pH, acidity, sugars, alcohol insoluble solids (A.I.S.), pectic substances, chlorophylls, pheophytins, objective measurements of firmness and color and organoleptic evaluation were carried out on fresh and frozen peas after processing and after storing at −20C for 6 months. The analysis pointed out that blanching and freezing caused losses mainly of dry matter, acidity and sugar content. Only a small pheophytinization of chlorophylls took place so the color was stable, as confirmed by the objective measurements and by organoleptic evaluations. After 6 months of storage at −20C only small changes in the chemical composition were observed. The organoleptic characteristics of color, flavor, texture and appearance were acceptable both after freezing and after cold storage of six months. Only the skin perceptibility was evaluated as slightly sufficient. The behavior of the cultivar was very similar during processing and cold storage.

INTRODUCTION

In frozen peas utilization, the organoleptic and nutritional features (Monzini 1964, 1977; Monzini *et al.* 1969; Monzini and Maltini 1980) must be considered as the principal aspects. The changes of color, firmness and flavor are dependent on the changes of the chemical composition of the peas during processing. In green vegetables the conversion of chlorophylls into pheophytins is responsible for the discoloration. Adams *et al.* (1983) observed that the major changes in green color of peas was due to the degradation of chlorophyll *a*, while chlorophyll

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b remained relatively stable; consequently, the color changed from green to yellow/brown because the carotenoid color became more evident.

Pectin composition greatly influenced the firmness of fresh and processed vegetables: Adams *et al.* (1983) pointed out that a loss of pectic substances in peas after blanching lead to changes of firmness in frozen peas.

The quality of frozen peas is also influenced by the cultivar (Dietrich *et al.* 1977; Anon. 1986; Crivelli *et al.* 1986).

This research examines the effects of freezing and storing on the chemical composition and sensory characteristics of four new lines of two different sizes of peas and one new cultivar.

MATERIALS AND METHODS

Peas and the Freezing Process

Peas (*Pisum sativum*) of the cultivar MARS (calibrated by sieves with a size of peas between 9.20 and 10.20 mm) and four lines, 20B, 34D, 36N, 41A (calibrated with a size of peas between 8.75 and 9.19 mm and between 9.20 and 10.20 mm) were used; the small size was indicated IV while the large size was indicated V.

Peas, harvested at the end of May, were supplied by the Istituto di Agronomia, Università Cattolica, Piacenza, Italy. Peas were stored for 12 h at 0C before processing, blanched in water 98C × 90 s), immediately cooled and frozen I.Q.F. (Individual Quick Freezing) in an air blast tunnel at −35C for 25 min. The frozen peas were packaged in polyethylene plastic bags, in cardboard boxes and stored at −20C for 6 months. Peas are indicated with the name of the cultivar or line followed by the size: MARSV, 20BIV, 20BV, etc.

Analytical Methods

The assay was conducted on fresh peas, after freezing and after 6 months of frozen storage. Peas were analyzed for pH, total titratable acidity, and dry matter following the AOAC methods (AOAC 1980).

Sugars were quantified by HPLC on a LiChrosorb NH_2 column (10 μm, 250 × 4 mm I.D.) eluting with acetonitrile/water (80/20), with a Shodex SE11 refractometric detector (Torreggiani *et al.* 1988).

Chlorophylls *a* and *b*, and pheophytins *a* and *b* were analyzed by a specific HPLC method utilizing a LiChrosorb RP-SelectB column (5 μm, 250 × 4 mm I.D.) eluted with a mixture of acetone/ethanol/water (70/17/13). The pigments were detected with a Spectrofluorimeter equipped with a PS 110 C Hg lamp power supply (λ Ex = 413 nm, λ EM = 669 nm) (Forni *et al.* 1988a,b).

Alcohol insoluble solids (AIS) were extracted from the peas according to the procedure of Forni *et al.* (1986).

The pectin content of peas was determined using the method of Forni *et al.* (1987), based on the enzymatic depolymerization of pectic substances in the AIS by Rohament-P polygalacturonase and quantifying the released galacturonic acid by HPLC on a RP-SelectB (Merck) column (5 μm, 250 × 4.5 mm I.D.), eluting with 0.25M of Hexadecyl-trimethylammonium bromide in 0.75M $NH_4H_2PO_4$ solution (pH = 6.8). The detector used was a Shodex Refractometer SE11.

Reported data are the average of four determinations.

Color

Color difference measurements were determined with a Hunterlab Color Difference Meter D25M. The readings were performed on 100 g of peas; reported data are the average of five determinations. From Hunter values of a, b and L the color difference delta E of the peas after six months of storage were calculated as $\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2}$ (Hunter 1975).

Firmness

The peas' firmness was evaluated by an Instron Model 1140 equipped with a Kramer Shear Press CS1 device. The chart and crosshead speed were 20 cm/min, with a maximum loading force of 500 kg on 50 g of peas. The results were the average of three measures and were expressed in kilograms. The skin firmness was measured with a piston of 1.5 mm diameter and a chart and crosshead speed of 20 cm/min, the maximum loading force was 500 g on 20 single peas and the results were expressed in grams.

The data were processed according to the analysis of variance and Duncan's multiple range test were used to determine statistically significant differences ($P \leq 0.01$), (Larmond 1977).

Sensory Evaluations

Sensory evaluations were carried out with a preference test (Larmond 1977) using a 9-point hedonic scale ranging from 1, dislike extremely, to 9, like extremely, and taking into account color, appearance, flavor, texture and skin toughness. The panel was composed of nine semitrained judges. Each tasting was repeated twice on subsequent days. Analysis of variance and Tukey's multiple range test were used to determine statistically significant differences ($P \leq 0.05$) (Snedecor 1956).

For tasting, 150 g of frozen peas were cooked for 5 min in 300 mL of boiling water with 1% NaCl, then cooled and tested at ambient temperature.

RESULTS

Dry Matter

Blanching and freezing caused losses of dry matter, with respect to the initial value, from 9% for 34DV peas to 19% for MARSV (Table 1). During storage the increases of dry matter ranged from 5% to 15% on the initial value.

pH, Total Tritable Acidity

Blanching and freezing of peas caused losses of acidity ranging between 32% and 70% with respect to the fresh peas' acidity (Table 1); the pH values consequently increased during processing. Further decreases in acidity, ranging from 20–30%, were noticed during storage.

Sugars

Sucrose was the only sugar observed in peas. Peas of differing lines and cultivar had different content of this sugar, ranging from 18.36 to 30.90, as already pointed out by Lee and Whitcombe (1945) and Lee *et al.* (1970). The reduction of the concentration of sucrose during blanching and freezing ranged from 7 to 30% of the sucrose content of fresh peas.

A.I.S.

The alcohol insoluble solids (A.I.S.) contents increased in peas during blanching and freezing, exhibiting little fluctuation during frozen storage (Table 2). The A.I.S. of the peas of size V was greater than the A.I.S. of peas of size IV.

Total Pectin

The amounts of total pectic substances in peas were very low (Table 2). The average of pectic substances was less than 2% of the A.I.S. After blanching and freezing, some peas exhibited a decrease and some others an increase in pectin content. Adams *et al.* (1983) observed differences in the behavior of the pectin content in fresh and frozen peas. Frozen storage produced increases of pectin in most of the peas.

Chlorophylls and Pheophytins

The chlorophyll and pheophytin contents of fresh peas and of frozen peas during storage at −20C are presented in Fig. 1. Chlorophyll *a* varied slightly during the freezing process and storage, while chlorophyll *b* was more stable. Pheophytins were observed only in processed peas, with a very low increase of

TABLE 1.
DRY MATTER, pH, ACIDITY AND SUCROSE CONTENT OF PEAS

Peas	Dry matter (%)	pH	Acidity* (meq/100g)	Sucrose* (%)
34DIV				
F**	23.26	6.52	13.42	25.05
C_o	20.48	7.04	5.13	20.69
C_6	20.01	7.46	3.45	20.53
34DV				
F	23.80	6.52	12.27	22.61
C_o	21.68	6.94	6.73	18.13
C_6	22.72	7.30	3.87	19.65
36NIV				
F	23.10	6.52	12.61	22.81
C_o	19.83	7.07	7.51	19.45
C_6	22.53	7.52	2.35	15.81
36NV				
F	24.40	6.50	12.17	18.36
C_o	22.12	7.00	5.39	13.74
C_6	23.40	7.31	3.08	15.44
41AIV				
F	25.95	6.55	10.73	22.82
C_o	21.34	6.92	5.81	17.83
C_6	22.46	7.35	2.80	16.40
41AV				
F	27.48	6.48	11.12	20.67
C_o	23.46	6.79	6.58	16.03
C_6	24.24	7.26	3.42	14.56
20BIV				
F	24.44	6.46	13.82	20.81
C_o	20.92	6.87	7.74	19.54
C_6	22.00	7.32	3.23	18.89
20BV				
F	24.63	6.41	13.29	21.00
C_o	21.40	6.80	7.97	18.16
C_6	21.69	7.30	3.37	18.56
MARSV				
F	22.91	6.43	12.97	30.90
C_o	18.48	6.74	8.80	24.46
C_6	18.16	7.36	3.08	28.05
R.c.v.***	0-0.35%		0.1-5%	1-9%

*= on dry matter
F**= fresh peas
C_o = frozen peas
C_6 = frozen peas after 6 mo of storage at -20 C
R.c.v.***= range of variation coefficient

the pheophytins after six months. Dietrich *et al.* (1977) reported that pheophytinization is mainly due to blanching process. Previous research reported the isomerization of chlorophyll *a* and *b* to chlorophyll *a'* and *b'* after freezing and frozen storage of peas (Forni *et al.* 1988b), but because the stereoisomers have

TABLE 2.
AIS AND PECTIN CONTENT OF PEAS

	AIS* (%)		Pectin** (mg/100g)	
Peas/size	IV	V	IV	V
34D				
F	68.56	73.40	0.87	1.36
C_o	78.65	80.34	1.24	0.42
C_s	81.82	76.94	1.25	0.88
36N				
F	73.80	78.46	1.50	0.78
C_o	83.22	85.05	0.87	0.56
C_s	80.67	84.54	1.18	0.71
41A				
F	72.59	77.06	0.48	1.22
C_o	80.54	82.44	0.64	0.58
C_s	79.71	85.86	1.00	0.68
20B				
F	66.56	73.88	1.13	1.52
C_o	76.78	87.02	0.23	1.73
C_s	79.51	84.96	0.94	1.62
MARS				
F		59.36		0.74
C_o		72.36		0.48
C_s		72.70		0.48
R.c.v.***	1- 1.8%		0.1-0.2%	

*= on dry matter
** as galacturonic acid on dry matter
F= fresh peas
C_o= frozen peas
C_s= frozen peas after 6 mo of storage at -20 C
R.c.v.***=range of variation coefficient

the same absorption spectrum as the natural chlorophylls, the isomerization did not influence the color of the peas. As has been observed by others isomerization occurs mainly during thermal treatments.

Firmness Evaluations

The firmness of frozen peas seed and of the skin is reported in Table 3. The largest peas were the firmest. After frozen storage an increase in firmness of peas was observed.

The regression between the A.I.S. content of the frozen peas and the total firmness was $Y = -4.76 + 6.52X$ ($R = 0.67$, $F = 99\%$). The regression between the AIS and the skin firmness was $Y = -3.18 + 12.3X$ ($R = 0.73$, $F = 99\%$).

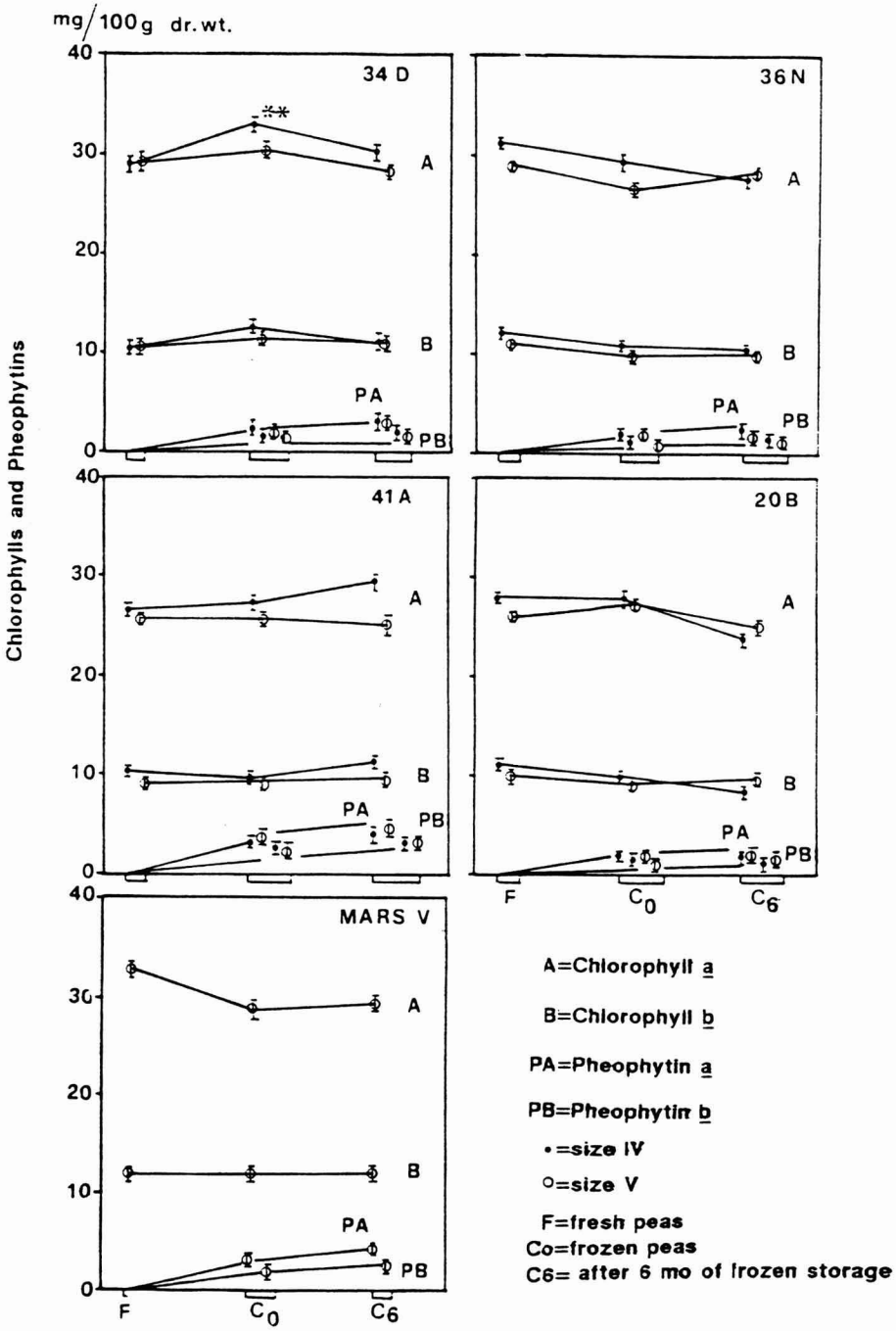

FIG. 1. CHLOROPHYLLS AND PHEOPHYTINS OF FRESH AND FROZEN PEAS DURING STORAGE AT −20C

**Confidence limits (p = 95%)

TABLE 3.
FIRMNESS EVALUATIONS OF FROZEN PEAS AFTER FREEZING (0 MONTHS) AND AFTER 6 MONTHS OF STORAGE AT −20C

Peas	Seed firmness (Kg)		Skin firmness (g)	
	0 mo	6 mo	0 mo	6 mo
34DIV	105bc*	123bc	184ab	196abc
34DV	112a	131ab	204a	213ab
36NIV	107b	120cd	183b	176e
36NV	102cd	134a	200ab	210abc
41AIV	99d	113de	147c	194bcd
41AV	104bc	111e	180b	191cd
20BIV	99d	128ab	187ab	199abc
20BV	102cd	132a	194ab	215a
MARSV	61e	73f	90d	115e

* :Means with different subscript in the same row are different ($P \leq 0.01$).

Color

The Hunter L,a,b color values of frozen peas and the delta E between frozen peas before and after frozen storage are presented in Table 4. The color did not change for most of the peas. 34DV, 41AIV and MARSV exhibited delta E values greater than the delta E of the other lines.

Organoleptic Evaluations

The color of frozen peas after freezing was acceptable, without significant differences among the peas. After 6 months of frozen storage the peas' color was judged acceptable, although some differences were found between lines. The appearance of the peas was acceptable, except for 34DV and 41AIV because there was shrinkage. After 6 months of frozen storage, no statistically significant differences were noted in appearance; however, there was an improvement in acceptability.

The flavor was acceptable both at 0 months and 6 months. The pulp firmness of peas was judged acceptable both at 0 and 6 months except for MARSV, which had become soft. The skin of MARSV and 20 BIV were the least perceptible, while for the other peas the skin perceptibility was more or less similar and slightly acceptable.

The results of the organoleptic evaluations suggested that peas retained good quality after frozen processing and frozen storage, especially for color, flavor and firmness.

