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JOURNAL OF FOOD PROCESSING AND PRESERVATION

D.B. LUND
EDITOR

FOOD & NUTRITION PRESS, INC.

OLUME 18, NUMBER 3

AUGUST 1994

JOURNAL OF FOOD PROCESSING AND PRESERVATION

All articles for publication and inquiries regarding publication should be sent to Dr. D.B. Lund, Rutgers, The State University, 104 Martin Hall, P.O. Box 231, New Brunswick, New Jersey 08903 USA. There are no page charges for publication in the *Journal of Food Processing and Preservation.*

All subscriptions and inquiries regarding subscriptions should be sent to Food & Nutrition Press, Inc., P.O. Box 374, Trumbull, CT 06611 USA.

One volume of six issues will be published annually. The price for Volume 18 is $143.00, which includes postage to U.S., Canada, and Mexico. Subscriptions to other countries are $163.00 per year via surface mail, and $172.00 per year via airmail.

Subscriptions for individuals for their own personal use are $113.00 for Volume 18, which includes postage to U.S., Canada, and Mexico. Personal subscriptions to other countries are $133.00 per year via surface mail, and $142.00 per year via airmail. Subscriptions for individuals should be sent directly to the publisher and marked for personal use.

The *Journal of Food Processing and Preservation* is listed in *Current Contents/Agriculture, Biology & Environmental Sciences (CC/AB).*

The *Journal of Food Processing and Preservation* (ISSN:0145-8892) is published bimonthly by Food & Nutrition Press, Inc.—Office of Publication is 2 Corporate Drive, Trumbull, Connecticut 06611 USA.

Second class postage paid at Bridgeport, CT 06602.

POSTMASTER: Send address changes to Food & Nutrition Press, Inc., P.O. Box 374, Trumbull, Connecticut 06611 USA.

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VOLUME 18
NUMBER 3

Editor: D.B. LUND

FOOD & NUTRITION PRESS, INC.
TRUMBULL, CONNECTICUT 06611 USA

ISSN 0145-8892

Printed in the United States of America

CONTENTS

FNP PUBLICATIONS IN FOOD SCIENCE AND NUTRITION

Journals

JOURNAL OF FOOD LIPIDS, F. Shahidi
JOURNAL OF RAPID METHODS AND AUTOMATION IN MICROBIOLOGY, D.Y.C. Fung and M.C. Goldschmidt
JOURNAL OF MUSCLE FOODS, N.G. Marriott and G.J. Flick, Jr.
JOURNAL OF SENSORY STUDIES, M.C. Gacula, Jr.
JOURNAL OF FOODSERVICE SYSTEMS, C.A. Sawyer
JOURNAL OF FOOD BIOCHEMISTRY, J.R. Whitaker, N.F. Haard and H. Swaisgood
JOURNAL OF FOOD PROCESS ENGINEERING, D.R. Heldman and R.P. Singh
JOURNAL OF FOOD PROCESSING AND PRESERVATION, D.B. Lund
JOURNAL OF FOOD QUALITY, J.J. Powers
JOURNAL OF FOOD SAFETY, T.J. Montville
JOURNAL OF TEXTURE STUDIES, M.C. Bourne

Books

MEAT PRESERVATION: PREVENTING LOSSES AND ASSURING SAFETY, R.G. Cassens
S.C. PRESCOTT, M.I.T. DEAN AND PIONEER FOOD TECHNOLOGIST, S.A. Goldblith
FOOD CONCEPTS AND PRODUCTS: JUST-IN-TIME DEVELOPMENT, H.R. Moskowitz
MICROWAVE FOODS: NEW PRODUCT DEVELOPMENT, R.V. Decareau
DESIGN AND ANALYSIS OF SENSORY OPTIMIZATION, M.C. Gacula, Jr.
NUTRIENT ADDITIONS TO FOOD, J.C. Bauernfeind and P.A. Lachance
NITRITE-CURED MEAT, R.G. Cassens
POTENTIAL FOR NUTRITIONAL MODULATION OF AGING, D.K. Ingram *et al.*
CONTROLLED/MODIFIED ATMOSPHERE/VACUUM PACKAGING OF FOODS, A.L. Brody
NUTRITIONAL STATUS ASSESSMENT OF THE INDIVIDUAL, G.E. Livingston
QUALITY ASSURANCE OF FOODS, J.E. Stauffer
THE SCIENCE OF MEAT AND MEAT PRODUCTS, 3RD ED., J.F. Price and B.S. Schweigert
HANDBOOK OF FOOD COLORANT PATENTS, F.J. Francis
ROLE OF CHEMISTRY IN THE QUALITY OF PROCESSED FOODS, O.R. Fennema, W.H. Chang and C.Y. Lii
NEW DIRECTIONS FOR PRODUCT TESTING OF FOODS, H.R. Moskowitz
PRODUCT TESTING AND SENSORY EVALUATION OF FOODS, H.R. Moskowitz
ENVIRONMENTAL ASPECTS OF CANCER: ROLE OF MACRO AND MICRO COMPONENTS OF FOODS, E.L. Wynder *et al.*
FOOD PRODUCT DEVELOPMENT IN IMPLEMENTING DIETARY GUIDELINES, G.E. Livingston, R.J. Moshy, and C.M. Chang
SHELF-LIFE DATING OF FOODS, T.P. Labuza
ANTINUTRIENTS AND NATURAL TOXICANTS IN FOOD, R.L. Ory
UTILIZATION OF PROTEIN RESOURCES, D.W. Stanley *et al.*
VITAMIN B_6: METABOLISM AND ROLE IN GROWTH, G.P. Tryfiates
POSTHARVEST BIOLOGY AND BIOTECHNOLOGY, H.O. Hultin and M. Milner

Newsletters

MICROWAVES AND FOOD, R.V. Decareau
FOOD INDUSTRY REPORT, G.C. Melson
FOOD, NUTRITION AND HEALTH, P.A. Lachance and M.C. Fisher
FOOD PACKAGING AND LABELING, S. Sacharow

DISPERSED PHASE PARTICLE SIZE EFFECTS ON WATER VAPOR PERMEABILITY OF WHEY PROTEIN-BEESWAX EDIBLE EMULSION FILMS

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Accepted for Publication November 1, 1993

ABSTRACT

Lipid particle size and distribution were found to significantly affect the water vapor permeability (WVP) properties of whey protein/beeswax edible emulsion films. Films oriented with the lipid-enriched side facing the high relative humidity environment during WVP testing exhibited lower WVPs. Particle size distributions were determined before and after film formation using laser light scattering and scanning electron microscopy, respectively. Decreasing mean emulsion particle diameters correlated well with linear decreases in average film WVP values. Whey protein chain crosslinkage at beeswax particle interfaces resulted in lowered WVPs as interfacial areas increased with decreasing particle diameters, providing greater opportunity for protein-beeswax interaction. Several models were applied and interaction factors were compared for films possessing different mean particle diameters.

INTRODUCTION

The properties of edible films have been reviewed by Kester and Fennema (1986), Guilbert (1986) and Krochta (1992). Effective edible emulsion films can retard degradative mass transfer in food systems to prolong food product

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Journal of Food Processing and Preservation **18** (1994) 173–188.

shelf-life and improve product quality. Edible films can also decrease amounts of disposable synthetic packaging materials and improve their recyclability.

Ukai *et al.* (1976) patented the use of coatings containing an aqueous water-soluble high polymer and a hydrophobic substance in an aqueous emulsion or suspension for coating agricultural food products. Ukai *et al.* (1976) identified the potential of protein-lipid emulsion coatings as effective mass transfer barriers; however, regulation of mass transfer based on lipid particle size and distribution in films was not explored.

The interactions between proteins and lipids at interfaces, such as in emulsions, have been studied extensively (Phillips 1981; Jost *et al.* 1986; Horbett and Brash 1987; Barfod *et al.* 1989; Le Meste *et al.* 1991). Homogenization and lipid particle size affect the functionality of a variety of colloidal systems. For example, formation and firmness of heat-induced gels were improved with increasing emulsion fineness and homogeneity (Jost *et al.* 1986).

Models of electrical and heat conductivity have been applied to model mass transfer through multicomponent systems. The majority of these models assume no interaction between the continuous and the dispersed phase (Crank 1975). Rogers (1965, 1985) modeled interfacial effects on barrier properties of polymeric materials assuming that crystalline domains of the film were not available for sorption or diffusion of water vapor. The crystalline domains were also hypothesized to act as crosslinking regions for the interfacial polymer chains of the amorphous polymer. The magnitude of this crosslinking effect increased as the crystalline domain size decreased at constant total crystalline volume fraction. This model has not been previously applied to composite edible films formed from emulsions of proteins and lipids.

The objective of this study was to investigate the effect of lipid particle size and distribution on the water vapor permeability (WVP) properties of whey protein/beeswax edible emulsion films. The origin of these effects was (1) hypothesized by utilizing knowledge of protein-lipid interactions at interfaces, (2) verified through the application of several classical models and (3) quantified using interaction parameters provided by the Rogers' model (1965, 1985).

MATERIALS AND METHODS

Materials

BiPRO whey protein isolate for films was supplied by Le Sueur Isolates (Le Sueur, MN). White beeswax was purchased from Aldrich Chemical Company, Inc. (Milwaukee, WI). Films were plasticized using sorbitol from Fisher Scientific, Inc. (Fair Lawn, NJ). Sodium chloride, utilized for the formation of

saturated salt solutions, was purchased from Fisher Scientific, Inc. (Fair Lawn, NJ). All chemicals were laboratory grade.

Film Formation

Aqueous solutions of 10% (w/w) whey protein isolate (WPI) were prepared and heated at 90C for 30 min in an oil bath (Haake Model No. N4B, Catalog No. 13-874-119C, Fisher Scientific, Inc., Fair Lawn, NJ). Solutions were cooled to room temperature and a vacuum was applied to remove dissolved air. Sorbitol was added at 2.8% (w/w) relative to the original weight of the water, to yield films with 78% WPI22% sorbitol, total solids basis. For the formation of emulsion films, the whey protein/sorbitol solutions were then reheated to 75C and sufficient beeswax (BW) was added to produce 56% WPI/28% BW/16% sorbitol total solids emulsions. After melting the beeswax, the mixture was homogenized to different extents using an Ultra-Turrax T-25 homogenizer (Ultra-Turrax, Model T25, IKA-Works, Inc., Cincinnati, OH) and an APV Rannie Mini-Lab homogenizer (Type 8.30H, Minneapolis, MN). Homogenization levels were as follows: (#1) 1.5 min red setting ($\approx$13,500 RPM) and 1.0 min blue setting ($\approx$20,500 RPM) (Ultra-Turrax); (#2) 1.5 min red and 4.0 min blue (Ultra-Turrax); (#3) level #2 (Ultra-Turrax) followed by 1 pass at 0, 200 and 300 bars (APV Rannie); (#4) level #2 (Ultra-Turrax) followed by 1 pass at 0, 200, 300, 400 and 600 bars (APV Rannie); (#5) same as level #1; however, the Ultra-Turrax RPM were adjusted upward; and (#6) same as level #2; however, the Ultra-Turrax RPM were adjusted upward. Particle size distributions following homogenization proved more relevant than homogenization conditions for data analyses.

Film Casting

Films were cast on 14.7 cm (internal diameter) rimmed, smooth polymethylmethacrylate (Plexiglas) plates located on level granite slabs. To minimize film thickness variations, 2.625 g total solids were applied to each plate. Six films were prepared for each film type. The WPI-sorbitol solutions and WPI-BW-sorbitol emulsions were spread evenly with a bent glass rod and allowed to dry for approximately 18 h at 40% relative humidity (RH) and 23C. Dried films could be peeled intact from the casting surface.

Film Thickness Measurement

After WVP testing, film thicknesses were measured to the nearest 0.0001 in. at five random positions around the film using a micrometer (L.S. Starrett Co., Series 436, Catalog No. T436RL-1, Athol, MA). Individual film thickness

measurements varied up to 5%. Average values of five thickness measurements per film were used in all WVP calculations.

Water Vapor Permeability Determination

The WVP Correction Method was utilized (McHugh *et al.* 1993). Plastic desiccating cabinets (Fisher Scientific, Inc., Catalog No. 08-647-28, Fair Lawn, NJ) containing motors (Bodine Motor, Model No. 574, Minarik Electric Co., Fresno, CA) with variable speed controllers (Motor Master, Series 20000, Minarik Electric Co., Fresno, CA) and fans (Refrigeration Supply House, Model No. 607601-01, Sacramento, CA) were placed in a 23C controlled temperature room. Fan speeds were set to achieve air velocities of 152 m/min in the cabinets. Each cabinet contained a hygrometer (Airguide, Model No. 605, Chicago, IL) to monitor the RH conditions. Prior to each experiment, cabinets were equilibrated to 0% RH using calcium sulfate desiccant (Drierite, Fisher Scientific, Inc., Fair Lawn, NJ).

Circular test cups were made out of polymethylmethacrylate (Plexiglas). The external base cup dimensions were 8.2 cm diameter and 1.25 cm tall. The area of the cup mouth was 19.6 cm^2, and the cup well depth was 1.1 cm. Cup walls were sufficiently thick to render the cup impermeable to water vapor. A film was sealed to the cup base with an 8.2 cm diameter and 0.60 cm tall polymethylmethacrylate (Plexiglas) ring containing a 19.6 cm^2 opening using four screws symmetrically located around the cup circumference. Both sides of the cup contacting the film were coated with silicon sealant (High Vacuum Grease, Dow Corning, Midland, MI).

Deionized water or an equivalent amount of sodium chloride saturated salt solution was placed in the bottom of the test cup to expose the film to a high percentage RH inside the test cups. Next, films were mounted on the cups. The distance between the solution and the film was determined both before and after each experiment to the nearest 0.001 in. using a micrometer (Lufkin Rule Co., Model No. 515, Saginaw, MI). Average stagnant air gap heights were used in WVP calculations.