TABLE 4.
DELTA E OF FROZEN PEAS AFTER SIX MONTHS OF STORAGE AT −20C

Peas	34DIV	34DV	36NIV	36NV	41AIV	41AV	20BIV	20BV	MARSV
Delta E	0.77	2.70	1.57	1.61	2.08	3.67	1.27	0.37	3.68

TABLE 5.
ORGANOLEPTIC EVALUATIONS OF PEAS DURING STORAGE AT −20C

Peas	0 months					6 months				
	C**	A	F	PF	SP	C	A	F	PF	SP
34DIV	7.33	5.60^{ab*}	6.87	6.47^{a}	4.93	6.80^{ab}	5.67	6.33^{ab}	6.47^{abc}	5.07^{bc}
34DV	7.00	4.87^{b}	6.67	6.93^{a}	4.93	6.60^{abc}	5.60	6.33^{ab}	6.00^{abc}	4.80^{c}
36NIV	6.87	5.67^{ab}	7.00	7.00^{a}	5.53	6.80^{ab}	5.87	6.80^{a}	6.80^{ab}	5.80^{bc}
36NV	6.53	5.40^{ab}	6.60	6.87^{a}	5.47	6.73^{ab}	6.47	6.73^{a}	6.87^{ab}	5.47^{bc}
41AIV	6.60	4.73^{b}	6.60	6.80^{a}	6.20	6.60^{abc}	5.60	6.80^{a}	7.00^{a}	5.73^{bc}
41AV	6.47	5.13^{ab}	6.13	6.53^{a}	5.13	6.00^{c}	6.00	5.73_{b}	6.00^{abc}	4.60^{c}
20BIV	6.40	5.53^{ab}	6.87	6.67^{a}	5.60	6.80^{ab}	6.07	7.00^{a}	6.93^{ab}	6.27^{b}
20BV	7.00	6.33^{a}	7.07	7.20^{a}	5.60	6.33^{bc}	6.33	6.80^{a}	6.93^{ab}	5.73^{bc}
MARSV	6.67	5.60^{ab}	6.67	4.93^{b}	7.47	7.07^{a}	5.60	6.53^{ab}	5.53^{c}	7.67^{a}

**:C=color - A=appearance - F=flavor - PF=pulp firmness - SP=skin perceptibility
* :Means with different subscript in the same row are different ($P \leq 0.05$).

CONCLUSION

The blanching and freezing processing of peas caused losses in dry matter, due to the fact that water soluble components, like sugars or acids, were released into the hot blanching water.

A small quantity of chlorophylls was converted into the respective *a'* and *b'* stereoisomers, and also into the respective pheophytins during blanching and freezing. The low chlorophyll conversion during storage was associated with the low acidity of the stored frozen peas. Correspondingly, the color of peas was stable as confirmed by the objective measurements and by organoleptic evaluations.

The organoleptic quality of frozen peas was judged acceptable at 0 months of frozen storage for color, flavor and texture and slightly acceptable for appearance and skin perceptibility.

Six months of frozen storage at −20C produced fewer effects than the blanching and freezing process. Acidity decreased, while dry matter and sucrose exhibited an increase in few lines of peas. There were no significant changes in A.I.S., pectin chlorophylls or pheophytins, so the peas showed small texture and color modifications.

The stored frozen peas were judged acceptable by the panel test, appearance improved and only skin perceptibility of 34DV and 41AV remained slightly acceptable. In accordance with the low A.I.S. content and with the low instrumentally measured firmness, MARSV was judged softer than the other lines of peas.

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STUDIES ON THE IMPROVEMENT OF FUNCTIONAL AND BAKING PROPERTIES OF WHEAT-CHICKPEA FLOUR BLENDS[1]

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ABSTRACT

Studies on the improvement of functional and baking properties of wheat-chickpea flour blends with addition of gluten (1.5, 3.0, 4.5 and 6.0%) and sodium steroyl-lactylate (SSL) 0.5%, were carried out. Chickpea flour at 10 and 20% level improved the dough stability and degree of softening but were adversely affected at 30% level of blending. Loaf volume, specific volume and crumb softness of bread decreased with the increase in chickpea flour in the blends. Addition of gluten at 3% level was found to effectively improve the rheological, loaf volume and crumb characteristics of wheat chickpea flour blends. Puffing ability and softness of chapaties deteriorated with increase in chickpea flour beyond 20% level, were improved by the addition of SSL. Cookie spread factor decreased and softness increased with increase in chickpea flour. Addition of SSL improved the spread factor up to 10% level of chickpea flour supplementation whereas softness of cookies improved progressively with the increase in chickpea flour.

INTRODUCTION

Supplementation of legume flours with cereal flours has great potential in developing countries for improving the nutritional value of different baked products. A good number of studies on the supplementary value of legumes, particularly with respect to soyabean are available (Tsen *et al.* 1971; Tsen and Tang 1971; Tsen and Hoover 1973; Marnett *et al.* 1973; Fellers *et al.* 1976; Ebeler and Walker 1983; Lindell and Walker 1984).

Hallab and Khatchadourian (1974) found that the supplementation of chickpea flour to wheat flour at levels of 30% and above adversely affected taste and ac-

[1]Contribution No. FTR-18, Department of Food Science & Technology, Punjab Agricultural University, Ludhiana, India.

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ceptability of bread. Luzfernandez and Berry (1989) also reported that supplementation with chickpea flour had an adverse effect on the rheological and baking properties of wheat flour. However, chickpea could advantageously be used for the enrichment, if the adverse effects of its supplementation on the quality of baked products could be overcome. The present study was, therefore, undertaken with the objective to improve the functional and baking quality of wheat-chickpea flour blends.

MATERIALS AND METHODS

Preparation of Samples

Wheat variety WL-1562 was procured from 1989–90 harvest. The cleaned samples were tempered at 14% moisture for 48 h and milled in a Buhler Pneumatic Mill (MLU, 202) to 72% extraction. Chickpea variety GL-769 was conditioned to 10% moisture and kept under the sun for 3 h, dehusked and ground in a stone grinder. The flour so obtained was passed through a 48 mesh sieve. The chickpea flour was blended at the 10, 20 and 30% levels with wheat flour.

An experimental stone grinder with adjustable gap between the top revolving stone disc and fixed bottom was used for producing wheat meal which was passed through a 48 mesh sieve.

Extraction of Gluten

Gluten was extracted from wheat flour by the hand washing method (AACC 1976) and was used at levels of 1.5, 3.0, 4.5 and 6.0% on dry weight basis to study the rheological and baking properties.

Rheological Properties

The rheological properties were studied using the farinograph by AACC (1976) method (54-21).

Baking Performance

The remix procedure by Irvine and McMullan (1960) was adopted for testing baking performance of blends. The bread formula for each loaf consisted of 250 g flour, 6.25 g yeast, 3.75 g salt, 5.0 g sugar and water as required to obtain optimum consistency. A single gluten preparation was used for all samples. The dough was fermented for 1.15 h at 30C at 85% RH. The loaves were mechanically molded, proofed and baked at 450F for 30 min. The loaf volume was measured by the rapeseed displacement method. The loaves were evaluated for various sensory attributes as described earlier (Singh *et al.* 1990). The firmness of bread

was studied with the help of the Instron Universal testing machine (Model-1111) with the following settings: bread slice thickness, 12 mm; drive speed, 100 mm/min; chart speed, 200 mm/min; force range 0–2000 grams (g); compression, 6 mm; dye size, 15 mm. The firmness of the bread slices was measured in 'g' force required for 6 mm compression at the center. The pasting properties of bread crumb were studied using the Brabender Visco-amylograph. Bread crumb (130 g) was suspended in 320 mL distilled water and agitated in a laboratory blender for one minute. The slurry was transferred to the Visco-amylograph bowl with 100 mL additional water and heated from 25 to 95C held at 95 for 10 min and cooled to 50C.

Chapati Making and Evaluation

To study the effect of blending chickpea flour in wheat meal on chapaties the method of Austin and Ram (1971) with some modifications was used. Flour (100 g) was optimally mixed with adequate quantities of water to get a dough of moderate stiff consistency. The dough was kept at 30C and 95% RH for 30 min. The dough was divided into 25 g portions and rounded off between hands into a round ball. It was rolled into a chapati of 15 cm diameter. The chapaties were baked on an iron plate at 220C. When one side of chapati was baked (about 30 s) it was turned and other side was baked for about same time. It was again turned and made to puff with clean cloth by pressing and moving. The total time varied between 1 to 2 min. The effect of addition of SSL (0.5%) on chapati making was also studied. The texture of chapaties was studied after one hour of baking by the Instron Universal Testing Machine using the same settings as described earlier except that the dye size was 3.5 mm.

Chapaties from each lot were placed on a hollow cell having a diameter of 15 mm and punctured at 2 places. The mean peak height of the curves, i.e., force in 'g' required to puncture the chapaties was reported. Chapaties were organoleptically evaluated by 6 semi-trained panelists after one hour of baking used a 9-point hedonic.

Cookies Preparation and Evaluation

Cookies were prepared according to the method (AACC 1976) method (10-50D) with the addition of 0.75% ammonium bicarbonate.

The Instron Universal Testing Machine was used for the crushing test which simulated the shattering of the cookies in the mouth during chewing with the molars. The 50 kg compression load cell was used with a drive speed of 20 mm/min and chart speed of 200 mm/min. A 15 mm diameter flat, circular probe was centered on middle of an individual cookie which was compressed to 6 mm. Mean force for five cookies was reported.

Statistical Analysis

The samples were statistically analyzed using two factors in a randomized block design as described by Steel and Torrie (1960) using HP-1000 computer system.

RESULTS AND DISCUSSION

Rheological Properties

Farinograph water absorption, dough development time and dough stability decreased significantly, whereas degree of softening increased significantly beyond the 20% level of blending chickpea flour (Tables 1 and 2). Similar changes in dough characteristics with blending of chickpea flour were observed by Luzfernandez and Berry (1989). However, Shehata and Fryer (1970) reported little ef-

TABLE 1.
EFFECT OF SUPPLEMENTATION WITH GLUTEN ON RHEOLOGICAL PROPERTIES OF WHEAT-CHICKPEA FLOUR BLENDS

Chickpea Flour (%)	Characteristics	Gluten (%) 0	3.0	4.5	6.0
0	Water absorption (%)	55.5	56.5	58.0	61.0
	Dough development time (min)	4.5	4.8	5.1	6.2
	Dough stability (min)	5.2	5.7	8.7	9.0
	Degree of softening (BU)	85	75	50	40
10	Water absorption (%)	56.0	57.0	57.8	60.4
	Dough development time (min)	4.6	4.7	5.0	5.8
	Dough stability (min)	7.5	7.8	8.6	12.1
	Degree of softening (BU)	70	65	60	50
20	Water absorption (%)	55.0	56.2	57.0	60.0
	Dough development time (min)	4.2	4.6	4.9	5.5
	Dough stability (min)	7.0	6.5	6.8	11.5
	Degree of softening (BU)	90	85	80	60
30	Water absorption (%)	53.2	55.4	56.7	59.5
	Dough development time (min)	3.9	4.5	4.8	5.2
	Dough stability (min)	3.5	3.8	4.0	6.0
	Degree of softening (BU)	100	95	90	75

TABLE 2.
SIGNIFICANT TERMS (F-VALUES) FROM ANALYSIS OF VARIANCE OF DATA IN TABLE 1

Source of Variation	d.f.	F-values			
		Water Absorption	Dough Dev. time	Dough Stability	Degree of Softening
Replicates	2	NS	NS	NS	NS
Treatment combinations	15	228.5**	77.4**	681.6**	59.7**
Chickpea levels	3	111.4**	51.1**	1743.0*	145.0**
Gluten levels	3	1006.9**	315.5**	1378.8**	130.7**
Interaction	9	8.0**	6.7**	95.4**	7.5**

**Significant at p = 0.01
NS = Not significant

fect on dough stability and departure time upon supplementing chickpea flour at 20% level. The changes in dough characteristics upon addition of chickpea flour are attributed to dilution of gluten forming proteins causing weakening of doughs. Competition between chickpea and wheat flour proteins for water of hydration and variation in their hydration behavior due to differences in the nature of proteins may be another reason for the change in dough characteristics. The deterioration in farinographic characteristics with chickpea supplementation was significantly overcome with the addition of gluten and the extent of improvement was proportional to the quantity of gluten added.

Baking Properties

Baking absorption, loaf volume and specific loaf volume decreased significantly with the increase in the level of chickpea flour in the blends (Tables 3 and 4). The crumb became increasingly harsh and dry and crust color progressively darkened with the increase in chickpea flour. The crust color of breads produced from wheat flour supplemented with 30% chickpea flour was unacceptable. With the increased level of chickpea flour in the blends, the force required to compress the bread crumb increased significantly which indicated a decreased softening of the bread crumb. However, softening of crumb was significantly improved with the addition of gluten. Shehata and Fryer (1970) also reported deterioration

TABLE 3.
EFFECT OF SUPPLEMENTATION WITH GLUTEN ON THE PASTING AND BREAD MAKING PROPERTIES OF WHEAT-CHICKPEA FLOUR BLENDS

Chickpea Flour (%)	Characteristics	Gluten (%)				
		0	1.5	3.0	4.5	6.0
0	Baking absorption (%)	58.1	59.6	60.3	62.0	64.5
	Loaf volume (cc)	1045	1110	1190	1330	1420
	Specific loaf volume (cc/g)	3.0	3.2	3.5	3.9	4.1
	Firmness (g)	1005	815	670	590	450
	Crumb peak viscosity (BU)	1075	980	760	640	580
	Total scores (100)	65.0	69.2	80.0	86.5	80.5
10	Baking absorption (%)	57.1	58.3	58.6	59.8	60.2
	Loaf volume (cc)	1040	1095	1140	1275	1375
	Specific loaf volume (cc/g)	2.9	3.1	3.3	3.6	3.8
	Firmness (g)	1050	890	795	680	570
	Crumb peak viscosity (BU)	870	760	650	600	560
	Total scores (100)	60.7	64.5	72.2	78.5	77.5
20	Baking absorption (%)	56.0	56.5	57.0	57.5	58.5
	Loaf volume (cc)	720	820	870	1000	1105
	Specific loaf volume (cc/g)	2.1	2.4	2.6	2.9	3.2
	Firmness (g)	1200	1030	900	800	680
	Crumb peak viscosity (BU)	775	590	565	530	470
	Total scores (100)	33.0	38.7	45.0	58.0	61.5
30	Baking absorption (%)	54.2	55.3	56.4	57.8	58.2
	Loaf volume (cc)	670	740	810	880	1045
	Specific loaf volume (cc/g)	1.9	2.2	2.4	2.6	3.0
	Firmness (g)	1340	1180	1020	940	780
	Crumb peak viscosity (BU)	550	525	480	420	380
	Total scores (100)	22.0	26.5	34.5	42.0	48.5

TABLE 4.
SIGNIFICANT TERMS (F-VALUES) FROM ANALYSIS OF VARIANCE OF DATA IN TABLE 3

Sources of Variation	d.f.	F-values					
		Baking Absorption Volume	Loaf Volume	Specific Loaf	Firmness	Crumb Viscosity	Total Scores[a]
Replicates	2	NS	NS	NS	NS	NS	NS
Treatment combinations	19	337.1**	821.5**	76.9**	762.1**	591.0**	1326.9**
Chickpea level	3	1363.8**	3262.5**	297.4**	1630.1**	1836.1**	6647. **
Gluten level	4	666.6**	1438.8**	139.8**	2384.5**	1187.8**	1218.7**
Interaction	12	33.9**	5.5*	0.8	4.3*	80.8**	32.8**

[a]Degrees of freedom for replicates, 5; treatment combination, 19; chickpea level, 3; gluten level, 4; interaction, 12.
*Significant at p = 0.05, **Significant at 0.01, NS = Not significant

of crust color with the addition of chickpea flour, however, they reported that supplementation of 20% chickpea flour had no effect on the overall acceptability of Egyptian bread. Hallab and Khatchadourian (1974) reported that at or above the 30% level of chickpea flour blending, taste and overall acceptability were adversely affected. The darkening of crust color is attributed to the increased Maillard reaction in the crust.

Peak viscosity of bread crumb progressively decreased by the addition of chickpea flour which might have been due to the reduced ability of chickpea flour granules to swell before physical breakdown and to reduced starch content. Similar observations with respect to wheat-bean composite flours were made by Deshpande *et al.* (1983). Addition of gluten to the wheat and chickpea flour blends improved the bread loaf volume, specific loaf volume, crumb softness and acceptability. The improvement was greater with higher addition of gluten up to 6%. Overall, the desired results could be obtained with the addition of gluten and chickpea at levels as low as 3 and 10%, respectively.

Chapati Making

Water absorption of whole wheat meal decreased with the increase in the level of chickpea flour which was unaffected by addition of SSL (Tables 5 and 6). The replacement of wheat flour with more than 10% chickpea flour lowered the puffing ability of dough. This was improved with the addition of SSL. This improvement was noticeable up to 20% replacement of wheat flour with chickpea flour. Puffing ability of the dough improved upon addition of SSL because the surfactant facilitated air incorporation in the dough during mixing (Junge and Hoseney 1981; Junge *et al.* 1981).

Puncture force for chapaties measured by Instron Universal Testing machine increased significantly with the increase in level of chickpea flour in blends. The lower puncture force indicated softer chapaties. The differences in puncture force between control and chickpea flour blended chapaties were significant. The puncture force was significantly reduced by addition of SSL indicating improvement in texture of chapaties upon addition of SSL making them more tender. Sidhu *et al.* (1989) also reported that addition of surfactants at 0.5% level increased the tenderness of chapaties.

Overall acceptability of chapaties was significantly reduced with the supplementation of chickpea flour. However, good quality chapaties could be prepared from a 20% chickpea and wheat meal blend by the addition of 0.5% SSL.

Cookie Making Properties

Cookie spread factor decreased with the increase in level of chickpea flour; however, this decrease was significant only beyond 20% blending of chickpea

TABLE 5.
EFFECT OF ADDITION OF SSL ON THE CHAPATI MAKING PROPERTIES OF WHEAT-CHICKPEA FLOUR BLENDS

Chickpea Flours (%)	Treatment	Characteristics			
		Water Absorption (%)	Puffing	Puncture Force (g)	Total Scores (9.0)
0	Without SSL	70	Full	360	8.0
	With SSL	70	Full	260	8.3
10	Without SSL	68	Full	340	7.0
	With SSL	68	Full	300	7.5
20	Without SSL	64	Partial	520	5.4
	With SSL	64	Full	480	6.3
30	Without SSL	60	Partial	540	4.0
	With SSL	60	Full	525	5.4

TABLE 6.
SIGNIFICANT TERMS (F-VALUES) FROM ANALYSIS OF VARIANCE OF DATA IN TABLE 5

Source of Variation	d.f.	F-values	
		Puncture Force	Total Score[a]
Replicates	5	NS	NS
Treatment combination	7	955.7**	69.9**
Chickpea level	3	2068.6**	146.7**
SSL level	1	362.4**	38.0**
Interaction	3	48.6**	NS

[a]Degrees of freedom for replications, 9; treatment combination, 7; chickpea level, 3; SSL level, 1 and interaction, 3.

**Significant at p = 0.01, NS = Not significant

TABLE 7.
EFFECT OF ADDITION OF SSL ON THE COOKIE MAKING PROPERTIES OF WHEAT-CHICKPEA FLOUR BLENDS

Characteristics	Treatment	Chickpea Flour (%)					
		0	10	20	30	40	50
Cookie spread (w/t)	Without SSL	5.7	5.6	5.5	5.3	5.2	5.0
Instron force (g)		30.3	28.2	22.0	16.0	13.2	10.5
Total scores (9.0)		8.2	8.6	8.0	7.6	7.0	6.6
Cookie spread (w/t)	With SSL	6.1	5.6	5.5	5.4	5.0	4.8
Instron force (g)		28.5	26.3	20.0	14.5	9.5	7.5
Total scores (9.0)		8.5	8.7	8.0	7.4	7.0	6.3

flour (Tables 7 and 8). Addition of SSL at 0.5% to the control flour significantly improved the spread factor of cookies. Instron force required to compress the cookies decreased with the increase in the level of chickpea flour. All the blends differed significantly from each other with respect to cookie spread, possibly due to differences in effective thickness and the dilution of gluten proteins by the addition of chickpea flour. The lesser compressing force indicated softer cookies.

TABLE 8.
SIGNIFICANT TERMS (F-VALUES) FROM ANALYSIS VARIANCE OF DATA IN TABLE 7

Source of Variation	d.f.	F-values		
		Cookie spread	Instron force	Total score[a]
Replicates	5	NS	NS	NS
Treatment combination	11	61.1	572.8**	24.15**
Chickpea level	5	117.8**	1223.0**	52.3**
SSL level	1	NS	159.2**	NS
Interaction	5	160**	5.4*	NS

[a]degrees of freedom for replicates, 9; Treatment combination, 11; chickpea level, 5; SSL level, 1; Interaction, 1.
*Significant at p = 0.05; **Significant at p = 0.01; NS = Not significant

The softness of cookies increased with the addition of SSL as indicated by decrease in Instron force.

Top grain of cookies was slightly improved with addition to chickpea flour up to 10%. Beyond this level the top grain was unacceptable. Texture of cookies improved up to the 30% level beyond which texture was not liked much. Addition of SSL improved the top grain of cookies prepared from the control or with supplementation of 10% chickpea flour. Beyond 20% level, SSL did not show any improvement in top grain. Improvement in spread factor and top grain by addition of SSL has also been reported by Tsen *et al.* (1975). However, the addition of SSL did not bring about any improvement in the sensory quality of cookies as seen from the sensory scores.

Overall, the addition of SSL and supplementation of chickpea at 20% produced cookies as good as the control and with better texture.

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ASSESSMENT OF THE EXPONENTIAL MODEL TO SOUR CHERRY JUICE FILTRATIONS

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ABSTRACT

De La Garza and Boulton's "Exponential model" of wine filtrations simulated the data satisfactorily in the cake filtration of sour cherry juice. There was an inverse relation between the filtration rate and the resistance. Increase in the amount of precoating decreased the filtration rate. However, increase in filter aid dose increased the filtration rate. Further increase in filter aid dose and pressure over some limiting value gave no further increases infiltration rate.

INTRODUCTION

Solid-liquid filtration may be defined as that unit operation in which the insoluble solid component of a solid-liquid suspension is separated from the liquid component by passing the suspension through a porous medium.

The flow of the filtrate may be brought about by means of gravity alone (gravitational filtration), by the application of a pressure greater than atmospheric upstream of the medium (pressure filtration), by applying a vacuum downstream of the medium (vacuum filtration) or by means of centrifugal force (centrifugal filtration).

There have been major developments in filtration during recent years (Brennan *et al.* 1981; Bayindirli *et al.* 1989).

In fundamental sour cherry juice production process, washing and sorting, milling, mash treatment, pressing, screening, depectinization, clarification, filtration and pasteurization are the main steps.

Model studies on filtration in food industry are extremely limited (i.e., De La Garza and Boulton 1984; Bayindirli *et al.* 1989). Sperry's model (Sperry 1917) has formed the basis of the filtration theory (Orr 1977). Sperry's equation is useful for filtrations in which the solids being removed are rigid, or when the cake being formed with a filter aid is rigid, in what is referred to as a "surface collection" mechanism (De La Garza and Boulton 1984). Clogging of the filter medium

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due to the slimy and gelatinous particles is the main problem in filtration of liquid foods. Therefore, Sperry's equation is completely unsatisfactory for food filtrations.

In cake filtrations, filter aids which are inert particles are used in a number of ways to retard filter fouling. They can be used as a precoat before the slurry is filtered. They can also be added to the slurry prior to filtration (Tressler and Joslyn 1971). Usage of both precoating and filter aid addition are necessary, because the filter medium is not exposed to the gelatinous particles of the non-filtered liquid by means of precoating. When the filter medium is precoated, more clear juice is obtained. Addition of rigid filter aid particles surround the particles within the slurry. In this case, porosity of the cake formed increases. As a result, filtration resistance decreases (Bayindirli *et al.* 1989).

Particle size of the filter aid also affects the filtration resistance. Since beds formed by larger particles have greater void volumes, obviously flow rates decrease with decreasing particle size, i.e., using filter aid with larger particle size is necessary for higher flow rate (Bayindirli *et al.* 1989).

Sperry's model was modified by De La Garza and Boulton (1984) to develop mathematical models for wine filtrations. Two new mathematical models which are "Exponential" and "Power" models have been developed. These filtration rate equations are described, respectively, as:

$$\frac{dV}{dt} = \frac{\Delta P\ A}{\mu} \frac{1}{(R_m \exp(k\ V/A))} \tag{1}$$

and

$$\frac{dV}{dt} = \frac{\Delta P\ A}{\mu} \frac{1}{(R_m + \alpha\ c\ (V/A)^n)} \tag{2}$$

where ΔP is the pressure difference across the filter; A is the filtration area; μ is the viscosity of the filtrate; c is the concentration of the solids being collected; t is the time and V is the volume of the filtrate collected; R_m and α are the medium and specific cake resistances, respectively; k and n are positive constants.

Filtration resistance occurs immediately upon filtration in the exponential model; whereas, in the power model, resistance to filtration does not occur until some initial filtrate volume has passed, at which time rapid fouling occurs.

Modeling of apple juice filtrations has been studied (Bayindirli *et al.* 1989). De La Garza and Boulton's "Exponential model" has been found to be satisfactory for apple juice filtrations. The other filtration models were not appropriate. De La Garza and Boulton's "Power model" has not been used since a constant value could not be found for parameter n, to describe all data (Bayindirli *et al.* 1989).

The purpose of this study is to evaluate the validity of "Exponential Law" approximation to sour cherry juice filtration and to investigate the effects of certain process parameters on filtration rates.

MATERIALS AND METHODS

In the experiments, sour cherries from a local variety (Afyon) were used. They were washed, sorted, seeds and stems were removed. Then, juice extraction was done with hand pressing. This was adequate for this study because only a theoretical model was tested and the effects of certain process parameters on filtration rate were investigated. Industrial application was not simulated. After the juice was extracted, it was filtered directly without any prefiltration, depectinization and clarification.

Filtration equipment was composed of three major parts: Pressure supply, reservoir and filtration unit. The filtration unit (storage vessel) was pressurized with a regulated nitrogen supply. The applied pressure difference, ΔP, was set to be constant for each run of the experiment with the help of a pressure regulator (Matheson, Model 9-350, USA). A stainless steel storage vessel with 2.5 L capacity had been placed on top of a magnetic stirrer to prevent settling. The filtration cell holder (Spectra/Por, Spectrum Medical Ind., New York) was originally manufactured for ultrafiltration studies. In this study, instead of ultrafiltration membranes filter media was used. The filtration area of the cell holder was 30.2 cm^2. The filtrate was collected in a graduated cylinder.

Unless otherwise specified, experiments were performed with fabric filter medium at about 21C, under 0.65 atm. gauge pressure.

The fabric filter medium was tightly woven synthetic cloth manufactured locally and used in beet sugar production industry. Both precoating and filter aid addition by using a filter aid with largest particle size available was employed, since this is better for a higher filtration rate, as stated by Bayindirli *et al.* (1989). The filter aid, which was flux calcined diatomaceous earth with 20 μm particle size and 0.60 m^2/cm^2 specific surface area (Turkish Sugar Factories, Inc., Ankara), was used both for precoating and filter aid addition.

In the first set of experiments, effects of the precoating amount were studied; 0.005 g/cm^3 juice was used for aid dose. For precoating, 0.05, 0.10 and 0.25 g/cm^2 filter areas were tried. In addition, one of the experiments was performed without using precoating.

In the second set, the filter aid dose was changed and the other parameters were kept constant. An optimum amount of precoating found in the first set was used in this case. For aid dose, 0.002, 0.005 and 0.010 g/cm^3 juice were tried.

In the third set, optimum amounts of precoating and filter aid dose found in first and second sets of the experiments were used. Several pressure differences (0.20, 0.65, 1.00 and 1.50 atm. gauge pressures) were tried to see the effects of pressure on the filtration rate.

For precoating of the medium, the storage vessel was filled with water containing diatomaceous earth, and then this mixture was forced to pass through the filter medium. After that, filter aid was added to the juice to be filtered and stirred vigorously with magnetic stirrer to produce a homogeneous mixture. Next, this mixture was filled into the storage vessel and the graduated cylinder was placed under the filtration unit. This mixture was then forced from the reservoir to the filter unit with the help of a nitrogen supply. Filtration was achieved through the precoated medium in the filter unit. Filtrate was collected in the graduated cylinder and volume of the filtrate as a function of time was recorded.

Since sour cherry and apple juices show similarities, the "Exponential model," which was satisfactory for apple juice filtration, was also employed in the case of sour cherry juice filtration. According to this model:

$$R = \frac{dt}{dV} \frac{\Delta P\ A}{\mu} \tag{3}$$

and total resistance can be defined as:

$$R = R_m \exp (k\ V/A) \tag{4}$$

The exponential cake resistance coefficient, β, is defined as:

$$\beta = k/A \tag{5}$$

Substituting the Eq. (5) into Eq. (4) and taking the natural logarithm of both sides;

$$\ln R = \ln R_m + \beta\ V \tag{6}$$

Constant pressure filtration was achieved in each run of the experiments by means of a pressure regulator as mentioned before. Filtration area, A, was a constant also. Filtrate viscosity, μ, was measured by using Cannon-Fenske capillary viscometer. The increments in volume, ΔV, and in time, Δt, between successive points were calculated from the filtrate volume-time data taken during each experiment. The resistance, R, at each interval mid-point was calculated from Eq. (3) (McCabe and Smith 1976; De La Garza and Boulton 1984). The model parameters ln R_m and β were determined from the ln R versus V data by using the least square analysis.

All the experiments were done in duplicate. The maximum variation was only about $\pm 5\%$ in the filtrate volumes in the time course of the replicate filtration experiments.

RESULTS AND DISCUSSION

The effects of precoating amount, filter aid dose and pressure on filtration resistance are shown in Fig. 1. The values of the model parameters R_m and β, which were obtained by using linear regression, were summarized in Table 1.

Equation (1) was solved for V at any time t and the filtrate volumes were calculated by using the previously determined values of R_m and β. Then, the calculated filtrate volumes were plotted against time for each case (Fig. 2).

There was an inverse relation between the filtration rate and resistance. In the experiments where the resistance was lower, higher filtrate volumes were achieved.

When the filter medium was precoated, plugging of the medium by gelatinous particles was retarded since slimy particles were not in contact with the filter medium. However, medium resistance, R_m, increased as precoating thickness increased (Table 1), because medium resistance represents the resistance due to the precoat thickness in addition to the filter medium. Total resistance, R, decreased and so filtration rate increased with decreasing amounts of precoating, since the depth of the packed bed is directly proportional to the resistance from the fluid mechanics [Fig. 1(a) and 2(a)]. Cloudy juice that can be observed with eyes was obtained when precoating amount was below 0.10 g/cm^2.

Increase in the filter aid concentration caused a decrease in the exponential cake resistance coefficient, β, (Table 1). When the filter aid concentration was increased, clogging the channels of the filter cake decreased since filter cake with a higher void fraction formed. As a result, filtration resistance decreased [Fig. 1(b) and 2(b)]. Increasing the aid concentration beyond 0.005 g/cm^3 gave no significant increase in filtration rate. Therefore, 0.005 g/cm^3 filter aid addition was optimum for sour cherry juice filtration.

Pressure is a quite effective parameter in the case of sour cherry juice filtration, since the juice contains deformable, slimy and gelatinous particles; 0.65 atm. gauge pressure was optimum since the filtration resistance was minimum in this case [Fig. 1(c)]. Increasing the pressure above this point caused an increase in R_m but a decrease in β values (Table 1). However, since the effect of R_m on total resistance is less than the effect of β, no significant change was observed in terms of flow rates [Fig. 2(c)]. Increase in the filtration resistance as pressure increases was most probably due to the formation of a denser filter cake under too high pressures. When the upstream pressure was 0.20 atm. gauge, both R_m and β was maximum (Table 1). The flow rate was too low [Fig. 2(c)] because this pressure was not enough to force the slurry to pass through the precoated medium.

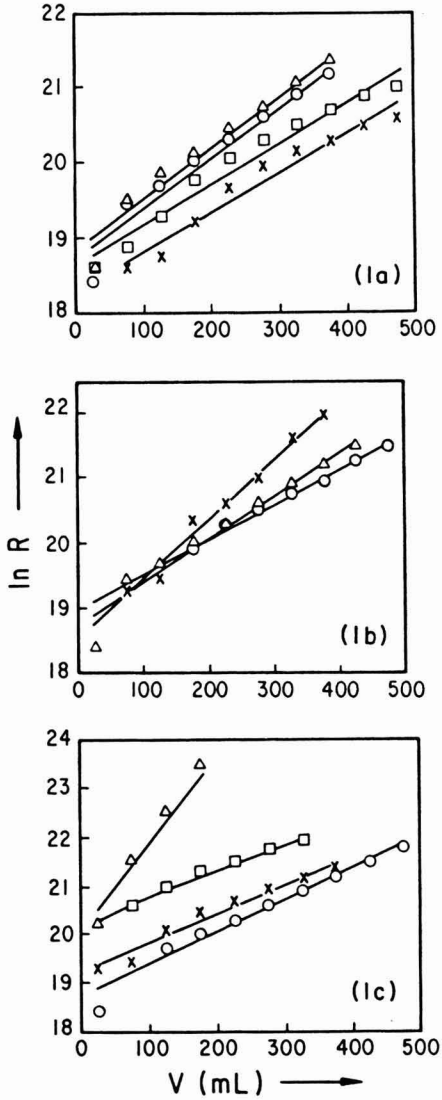

FIG. 1. EFFECT OF PRECOATING AMOUNT, FILTER AID DOSE AND PRESSURE ON FILTRATION RESISTANCE

(a) Effect of precoating amount on resistance (△ = 0.25 g/cm², ○ = 0.10 g/cm², □ = 0.05 g/cm², × = no precoating);
(b) Effect of filter aid dose on resistance (△ = 0.005 g/mL, × = 0.002 g/mL, ○ = 0.010 g/mL);
(c) Effect of pressure difference on resistance (○ = 0.65 atm. gauge), △ = 0.20 atm. gauge, × = 1.00 atm. gauge, □ = 1.50 atm. gauge).
Diatomaceous earth with 20μm particle size and fabric filter medium were employed in all the experiments
Temperature was 21C. Filter aid dose was 0.005 g/mL in Fig. 1a and 1c; precoating was 0.10 g/cm² in Fig. 1b and 1c; pressure difference was 0.65 atm. gauge in Fig. 1a and 1b.
Symbols represent the experimental data points and the solid lines are the proposed exponential model.

TABLE 1.
EXPERIMENTAL CONDITIONS AND EXPONENTIAL MODEL PARAMETERS FOR SOUR CHERRY JUICE FILTRATION

Fig. Number	Experimental Conditions	Model Parameters		
		Rm (1/cm)	β (1/cm^3)	r[1]
1a	0.25 g/cm^2 precoating	1.5E+08	0.007	0.98
1a	0.10 g/cm^2 precoating	1.3E+08	0.007	0.98
1a	0.05 g/cm^2 precoating	1.2E+08	0.005	0.98
1a	No precoating	8.9E+07	0.005	0.98
1b	0.005 g/cm^3 filter aid	1.3E+08	0.007	0.98
1b	0.002 g/cm^3 filter aid	1.1E+08	0.009	0.99
1b	0.010 g/cm^3 filter aid	1.7E+08	0.005	0.99
1c	ΔP = 0.65 atm. gauge	1.3E+08	0.007	0.98
1c	ΔP = 0.20 atm. gauge	5.2E+08	0.018	0.97
1c	ΔP = 1.00 atm. gauge	2.2E+08	0.006	0.98
1c	ΔP = 1.50 atm. gauge	5.0E+08	0.005	0.99

[1]Correlation coefficient.