After assembly, the test cups containing mounted films were inserted into preequilibrated 0% RH desiccator cabinets. Within 2 h, steady state was achieved. Five weights were then taken for each cup at greater than 3 h intervals. Four replicates of each film were tested. Regression coefficients for weight loss as a function of time were greater than 0.99. Inner film surface RH and corrected WVP were calculated using the WVP Correction Method to account for water vapor transfer through the stagnant air layer in the cups (McHugh *et al.* 1993). Inner surface film RH values varied by 5%.

Inner film surface RH has been shown to exhibit an exponential effect on the WVP of edible films (McHugh *et al.* 1993; McHugh and Krochta 1994).

Model applications required determination of film WVP values under equivalent RH conditions. The WVPs of 78% WPI/22% sorbitol amorphous films containing no beeswax were determined at an average RH of 80.2% at the film underside in test cups. These WVP values were utilized in all models; therefore, WVPs for emulsion films were determined by interpolation to 80.2% RH conditions. Using the WVP Correction Method (McHugh *et al.* 1993) sodium chloride saturated salt solution resulted in a calculated average RH of 72%, and water resulted in a calculated average RH of 92% at emulsion film undersides in test cups. An exponential relationship was assumed between film WVPs and inner surface RHs (McHugh *et al.* 1993; McHugh and Krochta 1994) for interpolation to 80.2% RH. Emulsion films exhibited a constant difference of 0.15 g-mm/kPa-h-m^2 between WVP values obtained using water and those interpolated to 80.2% RH. Therefore, a factor of 0.15 g-mm/kPa-h-m^2 was subtracted from all WVP values obtained using water to correct WVP values to 80.2% RH conditions.

Malvern Laser Particle Size Analysis

A Malvern MasterSizer IM 100 laser light scattering instrument was used to measure mean particle size and particle size distribution of beeswax droplets in 56% WPI28% BW/16% sorbitol emulsions (Malvern Instruments Ltd., Malvern, England).

Scanning Electron Microscopy

Films were fractured under liquid nitrogen and dehydrated under vacuum for three days. Next, films were sectioned and mounted on aluminum stubs using double stick tape and silver paint. Sputter coating with gold-palladium alloy was performed at 20 mA. An ISI DS-130 scanning electron microscope was used to examine film microstructure.

Statistical Analysis

StatView 4.0 was used for all statistical analyses (Abacus Concepts, Berkeley, CA). Analyses of variance, Fisher PLSD multiple comparisons, and regression analyses were performed.

Model Applications

The applicability of several composite film transport models to edible protein-lipid emulsion films was examined. Emulsion films were composed of the 78% protein/22% sorbitol amorphous continuous phase (phase A), and the

28% crystalline beeswax dispersed phase (phase B). Several models of varying complexity have been developed to analyze dispersed systems; however, such models have never been applied to analyze mass transport through edible emulsion films.

Maxwell Model. The Maxwell model assumes that (1) the dispersed phase particles are spherical, (2) interaction between particles is negligible, and (3) continuous phase properties are unaffected by the dispersed phase. In addition, the Maxwell model does not consider the size or distribution of the dispersed particles (Crank 1975). Assuming no diffusion and no solubility in the dispersed beeswax phase, B, due to its extremely low WVP, 0.05 g-mm/m^2-d-kPa (McHugh and Krochta 1994), Eq. 1 can be derived. X_A and P_A are, respectively, the volume fraction and the WVP of the amorphous continuous 78% whey protein/22% sorbitol phase, A. P_C is the predicted WVP of the composite film.

$$P_C = \frac{2(X_A)^2 P_A}{3 - X_A} \tag{1}$$

Tortuosity Model. Nielsen (1967) later developed a basic tortuosity model, which accounts for the diffusion of molecules by a tortuous path through the film due to the dispersed phase. Once again, this model assumes that the diffusivity through the dispersed phase, B, is 0. The tortuosity factor, T, is defined in Eq. 2.

$$T = \frac{\text{average distance a molecule must travel to get through a film}}{\text{thickness of the film}} \tag{2}$$

In addition to the tortuosity factor, permeability is reduced due to a reduction in the volume fraction of penetrable material (Nielsen 1967). The WVP of the composite, Pc, can be calculated using Eq. 3. Similar to the Maxwell model, the tortuosity model does not consider possible interactions between the dispersed and the continuous phases.

$$P_C = \frac{X_A P_A}{T} \tag{3}$$

If it is assumed that the dispersed phase, B, is present in spherical or cubic particles that are uniformly dispersed in the polymer, the applicable tortuosity factor can be calculated using Eq. 4 (Nielsen 1967). L is the length of a face of the dispersed particle. W is the thickness of the dispersed particle. X_B is the volume fraction of the dispersed phase.

$$T = 1 + \left(\frac{L}{2W}\right)X_B \tag{4}$$

Interaction Model. The model developed by Rogers (1965, 1985) for amorphous polymers with crystalline domains not only accounts for the tortuosity effects described using the tortuosity model, but also considers phase interactions. The interaction model assumes that the crystalline dispersed phase is not available for sorption or diffusion of penetrants; therefore, mass transfer only occurs through the continuous amorphous polymer phase. The crystalline dispersed phase also acts as a crosslinking region for polymer chains that enter and leave from the surrounding amorphous phase, where sorption and diffusion occur. The magnitude of the crosslinking effect increases as crystallite size decreases at constant crystalline total volume fraction. The interaction effects were quantified through the use of interaction factors, $\beta \geq 1$ and $\beta' \geq 1$, which represent chain immobilization factors for the diffusivity and solubility coefficients, respectively. To simplify this analysis, β and β' will be combined into one interaction factor, $\beta \geq 1$. The composite film WVP, P_c can then be approximated using Eq. 5.

$$P_C = \frac{X_A P_A}{T\beta} \tag{5}$$

RESULTS AND DISCUSSION

Effects of Homogenization and Orientation on WVP

Increased homogenization from level #1 through level #6 resulted in significant reductions in WVP values (Table 1). This effect has not been previously observed in edible film systems.

An effect of film orientation in the test cup on WVP of emulsion films was also observed (Table 1). Emulsion films oriented with the lipid-enriched side facing down (towards the inner, high relative humidity environment of the cup) exhibited significantly lower WVPs than films oriented with the lipid side up. This is due to the hydrophilic nature of whey protein based films and the exponential effect of relative humidity on their WVP properties (McHugh *et al.* 1993; McHugh and Krochta 1994). When the lipid-enriched side of the film faced the high relative humidity environment, the protein side of the film experienced a lower relative humidity, lowering its contribution to the overall

TABLE 1.

EFFECT OF HOMOGENIZATION AND ORIENTATION ON THE WATER VAPOR PERMEABILITY OF 56% WHEY PROTEIN/28% BEESWAX/16% SORBITOL EDIBLE EMULSION FILMS AT 23 C

Homogenization Level*	Thickness (mm)	Relative Humidity♦ (%)		Water Vapor Permeability♦ (g-mm/kPa-h-m^2)	
		Down♣	Up♣	Down♣	Up♣
Level #1	0.19	93	87	1.17^c	2.13^a
Level #2	0.17	94	93	0.86^d	1.11^c
Level #3	0.16	94	93	0.78^d	1.43^b
Level #4	0.16	94	94	0.72^d	0.81^d
Level #5	0.14	96	96	0.41^e	0.53^e
Level #6	0.14	98	97	0.22^f	0.52^e

All values reported are means of at least three measurements.

* Level #1: 1.5 min on red and 1.0 min on blue (Ultra-Turrax); Level #2: 1.5 min on red and 4.0 min on blue (Ultra-Turrax); Level #3: Level #2 (Ultra-Turrax) followed by 1 pass each at 0, 200 and 300 bars (APV Rannie); Level #4: Level #2 (Ultra-Turrax) followed by 1 pass each at 0, 200, 300, 400 and 600 bars (APV Rannie); Level #5: Level #1 with RPM adjusted on Ultra-Turrax; Level #6: Level #2 with RPM adjusted on the Ultra-Turrax.

♦ Relave humidity at the inner surface of the film and water vapor permeability values were corrected for test cup stagnant air effects using the WVP Correction Method (McHugh et al. 1993).

♣ Down and Up refer to the lipid-enriched side of the film facing the inside high relative humidity in the cup (Down) versus the outer 0% relative humidity of the cabinet (Up)

a,b,c,d,e,f indicate significant differences at $p<0.05$ using Fisher's PLSD multiple comparison test.

film WVP. These results demonstrate the importance of reporting film orientation along with relative humidity, film thickness, and WVP data when examining films containing hydrophilic materials.

Emulsion creaming during film dehydration resulted in the nonisotropic nature of emulsion films. After drying, the shiny film side facing the casting surface resembled WPI/sorbitol films, thus indicating the presence of elevated protein content; conversely, the dull side facing the room resembled pure lipid films, thus indicating the presence of elevated lipid content. Although emulsion separation occurred within these film systems, bilayer films were not formed. Discrete lipid particles remained within the protein matrix, as shown by scanning electron microscopy.

These results also identified the importance of particle size analyses following homogenization in order to ascertain the degree of emulsification. Variability within the homogenizer performance can produce emulsions with different size distributions even at similar homogenization levels. As discussed below, our data indicate that particle sizes, not equipment homogenization levels, were relevant for analyses and prediction of water barrier properties.

Particle Size Analyses

Laser light scattering was utilized to examine the particle size distributions of emulsions prior to film formation. As homogenization increased from level #1 to level #6, the particle size distributions shifted to lower particle diameter ranges (Fig. 1).

Scanning electron microscopy (SEM) was employed as a means to determine approximate particle sizes in emulsion films. Figures 2 and 3 show cross sections of typical level #3 and level #4 emulsion films. Examination of multiple electron micrographs confirmed that particle sizes in films were similar to those in emulsions (Fig. 1). Thus, lipid particles did not coalesce to a great extent during film formation. Orientation effects on WVP were attributed predominantly to creaming of lipid particles. In the future, image analyses of electron micrographs may provide a means to more accurately correlate liquid emulsion and emulsion film particle sizes. For the purpose of this study, correlations and calculations utilized particle size measurements obtained from laser light scattering analyses (Fig. 1).

Scanning electron microscopy also revealed that the beeswax in emulsion films was in a crystalline state and that the lipid particles were dispersed in a protein/sorbitol continuous matrix.

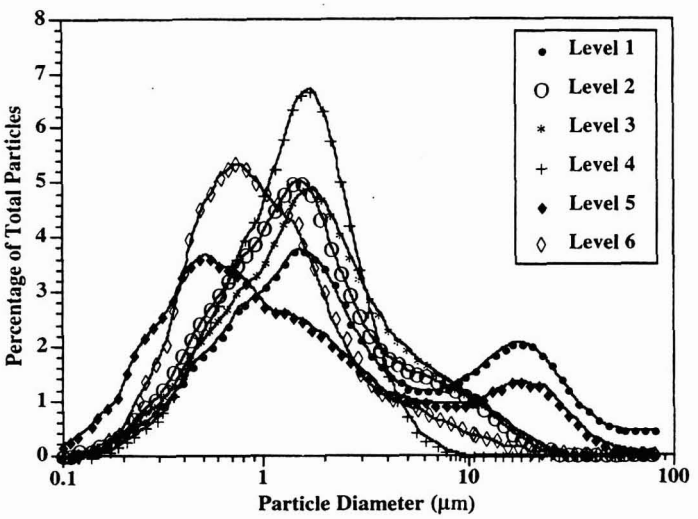


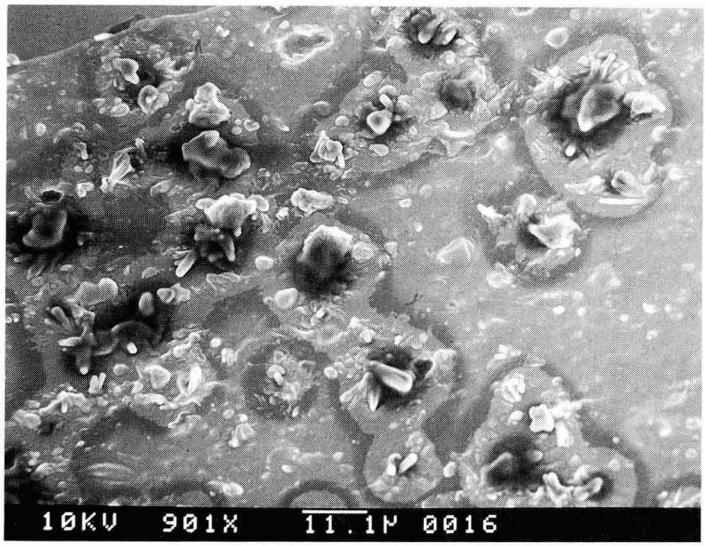
FIG. 1. PARTICLE SIZE DISTRIBUTIONS OF 56% WHEY PROTEIN/28% BEESWAX/16% SORBITOL EMULSIONS HOMOGENIZED AT LEVELS #1 THROUGH #6 PRIOR TO FILM FORMATION

(#1) 1.5 min on red and 1.0 min on blue (Ultra-Turrax); (#2) 1.5 min on red and 4.0 min on blue (Ultra-Turrax); (#3) Level #2 followed by 1 pass at each 0, 200 and 300 bars (APV Rannie); (#4)Level #2 followed by 1 pass each at 0, 200, 300, 400 and 600 bars (APV Rannie); (#5) Level #1 with RPM adjusted on Ultra-Turrax; (#6) Level #2 with RPM adjusted on the Ultra-Turrax.

Correlations Between Mean Particle Size and WVP

The relationship between mean particle diameter and average WVP was examined (Fig. 4). WVP values obtained using up and down orientations were averaged and mean particle diameters calculated from laser light scattering measurements (Fig. 1). Decreased mean particle diameters resulted in linear decreases in WVP values with a regression coefficient of 0.96.

The decrease in WVP with decreased mean particle diameters was hypothesized to originate due to the increased interfacial areas provided by small particles at constant lipid content. The exact nature of protein/lipid reactions at interfaces remains unknown, and conflicting opinions can be found in the literature. LeMeste *et al.* (1991) used electron spin resonance spectroscopy to examine the interaction between milk proteins and fatty acids. The milk protein and lipid were found to interact through their polar groups. The milk protein was depicted sitting on the surface of the lipid droplets. No evidence of protein or lipid reorganization at the interface was observed. Phillips (1981), on the



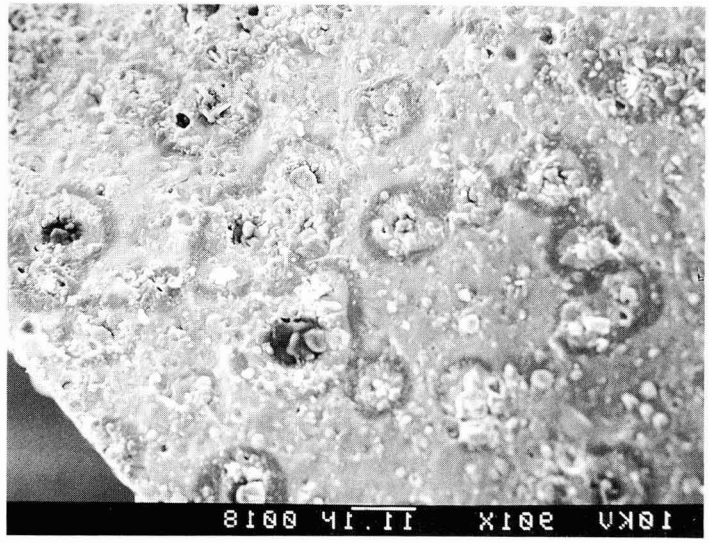


FIG. 2. HIGH MAGNIFICATION SCANNING ELECTRON MICROGRAPH OF 56% WHEY PROTEIN/28% BEESWAX/16% SORBITOL EMULSION FILM CROSS SECTION
Homogenized to Level #3.
1.5 min red, 4 min blue (Ultra-Turrax) and 1 pass at each 0, 200 and 300 bar (APV Rannie).
White bar length is 11.1 mm.

FIG. 3. HIGH MAGNIFICATION SCANNING ELECTRON MICROGRAPH OF 56% WHEY PROTEIN/28% BEESWAX/16% SORBITOL EMULSION FILM CROSS SECTION
Homogenized to level #4.
1.5 min red, 4 min blue (ultra-Turrax) and 1 pass at each 0, 200, 300, 400 and 600 bar (APV Rannie). White bar length is 11.1 mm.

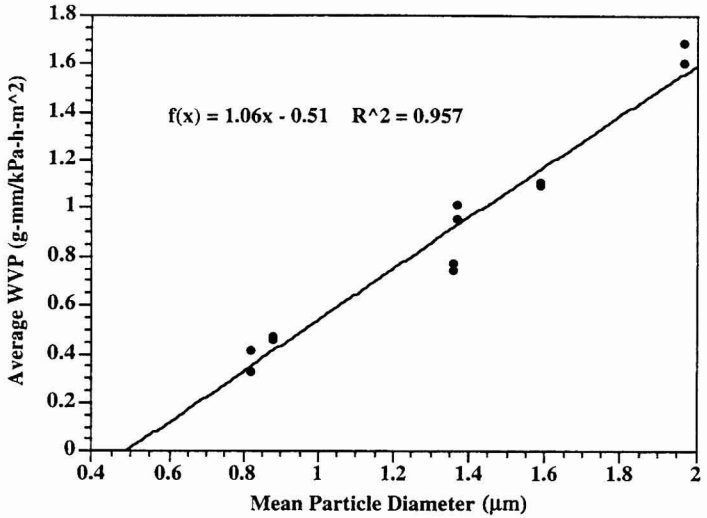


FIG. 4. CORRELATION BETWEEN MEAN EMULSION PARTICLE SIZE AND AVERAGE WATER VAPOR PERMEABILITY FOR 56% WHEY PROTEIN/28% BEESWAX/16% SORBITOL EDIBLE EMULSION FILMS AT 23C

other hand, found that flexible, β-casein molecules lay in the plane of the water/oil interface in emulsion systems with protein chains, loops and tails protruding into both phases. Globular lysozme protein behaved in a similar manner. Barfod *et al.* (1989) developed a similar model of lipid-protein interactions in an oil-water emulsion interface. Protein molecule chains, loops and tails interacted at the interface to stabilize the lipid particles. We hypothesize that whey protein-beeswax interfacial interactions in emulsion films result in reduced protein phase interfacial mobility and decreased WVP values.

Application of Models

The results of applying the Maxwell model (1873), the tortuosity model (Nielsen 1967), and the interaction model (Rogers 1965, 1985) were used to explain and quantify the effects of particle size on WVP (Table 2). WVP values were calculated by averaging values from different film orientations and interpolating WVP values to 80.2% relative humidity conditions.

Based on a WVP of 2.16 g-mm/kPa-h-m^2 for 78% WPI/22% sorbitol films, the Maxwell's model predicted that the WVP for the 56% WPI/28% BW/16% sorbitol emulsion films would be a 0.98 g-mm/kPa-h-m^2 (Eq. 1) for all emulsion films. Experimentally, water vapor permeability was found to vary with average

TABLE 2.

MODEL COMPARISON FOR THE EFFECT OF PARTICLE SIZE ON WATER VAPOR PERMEABILITY OF 56% WHEY PROTEIN/28% BEESWAX/16% SORBITOL EDIBLE EMULSION FILMS AT 23 C

Homogenization Level*	Mean Diameter (μm)	Average Experimental Water Vapor Permeability (80.2%RH)♦	Maxwell's Model P_C equals	Tortuosity Model Assuming T=1.14, P_C equals	Interaction Model Assuming T=1.14, β equals
78% WPI/22% S		2.16			
1	1.97	1.50	0.98	1.36	0.91
2	1.37	0.84	0.98	1.36	1.62
3	1.59	0.95	0.98	1.36	1.43
4	1.36	0.61	0.98	1.36	2.23
5	0.88	0.23	0.98	1.36	4.25
6	0.82	0.23	0.98	1.36	5.91

* Level #1: 1.5 min on red and 1.0 min on blue (Ultra-Turrax); Level #2: 1.5 min on red and 4.0 min on blue (Ultra-Turrax); Level #3: Level #2 (Ultra-Turrax) followed by 1 pass each at 0, 200 and 300 bars (APV Rannie); Level #4: Level #2 (Ultra-Turrax) followed by 1 pass each at 0, 200, 300, 400 and 600 bars (APV Rannie); Level #5: Level #1 with RPM adjusted on Ultra-Turrax; Level #6: Level #2 with RPM adjusted on the Ultra-Turrax.

♦ Average of Up and Down Oriented Water Vapor Permeability values (g-mm/kPa-h-m^2) calculated using the WVP Correction Method (McHugh et al. 1993). WVP values were then interpolated to 80.2% relative humidity as described in the Methods section of this paper.

particle diameter. Therefore, the Maxwell model proved insufficient to describe protein-lipid edible film WVP properties.

Next, the tortuosity model (Nielsen 1967) was investigated. A tortuosity of 1.14 was calculated (Eq. 4). This tortuosity value was then utilized in Eq. 3 to test the efficacy of the tortuosity model. Once again, a constant value was observed, 1.36 gmm/kPa-h-m^2, due to the model's inability to account for particle size effects.

Finally, the interaction model (Rogers 1965, 1985) was applied. Once again a constant tortuosity value of 1.14 was assumed. The resulting interaction factor, β, was calculated using Eq. 5. The interaction factor, β, increased as particle size decreased (Table 2). According to Rogers (1965), the logarithm of the interaction factor should correlate linearly with the particle diameter squared. This was verified for the 56% WPI/28% BW/16% sorbitol edible emulsion films (Fig. 5). It was concluded that immobilization of proteins at the lipid interface in emulsion films was responsible for the decrease in WVP observed with decreasing particle diameters.

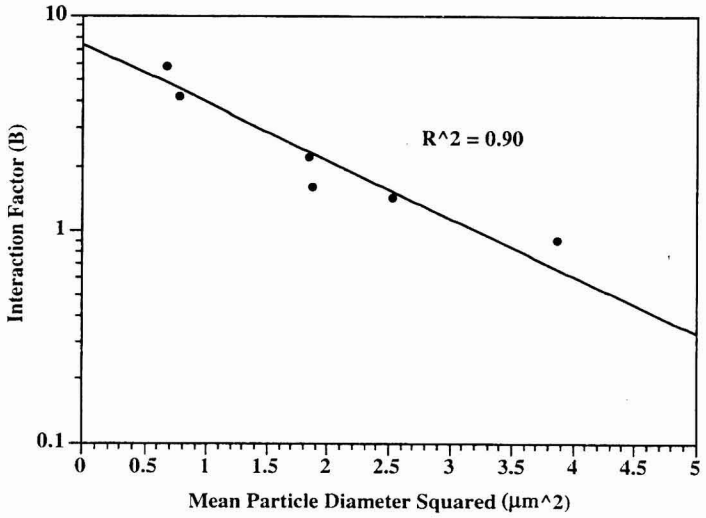


FIG. 5. CORRELATION BETWEEN THE LOGARITHM OF INTERACTION FACTORS AND SQUARED MEAN PARTICLE DIAMETERS FOR 56% WHEY PROTEIN/28% BEESWAX/16% SORBITOL EDIBLE EMULSION FILMS AT 23C

Protein-lipid edible emulsion film systems exhibit barrier properties dependent on the emulsion mean particle size. Film barrier properties improved as emulsion particle size decreased. By controlling lipid particle size and

distribution in emulsion films and coatings, moisture loss, respiration and oxidation in foods can be regulated. Furthermore, the lipid and aroma barrier properties of the films may be controlled, as could their mechanical properties.

ACKNOWLEDGMENTS

This research was supported by a grant from the California Dairy Foods Research Center and a gift from Nabisco Brands, Inc.

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EFFECTS OF SEMPERFRESH™ AND JOHNFRESH™ FRUIT COATINGS ON POSTSTORAGE QUALITY OF "ANKARA" PEARS

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Accepted for Publication November 16, 1993

ABSTRACT

Pears were coated with different concentrations of Semperfresh or with Johnfresh edible coatings after storage for five months at 0C to determine the effects of coatings on poststorage quality. Coating levels were effective for extending the poststorage life and delaying ripening. Both Semperfresh and Johnfresh treated pears had better color, firmness, ascorbic acid, titratable acidity, soluble solid retention than control pears. Although Semperfresh was effective for the reduction of respiration and weight loss, Johnfresh was determined to be the most efficient coating for the same purpose.

INTRODUCTION

Edible coatings have been shown to be efficient to preserve quality of some fruits in storage. A modified atmosphere is produced within the fruit after the application of the coating, which results in a decrease in respiration and delay in the ripening of fruits. SPE coatings (sucrose esters of fatty acids, sodium carboxymethyl cellulose and monodiglycerides of fatty acids) have been used with bananas (Banks 1984; Marchal *et al.* 1987), apples (Santerre *et al.* 1989; Drake *et al.* 1987) and found effective for delaying the ripening of fruits. When SPE were tried on pears after cold storage, it was found that they were effective in reducing the ripening rate, but 1.2% and 1.5% concentrations caused uneven coloration of pears (Van Zyl *et al.* 1987). Meheriuk and Lau (1988) showed that SPE delayed the ripening, but loss of ripening capacity was also observed. Semperfresh (Semper Bio Technology, Reading, UK) is an improved formulation of earlier SPE products. It contains a higher proportion of short chain unsaturated fatty acid esters than other SPE products (Drake *et al.* 1987).

Journal of Food Processing and Preservation **18** (1994) 189–199.

"Ankara" pears are generally stored in cold storage where they do not ripen; however, they show abnormal ripening behavior during subsequent storage at higher temperatures. This is due to the sudden increase in respiration rates with resulting increases in internal carbondioxide concentration and physiological disorders. This abnormal ripening behavior causes problems marketing at high temperatures and low %RH (Pekmezci 1978).

Fruit coating, a recent development in Turkey, may be efficient to minimize these problems. Semperfresh and Johnfresh (SC Johnson, Istanbul, Turkey) are available in Turkey to use with pears. The purpose of this study was to determine the effects of coatings on quality and to prolong the poststorage life of "Ankara" pears.

MATERIALS AND METHODS

"Ankara" pears (a local variety) were harvested in late September from the orchard of the Faculty of Agriculture at Ankara University (Ankara, Turkey). Pears were harvested with soluble solids and firmness values of 9–10% and 7.2–8.0 kg, respectively. Just after harvest they were stored in cold storage (0–1C and 85–90% RH) for a period of five months prior to coating.

Pear Treatments

Pears were coated with 0.5%, 1.0%, 1.5% (w/v) concentrations of Semperfresh (sucrose polyester base coating). Johnfresh (carnauba wax and shellac based coating) were used as they were supplied from the manufacturer without any dilutions. Coatings were applied by dipping pears for 5 s in the solutions. Control pears were dipped only in water. Pears were divided into 5 lots of 12 kg each. After treatment pears were stored at 20C for 15 days and analyzed for firmness, color, titratable acidity, soluble solids, ascorbic acid weight loss, ash and alcohol. Respiration rate was determined for pears stored at 15C. Three replications per treatment were used during the analysis.