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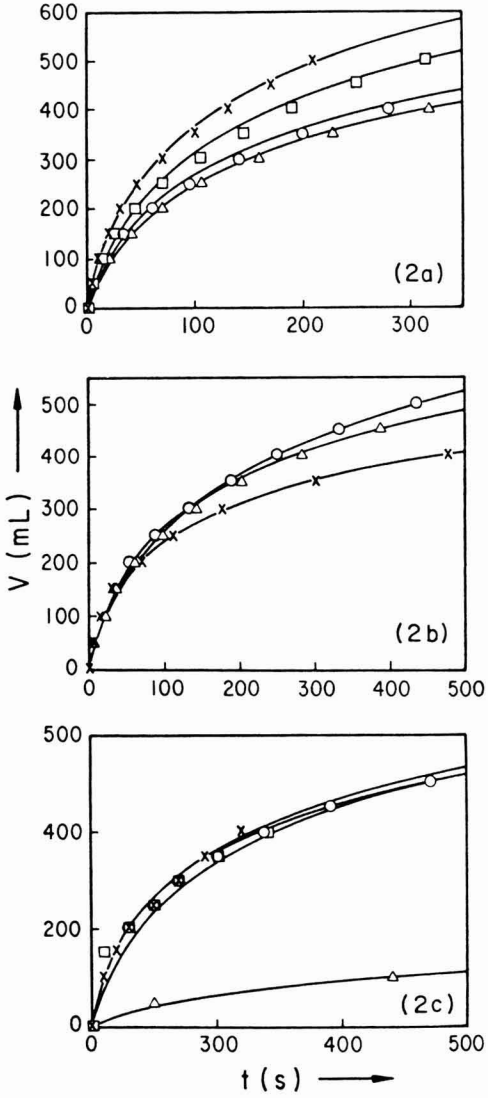

FIG. 2. FILTRATE VOLUME VERSUS TIME
(a) Effect of amount of precoating on filtration rate;
(b) Effect of filter aid dose on filtration rate;
(c) Effect of pressure difference on filtration rate.
Experimental conditions and symbols for Fig. 2a, b and c are the same as those of Fig. 1a, b and c, respectively.

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SENSORY, CHEMICAL AND BACTERIOLOGICAL STABILITY OF FRANKFURTERS CONTAINING MILK PROTEINS AND CORN GERM PROTEIN FLOUR[1]

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ABSTRACT

Shelf stability of frankfurters extended with milk proteins and corn germ protein flour (CGPF) stored at 1–2C for 45 days was studied by water activity, sensory evaluation, bacteriological tests, and chemical determinations of total volatile nitrogen (TVN), ammonia nitrogen, and TBA values. The incorporation of milk proteins and CGPF affected shelf stability of frankfurters as determined by sensory characteristics, bacteriological test, and chemical analysis. After 45 days of storage, the frankfurters with CGPF added had a stronger atypical aroma and atypical flavor than the all-meat control and products containing milk proteins. However, the atypical notes were relatively low, up to 10.2 on a 60-point scale, and should be considered as weak and acceptable. The all-meat control had a juicier mouthfeel than the other frankfurters. CGPF-containing frankfurters contained an intermediate count (log 3–4 CFU/g) of psychrotrophic bacteria, whereas the other products contained a low bacteria count (log 0–2 CFU/g) after 45 days of storage. There was no formulation treatment effect on TBA values. TVN in frankfurters increased with increasing storage time. The ammonia contents of treatment and control frankfurters were not different during 45 days of storage.

INTRODUCTION

Evaluation of product stability to test shelf-life limits and potential pathogens is a requirement for the development of any new food product. Unlike whole steaks of roasts, in which the interior of the product is essentially sterile or has a very low microbial count, comminuted meat products contain microorganisms as a result of handling, incorporation of contaminated raw material and ingredients, and contact with equipment.

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Moisture availability is a primary requirement for bacterial growth. Water activity (a_w) is an intrinsic parameter of a food system. It influences the growth and metabolic activity of microorganisms that cause the deterioration of foods.

A variety of methods have been used, based on measurements of deteriorative changes associated with sensory quality, microbial enumeration, and chemical changes. Indications of spoilage are found not only through culturing and counting microorganisms, but also by changes in color, odor, and texture. The traditional sensory evaluation is costly, because of its requirement for a special training program. Microbiological tests and chemical analyses, such as total volatile nitrogen (Cobb III *et al.* 1973; Pearson and Muslemuddin 1968), ammonia nitrogen (Gardner and Stewart 1966), and biogenic amines (Edwards *et al.* 1985; Vallejo-Cordoba *et al.* 1987), are important criteria of food storage stability.

The spoilage of meat and poultry at low temperatures is because of microbiological action and not because of tissue autolysis (Ingram and Dainty 1971; Jay 1972; Shewan 1976). The end result of bacterial metabolism is the formation of volatile chemicals, including off-odor compounds.

The total soluble nitrogenous compounds in meat are about 1.65%, including 0.35% amino acids, 0.55% creatine, and 0.75% other minor components (ICMSF 1980). Of the soluble nitrogenous substances, the amino acids are most readily metabolized by microorganisms. Most of these metabolic reactions result in the release of ammonia or amines, which create the odor of putrefied meat (Banwart 1981). Putrefaction of proteins or amino acids (nitrogenous compounds) to ammonia by microbial proteases also results in adverse changes in flavor.

Spoilage may also be a function of lipid oxidation. Oxidative rancidity in meats is the result primarily of autoxidation or the action of tissue lipases. Lipid oxidation is a primary cause of deterioration in meat products. Oxidative rancidity of meat fats can directly influence some characteristics, i.e., color, odor, flavor, texture, nutritive value, and safety (Pearson *et al.* 1983). Various mechanisms for the development of rancid flavors in meat products were reviewed by Asghar *et al.* (1988).

Limited information has been reported on the comparative shelf-lives of comminuted meats containing milk proteins and corn germ protein flour (CGPF). Functional properties of CGPF in model system have been studied: water retention and solubility in comparison with soy flour, concentrate and isolate (Wang and Zayas 1991); emulsifying properties (Lin and Zayas 1989); emulsifying capacity and emulsion stability in comparison with nonfat dry milk, sodium caseinate and whey protein concentrate (Hung and Zayas 1991); fat binding capacity (Lin and Zayas 1987).

The following aspects of CGPF utilization in meats have been studied: functionality of CGPF in beef patties (Brown and Zayas 1990); functionality of CGPF in comminuted meats (Zayas and Lin 1989a); proximate and amino acid composition of CGPF and meat products containing CGPF (Zayas and Lin 1989b).

Incorporation of CGPF in the formulations of frankfurters increased yield and decreased cooking losses, improved juiciness and textural properties as a result of increased water holding capacity, emulsifying capacity and fat binding capacity. Researchers in the Department of Foods and Nutrition, KSU have developed the commercial process and formulations of CGPF utilized in comminuted meats. CGPF can be utilized as an extender and replacer of meat proteins in comminuted meats, and this is commercially feasible.

The objective of this study was to investigate the effect of milk proteins and CGPF incorporated in comminuted meat products on their quality characteristics during prolonged storage, up to 45 days at 1–2C, including water activity measurement; sensory evaluation (aroma, flavor, and juiciness); bacteriological tests; and chemical determinations (total volatile nitrogen, ammonia nitrogen, and TBA values).

MATERIALS AND METHODS

Studies of frankfurters containing milk proteins and CGPF as powdered ingredients were conducted to test storage stability. Nonfat dry milk (NFDM), whey protein concentrate (WPC, RH-34HM), and sodium caseinate (SC) were obtained from Ridgeview, La Crosse, WI. CGPF was obtained from the dry corn milling industry.

Product Manufacture

Fresh meats (50/50 beef trim and boneless picnics), obtained from the meat lab of Kansas State University, were ground through a 9.38 mm plate and frozen at −18C until use. Coarsely ground meats were mixed and then reground twice through a 4.69 mm plate. To maintain desired proportions of protein and moisture, higher levels of water were added to experimental formulations. Water was added to the weight of meat in the formulations at the level of 25.0% for controls. Four experimental treatments were conducted: 3.5% CGPF, 28.5% water added; 3.5% NFDM, 28.5% water added; 2.0% SC, 27.0% water added. The formulation contained salt (2.0%); Prague powder (0.25%, 156 ppm in comminuted meats) containing 6.25% sodium nitrite (Griffith Lab., Alsip, IL); ascorbic acid (0.1%); and sugar (2%).

The finely ground meats were mixed with salt and ⅓ of the water for 2 min in a Hobart bowl mixer equipped with a dough hook, then the Prague powder, ascorbic acid, and ⅓ of the water were added to the blend and mixed for 1 min. Final blending involved a 2 min mixing with the seasoning, sugar, extenders (CGPF or milk proteins), and ⅓ of the water immediately before processing. The preblend was comminuted through a Mincemaster emulsion mill (Griffith GL-86, Griffith Design and Equipment Co., Chicago, IL; with 1.7 mm plate).

The batter was stuffed into 24 mm-diameter cellulose casings formed in links 11 cm in length, and heat processed in a smokehouse. A four-stage heating cycle was set in which the frankfurters were preheated at 48C for 10 min, followed by 55C for 30 min, 55C for 5 min smoking, and 80C until an internal temperature of 70C was reached. A cold shower (6–7 min) was finally applied to reduce the internal product temperature to 20C or below. Additional chilling to 4C was also applied. After peeling, the frankfurters were vacuum-packaged and held at 1–2C for 45 days. Shelf-stability tests were conducted after 1, 30, and 45 days of storage, including water activity, sensory evaluation, bacteriological tests, and chemical determinations.

Water Activity

Water activity (a_w) was measured using an NT-3 Nanovoltmeter with thermocouple psychrometer device model SC-10A (Decagon Devices, Inc., Pullman, WA). The instrument was calibrated according to the directions in the operation manual. Frankfurters were chopped for 2 min and packed by volume (75%) into the sample cups, which were placed into the chambers. Readings were obtained after the mV digital signals stabilized and were converted by drawing of a standard curve, using NaCl solution (0.1–1.0 M) as a standard.

Sensory Evaluation

Sensory characteristics of aroma, flavor, and juiciness of control frankfurters and frankfurters containing one of three milk proteins or CGPF were determined by a professional sensory panel, Sensory Center, KSU. A 12-member panel was trained first according to panelist instructions in three sessions to be familiar with the product parameters of aroma, flavor, and juiciness. Aroma and flavor included meaty, smoky-cured, and atypical aroma; meaty, smoky-cured, sweet, salty, spicy, and atypical flavor.

Frankfurters were randomly selected from vacuum packages stored at 1–2C and cooked in boiling distilled water for 1 min. Two 2 cm segments, excluding 2 cm of each end, were presented to each panelist in a warmed custard cup (coded with a 3-digit random number) covered with a watch glass and kept warm on an electric warming tray before serving. The evaluation order was aroma first, then juiciness and flavor. Samples were presented randomly to the panelists and evaluated under masking light (red) in individual booths. Sliced Golden Delicious apples and distilled water were provided for removal and rinsing of residual flavor from the mouth. An unstructured intensity scale with 60 points (15 cm) from 0 = weak to 30 = medium to 60 = strong was used. Five sets of samples were evaluated in each session, including four treatment products and a control, with evaluations conducted at 1, 30, and 45 days of storage.

Bacteriological Tests

Samples of frankfurters (10 g) were removed aseptically and placed into sterile whirl-pak bags. Sterile saline solution (90 mL) was added for a 1:10 dilution, and samples were homogenized for 1 min in a Stomacher blender. Five consecutive dilutions of the sample were made with duplicate pour plates. Appropriate dilution aliquots were drawn with sterile pipets and placed into the center of disposable petri dishes. Media stored at 45C were poured in petri dishes and carefully rotated for complete distribution.

1. Psychrotrophic Test. This test was conducted on Standard Plate Count agar (Difco Laboratories, Detroit, MI). The agar (23.5 g) was suspended in 1 L distilled water and heated to boiling to dissolve completely. It was sterilized in an autoclave at 15 lb pressure and 121C for 15 min and stored in a water bath to stabilize at 48C before using (Difco 1984). Petri dishes were inverted and incubated at 7C for 10 days. Number of Colony Forming Units (CFUs) were counted with the aid of a Quebec Colony Counter and a 10X dissecting scope. Number of CFUs were multiplied by the dilution factor to determine CFUs/g.

2. Fecal Coliform Test. The coliform test was conducted on Violet Red Bile agar (Difco Laboratories, Detroit, MI). Violet Red Bile agar (41.5 g) was suspended in 1 L distilled water and heated to boiling for not more than 2 min to dissolve. It was cooled in a water bath (48C) for use (Difco 1984). For obtaining fecal coliform counts, dilutions were pour plated with Violet Red Bile agar allowed to solidify and overlaid with the same media. The petri dishes were inverted and incubated at 35C for 24 h. A positive CFU is a purplish red colony 0.55 mm or larger with a halo or 1–2 mm in diameter and surrounded by a reddish zone of precipitated bile (Klein and Fung 1976).

Chemical Determinations

Three determinations were conducted to test chemical changes of frankfurters extended with milk proteins and CGPF during prolonged storage.

1. Total Volatile Nitrogen. Total volatile nitrogen was determined by steam distillation using a microKjeldahl distillation apparatus (Cobb III *et al.* 1973). Samples of frankfurters were blended in a blender, and 20 g were taken and mixed with 40 mL of 7% trichloracetic acid (TCA). The mixture was homogenized in a Waring Blender and filtered through Whatman #1 filter paper. The clear filtrates were refrigerated until tested for total volatile nitrogen. Saturated Na_3PO_4 (5 mL) was added to filtrates (5 mL), and subsequently, steam distillation was employed. Volatile nitrogen was trapped in 5 mL of boric acid solution (50 mL

3.1% H_3BO_3 solution with 1 mL mixed indicator). During distillation, up to 30 mL of distillate was collected. Values were reported as mg nitrogen per 100 g meat.

2. Ammonia Nitrogen. Ammonia nitrogen was determined using the colorimetric method (AOAC 1984; 18.027) with minor modification. Colorimetric absorbance (680 nm) readings obtained from a Perkin-Elmer Spectrophotometer (Coleman model 124 D) were converted to quantities of ammonia nitrogen by use of a standard curve. Values were reported as mg nitrogen per 100 g meat.

3. Thiobarbituric Acid (TBA) Analysis. Oxidative rancidity was determined with the use of the 2-thiobarbituric acid test. TBA values were determined using a modification (Kuntipanti 1978) of the extraction procedure of Witte *et al.* (1970). Colorimetric absorbance at 529.5 nm obtained from a Perkin-Elmer ultraviolet spectrophotometer was converted to malonaldehyde using a standard curve. TBA values were expressed as mg malonaldehyde per 1000 g meat.

Statistical Analyses

A complete randomized block (CRB) design was followed, and four replications were independently processed. Analysis of variance (Steel and Torrie 1980) was conducted to test the differences in water activity, bacteriological counts, and chemical determinations. The least significant difference (LSD) at a 5% level of probability was used to separate means. For sensory evaluation, a split-plot design was used to test the effects of extenders on aroma, flavor, and juiciness. Analysis of variance and LSD tests were conducted by the methods of Steel and Torrie (1980). Data analyses were done with the Statistical Analysis System (SAS 1982) program.

RESULTS AND DISCUSSION

Water Activity

Water activity is widely accepted for predicting shelf-life of food products. Water activity was not affected by treatment ($P > 0.05$) at 1, 30, and 45 days of storage. All frankfurters had similar water activity at 1, 30, and 45 days ranging from 0.9734–0.9752, 0.9744–0.9756, and 0.9749–.09771, respectively. Kitic *et al.* (1986) indicated that the water activity range for microbial growth was from about 0.57 to 0.97. The water activities of experimental samples were in this range. Although there was a trend of increase in water activity from 1 to 45 days of storage, the increase was not significant ($P > 0.05$).

Sensory Evaluation

The meaty aroma profile of frankfurters for 45 days storage is presented in Fig. 1. The general trend in meaty aroma was a slight decrease over the storage period, except frankfurters containing SC, which increased between 1 and 30 days, then decreased after 45 days of storage. However, there was no difference ($P > 0.05$) in meaty aroma over the time of storage. The extenders added in formulations of frankfurters affected ($P < 0.05$) the sensory characteristics of meaty aroma. The control frankfurters tested after 1 day of storage had a stronger ($P < 0.05$) meaty aroma than the frankfurters with extenders, except those with

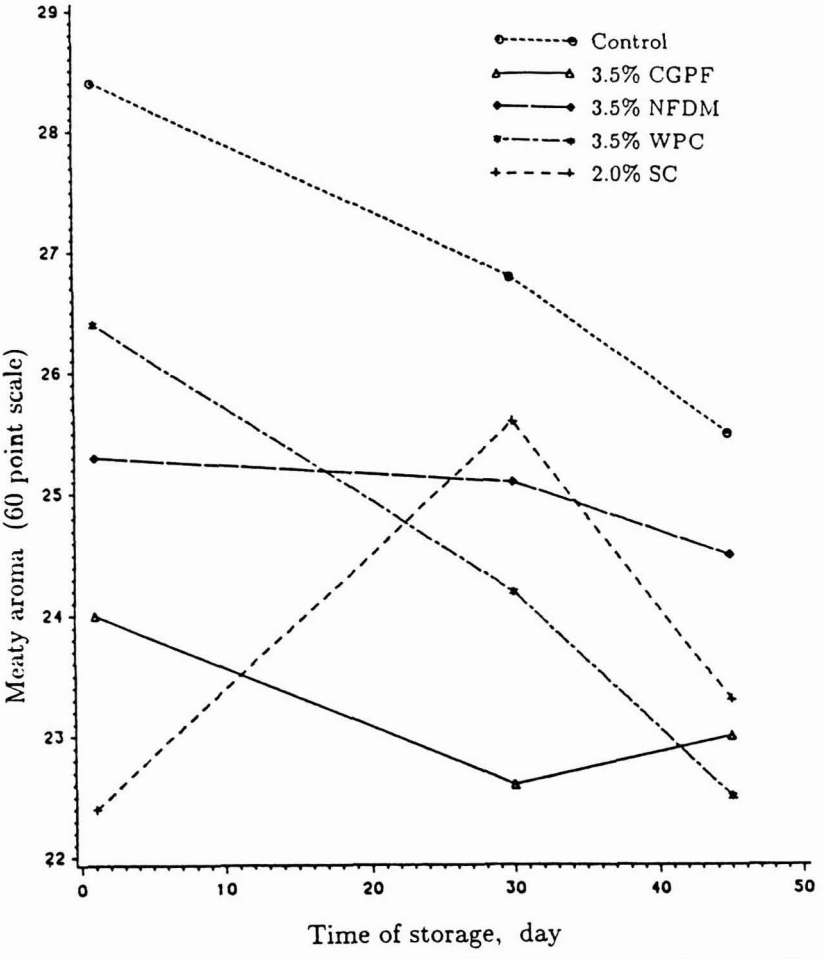

FIG. 1. EFFECT OF STORAGE ON MEATY AROMA OF FRANKFURTERS CONTAINING EXTENDERS

WPC added (Fig. 1). For smokey-cured aroma, no significant changes ($P > 0.05$) occurred during 45 days of storage and also no treatment effects ($P > 0.05$) were observed.