Quality Evaluations

Skin color was measured with an UV Visible Spectrophotometer (Shimadzu UV 2100, Japan), standardized with a white reference (L:74.776, a: 0.175, b: −9.553). Three values were determined around the circumference of each pear and three pears were used with each treatment. *Firmness* was measured by using a fruit hardness tester (Everwell CF 371, Japan) with a plunger of 8 mm diameter at three locations along the equatorial plane of 8 pears per treatment

after removal of 1.27–1.90 cm diameter disk of peel. *Titratable acidity* was measured using 6 pears for each treatment per replication. Fruit was pressed with an electrical juice presser (Arcelik Robo-Press ARK71 RF). A 10-ml portion of pressed juice was diluted to 250 ml with distilled water. A 50-ml portion of juice was titrated with 0.1 N NaOH to a pH = 8.1. Results were expressed as % malic acid. *Ash content* was determined by an AOAC method (AOAC 1975). *Ascorbic acid* was determined by 2,6 dichloroindophenol method (AOAC 1975). *Weight loss* was determined by weighing individually the randomly selected same 10 pears for each treatment immediately after coating and then at 4th, 9th and 16th days with a laboratory balance (Mettler 163, Germany), and the results are expressed as percent loss by weight. *Respiration* was measured by IR Gas analyzer (Servomex PA 404). Pears were stored in sealed jars for 6 h. Then carbon dioxide, which was present in the jar due to respiration of the pears, was withdrawn by the syringe of the gas analyzer immediately after piercing the jar lids with the syringe. Each treatment per replication contained four pears in each jar. *Alcohol* was determined by an AOAC method (AOAC 1975).

Analysis of variance was used to determine statistical relationships among treatments. Significance was determined at p = O.05 level for all analysis. Treatment means were compared by Duncan's New Multiple Comparison Method.

RESULTS AND DISCUSSION

Respiration rates increased when the pears were removed from cold storage (respiration rate was 3.203 ml CO_2/kg h)(Fig. 1). The effects of coatings in reducing the respiration rates may be related to the reduction in the climacteric peak. Johnfresh fruit coating reduced the climacteric peak more effectively than different concentrations of Semperfresh. Treatments with 1.0% and 1.5% Semperfresh were efficient to reduce respiration but 0.5% treatment was not significantly different from the control. Banks (1985) also showed that internal oxygen content of "Cox Orange Pippin" apples did not reduce when they were coated with SPE. Even 1.5% concentrations were not as efficient as Johnfresh fruit coating. Ineffective coverage of pears by Semperfresh coating may have caused this result. "Ankara" pears contain high concentration of surface waxes compared to other pears, and these waxes may have prevented effective coating of surface by SPE, thus reducing the effect on respiration and ripening. The effectiveness of Johnfresh fruit coating for reduction of respiration may be related to its shellac content, as coatings with high amount of shellac were reported to have low oxygen permeability (Hagenmaier and Shaw 1992).

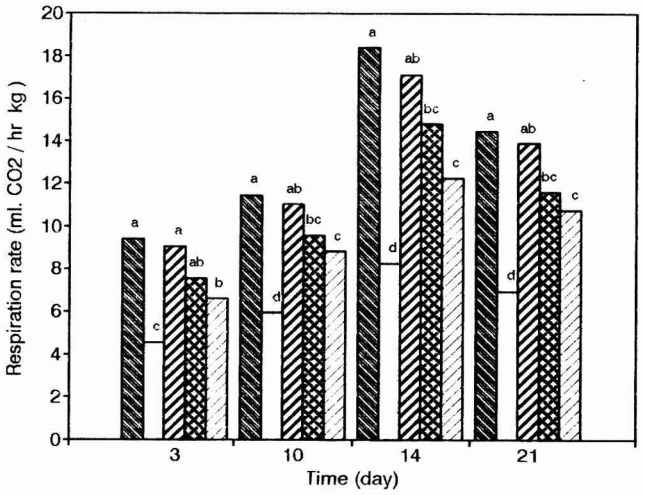

FIG. 1. VARIATION OF RESPIRATION RATE OF PEARS WITH WAX TREATMENTS

(▩): Control, (▔): Johnfresh, (▨) Semperfresh (0.5%), (▩): Semperfresh (1.0%), (▨): Semperfresh (1.5%). Bars with the same letters (i.e., a,b,c) within time are not significantly different at the p = 0.05 level.

Johnfresh coated pears had higher firmness values than others, as expected knowing the efficiency of this coating for reduction of respiration. Generally the desired firmness of "Ankara" pears for the consumers is 2.61 kg (Pekmezci 1978). Control pears passed this point within 10 days. Pears coated with Johnfresh or 1.5% Semperfresh were still firm at this point showing the effectiveness of coatings on firmness retention (Table 1). Treated pears had higher color retention compared to the control. This can be detected by the slow decrease in hue angle during storage (Table 2). Hue angle is expressed as arc tan (b/a). The decrease in hue angle is inversely related with ripening. Hunter "a" values of "Ankara" pears increased during storage and control pears had higher "a" values meaning that control fruits lost green color quickly (Table 3). There was no significant difference in Hunter "b" and lightness values between coated and uncoated pears (Table 3). It was also shown that 0.5% and 1% concentrations of Semperfresh delayed the development of yellow color of cold-stored Bartlett pears, but pears coated with 1% Semperfresh remained green even after 4 days of storage at 20C (Dinamarca *et al.* 1989). In the study pears treated with 1.5% Semperfresh had the most green color when they were ripened, but uneven coloration (green-yellow) was detected. This defect in appearance was observed in 1.2% and 1% Semperfresh in studies of Van Zyl *et al.* (1987). Meheriuk and Lau (1988) also showed that a blotchy appearance

TABLE 1.
VARIATION OF FIRMNESS OF "ANKARA" PEARS
AFTER COLD STORAGE AT 20C (KG)

Treatment	Storage time (days)		
	0	10	15
Control	4.58	2.09 a	0.93 a
SF (0.5%)	4.58	2.39 ab	1.12 a
SF (1.0%)	4.58	2.65 bc	1.35 b
SF (1.5%)	4.58	2.90 c	1.60 c
Johnfresh	4.58	3.49 d	1.69 c

* means in a column followed with different letters are significantly different (p=0.05).

** SF=Semperfresh

TABLE 2.
VARIATION OF HUE ANGLE OF "ANKARA" PEARS
AFTER COLD STORAGE AT 20C

Treatment	Storage time (days)		
	0	7	14
Control *** a	122.06	111.13	98.20
SF (0.5%) ab	122.06	112.57	102.98
SF (1.0%) bc	122.06	118.14	110.26
SF (1.5%) c	122.06	119.10	113.71
Johnfresh bc	122.06	114.02	110.08

*** means coatings with different letters are significantly different (p=0.05).

TABLE 3.
VARIATION OF COLOR IN "ANKARA" PEARS
AFTER COLD STORAGE AT 20C

	Coating type	Storage time (days)		
		0	7 *	14
Hunter a value	Control **	-8.541	-5.830 a	-2.335 a
	SF (0.5%)	-8.541	-6.122 a	-5.353 a
	SF (1.0%)	-8.541	-6.383 a	-3.558 b
	SF (1.5%)	-8.541	-7.907 a	-6.293 b
	Johnfresh	-8.541	-6.318 b	-5.491 b
Hunter b value	Control	13.635	15.089 a	16.220 a
	SF (0.5%)	13.635	14.731 a	15.438 a
	SF (1.0%)	13.635	14.340 a	14.504 a
	SF (1.5%)	13.635	14.210 a	14.330 a
	Johnfresh	13.635	14.179 a	14.248 a
Lightness	Control	40.288	43.052 a	48.675 a
	SF (0.5%)	40.288	40.732 a	48.360 a
	SF (1.0%)	40.288	40.760 a	45.902 a
	SF (1.5%)	40.288	41.200 a	43.368 a
	Johnfresh	40.288	40.942 a	44.961 a

* means in a column followed by different letters are significantly different (p=0.05).

** SF=Semperfresh

occured in many of the SPE-treated pears. Reduced internal levels of oxygen and increased internal levels of carbon dioxide have been implicated in the retention of green color (Trout *et al.* 1953). This is contrary to our results. Although Johnfresh was more effective in reduction of respiration than Semperfresh, 1.5% concentration of Semperfresh was more effective for retention of green color. Color retention in the Semperfresh-treated fruit may not

be related to modified atmosphere in the fruit but may be due to direct interference of the coating with chlorophyll degradation processes or effects on chloroplast structure due to the penetration of coating into the fruit.

TABLE 4.
COMPOSITIONAL CHANGES IN "ANKARA" PEARS
AFTER COLD STORAGE AT 20C

Composition	Coating type	Storage time (days)		
		0	5	13
Ascorbic acid (mg /100 g)	Control**	5.735	4.563 c*	2.352 d
	SF (0.5%)	5.735	4.942 bc	3.147 c
	SF (1.0%)	5.735	5.378 ab	4.509 b
	SF (1.5%)	5.735	5.523 a	5.000 ab
	Johnfresh	5.735	5.669 a	5.353 a
Soluble solids (%)	Control	13.3	13.84 a	14.35 a
	SF (0.5%)	13.3	13.63 b	14.20 b
	SF (1.0%)	13.3	13.60 b	13.95 b
	SF (1.5%)	13.3	13.60 b	14.00 b
	Johnfresh	13.3	13.60 b	13.90 b

* means in a column followed by different letters are significantly different (p=0.05)

** SF=Semperfresh

Semperfresh with 1% and 1.5% concentrations and Johnfresh fruit coating were effective for reducing the rate of ascorbic acid loss (Table 4). Ascorbic acid retention by means of coating is related to the reduction of respiration of fruits. Concentration of 0.5% was not significantly different from the control for retention of ascorbic acid. That was an expected result, as 0.5% concentration was not effective for reduction of respiration. SPE coatings were also shown to be effective for retention of ascorbic acid of mangoes (Dhalla and Hanson 1988).

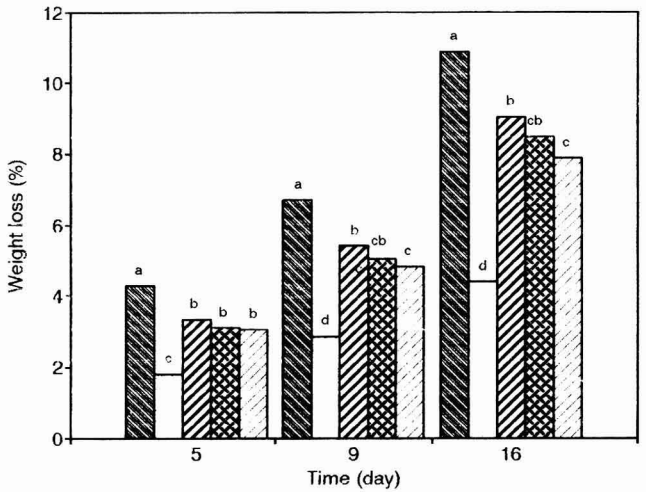

FIG. 2. VARIATION OF WEIGHT LOSS OF PEARS WITH DIFFERENT WAX TREATMENTS (▨): Control, (▔): Johnfresh, (▨) Semperfresh (0.5%), (▩): Semperfresh (1.0%), (▨): Semperfresh (1.5%). Bars with the same letters (i.e., a,b,c) within time are not significantly different at the p = 0.05 level.

Coatings were effective for reduction of weight loss of "Ankara" pears (Fig. 2). Increasing the concentration of Semperfresh reduced the weight loss, but the reduction weight loss was not in great amounts. Johnfresh fruit coating was the best coating for reduction of weight loss. Hagenmaier and Shaw (1992) showed that SPE coatings have higher water vapor permeability than commercial waxes. This may be related to the hygroscopic characteristics of SPE. Although SPE coatings act as a barrier for gas transfer by plugging lenticels, transpiration takes place from the cuticle, which may be increased by hygroscopic characteristics of coatings, especially during marketing at high temperatures and low relative humidity (Smith *et al.* 1987). Alcohol detected in treated pears was not significantly different from untreated ones (data not shown), indicating that coatings did not reduce the oxyyen transfer into the fruit completely and anaerobic fermentation did not take place.

Coated pears displayed high acidity and low soluble solids. Although there was not much difference between coatings, the results showed that the pears did not ripen during storage. When coating is applied on fruits at advanced maturity, Semperfresh does not have any effect on the change of acidity (Santerre *et al.* 1989). Titratable acidity and soluble solid variation of pears are given in Fig. 3 and Table 4, respectively. Ash content of treated pears did not change significantly from untreated ones (data not shown).

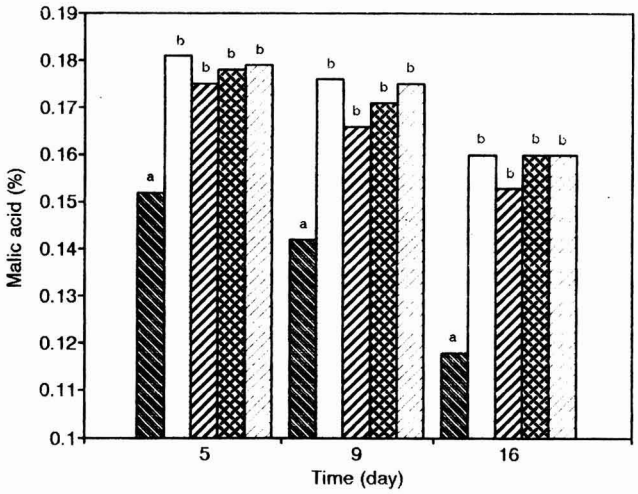

FIG. 3. VARIATION OF ACIDITY OF PEARS WITH DIFFERENT WAX TREATMENTS ( ): Control, ( ): Johnfresh, ( ) Semperfresh (0.5%), ( ): Semperfresh (1.0%), ( ): Semperfresh (1.5%). Bars with the same letters (i.e., a,b,c) within time are not significantly different at the p = 0.05 level.

Determination of the optimum concentration of Semperfresh is a significant factor for delaying the ripening of fruits. Even for different species of the same fruit there is an optimum concentration, depending on the respiration rate and structure of fruit. The optimum concentration for some species of fruits is sometimes not the same as the concentration recommended by the company. The recommended concentration of Semperfresh for pears is 0.8%. For "Ankara" pears 0.5% Semperfresh was determined to be not efficient for delaying the ripening. Coatings with 1% and 1.5% concentrations had similar effects on ripening parameters, but most of the pears coated with 1.5% Semperfresh ripened with uneven color. That is why 1% may be selected as the optimum concentration of Semperfresh for "Ankara" pears.

Post storage life of "Ankara" pears increased due to the application of coatings. There was an increase of 10%, 20%, 30% and 30% in poststorage life for 0.5%, 1.0% and 1.5% concentrations of Semperfresh and Johnfresh coated pears, respectively.

CONCLUSIONS

Coatings with 1% and 1.5% concentrations of Semperfresh were effective for delaying ripening of "Ankara" pears. Coated pears had higher color,

firmness, acidity and soluble solid retention. Since 1.0% and 1.5% concentrations affected most of the ripening parameters similarly and many pears treated with 1.5% concentrations showed uneven coloration, 1% is selected as the optimum concentration of Semperfresh for "Ankara" pears.

When the efficiency of Semperfresh was compared to Johnfresh fruit coating, Johnfresh was determined to be the effective coating for reduction of the weight loss. There was no significant difference between Johnfresh coating and Semperfresh in other quality factors. That is why the cost of these coatings should also be considered before deciding the best coating for "Ankara" pears.

ACKNOWLEDGMENT

ASC Celebi Ltd. and SC Johnson provided Semperfresh and Johnfresh coatings.

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EFFECTS OF CARRAGEENANS AND XANTHAN GUM ON THE TEXTURE AND ACCEPTABILITY OF LOW FAT FRANKFURTERS

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Accepted for Publication December 1, 1993

ABSTRACT

The effects of fat level, kappa and iota carrageenans, and xanthan gum on the composition, hydration, textural properties, color, and sensory properties of pork/beef frankfurters were evaluated. Cooking losses and pH were not affected by fat levels or gums. Reheating losses were lower in the high fat products compared to low fat treatments. Iota reduced the water holding capacity of low fat products. Xanthan gum provided the largest increase in color lightness (L) of raw low-fat products and also of cooked products that were stored frozen. Redness (a) increased due to cooking and also due to freezing. Product hardness, springiness, and chewiness were reduced by iota and xanthan gums. All the products were equally liked by the sensory panel.

INTRODUCTION

Since the North American diet is high in fat (Kinsella 1987, 1988), a reduction in calories derived from fat and saturated fat has been recommended by different medical associations (Hudnell *et al.* 1991). In meat products, fat contributes to flavor, texture and mouth feel; therefore fat reduction by itself can significantly lower the acceptability of meat products (Huffman and Egbert 1990). One of the major aspects of lower acceptability is due to an increase in the toughness of the meat product (Paul and Foget 1983; Barbut and Mittal

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Journal of Food Processing and Preservation **18** (1994) 201–216.

1989). There have been attempts to develop low-fat frankfurters by replacing some of the fat with soy proteins (Sofos and Allen 1977), carbohydrate gums (Foegeding and Ramsey 1986) or by modifying the processing conditions (Hand *et al.* 1987). The effects of adding gums such as iota and kappa carrageenans, guar gum, locust bean gum, xanthan gum, methylcellulose, and a mixture of locust bean gum and kappa-carrageenan to low fat meat batters have been reported by Foegeding and Ramsey (1986). Xanthan and guar gums at a 0.2% level significantly affected the texture profile analysis parameters. Increasing the concentration of xanthan gum decreased batter hardness without affecting batter stability. Sensory evaluation indicated that low-fat frankfurters (12% fat) were as acceptable as control frankfurters (27% fat). Claus and Hunt (1991) characterized the sensory and processing characteristics of low-fat (10%), high added-water (30%) bologna formulated with texture-modifying ingredients. Dry ingredients were incorporated (2–5%) and the test bologna were less firm than the high fat (30%) control; however, they were firmer than the low-fat control. Fiber-containing bologna was more grainy in texture and less juicy than the high-fat control. Low-fat bologna was darker than high-fat bologna. Sugar beet pulp fiber and oat fiber resulted in greater cooking losses than the low-fat control. In hamburgers, a more recent approach has been to use a mixture of iota carrageenan and hydrolyzed vegetable protein. This has been found to be successful in the production of low-fat hamburgers for some fast food chains (Egbert *et al.* 1991).

Overall, gums are used by the food industry to enhance various textural characteristics of food products. One group of gums gaining much popularity presently is the carrageenans, which are used for their good water binding ability, texture and low flavor profile. In solution, kappa carrageenan forms a strong rigid opaque gel (FMC 1988), while the addition of potassium increases gel strength but also forms a more brittle gel. On the other hand, iota forms an elastic clear gel, and the addition of calcium results in a more condensed helical structure. Much work has been done on their interactions with dairy proteins; however, not much has been done in the meat area. Barbut and Mittal (1989) examined the rheological and gelation changes resulting from the addition of either iota or kappa carrageenans and potassium/calcium ions on the properties of reduced fat (14.8% fat) meat batters. The highest rigidity modulus (G) value obtained during cooking was observed in the iota treatment, followed by the iota + Ca^{++}, kappa + K^{+}, low fat, and the high fat (27%) treatments. The increase in G observed by the addition of kappa and iota was due to their ability to form thermally reversible gels. The addition of carrageenans further decreased the brittleness of the reduced fat products. Thus, the objectives of this study were to determine the effects of kappa and iota carrageenans and xanthan gum on reduced fat pork/beef frankfurters and to compare them to products prepared with the average fat level currently used by the industry.

MATERIALS AND METHODS

Frankfurter Preparation

Five different pork/beef frankfurters were prepared in two separate trials. Fresh pork and beef lean leg meat and pork back fat were obtained from the University of Guelph abattoir. The postrigor meat was ground through a 9 mm plate to obtain a homogeneous mass prior to chemical analysis of the raw materials (AOAC 1990). The meat and fat were frozen, separately in polyethylene bags and kept frozen for up to 2 weeks at −20C prior to use. The lean meat block (80% pork, 20% beef) was chopped (Schneidemeister, SMK40, Alexanderwerk, Germany) with all the salt (2.5%), at the high speed setting for 1 min, and kept in the bowl chopper for 5 min to allow some time for protein extraction under high salt concentration. Then the pork back-fat, seasoning, cure (1% dextrose, 0.25% white pepper, 0.07% nutmeg, 0.05% sodium erythorbate, and 0.015% sodium nitrite; supplied by Griffith Laboratory, Scarborough, Ontario) and water, were added. In the reduced fat products, water was added to the meat block to substitute for the fat and to come up with the same protein level as in the high fat batter. The three gums used in this experiment (0.5% iota or kappa carrageenan and xanthan, supplied by Davis Germantown, Scarborough, Ontario) were rehydrated (1:10) before adding to the product. The batters were then chopped for an additional 3.5 min; batter temperature did not exceed 13C. The batters were later tumbled (10 G Tabletop Vacuum Tumbler, Lyco, Columbus, WI) for 45 s under vacuum (2000 Pa) to remove small air bubbles. The batters were stuffed into 26 mm cellulose casings (Union Carbide, Chicago IL) and after 1 h storage in a cooler, they were smoked and cooked (drying at 50C for 10 min, application of liquid smoke, heating in steps: 60, 70, and 77C and gradually increasing the relative humidity up to 100% for 5 min at the end when product temperature reached 69C, which occurred within 1.2 h). The frankfurters were then cold showered for 5 min, stored overnight at 3C, peeled the next day, vacuum packaged and stored at 3C.

Quality Evaluation

The textural properties of the cooked products were evaluated using the Textural Profile Analysis (TPA) test (Bourne 1978) employing a universal testing machine (model 4204, Instron Corp., Burlington, Ontario, Canada) equipped with a 1 kN load cell. The center cores of five cooked samples per treatment were cut (2.0 cm in diameter) and compressed twice to 75% of their original height. The cross sectional area of the samples was calculated and values of hardness were expressed as N/cm^2. A cross head speed of 200 mm/min and a chart speed of 20 mm/min were used. The TPA parameters,

namely hardness (first bite, the force required to produce the first compression), cohesiveness (ratio of the area of the second force-displacement curve to the area of the first curve), springiness (distance the sample recovered in height after the first compression, cm), gumminess (hardness × cohesiveness), and chewiness (gumminess × springiness) were computed. Warner Bratzler shear force was measured on five cores (20 mm in diameter) obtained from the cooked samples. The Warner Bratzler cell was attached to the universal testing machine and shearing speed was 100 mm/min. The color of the raw and cooked products was determined by Pacific Scientific Color System (Silver Spring, MD) and expressed as the Hunter "L" (lightness), "a" (redness) and "b" (yellowness). Water holding capacity (WHC) was determined as the ratio of sample (n = 5/treatment) mass after being placed under vacuum of 63.5 kPa at room temperature for 4 h, minus the mass of the dried matter, to the initial sample mass. Free water was determined by pressing (5520 kPa) 0.5 g samples (n = 5/treatment) placed in between filter papers and expressed as the ratio of the "squeezed" water to the initial moisture content of the sample. Cooking loss was the change in mass during cooking divided by the initial mass. Similarly, reheating loss was the change in mass during reheating divided by the unheated mass.

Sensory Analysis

Sensory analysis was performed by graduate students and staff in our Food Science Department. The panelists were experienced in sensory evaluation of various food products. The cooked samples were identified by 3-digit random numbers placed on a 29 cm diameter round plate. The samples were reheated in a covered container in a microwave oven for 30 s and served at 45C. The panelists were seated in individual booths and provided with cold water and unsalted crackers to allow rinsing and cleaning between samples. Evaluation of the attributes were recorded by the panelists on scales typical of descriptive sensory analysis (Stone *et al.* 1974). The semi-structured linear scales on ballots were later coded on a 10 point basis. The panelists were asked to first evaluate the color of the samples and then to taste them. Attributes included color (1 = pale, 10 = dark), tenderness (1 = very tender, 10 = very tough), juiciness (1 = very dry, 10 = very juicy) and overall acceptability (1 = dislike, 10 = like: hedonic scale).

Stress Relaxation Analysis

Stress relaxation testing was performed using the universal testing machine with 1 kN load cell following the procedure described by Mohsenin (1986). The chart speed was set at 3.3 mm/s for the first 60 s and then changed to 0.33 mm/s for the remaining 480 s. The sample was compressed to 50% of its

original height and the force-time curve was recorded. Five measurements were taken for each of the treatments in each trial.

The stress relaxation data have traditionally been described in terms of a discrete linear-Maxwell model (Mohsenin 1986):

$$F(t) = E_o + \sum_{i=1}^{n} E_i \, e^{-\frac{t}{\tau_i}} \quad (1)$$

where F(t), E_0 and E_i are the decaying parameters (force or stress), τ_i relaxation times and t is time. It has been demonstrated (Mohsenin 1986) that a model having two to four Maxwell elements is sufficient to describe experimental data with a high degree of fit. For many foods usually 2–3 terms involving 4–7 constants are sufficient. The experimental data were fitted in a three term model using the method of Mohsenin (1986) and the computer program of Rudra (1987). Thus, E0 is the equilibrium force or stress, E1, E2, and E3 are the elastic moduli of first, second and third elements, and $\tau 1$, $\tau 2$, and $\tau 3$ are the relaxation times representing the viscous components of the Maxwell elements.

Data Analysis

The experiment was designed as a complete randomized block experiment with two replications. Statistical analyses were performed using the Statistical Analysis System (SAS 1988). The General Linear Model ANOVA procedure for the analysis of variance and Duncan's multiple range test for ranking were used.

RESULTS AND DISCUSSION

Composition

Table 1 illustrates the effects of fat level and gums on the composition of raw and cooked pork/beef frankfurters. Cooking resulted in a small change in composition due to small evaporation losses (3.8–4.7%, Table 2). Moisture retention was high and the changes in moisture content amounted to 1–2% (Table 1). The high-fat product was formulated to contain 28% fat. This level was found in a previous survey (Mittal and Barbut 1993) to be representative of a high-fat level in Canadian frankfurters on the market. The reduced fat formulations were targeted to have 50% less fat (14%). Fat was replaced by water (Table 1, see moisture content of raw batters) and gums (0.5%). The fat levels in the cooked products (high and low fat) came close to the targeted fat

TABLE 1.
EFFECTS OF FAT LEVELS AND GUMS ON THE COMPOSITIONS OF RAW AND COOKED PORK/BEEF FRANKFURTERS

Treatment			Chemical Composition (%)							
			Raw				Cooked			
#	Fat	Gum (0.5%)	Moisture	Fat	Protein	Ash	Moisture	Fat	Protein	Ash
1	High	-	54.88^{d}	28.51^{a}	14.31^{a}	2.96^{a}	54.21^{d}	27.12^{a}	14.79^{a}	2.64^{a}
2	Low	-	69.22^{a}	14.25^{b}	14.88^{a}	2.86^{b}	68.60^{ab}	14.28^{b}	15.30^{a}	2.41^{d}
3	Low	Kappa	68.40^{bc}	14.25^{b}	14.36^{a}	2.94^{a}	67.88^{bc}	14.26^{b}	15.12^{a}	2.58^{b}
4	Low	Iota	69.67^{a}	13.90^{b}	14.86^{a}	2.95^{a}	69.00^{a}	13.43^{b}	15.22^{a}	2.47^{c}
5	Low	Xanthan	68.20^{c}	14.49^{b}	14.28^{a}	2.92^{ab}	67.54^{c}	14.18^{b}	15.16^{a}	2.47^{c}

$^{a-d}$ Means (n = 6, both replications) followed by a different letter within the same column are significantly different ($P<0.05$).

levels. The gum-containing treatments showed similar fat levels but somewhat different water contents due to the different moisture binding characteristics of the gums. Since all the formulations were stable, cooking did not change much the absolute amounts of protein, ash and fat in the cooked products. However, their relative amounts were changed due to some moisture evaporation during cooking. There was no appreciable change in protein (14.79–15.30%) and ash (2.41–2.64%) contents among the cooked treatments.