The effect of storage on atypical aroma of frankfurters containing extenders did not differ ($P > 0.05$) during the storage period (Fig. 2). However, after 45 days of storage, the frankfurters extended with CGPF revealed a stronger atypical aroma ($P < 0.05$) when compared with the all-meat control and the other treatment samples. This study confirmed that addition of CGPF in a sausage formulation results in the corny aroma of frankfurters observed by Zayas and Lin (1989b).

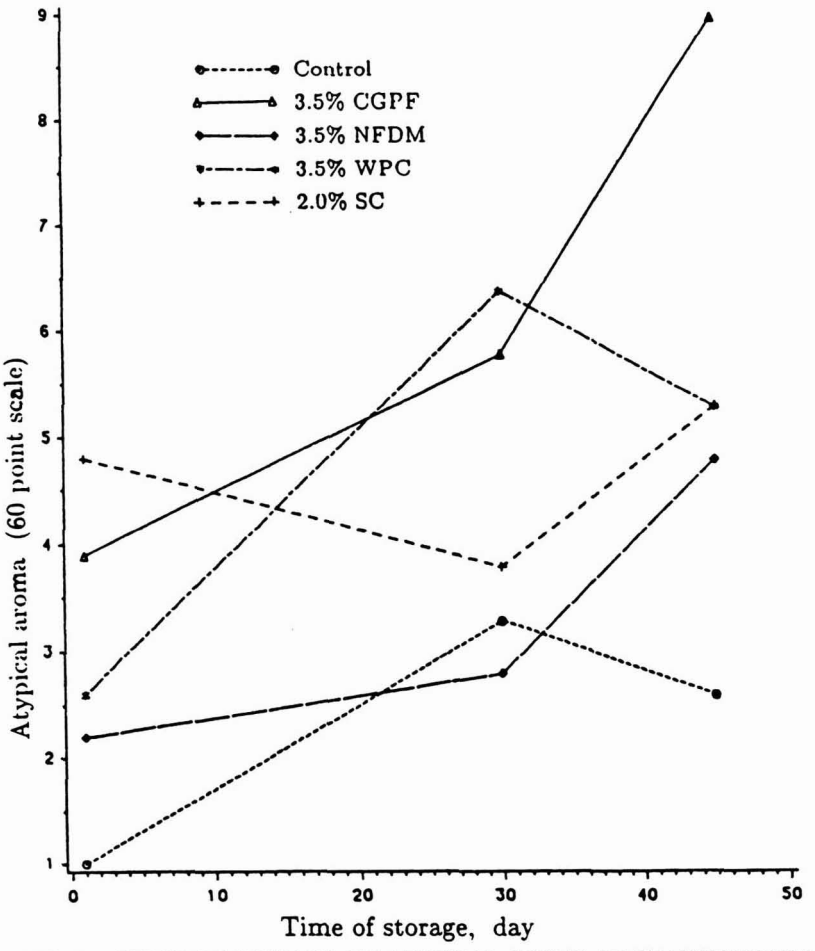

FIG. 2. EFFECT OF STORAGE ON ATYPICAL AROMA OF FRANKFURTERS CONTAINING EXTENDERS

The atypical aroma scores, up to 9 on a 60 point scale, should be recognized as acceptable.

Juiciness of frankfurters did not change ($P > 0.05$) during 45 days of storage (Fig. 3). However, the all-meat controls had a juicier mouthfeel ($P < 0.05$) than all products with extenders at 1, 30, and 45 days of storage. The juiciness of frankfurters with extenders was similar over the time of storage, except those with NFDM added at 45 days, which showed less juiciness.

No storage effects were found ($P > 0.05$) on meaty flavor for any of the frankfurters during 45 days of storage at 1–2C. However, frankfurters with CGPF had lower meaty flavor score ($P < 0.05$) throughout the time of storage, except

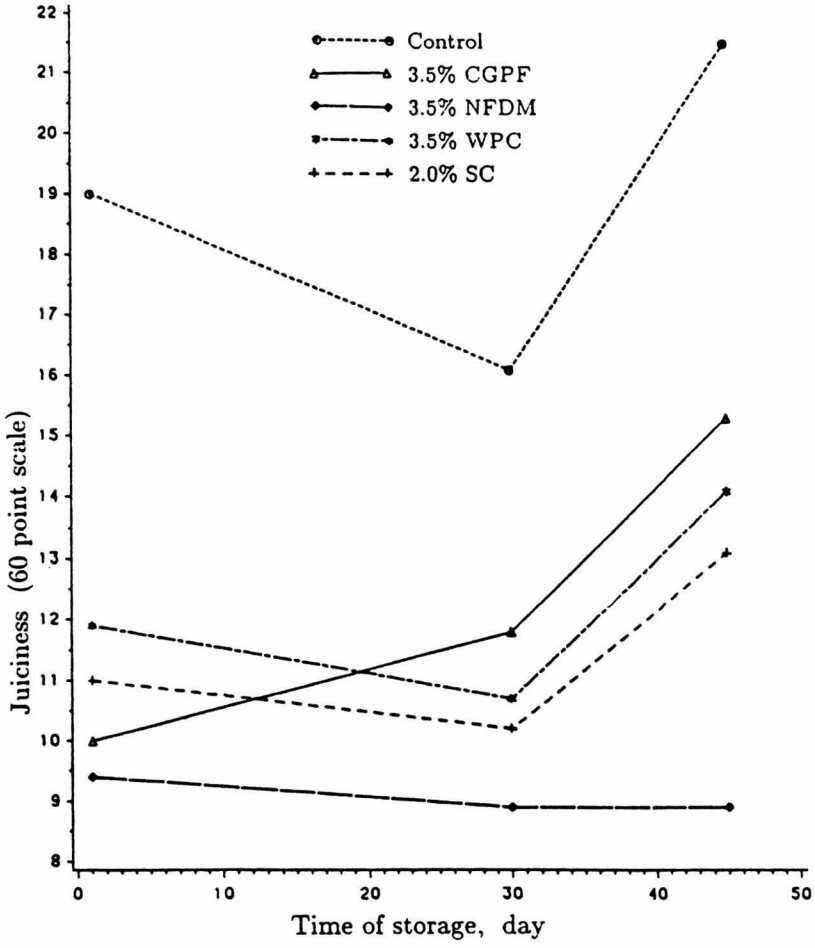

FIG. 3. EFFECT OF STORAGE ON JUICINESS OF FRANKFURTERS CONTAINING EXTENDERS

samples containing SC and NFDM and tested at 1 day of storage. This might have been due to the masking effect of the corny flavor from added CGPF. Control frankfurters had a stronger meaty flavor ($P < 0.05$) than the products with extenders at 1 and 45 days of storage (Fig. 4).

Although the smoky-cured flavor scores of frankfurters containing CGPF were lower than those of the other products during 45 days of storage, the differences were not significant ($P > 0.05$) among all-meat control and frankfurters with extenders. The numerical scores for smoky-cured flavor of all products were in the range 26–31 on the 60 point scale. During 45 days of storage, a trend toward

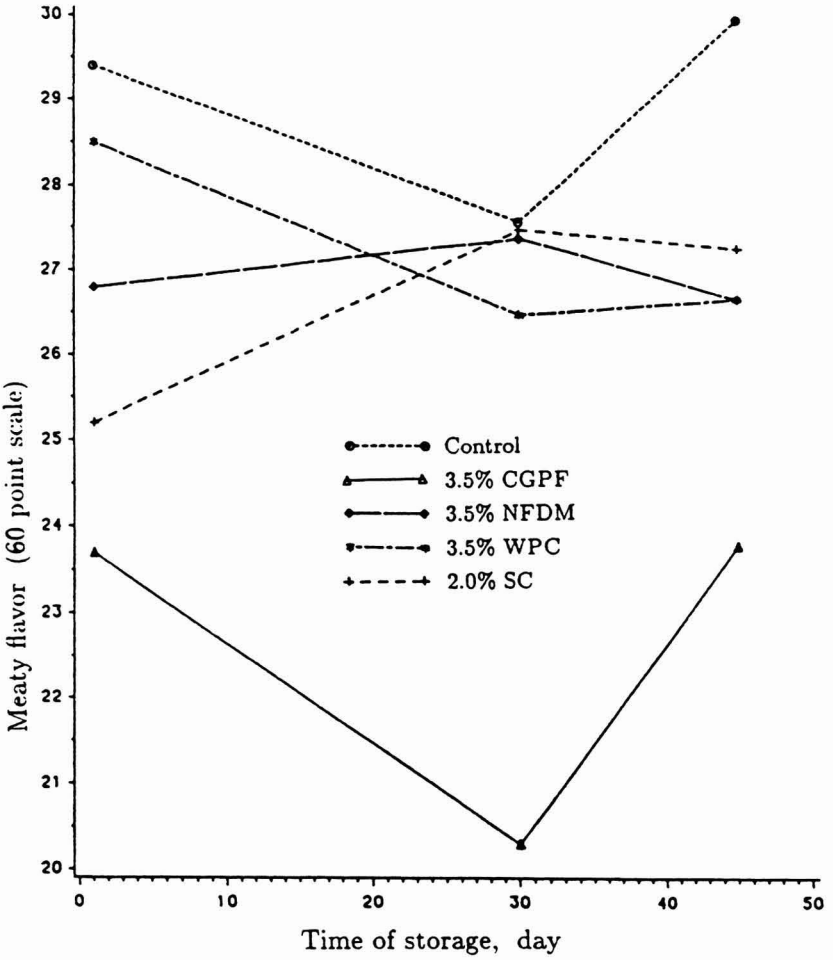

FIG. 4. EFFECT OF STORAGE ON MEATY FLAVOR OF FRANKFURTERS CONTAINING EXTENDERS

increase of sweetness and saltiness was observed for all-meat control and frankfurters with extenders. There was no difference ($P > 0.05$) among formulation treatments and the control sample at 1, 30, and 45 days in terms of sweet and salty flavors. Spicy flavor was not different ($P > 0.05$) among the groups of frankfurters at 1, 30, and 45 days of storage.

No storage effect was found on atypical flavor of frankfurters with different formulations, but the treatment effect on atypical flavor was significant ($P < 0.05$), when evaluated at the storage times of 1, 30, and 45 days (Fig. 5). As compared with control frankfurters at day 1, atypical flavor of frankfurters ex-

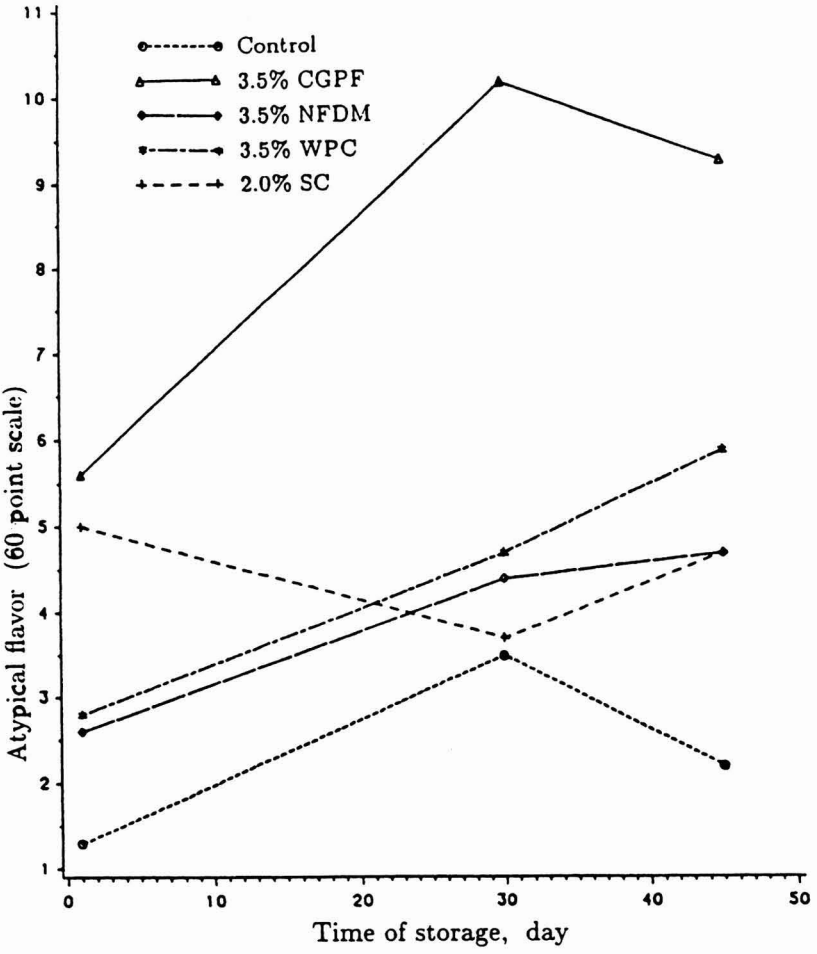

FIG. 5. EFFECT OF STORAGE ON ATYPICAL FLAVOR OF FRANKFURTERS CONTAINING EXTENDERS

tended with CGPF was stronger ($P < 0.05$) whereas that of frankfurters with milk proteins (NFDM and WPC) was not different ($P > 0.05$), except for the formulation with SC. The atypical flavor of CGPF-containing frankfurters developed from day 1 to day 30 of storage, increasing from 5.6 to 10.2. The latter score on a 60-point scale would be considered as weak and acceptable, although it could be detected ($P < 0.05$) by sensory evaluation.

Bacteriological Tests

Psychrotrophs, which grow at 1–10C, usually cause spoilage at refrigeration temperature. Mean values of logarithmic psychrotroph enumeration per 1 g for 4 treatments and control frankfurters during 45 days of storage are presented in Fig. 6. At day 1, psychrotrophs were undetected at the lowest dilution for samples containing WPC and CGPF, whereas the other samples (containing NFDM or SC and control) had a relatively low enumeration in the range of log 1–2 CFU/g, indicating that the products were microbiologically safe. This might have been due to the low initial microbial loads and/or the successful thermal processing to 70C internally, which inactivated most of the microorganisms. After 30 days of storage, the number of psychrotrophs did not increase ($P > 0.05$) for any products, and no treatment effects ($P > 0.05$) were found on psychrotroph enumeration. After 45 days of storage, CGPF-containing frankfurters had a higher log number ($P < 0.05$) of psychrotrophs in the range of 3–4 CFU/g. After prolonged storage, the higher intensities of atypical aroma (Fig. 3) and atypical flavor (Fig. 5) might result from the larger population of microorganisms. The other four products contained psychrotrophs ranging from log 2 to log 2.5 CFU/g, and no differences ($P > 0.05$) were observed among them (Fig. 6). According to the microbiological scale developed by Fung *et al.* (1980) to group and rank the data, only frankfurters extended with CGPF fell in the intermediate count region (log 3–4 CFU/g), and the other frankfurters fell in the low count region (log 0–2 CFU/g).

No fecal coliforms were noted in any frankfurters throughout 45 days of storage. Coliforms are heat sensitive and easily destroyed by the temperature reached during heat treatment. Our results indicated that adequate heat treatment without contaminated equipment or without postprocessing contamination were accomplished (Davis 1969)

Chemical Determinations

Storage stability is a critical factor for comminuted meats containing extenders. Chemical indices of shelf stability used for vacuum-packaged frankfurters stored at 1–2C for 45 days were TVN, ammonia nitrogen, and TBA values. TVN and ammonia nitrogen tests were carried out to further confirm the data from the sensory and bacteriological tests. Total volatile nitrogen (Cobb III *et al.* 1973; Pear-

son and Muslemuddin 1968; Pearson 1970) and ammonia nitrogen (Gardner and Stewart 1966; Vallejo-Cordoba *et al.* 1987) have been used as spoilage indices in meat and fish products.

Total Volatile Nitrogen. The profile of TVN is presented in Fig. 7. Generally, TVN of frankfurters increased with increasing storage time up to 45 days. The increase was in the range from 5.30–5.80 mg N/100 g sample shortly after processing to 6.26–7.31 mg N/100 g sample after 30 days of storage and 7.20–8.01 mg N/100 g sample after 45 days of storage. An increase ($P < 0.05$) of TVN

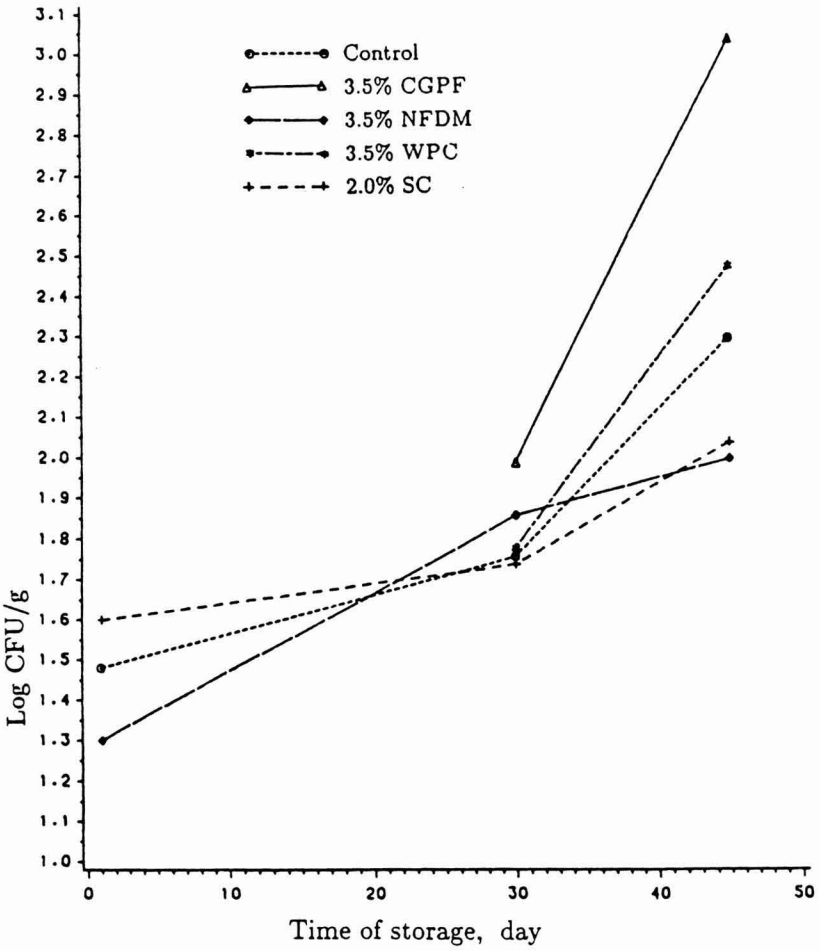

FIG. 6. EFFECT OF STORAGE ON PSYCHOTROPH COUNTS OF FRANKFURTERS CONTAINING EXTENDERS

was noted between 1 to 30 days of storage for both treatment and control frankfurters. However, no treatment effects ($P > 0.05$) with different extenders were found on the amount of TVN during 45 days of storage.