Hydration Properties and Color

The effects of fat level and gums on hydration properties and pH are shown in Table 2. WHC and free (or squeezed) water values were lower in the high-fat product compared to the low-fat treatments. This is basically because fat reduction was achieved by water addition, and hence the reduced fat products contained more moisture (Table 1). Iota carrageenan reduced the WHC of the raw low-fat formulation, but the other gums had no effect. Similarly, the amount of free water in the low-fat products was not affected by adding the gums; however, iota carrageenan treatment had a higher value for free (squeezed) water compared to the product with xanthan gum. Similar results were reported by Barbut and Mittal (1989), in which liquid losses were higher in the low-fat treatments compared to the high-fat batters. Overall, iota was noted to allow more moisture to be released as compared to the kappa treatment.

Cooking losses were not appreciably affected by fat level or gums except the kappa treatment, which showed slightly higher cooking losses. However, reheating losses were lower in the high-fat product compared to the low-fat treatments. This might have been due to the initial lower moisture content in this product. Xanthan gum reduced reheating losses considerably, and resulted in the same loss level as the high-fat product. Both carrageenan gums slightly increased the reheating losses compared to the low-fat control.

pH values were not affected by fat level or gums and showed a small variation (5.94–6.01). This is not surprising because none of this gum is acid/basic in nature.

Table 3 shows the effects of fat level and gums on the color of raw and cooked pork/beef frankfurters. The "L" value (lightness) of the high-fat product was the highest in both the raw and cooked state. That was expected, since a higher proportion of the white fat would produce a lighter product. The addition of the three gums resulted in lighter meat batters in the raw state. The redness values ("a") of the low-fat meat batters were higher compared to the high-fat raw formulation. That was due to the fact that less fat was present and more of the lean meat portion (containing myoglobin) was visible per unit area. It should be mentioned that the lean meat portion was similar in all the formulations (Table 1), so the higher "a" values has to do with some dilution or physical

TABLE 2.
EFFECTS OF FAT LEVELS AND GUMS ON HYDRATION PROPERTIES AND pH OF PORK/BEEF FRANKFURTERS

Treatment			Water Holding Capacity (%)	Free Water (%)	Cooking Loss (%)	Reheating Loss (%)	pH
#	Fat	Gum (0.5%)					
1	High (28%)	--	46.65^{c}	35.70^{c}	3.83^{b}	0.60^{c}	6.01^{a}
2	Low (14%)	--	60.34^{a}	48.15^{ab}	4.25^{b}	2.22^{b}	5.94^{a}
3	Low	Kappa	60.65^{a}	49.18^{ab}	4.74^{a}	2.68^{a}	5.96^{a}
4	Low	Iota	58.99^{b}	51.41^{a}	4.07^{b}	2.81^{a}	5.94^{a}
5	Low	Xanthan	60.37^{a}	44.62^{b}	3.90^{b}	0.89^{c}	5.96^{a}

$^{a-c}$ Means followed by a different letter within the same column are significantly different ($P<0.05$).

TABLE 3.
EFFECTS OF FAT LEVELS AND GUMS ON THE COLOR OF PORK/BEEF FRANKFURTERS

Treatment			Color of Raw Product			Color of Cooked Product			Color of Frozen Product		
#	Fat	Gum (0.5%)	"L"	"a"	"b"	"L"	"a"	"b"	"L"	"a"	"b"
1	High (28%)	--	65.83^{a}	6.33^{c}	14.93^{b}	62.58^{a}	11.18^{c}	12.06^{a}	56.08^{a}	12.13^{c}	11.67^{a}
2	Low (14%)	--	52.66^{e}	7.96^{a}	14.83^{b}	54.61^{b}	12.20^{b}	11.35^{c}	47.38^{e}	13.62^{a}	10.63^{c}
3	Low	Kappa	57.31^{c}	7.37^{b}	15.35^{a}	57.01^{b}	12.01^{b}	11.45^{c}	51.47^{c}	13.45^{a}	11.31^{b}
4	Low	Iota	54.53^{d}	7.80^{ab}	15.13^{ab}	54.99^{b}	12.80^{a}	11.35^{c}	48.24^{d}	13.55^{a}	10.67^{c}
5	Low	Xanthan	59.88^{b}	6.44^{c}	15.17^{ab}	55.39^{b}	11.41^{c}	11.73^{b}	54.78^{b}	12.65^{b}	11.31^{b}

$^{a-c}$ Means followed by a different letter within the same column are significantly different ($P<0.05$).

masking of the lean meat by the fat in the high-fat product. During cooking, "L" values slightly decreased in all the products except in the carrageenan treatments. That might have been due to a general decrease in "L" value (i.e., observed in all the products without carrageenan), which was compensated for by the formation of a gel by the carrageenans.

The increase in "a" values is the result of transforming the red meat pigment, myoglobin, into dinitrosylhemochrome (i.e., the pink cured meat pigment forming by myoglobin and nitrite) during cooking. Also, the low-fat products were more red compared to the high fat product, except for the xanthan gum treatment which also showed a low "a" value in the raw state.

Another color analysis was performed on the cooked and then frozen frankfurters, and was done to simulate the practice of freezing frankfurters either at home (i.e., by consumers, to increase the shelf-life of the product) or sometimes when frankfurters are sold in bulk. As a result of the freezing, the products became darker. That was indicated by lower "L" values, which came down by 6 points (on a 1–100 scale) in all the products except the xanthan gum treatment. The decrease in lightness is probably related to changes in water distribution within the product during freezing. Only the xanthan treatment, which was shown to bind the water better than other gums (i.e, lowest reheating and cooking losses among the low-fat products), did not show a change in "L" as a result of freezing. The redness values only slightly increased due to freezing. Yellowness ("b") was reduced after cooking in all the treatments. It further decreased slightly after freezing and thawing in all the products. The variations in "b" due to treatments for raw, cooked and frozen products were not appreciable.

Textural Properties

Table 4 summarizes the effects of fat level and gums on the textural properties of cooked pork/beef frankfurters. Fat level affected only shear force, which was higher for the low-fat product. This was probably due to the lower amount of fat, which allowed more binding among the meat particles. In the reduced fat products, gum addition resulted in a decrease in hardness compared to the low-fat product without any gum. That again might be explained by reducing the bind among the meat particles by the gums. Foegeding and Ramsey (1986) reported that fat reduction (25–10%) and later kappa and iota addition (0.2%) to the reduced fat formulations did not significantly affect hardness. When they added xanthan gum (0.2%) the results were similar to the results reported here; however when they added 0.5% xanthan, a significant reduction in hardness was observed. Springiness and chewiness were also reduced by iota-carrageenan and xanthan gum addition. Cohesiveness and gumminess were not affected by the gums. Overall, the gums tested here are suitable additives for

TABLE 4.
EFFECTS OF FAT LEVELS AND GUMS ON TEXTURAL PROPERTIES OF COOKED FRANKFURTERS

Treatment			Texture Profile Analysis					Shear Force
#	Fat	Gum (0.5%)	Hardness I (N/cm^2)	Springiness (cm)	Cohesiveness (ratio)	Gumminess (N/cm^2)	Chewiness (N/cm)	(N)
1	High (28%)	-	19.14^{a}	0.34^{ab}	0.17^{a}	3.21^{a}	1.15^{a}	13.07^{c}
2	Low (14%)	-	18.58^{ab}	0.37^{a}	0.17^{a}	3.19^{a}	1.16^{a}	16.10^{a}
3	Low	Kappa	17.74^{bc}	0.34^{ab}	0.18^{a}	3.13^{a}	1.07^{ab}	14.37^{b}
4	Low	Iota	17.07^{cd}	0.31^{bc}	0.17^{a}	2.88^{a}	0.91^{b}	14.86^{b}
5	Low	Xanthan	16.55^{d}	0.29^{c}	0.17^{a}	2.81^{a}	0.85^{b}	12.91^{c}

$^{a-d}$ Means (n = 10, both replications) followed by a different letter within the same column are significantly different ($P<0.05$).

frankfurters. Both of the carrageenans used here were previously found to affect the response of reduced fat meat batters to shear stress even before they went through their solubilization temperatures at around 70C (Rizzotti *et al.* 1983).

Effects of fat level and gums on the viscoelastic properties of cooked pork/beef frankfurters are shown in Table 5. E1, E2, E3, $\tau 1$, and $\tau 2$ were not affected by any of the treatments. It should be mentioned that since the standard deviations of E1 and E2, for the high-fat treatment were high, the differences among the treatment was negligible. Equilibrium modulus (E0) and $\tau 3$ values were lower for all the low-fat treatments with or without gums. Thus, viscoelastic properties were not sensitive to fat levels and gum applications.

Sensory Attributes

Table 6 shows the effects of fat level and gums on the sensory attributes of cooked pork/beef frankfurters. The high-fat product was perceived as the lightest product compared to the low-fat treatments. Similar results were measured objectively for all the low-fat versus the high-fat product (Table 3). Kappa-carrageenan and xanthan gum addition resulted in lighter (more pale) products compared to the low-fat product without gum. These evaluations are in agreement with the objective "L" values obtained for the cooked products. The increase in lightness can be explained by the formation of a gel by the gums. The high-fat product was scored more tender than the low-fat formulation without gums. The gums helped increase tenderness to the same level as the high-fat product; the most was achieved by using iota. Similar results were obtained for the objective textural measurements (Table 4). The panel did not find any significant differences in the degree of juiciness of the various formulations. All the products were found to be acceptable by the panel, indicating that acceptable reduced-fat products (down to 14%) can be manufactured successfully with or without gum addition. However, some beneficial effects of using gums were observed in this study. They include the reduction of toughness in the low-fat products when iota and xanthan gum were added. The resulting values for tenderness were the same as the high-fat control (Table 6). The same was determined, objectively, by the shear force results. The panel evaluating the reduced fat (14%) products tested here did not indicate tenderness to be significantly contributing to overall acceptability. Furthermore, gums like xanthan can be very beneficial in improving water holding ability during cooking and reheating. Overall, the use of certain gums currently on the market or the development of new fat substitutes that can more closely simulate the properties of fat (organoleptic and textural characteristics) would facilitate further developments in this area.

TABLE 5.
EFFECTS OF FAT LEVELS AND GUMS ON THE
VISCOELASTIC PROPERTIES OF COOKED FRANKFURTERS

	Treatment								
#	Fat (%)	Gum (0.5%)	E0 (N)	E1 (N)	E2 (N)	E3 (N)	$\tau 1$ (s)	$\tau 2$ (s)	$\tau 3$(s)
1	High (28%)	--	4.9[a]	12.04[a]	12.63[a]	4.89[a]	210[a]	29[a]	10.2[a]
2	Low (14%)	--	2.9[b]	4.38[a]	4.14[a]	3.97[a]	337[a]	50[a]	2.0[b]
3	Low	Kappa	2.7[b]	2.70[a]	3.69[a]	5.51[a]	494[a]	44[a]	2.2[b]
4	Low	Iota	2.6[b]	3.13[a]	3.84[a]	4.85[a]	404[a]	31[a]	2.3[b]
5	Low	Xanthan	3.2[b]	3.28[a]	3.71[a]	3.53[a]	513[a]	24[a]	2.0[b]

[a-b] Means (n = 10, both replications) followed by a different letter within the same column are significantly different ($P<0.05$).

TABLE 6.
EFFECTS OF FAT LEVELS AND GUMS ON THE SENSORY ATTRIBUTES OF COOKED FRANKFURTERS

Treatment			Sensory Attributes*			
#	Fat	Gum (0.5%)	Color	Tenderness	Juiciness	Overall Acceptability
1	High (28%)	--	5.36^{c}	6.36^{b}	7.56^{a}	9.05^{a}
2	Low (14%)	--	8.46^{a}	7.97^{a}	6.53^{a}	8.23^{a}
3	Low	Kappa	6.63^{b}	7.49^{ab}	7.21^{a}	7.01^{a}
4	Low	Iota	8.15^{a}	6.13^{b}	6.55^{a}	8.08^{a}
5	Low	Xanthan	5.47^{bc}	6.46^{ab}	7.42^{a}	8.03^{a}

* Scales: Color, 1 = pale, 10 = dark; tenderness, 1 = very tender, 10 = very tough; juiciness, 1 = very dry, 10 = very juicy; overall acceptability, 1 = dislike, 10 = like.

$^{a-d}$ Means (n = 24) followed by a different letter within the same column are significantly different ($P<0.05$).

Authors are thankful to the Ontario Pork Producers Marketing Board for financial support, and to Mr. Eden Huang, research associate, for assistance in data collection and experimentation.

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BIOGAS AS A GRAIN PROTECTANT AGAINST *CALLOSOBRUCHUS CHINENSIS* (BRUCHIDAE, COLEOPTERA)

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Accepted for Publication December 10, 1993

ABSTRACT

The effectiveness of biogas as a grain protectant against Callosobruchus chinensis *was evaluated. Biogas was found to have a quicker knock-down effect and resulted in complete mortality of adults in 16 h. Exposure of different life-stages of* C. chinensis *for five days using air-tight containers indicated that biogas was effective on all life-stages except the pupal stage. Continuous exposure of infested grains to a high concentration of biogas significantly reduced the level of infestation and loss in grain mass. The results of the study and the methodology developed are expected to meet the grain storage needs of farmers with small land-holdings.*

INTRODUCTION

In India due to the breaking up of joint families that once cultivated larger areas, a great majority of farmers have operational holdings of less than 2.0 ha

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Journal of Food Processing and Preservation **18** (1994) 217–227.