Ammonia Nitrogen. Spoilage organisms have been shown (Jay and Kontou 1967; Gill 1976) to subsist on readily available soluble nitrogenous substances, such as free amino acids and nucleotides. Ammonia is released by deamination of these nitrogenous compounds. A trend of increased ammonia nitrogen was observed from day 1 to day 30 and a decrease from 30 to 45 days during frankfurter storage (Fig. 8). This increase and decrease of ammonia nitrogen was similar

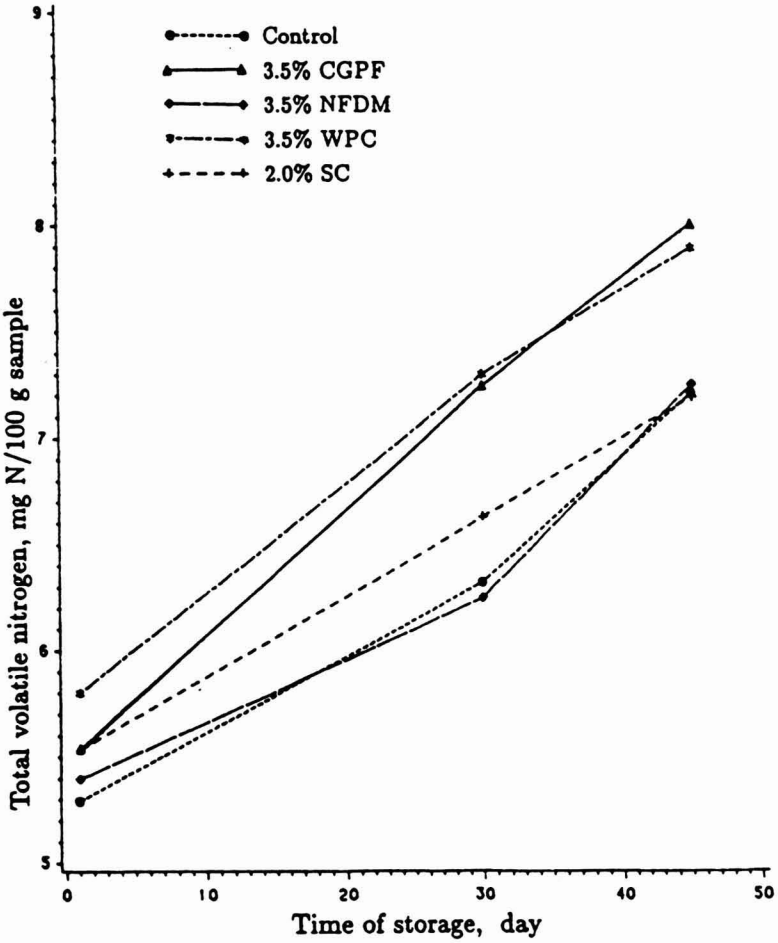

FIG. 7. TOTAL VOLATILE NITROGEN CONTENT OF FRANKFURTERS CONTAINING EXTENDERS

in the treatment and all-meat control frankfurters. The decrease in ammonia content at day 45 for vacuum-stored products might have been due to the solubilization of ammonia through the "skin" of frankfurters to the liquid between the frankfurter's surface and the vacuum-packaged film. In no case were the levels of ammonia nitrogen of any frankfurters higher than 7 mg N/100 g sample during 45 days of storage at 1–2C.

TBA Value. Among all the measurements for determining lipid oxidation in meat, the TBA value is correlated well with oxidative rancidity (Melton 1985).

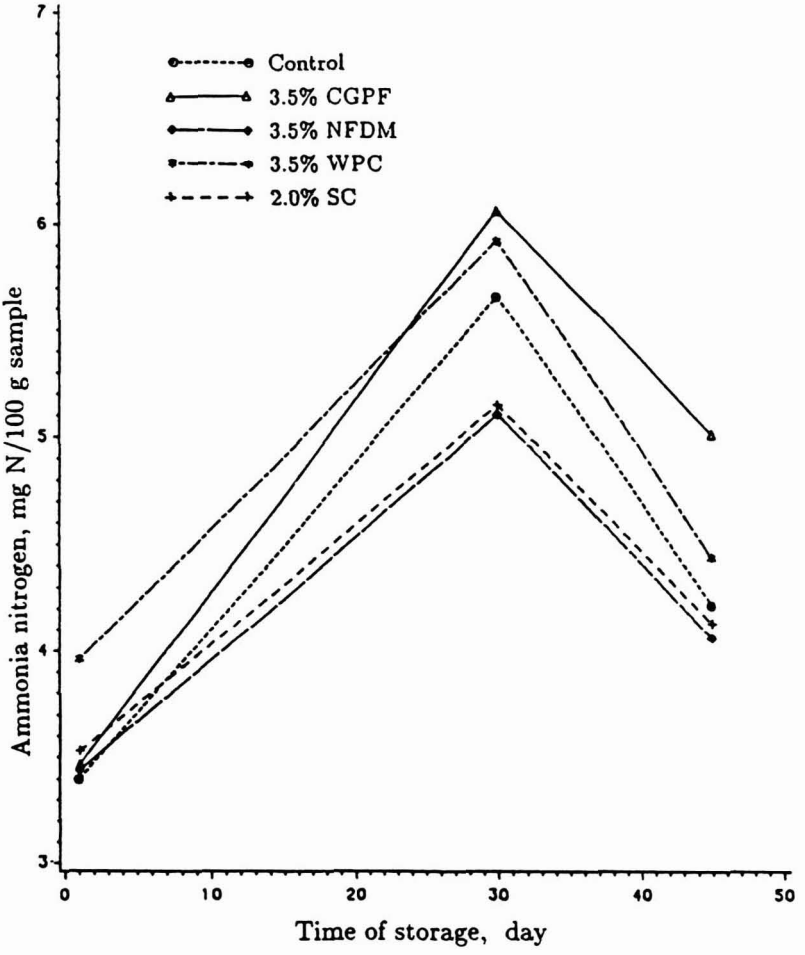

FIG. 8. AMMONIA NITROGEN CONTENT OF FRANKFURTERS CONTAINING EXTENDERS

Oxidative stability of the fat component in frankfurters is important in determining their quality. A general trend of decrease in TBA values during storage was observed (Fig. 9). The drawback of the TBA method is possible interference, because malonaldehyde, a secondary product of lipid oxidation, is very reactive with other meat constituents such as proteins, other carbonyl compounds, amino acids, and even urea (Witte *et al.* 1970; Tarladgis *et al.* 1960; Dillard and Tappel 1973). This interaction causes the formation of Schiff base fluorescent products, resulting in a decrease of TBA value (Gokalp *et al.* 1983). There was no formulation treatment effect ($P > 0.05$) on TBA values after 1, 30, and 45 days of storage.

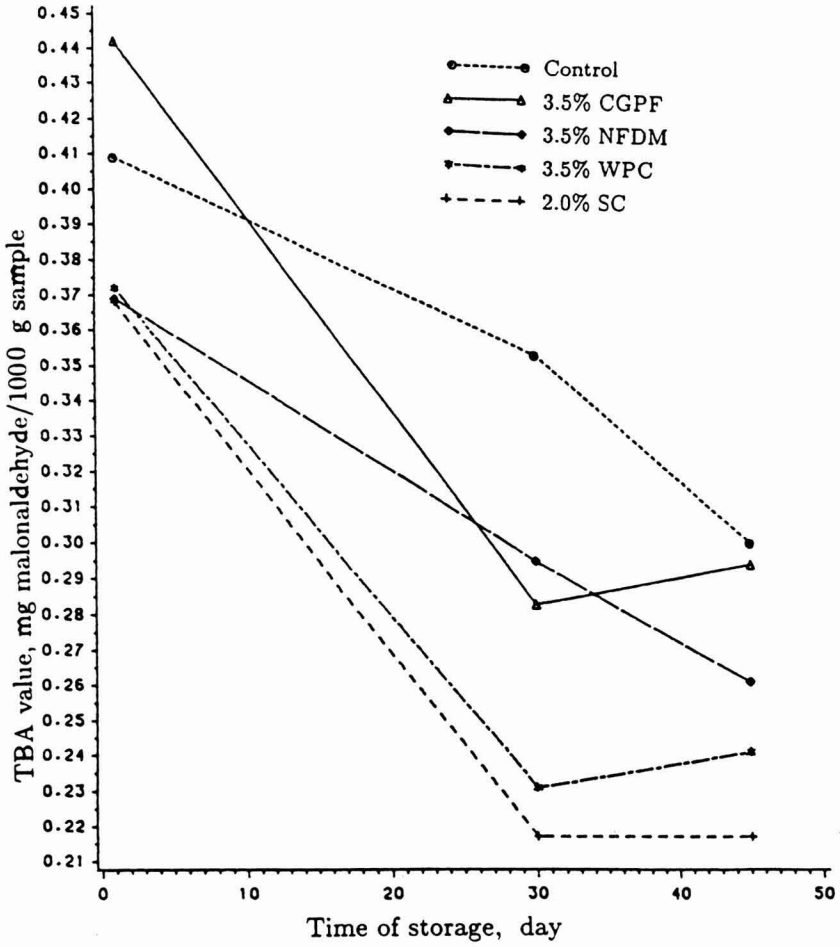

FIG. 9. TBA VALUES OF FRANKFURTERS CONTAINING EXTENDERS

CONCLUSIONS

The incorporation of milk proteins and CGPF affected shelf stability of frankfurters to some extent, as determined by sensory properties and bacteriological and chemical analyses. After 45 days of storage, frankfurters extended with CGPF had a stronger atypical aroma (up to 9.0 on a 60-point scale) than the other frankfurters, and they had a stronger atypical flavor than controls over 45 days of storage. Atypical flavor of CGPF-containing samples developed with time as compared with that of the control, especially from day 1 to day 30 of storage, increasing from 5.6 to 10.2. However, the atypical aroma and flavor were at the lower end of the scale, up to 9.0 and 10.2, respectively, and should be considered as weak and acceptable. Throughout storage, all-meat controls had a juicier mouthfeel than the frankfurters with extenders. After 45 days of storage, frankfurters extended with CGPF contained intermediate counts (log 3–4 CFU/g) of psychotrophic bacteria. The other frankfurters contained a low bacterial count (log 0–2 CFU/g). No fecal coliforms were detected for any samples over 45 days of storage. The low microbial load might have resulted from adequate heat treatment. Total volatile nitrogen increased from 1 to 30 days and then decreased slightly from 30 to 45 days. There was no formulation treatment effect on TBA values. Frankfurters extended with milk proteins and CGPF had good shelf-stability, including low to intermediate bacterial loads, low content of TVN (5.30–8.01 mg N/100 g sample) and ammonia nitrogen (3.40–6.07 mg N/100 g sample), low TBA values (0.217–0.442 mg MA/1000 g sample), and low atypical aroma and flavor (up to 10.2 on a 60-point scale). All treatment products were acceptable and apparently safe to be consumed after 45 days of storage.

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BAKING AND STORAGE STABILITY PROPERTIES OF HIGH FIBER BREADS CONTAINING COMPARABLE LEVELS OF DIFFERENT FIBER INGREDIENTS

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ABSTRACT

Textural changes and storage stability properties of high fiber yeast breads containing 13% dietary fiber (TDF, as is basis) from either white wheat bran, white wheat distillers' grains with solubles (DDGS), barley spent grains (BSG), soy fiber, or oat fiber were compared. Flour substitution levels ranged from 12% to 23%. There were approximately 100 colony forming units (CFU)/1000 g for the BSG containing breads and 10^6 to 10^7 CFU/1000 g for the other products. Mixing and baking properties for breads utilizing these ingredients varied widely. Breads containing wheat distillers' grains retained softness longer during the storage trial than did the other experimental products.

INTRODUCTION

Limited research has been conducted on the storage stability of foods containing distillers' grain materials. Aside from assessing lipid stability in distillers' grain ingredients (Bookwalter *et al.* 1984, 1988; Dawson *et al.* 1987; Rasco 1988), little else on the storage stability of this ingredient has been reported. Other recent studies with this ingredient have evaluated certain nutritional properties including protein quality (Ranhotra *et al.* 1982; Dong *et al.* 1987, 1990), vitamin content (Gazzaz *et al.* 1989), and fiber composition and profile (Dong and Rasco 1987; Sosulski and Wu 1988).

A problem arising in studies of the functional properties of high fiber formulated foods is that the results are difficult to compare. In most cases, the formulated products contain the same weight replacement (flour substitution level) of a given fiber ingredient. Formulations are not made so that the level of dietary fiber is

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constant (equal level of dietary fiber/serving). Although a comparison of products containing equal weight replacement may be of importance to a commercial baker, it is of limited interest to nutritionists or food scientists who wish to compare the product characteristics and healthful qualities of food items containing the same level of dietary fiber.

Little research has been conducted which directly compares the baking properties of high fiber formulated foods containing the same level of total dietary fiber (TDF) from various fiber ingredients, and/or the effect these fiber ingredients may have on the storage stability of formulated foods. Our primary interest in this study was to compare a distillers' grain product made from wheat with other fiber ingredients in yeast breads.

MATERIALS AND METHODS

Yeast breads containing the following fiber ingredients were prepared: distillers' dried grains with solubles from white wheat (% TDF = 56%) (Rasco *et al.* 1987a), white wheat bran (% TDF = 45%) (Fisher Mills, Harbor Island, WA), soy cotyledon fiber (% TDF = 87%) (Fibrim Soy Fiber 100, Ralston Purina Co., St. Louis, MO), oat fiber (80% TDF) (Better Basics Oat Fiber, No. 757, D. D. Williamson & Co. Inc., Modesto, CA), and barley fiber (% TDF = 45%) (brewer's spent grains, MGF 200, Coor's Food Products Co., Golden, CO). When more than one source of a fiber ingredient was commercially available, the one with the highest dietary fiber content was chosen for evaluation.

Experimental breads were prepared using a straight dough retail formulation containing the following as a weight percentage of the flour (Power Flour®, Fisher Mills Harbor Island, WA): 12–23% fiber ingredient, 1.6% salt, 7.9% granulated sugar, 1.6% active dry yeast, 3.2% unsalted butter, 3.5% homogenized 3.7% milk, and water as required for proper mixing (35–55%) based on farinograph values. This formulation was chosen because high levels of dietary fiber could be incorporated into it. The doughs were optimally mixed, then fermented for 90 min at 80F. The breads were baked at 400F for 18–20 min in a forced air convection oven. Breads used for sensory tests were held at room temperature and evaluated as fresh product within 1 day. Breads for microbiological studies were aseptically transferred to sterile plastic bags and stored at room temperature (22–25C) under fluorescent lighting.

Dietary Fiber Content

Substitution levels of each fiber ingredient corresponded to the same fiber substitution level as 20% replacement of flour with wheat DDGS. This level was chosen based on preliminary experiments and results of recent studies with distillers' grain materials (Rasco *et al.* 1987b, 1989b). The TDF content of each

ingredient and of the breads were measured using the procedure outlined in San Buenaventura *et al.* (1987). All fiber-containing breads evaluated in this study had comparable ($p > 0.05$) levels of dietary fiber, 13% TDF (w/w) as is basis, or 3.7 g dietary fiber per 1 ounce serving.

Mixing Properties of Fiber Containing Doughs

Rheological properties of doughs containing the fiber ingredients were evaluated at a 15% w/w replacement for bread flour (Power Flour®, Fisher Mills, Harbor Island, WA, a high gluten, bromated flour containing malted barley flour, potassium iodide, niacin, reduced iron, thiamin mononitrate and riboflavin) using the 50 g farinograph method (constant flour weight, variable dough weight) procedure (AACC 54-21; AACC 1983). Loaf volume was determined by rapeseed displacement tests; density was calculated by dividing the average loaf weight

TABLE 1.
LOAF VOLUME AND CRUMB GRAIN OF EXPERIMENTAL HIGH FIBER BREADS[A]

Product	Loaf Volume (cm^3)	Density[B] (g/cm^3)	Crumb Grain[C] Score
Control	264 ± 12	0.42	S
Bran, White Wheat	199 ± 6[b]	0.59	U
DDGS, White Wheat	206 ± 7[b]	0.57	S
Oat Fiber	210 ± 16[ab]	0.53	S
Barley Spent Grains	240 ± 7	0.53	Q - U
Soy Fiber	228 ± 8[a]	0.56	U

[A] For product formulations and fiber replacement levels, refer to text.