(marginal farmers) (Agarwal 1980; Datta and Sundaram 1978; Dewett and Verma 1978). On such lands, agriculture has been mainly for subsistence, which leaves the farmers hardly any marketable surplus (Datta and Sundaram 1978). Although this may be true with most of the crops, it may not be so with pulses, as most of the produce is marketed immediately after threshing. Pulses are the main source of protein among the vegetarians in India (Kurian 1981; Rita 1986; Vijayaraghavan 1981) and farmers face difficulty in storing pulses safely. Stored pulses are severely infested by bruchids (Bruchidae; Coleoptera), especially *Callosobruchus chinensis*, which shows specificity in attacking pulses (Jotwani *et al.* 1967; Krishnamurthy and Rao 1950; Rao and Majumdar 1964). This problem is further complicated, as the infestation by *C. chinensis* is invariably carried over from field to storage (Sangappa and Balaraju 1977; Usman 1957).

Though the conventional fumigants like ethylene dibromide and aluminium phosphide have proved to be very effective in the control of stored grain insects (Kashi 1981a,b; Muthu *et al.* 1954; Rajendran and Muthu 1985), there are many disadvantages in using them. They are known to have residual effects, pose handling and health hazards, and insects may develop resistance to them (Ames *et al.* 1980; Anon. 1965; Bond 1973, 1984; Kumar *et al.* 1981; Monro 1964; Monro *et al.* 1972; Price and Dance 1983). Moreover, fumigants are not locally produced and represent additional input cost for the farm community.

As a consequence, there has been a growing need for a safe and nontoxic alternative grain protectant. Efforts in this direction have brought forth a totally new concept of grain protection by way of modified atmosphere storage (MAS) of grains using carbon dioxide (CO_2) the most potential agent (Paster *et al.* 1991; Shejbal and Boislambert 1988).

In the recent past, biogas with methane and carbon dioxide as its chief constituents has emerged out as an important alternative source of energy (Price and Cheremisinoff 1981). In India, since its introduction in 1951, the installation of biogas plants have gained considerable popularity among farmers (Moulik 1982). Biogas is cheaper to produce and is readily available on farms (Sathianathan 1978). This paper explores the possibility of using biogas (with an underlying concept of MAS) as an alternative grain protectant against *C. chinensis* in rural areas and evaluates a viable technology that can be within the reach of a marginal farmer.

MATERIALS AND METHODS

The study was carried out in three stages. The preliminary comparative study was carried out in the laboratory and subsequent trials were conducted on farms with a biogas plant. The preliminary study with CO_2 and biogas utilized 500-ml glass jars filled with redgram *Cajanus cajan* grain. The jars were

provided with air-tight rubber stoppers with two self-sealing holes. Two plastic tubes (0.6 mm outer diameter) of suitable lengths introduced through these holes served as inlet for gas and outlets for air and excess gas, respectively. Carbon dioxide was drawn from a cylinder, while biogas was tapped from a biogas plant available on the farm.

A known number of freshly emerged adults of *C. chinensis* (less than 24 h old) reared in the laboratory were enclosed in a 50 × 50mm nylon gauze (100 mesh/cm^2) along with a few redgram grains. Two such pockets were placed in a jar, one each close to the top and bottom of the grain medium. About 15 L/min of CO_2 or biogas, as required, was released into the jar for 2 min to ensure complete saturation of the chamber. Immediately after the release of the gas, both the inlet and outlet tubes were pulled out and the mouth of the bottle with the airtight stopper was dipped in molten paraffin wax to ensure air-tightness. The adults of *C. chinensis* were exposed to the gases for periods varying from 1 to 25 h separately. Each treatment was replicated thrice.

At the end of each exposure period, the insects in each replicate were removed and transferred into separate glass vials with lids. Surviving and dead adult *C. chinensis* were counted after 24 h to exclude anesthetic effects of the gases. After averaging the values of top and bottom pockets of each replicate, the treatment means were calculated and expressed as percent adult survival.

Subsequent confirmatory trials were conducted using 200-L rotomoulded linear low density polyethylene (LLDPE) water-storage tanks. These bins (tanks) were rendered airtight by providing a gasket below the screw-top lid. Two tap-valves, one at top and the other at the bottom, were fitted to the side of the bin to serve as inlet and outlets. The bins were filled with redgram grains.

Infestation-free redgram grains held in polythene containers in 100-g portions were force infested with the bruchids. For this purpose, freshly emerged adults of *C. chinensis* raised in mass cultures at 30C and 70% RH on redgram, were used to ensure oviposition on all the grains. Based on the maturation duration of each lifestage of *C. chinensis* [egg stage: 3 days after oviposition (DAO); larvae stage: 10 DAO; pupa stage: 20 DAO; adults: 28 DAO] (Prabhakara 1979; Raina 1970), different developmental stages were exposed to biogas.

The infested grains (lOO g) containing a known stage of *C. chinensis* were enclosed in a 120 × 120 mm nylon gauze (100 mesh/cm^2) and tied with rubber bands. Pockets were placed close to the top and bottom of the grain medium. All treatments were replicated thrice and a set exposed to ambient air was used as a control.

Biogas was released into the grain filled bins containing a known life-stage of *C. chinensis* for a period of 2 min at the rate of 15–20 L/min and both tap-valves were closed immediately. The concentration of biogas inside the treated bin was monitored indirectly by measuring the concentration of CO_2 with

an Orsat gas analyzer. Carbon dioxide concentration inside the treated bin was measured at the beginning and the end of experimentation with each developmental stage. The CO_2 concentration in the bin during the experimental period was 23.0 ± 1.8%. The egg stage was exposed to biogas for four days and the rest were exposed for 5 days each.

The possible effects of biogas, if any, on the grains were determined by germination tests. Germination was determined for both biogas treated grains and an untreated control. The test was conducted by placing 100 sound grains between two layers of thick wet paper (Anon. 1985). Four replicates were prepared for both the treatments and incubated in an environmental chamber for a period of five days at 25C and 90–95% relative humidity and percent grain germination was estimated later.

The treated grains in different replicates of each stage and treatment were transferred to 200-ml polythene containers and were maintained in the laboratory until the emergence of adults. The effectiveness of biogas on each life stage was expressed as the number of adults emerged from treated samples and was compared with the control.

In the third and last trial with uninfested prefumigated redgram, into each of the LLDPE bins filled with grains, 200 pairs of freshly emerged *C. chinensis* were released. Biogas was released into the treatment bin for 5 min at a flow rate of 15 L/min at 10-day intervals for two months. Immediately before and after the release, the concentration of biogas within the treated bin was measured indirectly by determining the level of CO_2. Another bin without biogas treatment was used as a control. The average CO_2 percentage concentration in the bin during experimentation was 29.5 ± 8.7 (Fig. 1).

At the end of the experimental period, replicated 1000 grain samples were collected from the top and bottom levels of the bins. The percent mass loss in grains and infestation level due to bruchid infestation were estimated as follows:

$$\% \text{ Mass loss in grains due to infestation: } (C \times G)/B \times 100$$

$$\% \text{ Damaged grains (infestation level): } (G/A) \times 100$$

Where

A = sample size = 1000 grains;
B = mass of the sample;
C = loss in mass per grain due to infestation (D–E);
D = per grain mass of undamaged grains (H/F);
E = per grain mass of damaged grain (I/G);
F = number of undamaged grains in the sample;
G = number of damaged grains;
H = mass of undamaged grains;
I = mass of undamaged grains.

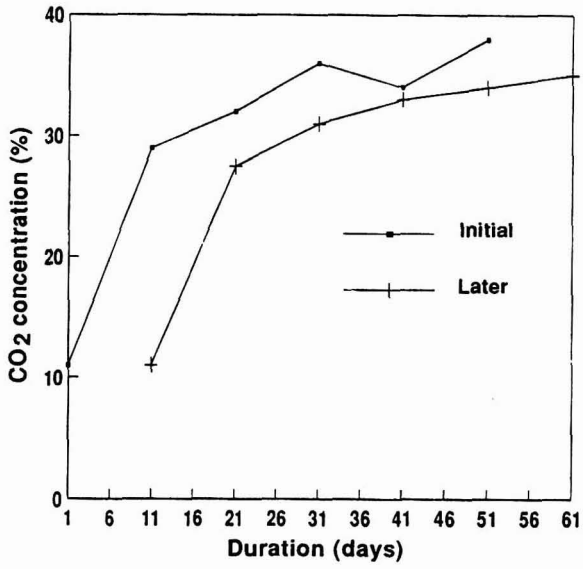

FIG. 1. LEVEL OF CARBON DIOXIDE INSIDE THE BIN

Correlation, regression, t test, chi-square and analysis of variance (for factorial experiment) were carried out wherever necessary as per Sundararaj *et al.* (1972).

RESULTS AND DISCUSSION

In the laboratory trials, though both CO_2 and biogas were effective on *C. chinensis* adults; they differed significantly with respect to their effectiveness ($t = 8.999$; $d.f. = 24$; $p < 0.05$). Adult survival decreased linearly with time when CO_2 was used, whereas it exhibited an inverse logarithmic function in the case of biogas (Fig. 2; CO_2: $r = -0.967$; biogas: $r = -0.950$; $n = 25$; $p < 0.001$).

Complete mortality of adults was observed after 22 h of exposure to CO_2 and 16 h in the case of biogas. Thus, biogas showed a quicker knockdown effect on *C. chinensis* adults, confirming the observations of Palaniswamy and Dakshinamurthy (1986) on the effectiveness of biogas as a stored grain protectant.

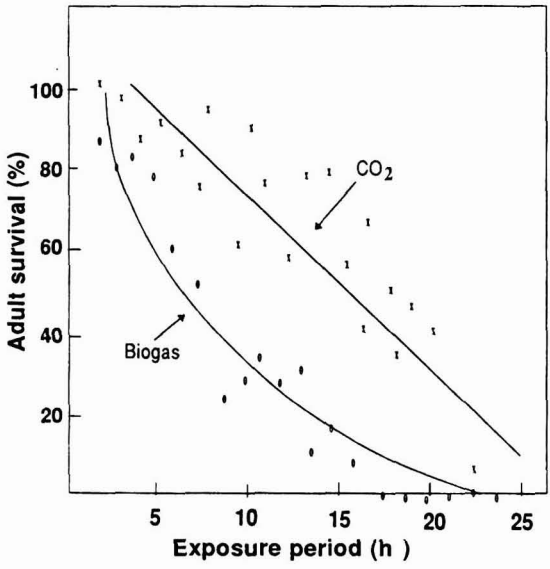

FIG. 2. EFFECTIVENESS OF CARBON DIOXIDE AND BIOGAS ON *C. Chinensis*
Regression equations for biogas: y = 104.7 − 3.4* log × and CO_2; y = 110.0 − 4.1* x.

The analysis of variance of the first on-farm trial, on the effectiveness of biogas on different developmental stages of *C. chinensis*, is given in Table 1. Highly significant differences were observed in the survival between the different developmental stages and in the effects of biogas and ambient air (control bins) ($p < 0.001$). Also, significant interactions were observed for: (a) developmental stages × treatment, and (b) bin position × treatment (Table 1). There were no significant differences in adult emergence due to bin position and no interaction between bin position and developmental stages of the insect. Figure 3 shows that biogas is effective on all developmental stages of *C. chinensis* except the pupal stage. In holometabolous insects, the pupal stage is quiescent (Imms 1963) and many insect species are known to survive for long periods even in the complete absence of air. This is because the extra-metabolic activity during this stage ceases and the basal metabolism continues anaerobically (Pruthi 1969; Wigglesworth 1953). The pupal stage of *C. chinensis* extends to about eight days (Prabhakara 1979; Raina 1970) and this period should be taken into account in application of biogas to stored grain.

However, the resistance of the pupal stage to biogas was overcome by repeated release of biogas over a two month period (Table 2). Even if the pupae are resistant to biogas atmosphere, future infestation of the grain by the adults

TABLE 1.
ANALYSIS OF VARIANCE FOR FIRST ON-FARM TRIAL WITH BIOGAS ON *C. chinensis*

Source	d.f.	S.S.	M.S.S.	F	Prob>F
Developmental stages A3	2	59417.14	86472.38	6.48	P < 0.001
Bin level ..B 1		38930.65	38930.65	2.92	
A * B	3	10462.59	3487.53	0.26	
Control Vs Treated ....C 1		821894.65	821894.65	61.62	P < 0.001
A * C	3	124112.65	41370.88	3.10	P < 0.05
B * C	1	93192.55	93192.55	6.99	P < 0.05
A * B * C	3	19746.71	6582.24	0.49	
Error C	32	426895.00	13339.06		

emerging subsequently will be prevented by successive releases of biogas. The results of the second on-farm trial confirm this. Significantly, the infestation by bruchids and the loss of grain mass due to infestation were almost negligible in bins treated with biogas as compared to the control (Table 2; $X^2 = 43.56$; d.f. = 1; $p < 0.05$).

No infestation of grain was observed in the bottom of both treated and control bins. Lack of infestation in the lower layer is due to the habit of the insect to congregate at the top layers of the grain bin where the resource for oviposition is abundant. Also, the bruchids show poor penetration ability in the grain medium, this ability being dependent on grain size (Rao and Majumdar 1964).

The germination test indicated no significant difference between germination of grains treated by biogas ($89 \pm 0.71\%$) and untreated ($91.0 \pm 1.23\%$) grains ($X^2 = 0.01$; d.f. = 1; $p = 0.05$).

The foregoing discussion clearly establishes the effectiveness of biogas against bruchids. The point to be noted here is that biogas is not a toxicant. The rationale behind the methodology is only to modify the atmosphere within the airtight storage bin with biogas. Within such an oxygen free condition further multiplication of the insect is prevented.