[B] Average value for three loaves from three baking trials. Calculated from loaf volume and weight data (see text).

[C] Crumb grain score was determined using a three point scale (Rubenthaler et al. (1965): S = satisfactory, Q = questionable, U = unsatisfactory.

[ab] Triplicate readings from three loaves from three baking trials. Values with the same superscript were not significantly different from each other by one way analysis of variance and Duncan's New Multiple Range test ($p < 0.05$) (Zar, 1984).

by the average loaf volume for each treatment. Crumb grain was evaluated using a three-point scale (satisfactory, questionable, unsatisfactory) (Rubenthaler *et al.* 1965).

Instrumental Color Analysis

Tristimulus colorimetric measurements were conducted as previously described (Rasco *et al.* 1987b) using a Model XL10 CDM colorimeter (Gardner Laboratory, Inc., Bethesda, MD) calibrated with a standard white tile (L = 94.3, a = −1.2, b = 2.9) and a luminescence C light source. The single readings were taken from three center slices of bread from different loaves; the mean values are reported in Table 2.

Microbiological Studies

Slices (10 g each) were aseptically removed from two loaves from separate batches of each type of bread at the sampling periods listed in Fig. 1. Samples were macerated in 100 mL of 0.1% sterile peptone broth and appropriately diluted using 0.1% sterile peptone broth. Pour plates for aerobic mesophiles and yeast/mold counts were prepared in duplicate for each of the serial dilutions using standard methods (FDA 1984). Plates were incubated at 22–25C and examined daily until visible colonies appeared (generally 48 h). Microbial data from both experiments were averaged (n = 4).

The average number of aerobic mesophiles (colony forming units per 100 gram, CFU/100 g) was also determined using standard bacteriologicala methods (FDA 1984). The number of aerobic mesophiles in the bread ingredients were as follows: 1,900 in bread flour, 300 in soy fiber, 2,300 in white wheat DDGS, 600 in white wheat bran, 20 in barley spent grains, and 1,600 in oat fiber.

RESULTS

The water absorption values for the fiber-containing doughs (15% w/w replacement), with the exception of the soy fiber containing doughs, were within 10% of that for the bread flour control. Absorbance values for the bread flour were 64%. Dough development times measured by farinograph were similar for all doughs (approximately 8 min) except for the one containing soy fiber (avg. 26 min). The stability of the barley spent grains dough (BSG) was less than that of the other doughs. Water absorbance values for the soy fiber doughs were very high, 99% absorption as determinedd by farinograph.

All experimental products had reduced loaf volumes ($p < 0.05$) relative to ...ol (Table 1) with the exception of the bread containing the BSG. The ...g BSG had the highest loaf volume in high fiber breads evaluated

TABLE 2.
COLOR PARAMETERS (L, a, b) FOR BREADS[a] CONTAINING VARIOUS FIBER INGREDIENTS

Product	Color Parameter[b] L	a	b	Hue Angle (Tan-1 b/a)	Color Difference from Control (ΔE)[c]	Color Difference from from Wheat Bran (ΔE)[c]
Control	73.37 ± 0.11	-2.97 ± 0.15	16.60 ± 0.17	79.8	na	18
White Wheat DDGS	55.4 ± 1.3	2.37 ± 0.32	17.50 ± 0.26	73.8	21	8
White Wheat Bran	63.0 ± 1.0	1.70 ± 0.10	19.10 ± 0.10	84.9	18	na
Soy Fiber	70.80 ± 0.5	-1.43 ± 0.06	15.87 ± 0.25	84.8	5	9
Oat Fiber	64.17 ± 0.91	0.10 ± 0.36	18.07 ± 0.41	86.7	12	2
Barley Spent Grains	43.10 ± 0.50	8.63 ± 0.23	15.57 ± 0.06	61.0	34	21

na = not applicable

[a] See text for description of fiber ingredients and bread formulations. Single readings from bread slices from three baking trials (n=3) were used to calculate the $\bar{X} \pm$ S.D.

[b] Measured using a Gardner Laboratory Inc. Colorimeter, Model XL10 CDM, a standard white color tile (L = 94.3, a = -1.2, b = 2.9) and a standard light source, luminescence C. L (100 = white, 0 = black), a (+ = red, − = green), b (+ = yellow, − = blue)

[c] Color difference (Δ E) calculated according to method of Clydesdale (1976). Hue angle (tan $^{-1}$ b/a) calculated from mean colorimeter values.

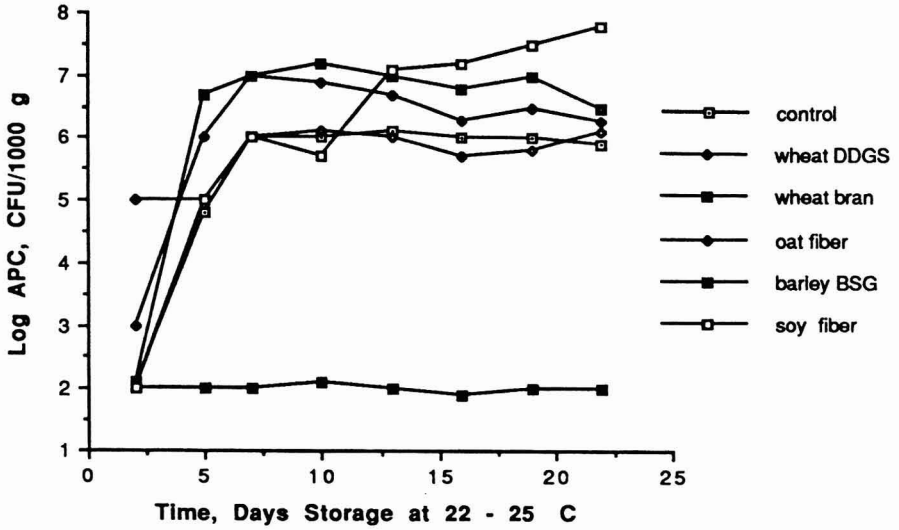

FIG. 1. AEROBIC PLATE COUNT (APC) OF HIGH FIBER BREADS HELD UNDER ASEPTIC CONDITIONS AT 22-25C

Breads were held under aseptic conditions for up to 22 days. Colony forming units (CFU/1000 g) are averages for duplicate samples from each of two experiments (n = 4).

in this study (91% of the control). The crumb grain scores were "satisfactory" (S) for breads containing wheat DDGC and oat fiber, "questionable" (Q) for the BSG bread, and "unsatisfactory" (U) for the soy and wheat bran breads.

The high fiber breads were darker and more red than the control (Table 2). The high fiber breads containing the barley BSG were darker than the other high fiber breads tested here. Relative to wheat bran, the color difference values (ΔE) of wheat DDGS, soy, and oat fiber containing breads were less than 10.

The wheat bran and wheat DDGS breads had visible flecks of bran present. Neither the DDGS nor the wheat bran were ground prior to incorporation into the bread doughs. The presence of the bran particles was less noticeable in the breads containing the DDGS than in the bread containing the wheat bran. The enzyme liquefaction treatment and postfermentative drying most likely softened the bran particles (Rasco *et al.* 1989a, 1990), resulting in a better crumb grain for the DDGS breads compared to the breads containing the wheat bran at the same fiber replacement levels (Table 1).

The effect of storage time on the number of aerobic mesophiles in high fiber breads is shown in Fig. 1. The predominent bacterial genera present were Gram positive rods which were microscopically confirmed as *Bacillus*. The initial number of aerobic mesophiles in the control and all fiber containing breads with the exception of the oat fiber bread ranged from 10^2 to 10^3 CFU/1000 g. After approximately 10 days storage, the number of aerobic mesophiles rose to approximately

10^6 CFU/1000 g in the control and then stabilized at this level during the rest of the storage study. The breads containing the BSG had a low microbial load initially (10^2 CFU/1000 g) and throughout 22 days of storage at 22–25C. The initial number of aerobic mesophiles in the breads containing the wheat DDGS or wheat bran were low (10^2–10^3 CFU/1000 g), increased rapidly by day 10 as seen for the control, and stabilized at 10^6–10^7 CFU/1000 g for the remaining 12 days of storage. The number of aerobic mesophiles in the oat fiber breads were 10^5 CFU/1000 g initially; after 22 days, these breads had levels of 10^6 CFU/1000 g. The greatest increase in the number of aerobic mesophiles was for the soy fiber bread increasing from 10^2 to 10^8 CFU/1000 g over the 22 day storage period.

Yeast and mold counts were low throughout the storage period for breads stored aseptically after baking. However, in the oat fiber containing breads, levels of up to 100 CFU/g were recovered from the breads by the end of two weeks. This may have been due either to a higher content of mold spores in this particular fiber ingredient or to a higher water activity in the baked product which encouraged more rapid mold growth. The contribution of yeasts and molds to the overall microbial population of the high fiber breads was low, except for those containing oat fiber breads. Visible mold colonies appeared on most of the aseptically stored breads by the end of the 22 day storage period.

DISCUSSION

The product quality of high fiber breads containing the same level of dietary fiber per serving was highly variable. In general, the performance of the wheat DDGS [20% flour replacement level, (w/w)] was comparable to or better than that of either wheat bran [24% flour replacement level (w/w)] or oat fiber [12% flour replacement level (w/w)]. In addition to the somewhat reduced substitution level relative to wheat bran, the higher level of ash, and soluble or suspended protein in the DDGS may have contributed to the relatively better baking properties of the DDGS breads relative to those containing wheat bran in the straight dough formulation used in this study. DDGS may have had less of a detrimental effect on the formation of a gluten network relative to breads containing the other fiber ingredients evaluated here. An improved gluten network may have been due to enhanced electrostatic interactions or through improved protein hydration (Rasco *et al.* 1990). The somewhat higher soluble protein content of wheat DDGS compared to wheat bran, increased levels of solubilized and possibly partially hydrolyzed wheat proteins, as well as higher concentrations of relatively low molecular weight polysaccharides, may have improved elasticity of the walls of the air cells in the dough during fermentation, leading to the formation of smaller, more uniform cells, and ultimately to a more desirable crumb grain in the finished

product. There are some indications that soluble macromolecular components in bread doughs can contribute to a soft grain and limitation of staling (Hoseney 1986). The high temperature liquefaction process and postfermentative drying treatment used in the manufacture of DDGS may have softened the fibrous components in DDGS, allowing for a faster hydration of DDGS than wheat bran during dough formation. The resultant softened texture of the bran component of the DDGS ingredient may have been a factor in the improved crumb grain score relative to the wheat bran bread. The bran in the DDGS may have been more pliable and would have had less of a tendency to puncture developing air cells.

There was a tendency for breads containing wheat bran, wheat DDGS, or oat fiber to retain a greater degree of softness during room temperature storage when compared to the control. The effect of added fiber on enhanced product softness was not observed for the breads containing the BSG. This evaluation was based on preliminary experiments using ranking test data from an experienced sensory panel (data not given). The observed differences in the softness between the fiber containing breads may have been due to the relatively large quantity of BSG [24% flour replacement level (w/w)] required to attain 3.7 g dietary fiber per 1 ounce serving.

Although the microbial load of freshly baked bread that has been properly handled is low, the product is not sterile. Mold spores (*Rhizopus sp., Penicillium sp., Neurospora sp., Aspergillus sp.*) and bacterial spores (in particular, *Bacillus sp*) survive baking. These spores can recover, germinate, and grow even in breads that have been stored aseptically because the water activity of bread is high (0.94 to 0.97) (Banwart 1979). Organisms detected in the high fiber breads were predominantly *Bacillus sp.*, which would have been introduced through the flour and possibly the fiber ingredient(s). Wheat DDGS had higher counts of aerobic mesophiles which were principally the yeast (added prior to fermentation) which had survived drying. Mold spores were more prevalent in the oat fiber (approx. 16 CFU/g) than in the other fiber ingredients tested here, and because of this, the oat fiber bread molded more quickly than the other aseptically held high fiber breads. We are uncertain as to whether different processing conditions for a dry-milled oat fiber ingredient, independent of chemical treatment, would have reduced the levels of mold spores for this ingredient. The bread containing the barley BSG had the lowest aerobic mesophile count and was also the least susceptible to microbial growth during storage. The microbial load of the BSG itself was low; BSG is recovered from beer manufacture and has undergone high temperature treatments during brewing and drying.

Mixing, baking and storage properties of breads containing high levels of fiber ingredients from wheat (bran and DDGS), barley (BSG), oat fiber and soy fiber (13% TDF as is basis) were examined. The wheat bran, wheat DDGS and oat fiber containing breads may retain softness better during storage at room temperature than the other high fiber breads. The BSG breads had the lowest number of aerobic mesophiles following aseptic storage at 22–25C for 22 days.

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CONTROL OF ENZYMATIC BROWNING IN RAW FRUIT JUICE BY FILTRATION AND CENTRIFUGATION[1]

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ABSTRACT

Filtration and centrifugation were investigated as means of preventing enzymatic browning in minimally processed fruit juices. The capacity of raw apple, grape and pear juices to undergo browning was associated with particulate fractions that could be removed by filtration with Bentonite and/or diatomaceous earth, microfiltration or ultrafiltration, depending on the commodity and cultivar. Centrifugation prevented browning in pear juice and in Granny Smith, Golden Delicious, and Red Delicious but not McIntosh apple juice, provided that foam was excluded and the relative centrifugal force was sufficient. A method for the preparation of nonbrowning, cloudy juices by recombination of juice supernatant or ultrafiltration permeate with particulates, heat-treated to inactivate polyphenol oxidase, was investigated.

INTRODUCTION

Conventionally, fruit and vegetable juices are preserved by pasteurization, which inactivates degradative enzymes and kills spoilage microorganisms but also may impart cooked flavors to heat-sensitive products. The food industry has given increasing attention in recent years to meeting consumer demands for "freshness" in processed foods by pursuing the development of minimally processed products (Albrecht 1986; Shewfelt 1987). Such characteristics might be achieved in raw fruit juices, cold-sterilized by membrane filtration, or preserved by refrigeration, if enzymatic processes associated with quality loss during storage such as

[1]Mention of brand or firm names does not constitute an endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

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enzymatic browning could be prevented (Borgstrom 1954; Moyer and Aitken 1980). Previously, we have investigated the use of various ascorbic acid (AA) derivatives, polyphenol oxidase (PPO) inhibitors, chelating agents, and cyclodextrins in delaying but not eliminating browning in juices during short-term storage (Sapers *et al.* 1989a,b,c).

An alternative approach to this problem is suggested by reports that, in many fruits, PPO activity is largely associated with particulate fractions (Mayer and Harel 1979; Vamos-Vigyazo 1981). The separation of such particulates from raw juices might be a sufficient step to eliminate or greatly reduce enzymatic browning during prolonged storage. Furthermore, with a molecular weight in excess of 30,000 daltons (although considerably smaller molecular masses have been reported in a few cases) (Vamos-Vigyazo 1981), even soluble forms of PPO might be separated from raw juices by ultrafiltration. Therefore, the objective of this study was to determine whether the capacity of raw fruit juices to undergo enzymatic browning during storage could be removed by centrifugation or filtration. Such treatments might form the basis of novel minimal processes for raw juices.

METHODS AND MATERIALS

Juice Preparation

To test the hypothesis that browning in raw juices could be prevented by removal of particulates, a model system was devised comprising raw juices, treated to remove particulate fractions and then stored under conditions favoring rapid oxidation. Juices were prepared from ripe apples, pears and grapes obtained at local food stores. Red and Golden Delicious apples were provided by ARS's Appalachian Fruit Research Station in Kearneysville, WV. The apples were cut into wedges with a kitchen appliance, dipped in 1% AA for ca. 30 s to prevent browning during juicing (omitted with Granny Smith apples because of their lesser tendency to brown), and drained. The wedges were juiced in an Acme Supreme Juicerator (Acme Juicer Mfg. Co., Lemoyne, PA), lined with a 6 × 56.5 cm strip of Whatman No. 1 filter paper. An additional 100 ppm AA was mixed into the freshly prepared juice to prevent browning during subsequent filtration and centrifugation steps (i.e., for several hours). Pear juice was prepared by the same procedure except that the fruit was sliced into cross cuts before dipping in 1% AA, draining and juicing. Grapes were cut in half before juicing with the Juicerator. Portions of these juices were treated by various filtration and centrifugation procedures; untreated portions containing AA, added during cutting and juicing, were used as controls.

Freshly prepared raw juices were analyzed for AA by the HPLC procedure of Liao and Seib (1990) to determine treatment residues. The juices were diluted

1:100 with an extraction solution containing 0.38% metaphosphoric acid and 70% mobile phase (with acetate omitted).

Filtration of Juices

In experiments to determine the effects of filtration on browning, the freshly prepared raw juices, with or without the addition of 0.1–2.0% Celite Analytical Filter Aid (CAFA; Manville Products Corp., Denver, CO), Bentonite (Sigma Chemical Co.), Celite 545 (Manville Products Corp.), talc (U.S.P., J. T. Baker Chemical Co., Phillipsburg, NJ), and silica gel (TLC, Mallinckrodt Chemical Works, St. Louis, MO), were filtered through Whatman No. 541 paper under suction. In separate experiments carried out with Granny Smith and Red Delicious juice, two other silica-derived adsorbents (BRITESORB® L900 and DP4250, The PQ Corp., Valley Forge, PA) were added at levels of 200–10,000 ppm. The suspensions were stirred for 10 min and then filtered through Whatman No. 541 paper under suction. In one trial, 0.4% Tween 80 (polyoxyethylene sorbitan monooleate, Sigma Chemical Co.) was dispersed in Golden Delicious juice prior to CAFA addition and filtration. A 5% slurry of Bentonite in water, dispersed with a Polytron homogenizer (Brinkmann Instruments Co., Inc., Westbury, NY), was added to some juice samples at levels corresponding to 0.1–0.5% Bentonite. The juice was then stirred for 5 min, mixed with 2% CAFA, and filtered, as described above. Juices that had been clarified by CAFA addition and filtration through Whatman No. 541 paper or by centrifugation (see below) were subjected to microfiltration through a Falcon 0.22 μm cellulose ester membrane or to ultrafiltration with a Minitan tangential flow system (Millipore Corp., Bedford, MA) through polysulfone membranes having molecular weight cut-off (MWCO) values of 30,000, 100,000 and 300,000 daltons.

Centrifugation

To determine the effects of centrifugation on the capacity of apple and pear juices to brown, 25 mL portions of raw juice were centrifuged in 30 mL polycarbonate Oak Ridge style centrifuge tubes at speeds of 1000–28,000 rpm (127–100,095 × g) for 10 min in a F-28/36 fixed-angle rotor of a Sorvall RC 28S Supraspeed Centrifuge (Du Pont Company, Wilmington, DE); all centrifugation was done at 4C. Since the presence of foam in freshly prepared juice was believed to interfere with centrifugation, some trials were carried out with juice added to centrifuge tubes by pipet rather than by graduated cylinder so that foam could be excluded.

Heat Inactivation of PPO in Juice Pellets

Juice particulates were isolated as pellets by centrifuging 25 mL portions of freshly prepared juice for 10 min at 28,000 rpm and decanting the juice super-

natants. The pellets were drained, rinsed with water, suspended in 5 mL water added to the centrifuge tubes, and heated for 5 min in a boiling water bath, at which time the suspension temperature was 87C. The heated pellet suspension was cooled and centrifuged for 10 min at 28,000 rpm. The new pellets were drained and dispersed in juice supernatant or ultrafiltration permeate, obtained by passing the juice supernatant through a 30,000 dalton MWCO membrane, with a serrated Teflon pestle tissue grinder (Thomas Scientific, Swedesboro, NJ) and evaluated for browning, as described below.

Evaluation of Capacity to Brown

Portions of treated juices and untreated controls (25 mL in 50 mL beakers containing 25 mm magnetic stirrer bars, covered with Parafilm M to prevent evaporation), were stirred at 300 rpm for as long as 14 h at room temperature (ca. 20C) on a multipoint stirrer (Cole-Palmer Instrument Co., Chicago, IL) to accelerate enzymatic browning if it were to occur. Samples were observed at frequent intervals for the onset of browning. The presence or absence of browning could be determined unambiguously by visual observation. No attempt was made to compare treatments on the basis of the extent of browning or time of onset since these were subject to sample-to-sample variability. Rather, the success of a treatment in preventing browning was judged on a "go, no-go" basis by the absence of browning in treated samples after at least 6 h of storage at 20C with stirring, usually followed by one or more days at 4C without stirring, when controls turned brown within 1 or 2 h at 20C. In some experiments, samples were stored at 4C without stirring, and observations were made daily to determine the presence or absence of browning.

RESULTS AND DISCUSSION

Residual AA in Raw Juices

Raw apple (Granny Smith, Golden Delicious, Red Delicious) and pear (Anjou, Bartlett) juices, prepared from wedges or slices dipped in 1% AA, were found by HPLC analysis to contain about 500 ppm AA (range of 436–695 ppm), irrespective of commodity or cultivar. When 2% AA was used as the dip, the juices contained about 1100 ppm AA (range of 847–1521 ppm). These levels were sufficient to delay browning in the raw juices until after the completion of filtration and centrifugation treatments.

Effects of Filtration on Browning

Preliminary observations of raw Granny Smith and Golden Delicious apple juices, clarified by the addition of CAFA (a diatomaceous earth) and filtration,

indicated that such juices did not undergo enzymatic browning during subsequent storage. To confirm and extend this observation, we investigated the ability of filtration with other filter aids and related products to eliminate the capacity of apple juice to brown (Table 1, Expt. A). CAFA proved to be the only material tested that appeared to prevent browning; i.e., juice filtered with CAFA did not brown after 3 h at 20C with stirring, followed by 36 h at 4C, when controls browned within 1 h at 20C. The BRITESORB® adsorbents, which were developed for the absorption of proteins from fruit juices, were ineffective in preventing browning in Granny Smith and Red Delicious juice.

Further experiments indicated that a level of CAFA addition of 2% was required for consistent prevention of browning (Table 1, Expt. B). Unfiltered juices

TABLE 1.
EFFECT OF FILTRATION ON ENZYMATIC BROWNING IN GOLDEN DELICIOUS APPLE JUICE[1]

Expt	Treatment[2]	Onset of browning (minutes at 20 C)
A	Unfiltered control	50
	Filtered	75
	Filtered with 1% CAFA	>180[3]
	Filtered with 1% Celite 545	75
	Filtered with 1% talc	120
	Filtered with 1% silica gel (TLC)	120
B	Unfiltered control	10
	Filtered	40
	1% CAFA	50
	Filtered with 1% CAFA	120
	2% CAFA	40
	Filtered with 2% CAFA	>180[3]
C	Filtered	5
	Filtered with 2% CAFA	>180[3]
	Filtered with 0.4% Tween 80 + 2% CAFA	5

[1] Filtered through Whatman No. 541 paper with suction.

[2] CAFA = Celite Analytical Filter Aid.

[3] No browning after additional 36 h at 4 C.

containing CAFA browned readily, demonstrating that the absence of browning in juices filtered with CAFA resulted from the removal of PPO and/or PPO substrates and not from the inhibition of PPO by the filter aid. The addition of Tween 80, a detergent known to solubilize bound PPO (Takeo 1965), prior to CAFA addition and filtration yielded a filtrate that underwent rapid browning (Table 1, Expt. C). These results suggest that the effectiveness of the CAFA-filtration treatment in preventing browning depends on the separation of particulate-bound PPO from the juice. Apparently, the other silica products did not interact with particulates to which PPO is bound, facilitating their separation.

Studies with other juices demonstrated that the CAFA-filtration effect is not generally applicable but depends on the commodity, and even the cultivar (Table 2). The treatment was effective with Granny Smith (no browning after 22 h at 20C) and Red Delicious juice (no browning after 7½ h at 20C followed by 16 h at 4C) but not McIntosh juice. Browning in pear juice could be greatly delayed but not usually prevented by the CAFA-filtration treatment. Presumably, the size

TABLE 2.
VARIATION IN THE RESPONSE OF FRUIT JUICES TO FILTRATION[1]

		Onset of browning (minutes at 20 C)	
Fruit	Variety	Unfiltered control	Filtration with[2] 2% CAFA
Apple	Granny Smith	30	>1320
	Red Delicious	1	>450[3]
	McIntosh	150	150
Pear	Anjou (sample 1)	30	180
	Anjou (sample 2)	90	>360[3]
	Bartlett	30	240
	Bosc	45	150
Grape	Thompson Seedless	5	>180[3]

[1] Filtered through Whatman No. 541 paper with suction.

[2] CAFA = Celite Analytical Filter Aid.

[3] No browning after additional 16 h at 4 C for juices of Red Delicious apple and Thompson seedless grape, and after 96 h at 4 C for Anjou pear juice (sample 2).

distribution or filtration characteristics of particulates to which PPO is bound will vary with commodity and cultivar.

Preliminary experiments indicated that browning in raw Golden Delicious apple juice could be prevented by addition of 0.5% Bentonite, followed by filtration through Whatman No. 541 paper; the speed of filtration could be improved by combining Bentonite with CAFA. Further studies demonstrated that McIntosh apple, Anjou pear and Bosc pear juices, which did not respond to filtration with CAFA alone, would not brown if pretreated with Bentonite before CAFA addition (Table 3). The amount of Bentonite required to prevent browning varied with each juice but never exceeded 0.5%. Under the conditions of this experiment, filtration was rapid,, and the filtered juices were only very slightly turbid. Bentonite is used as fining agent to remove haze-forming proteins from juices (Van Buren 1989). Our results indicate that Bentonite acts by coagulating particulates rather than by inactivating PPO.

Juices that did not respond to the CAFA-filtration treatment were subjected to microfiltration and ultrafiltration to determine the minimal filtration treatment required to prevent browning (Table 4). Filtration through a 0.22 μm membrane prevented browning in McIntosh apple juice but not in Bosc pear juice. Ultrafiltration through a 300,000 dalton MWCO membrane was effective with the latter juice. It is apparent that the particles removed by these treatments are many times larger than the molecular masses reported for PPO in apple and other fruits (Mayer

TABLE 3.
EFFECT OF BENTONITE ON BROWNING IN FILTERED APPLE AND PEAR JUICES

	Onset of browning (minutes at 20 C)					
	Juice					
	McIntosh apple		Anjou pear		Bosc pear	
Treatment[1]	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Unfiltered control	120	90	30	60	45	60
Filtered	120	90	150	90	45	120
Filtered, 0.1% Bentonite	180	120	540	>840	90	540
Filtered, 0.2% Bentonite	>840	120	>720	>840	240	>840
Filtered, 0.5% Bentonite	>840	>840	>720	>840	>780	>840

[1] 2% Celite Analytical Filter Aid added prior to filtration through Whatman No. 541 paper with suction.

TABLE 4.
EFFECTS OF MICROFILTRATION AND ULTRAFILTRATION ON BROWNING IN APPLE AND PEAR JUICE

Treatment	Onset of browning (minutes at 20 C) McIntosh apple	Bosc pear
Unfiltered control	150	45
Filtered with 2% CAFA	150	150
Filtered through 0.22 μm membrane[1]	>360	180
Filtered through 300,000 MWCO dalton membrane[1]	>360	>360[2]
Filtered through 30,000 MWCO dalton membrane[1]	>360	>360[2]

[1] Prefiltered through Whatman No. 541 paper with 2% CAFA before membrane filtration.

[2] No browning after additional 96 h at 4 C.

and Harel 1979; Vamos-Vigyazo 1981). Therefore, virtually all of the PPO in these juices must be bound to particulates.

While it has long been recognized that PPO in fruits is largely insoluble (Vamos-Vigyazo 1981) and that PPO is absent in clarified apple and pear juices (Biedermann 1956), there appears to have been no effort by juice processors to exploit these facts. Processes to obtain light-colored, cloudy apple juice by spraying the fruit with ascorbic acid solution prior to milling and then extracting, straining, deaerating, and pasteurizing the juice were developed in the 1940's (Pederson 1947; Atkinson and Strachan 1949). These processes depend on rapid handling of the crushed apples and juice to avoid product darkening. Various clarification and filtration or centrifugation procedures have been employed by processors to obtain clear juices (Smock and Neubert 1950; Moyer and Aitken 1980; Bump 1989; Kilara and Van Buren 1989). Although the ability of such procedures to remove preformed pigments resulting from enzymatic browning is recognized (Kilara and Van Buren 1989), their ability to prevent browning apparently has been overlooked.

The cold sterilization of fruit juices by filtration was first investigated and used in Europe more than 50 years ago (Charley 1939; Smock and Neubert 1950; Borgstrom 1954). Juices prepared by this process retain the characteristic flavor of the fresh fruit and are free of cooked taste but may be unstable due to the presence of active enzymes (Borgstrom 1954). More recently, ultrafiltration has been used to clarify fruit juices (Kirk *et al.* 1983; Swientek 1986; Thomas *et al.* 1986; Bump 1989). The potential use of such systems for cold sterilization

has been recognized (Barefoot *et al.* 1989). The influence of ultrafiltration on juice quality has been investigated (Drake and Nelson 1987; Padilla and McLellan 1989); however, because these studies employed heat-pasteurized products, no conclusions could be drawn about the exclusion of PPO or other enzymes by the ultrafiltration system. Heatherbell *et al.* (1977) observed haze and sediment formation during storage of unpasteurized juices that had been filtered through a 50,000 dalton MWCO membrane. They did not establish whether small amounts of enzymes or enzyme degradation products passing through the membrane were responsible. Kim *et al.* (1989) reported high recovery of added pectinase in apple juice retentates under high-temperature, short-time ultrafiltration conditions. Köseoğlu *et al.* (1990, 1991) employed ultrafiltration through 50,000 and 100,000 dalton MWCO membranes to remove degradative enzymes from raw citrus and vegetable juices which were subsequently cold sterilized and combined with heat-pasteurized pulp. They demonstrated the absence of pectinesterase activity in ultrafiltration permeates. Since the occurrence of enzymatic browning in fruit juice ultrafiltration permeates would have been obvious in the many ultrafiltration studies reported in the literature, it is highly unlikely that these permeates contained active PPO. Our data suggest that filtration through such highly retentive membranes might not be required to remove PPO from raw juice.

Effects of Centrifugation on Browning

Preliminary studies suggested that the capacity of Granny Smith apple juice to undergo browning could be eliminated by centrifuging at speeds of 8000 rpm (8200 × g) or higher. Subsequently, we recognized the importance of excluding foam produced during juicing from the centrifuge tubes (Table 5). Apparently, foam disrupted by centrifugation generates a low density particulate fraction containing bound PPO that remains in the supernatant. We also observed that the capacity of McIntosh apple juice to brown was not eliminated by centrifugation. The success of microfiltration with this juice (Table 4) indicates that the failure of centrifugation to prevent browning is due to the presence of PPO bound to low density particulates rather than to soluble PPO. Since the same situation may occur with other apple cultivars or in other fruit juices not studied herein, any attempt to use centrifugation to control browning in juices should be evaluated for each of the individual cultivars that may be processed. The centrifugation speed required to sediment particulates to which PPO is bound also varied with cultivar (Table 6). When care was taken to exclude foam, browning in Red Delicious juice could be controlled by centrifuging at 4000 rpm (2043 × g) while Golden Delicious juice required 6000 rpm (4596 × g).

Centrifugation in an atmosphere of inert gas to prevent aeration has been used

TABLE 5.
EFFECTS OF FOAM ON CONTROL OF BROWNING IN APPLE AND PEAR JUICE BY CENTRIFUGATION

Juice	Treatment[1]	Onset of browning in supernatant (minutes at 20°C)
Bosc pear	Control	30
	Centrifuged (foam included)	150
	Centrifuged (foam excluded)	>420
Red Delicious apple	Control	90
	Centrifuged (foam included)	240
	Centrifuged (foam excluded)	>600
McIntosh apple	Control	90
	Centrifuged (foam included)	240
	Centrifuged (foam excluded)	240

[1] Centrifuged 10 min at 28,000 rpm at 4 C.

to partially clarify apple juice (Moyer and Aitken 1980). According to Smock and Neubert (1950), centrifugation at 9000 × g will produce a cloudy juice that will remain free from sediment during storage. They suggested that high speed centrifuges be used before filtration to reduce the load on filters. There is no indication in the literature that the effect of centrifugation on browning described herein was recognized previously, probably since centrifuged juices were subsequently pasteurized.

TABLE 6.
EFFECT OF CENTRIFUGATION SPEED ON BROWNING IN APPLE JUICE

		Onset of browning (minutes at 20 C)	
Speed (rpm)[1]	Max. RCF[2] (x g)	Red Delicious	Golden Delicious
None	0	45-60	60-90
2000	511	90	90
4000	2043	>360	150
6000	4596	>420[3]	>360[3]
28,000	100,095	>420[3]	>420[3]

[1] Juices centrifuged for 10 min at 4 C.

[2] Relative Centrifugal Force.

[3] No browning after additional 72-96 hrs at 4 C.

Preparation of Nonbrowning Cloudy Juices

Particulate fractions in some fruit juices may make important contributions to product appearance, body and flavor. Therefore, the removal of such fractions by filtration or centrifugation to preclude browning would not be feasible. In this study, we have attempted to prepare a cloudy, "cold-blanched" fruit juice product by centrifuging the raw juice, filtering the supernatant through a 30,000 dalton MWCO membrane, heating the pellet to inactivate particulate-bound PPO, and recombining the supernatant or permeate and heated particulates. A similar approach was employed by Köseoğlu *et al.* (1990, 1991) for citrus and vegetable juices. According to Walker (1964), apple PPO is completely destroyed at 80C. We found that heating McIntosh and Winesap juice pellets, suspended in a small volume of water, to 87C completely eliminated their capacity to cause browning in reconstituted juice (Table 7). Had the heated particulates been recombined with supernatant instead of permeate, the McIntosh product but not the Winesap would have browned during storage. Earlier experiments with Red and Golden Delicious juices indicated that cloudy products prepared with supernatants and heated particulates would not brown (data not shown). Heat treatment did not adversely affect the dispersibility of the pellets in juice ultrafiltrate. Heat treatments to inactivate PPO in isolated juice particulates might be compatible with the goal of freshness, provided that the natural turbidity, body and flavor of the juice are not compromised. A major advantage of such treatments would be the collateral destruction of microorganisms in the juice particulates. The reconstitution of juice from heat-treated particulates and cold-sterilized supernatant or permeate, under aseptic conditions, could yield a sterile, shelf-stable product with the quality attributes of a fresh juice.

TABLE 7.
BROWNING IN APPLE JUICE RECONSTITUTED FROM ULTRAFILTRATE AND HEATED PELLET

	Onset of browning (days at 4 C)	
Treatment	McIntosh	Winesap
Control	1	4
Supernatant[1]	2	>6
Permeate[2]	>7	>6
Permeate and heated pellet[3]	>7	>6

[1] Centrifuged 10 min at 28,000 rpm.

[2] Through 30,000 dalton MWCO membrane.

[3] Pellet heated 5 min in boiling water bath.

Research is continuing on the extension of these approaches to other commodities and to the control of other enzymatic reactions that limit the storage life of fresh juice products.

CONCLUSIONS

The capacity of apple and pear juices to undergo enzymatic browning resides in particulate fractions that can be separated from the juices by filtration. The response of the juice to specific filtration procedures depends on the commodity and cultivar; browning in all juices tested would be eliminated by ultrafiltration through a 300,000 dalton MWCO membrane.

The capacity of McIntosh apple juice to brown was not removed by centrifugation at 28,000 rpm (100,085 × g) for 10 min. Centrifugation at lower speeds was sufficient to prevent browning in other apple juices tested, provided that foam was excluded from the sample before centrifuging.

A nonbrowning, cloudy apple juice can be prepared by recombining heat-treated juice particulates with juice supernatant or ultrafiltration permeate.

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