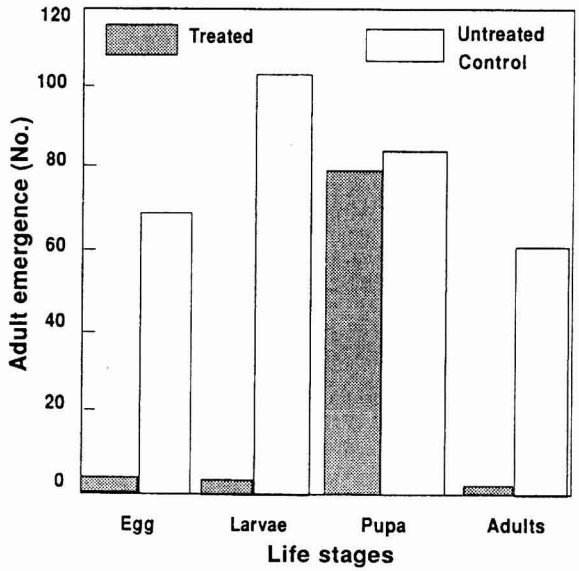

FIG. 3. EFFECT OF BIOGAS ON DIFFERENT DEVELOPMENTAL STAGES OF *C. Chinensis*

TABLE 2.
INFESTATION LEVEL AND MASS LOSS IN GRAIN DUE TO *C.chinensis*

	Untreated (Control)	Treated (Biogas)
% Infestation	5	nil
% Mass loss	17	1

CONCLUSIONS

The study shows the effectiveness of biogas as a grain protectant in airtight bins. Since the amount of biogas required for protection of small quantities of grain does not demand additional capital investment on the part of the farmers, surplus biogas produced on farm can be used for grain protection after domestic needs are met. Frequent release of surplus biogas when sufficient pressure is available should help marginal farmers prevent stored-grain insect problems.

ACKNOWLEDGMENTS

This study was funded by Indian Council of Agricultural Research (ICAR) New Delhi, India, under All India Coordinated Research Project on Post Harvest Technology. The encouragement given by Dr. Anwar Alam, Assistant Director General (Engg.), ICAR, New Delhi, Dr.R.P. Kachru, Project Coordinator, Central Institute of Agricultural Engineering, Bhopal and the Director of Research, University of Agricultural Sciences, Bangalore is gratefully acknowledged. We thank Valérie Orsat for reading through the manuscript, Peter Alvo for editorial input and the two independent reviewers for their useful comments and suggestions for improvements.

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COMPOSITION, FUNCTIONALITY AND SOME CHEMICAL AND PHYSICAL PROPERTIES OF EIGHT COMMERCIAL FULL-FAT SOY FLOURS

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Accepted for Publication January 14, 1994

ABSTRACT

Eight full-fat soy flours representing both wet and dry processing methods were evaluated for composition, functionality, and chemical and physical properties including proximate analysis, fatty acids, mineral profiles, presence of trypsin inhibitors (TI), protein dispersibility index (PDI), water and fat absorption, emulsifying capacity, foaming capacity and stability, viscosity, particle size, bulk density and shelf stability. All flours had acceptable TI levels. Functionality data and PDI results suggested potential ingredient applications. The flours were reasonably stable to lipid oxidation as indicated by peroxide values and organoleptic evaluation. Microscopy and laser particle sizing failed to support some manufacturers' claims of ultrafine particle size. Beverages made from all flours produced the so-called "catch throat" sensation.

INTRODUCTION

More than 95% of the U.S. domestic utilization of soybeans (*Glycine max*) is for production of oil, with the defatted meal being used mainly as animal feed. A small percentage of defatted soybean meal (less than 5%) is processed and used as ingredients in human foods in the form of grits, flours, flakes, concentrates and isolates. Full-fat soy flours are produced by a number of companies for a number of intended uses including reconstitution for use as soymilk, for tofu production and as ingredients in formulated foods such as

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Journal of Food Processing and Preservation **18** (1994) 229–252.

baked goods, pasta, seasonings, gravies, soups, dressings, confections, beverages, frozen desserts and cheese analogs (Anon. 1989a,b; 1991). Full-fat soy flours are particularly attractive as replacements for costlier or cholesterol-containing ingredients from animal sources, e.g., milk, eggs and animal fats (Duxbury 1987).

The behavior of full-fat soy flours in food systems depends on their functional properties. Important among these are their solubility/dispersibility in aqueous systems, emulsifying properties, water and fat absorption capacity and viscosity of aqueous dispersions. Their usefulness is also influenced by the bulk density, particle size distribution, chemical composition and shelf stability of the dry flours.

Functionality is affected to a degree by soybean variety, growing environment and other agronomic factors. However, since processors tend to obtain raw soybeans in the market as blends and mixtures of several varieties, these factors are difficult to isolate in the commercial products. It is possible to compare full-fat flours on the basis of the particular processes used in their manufacture.

Full-fat soy flours can be produced by either a wet or a dry process and possibly, by a combination of the two. In the wet processes, whole soybeans are thoroughly washed, soaked (usually overnight), drained and comminuted in fresh water to produce a slurry or soymilk. The soaking step may be omitted, as for example in the Oberg Process (Oberg 1978), wherein dry soybeans are ground in an atmosphere of live steam, thus affecting a savings in time and equipment and also inactivating the lipoxygenase enzymes responsible for inducing beany flavor. The comminuted material is filtered to remove insoluble solids, called okara, which consists of the hulls or seedcoats as well as cell walls and other insolubles.

In some processes, the okara is finely ground in special mills and added back to the soymilk. The soymilk can be subjected to vacuum treatment to remove volatile off-flavors or to pH adjustment and other manipulations. It is generally concentrated to about 20% solids and spray-dried (Shurtleff and Aoyagi 1979); at higher concentrations gelation may occur, making spray-drying impossible.

In the dry processes, cleaned soybeans are heated (80–165C or higher) to loosen the seedcoats and to inactivate antinutritional factors and enzymes. A cracking step may be employed to assist in hull removal. As in the case of wet processes, the hulls may be finely ground and added back. The cracked cotyledons are ground in an appropriate mill (Smith and Circle 1972; Snyder and Kwon 1987). Other variations of the wet and dry processes exist, some of which are proprietary in nature.

This study was undertaken to compare the composition, functionality, shelf stability and physical properties of eight full-fat soy flours produced by five different manufacturers using both wet and dry processes.

MATERIALS AND METHODS

Full-Fat Soy Flours

Eight different full-fat soybean products were obtained from five different manufacturers. The manufacturing processes differed, which could produce differences in product composition, functionality, shelf stability and physical properties. Although not all details of the processes are known due to the proprietary nature of some of them, the main features and manufacturers' claims of each product are described in Tables 1 and 2.

TABLE 1.
MAIN PROCESSING FEATURES AND MANUFACTURERS' CLAIMS FOR 3 WET-PROCESS FULL-FAT SOY FLOURS

Product	Other Process Features	Manufacturers' Claims
A	• Okara removed • Vac. deodorized • Emulsifier added • Spray-dried	• Fat encapsulated • Protein solubility
B-1	• Hot grind (Oberg) • Vac. deodorized • pH↑9.0↓7.0 • Spray-dried	• High fiber • Fat encapsulated
B-2	• Same as B-1, but okara removed	• Low fiber • Fat encapsulated

Product A was an "instant tofu mix" (Lot 19, 6/15/90) manufactured in Japan. It was made by the so-called Proton Method (Anon. 1963). Soybeans are soaked and processed to produce soymilk; the okara is removed and excluded. An emulsifier (unspecified) is added and the soymilk is deodorized, pasteurized at 90–100C for 1 min, concentrated under reduced pressure at 45C and spray-dried at inlet and exit air temperatures of about 160C and 90C, respectively. The powder is packaged in foil pouches and nitrogen flushed. The manufacturer promotes the product on the basis of the minimal heat treatment, added emulsifier, deodorization and nitrogen-flush packaging that provide good solubility, shelf stability and relatively bland flavor.

Product B was made by a modified Oberg Process (Oberg 1978). Whole, unsoaked beans are ground in an atmosphere of live steam. The hulls and okara

TABLE 2.
MAIN PROCESSING FEATURES AND MANUFACTURERS' CLAIMS
FOR 5 DRY-PROCESS FULL-FAT SOY FLOURS

Product	Other Process Features	Manufacturers' Claims
C-1	• Remove seedcoats • Heat to 120 C in fluidized bed • Grind to flour	• Enzyme active • Small particles
C-2	• Same as C-1, but heat to 165 C	• Enzyme inactive • Small particles
D-1	• Heat in ceramic bead heat exchanger • Grind to flour	• High fiber • Good shelf stability
D-2	• Remove seedcoats • Same as D-1	• Low fiber • Good shelf stability
E	• Highly proprietary	• Superfine particles

are ground finely in a colloid mill and returned to the soymilk to produce a high-fiber product (B-l) or removed to produce a low-fiber product (B-2). Soymilk is adjusted to pH 9.5 with NaOH, deodorized, acidified to pH 7.0 with HCl, concentrated to about 20% solids and spray dried (Oberg 1978; Anon. 1989a,b). The entire process requires a total of only 7 min from grinding to spray drying (Honer 1986). The product has been promoted on the basis of bland flavor and encapsulation of fat within the protein/carbohydrate matrix to give a shelf stability in excess of 10 months.

Product C was produced in so-called enzyme-active (C-1) and enzyme-inactive (C-2) forms (Gavin and Wettstein 1990). In the former, whole soybeans are preheated briefly at 120C in a fluidized bed whereas in the latter, preheating is carried out similarly at 165C. The preheated beans are cracked, dehulled, ground in a hammermill and then in a micromill system. The enzyme-active flour is intended for use as an ingredient in products whose manufacture involves further heating or bleaching (e.g., baked goods). The enzyme-inactive flour would be used in products that might receive little or no additional heating, (e.g., frozen desserts). The products are promoted as having excellent protein and fat solubility due to their small particle size (82% smaller than 30 μm; 99% smaller than 100 μm) and complete destruction of cellular structure (Gavin and

Wettstein 1990). It is also claimed that stability to lipid autoxidation is very high because the process does not destroy naturally occurring tocopherols.

Product D was produced by a patented process (Benson 1979; Duxbury 1987) whereby whole soybeans are dry-roasted by passing them through a bed of electrically-heated ceramic beads and then ground in a hammermill. The manufacturer claims that the process inactivates lipoxygenase and antinutritional factors without affecting tocopherols, resulting in a safe, mildly roasted, nutritive product with superior keeping quality over 12 months (Anon. 1986). A high-fiber product (D-l) is produced from whole soybeans, and a lower-fiber product (D-2) is produced using a dry dehulling step.

Product E was described by its Japanese manufacturer as a "superfine" full-fat flour produced by a dry milling process from dehulled/degermed soybeans. It is claimed that although okara is included, the very fine particle size (10–30 μm) eliminates the "dry throat" or "catch throat" sensation experienced when drinking other reconstituted okara-containing products (Anon. 1988). The flour is nitrogen-packed to ensure shelf stability. The process is not patented, but is proprietary.

Chemical Analysis

Proximate Analysis. The products were analyzed in triplicate for their moisture content (AOAC 14.004) and, on dry basis, for crude protein as N × 6.25 (AOAC 2.057), crude fat by Soxhlet (AACC 30-26), ash (AACC 08-16) (AOAC 1984; AACC 1976) and dietary fiber (Prosky *et al.* 1988).

Mineral Analysis. Duplicate 1-g samples of each product were weighed into tared 20-ml glass crucibles and dry-ashed at 485C for 12 h. The ash was taken up in 5 ml of 20% HCl; after 20 min, an additional 5 ml of water was added. These solutions were analyzed for Ca, Mg, Na, K, P, Fe, Al, Mn, Cu, Zn, B, Cd, Cr, Ni and Pb by inductively coupled plasma analysis in an ARL 3560-AES instrument (ARL Applied Research Laboratories, S.A., Ecublens, Switzerland). A multielement standard was used to recalibrate the instrument after analyzing each set of 10 samples. Results were expressed as mg of each element per 100 g of soy flour.

Fatty Acid Profiles. The Soxhlet fat extracts were analyzed for their fatty acid profiles. The solvent (petroleum ether) was removed by a rotary evaporator with a water bath temperature of 37C. The methyl ester derivatives were prepared according to Einig and Ackman (1987), with the exception of using 200 mg of oil extract instead of the suggested 20 mg. The fatty acid derivatives were stored at 4C in amber crimp vials and were analyzed within a week with a Hewlett Packard 5890 gas chromatograph with a flame ionization

detector (FID) and Hewlett Packard 7673A automatic injector (Palo Alto, CA). The carrier gas was helium and the samples were injected in split mode. The column was a DB 23 (J. and W. Scientific, Folsom, CA) with the dimensions of 30 m × 0.32 mm ID and 0.25 μm film thickness. The initial column temperature of 40C was increased at 15C/min to 160C, then at 5C/min to a final temperature of 220C. The length of each run was 27 min.

Trypsin Inhibitor Activity. The method used (Liu and Markakis 1989) is an improved colorimetric method using the substrate benzoyl-DL-arginine-p-nitroanilide hydrochloride (BAPA). The BAPA, porcine trypsin and Tris buffer (preset crystals) were obtained from Sigma Chemical Co., St. Louis, MO. A raw soybean reference, cultivar Hardin, was ground in a Micro-mill (TechniLab Instruments Inc., Pequannock, NJ). All samples were screened to pass a 50-mesh sieve and 0.5 g of each was suspended in 50 ml of distilled water in a 250-ml Erlenmeyer flask. The suspensions were shaken for 30 min in a G10 gyratory mechanical shaker (New Brunswick Scientific, New Brunswick, NJ) at 200 rpm. A 10-ml portion of the shaken suspension was transferred to a 30-ml test tube and destabilized by adding 10 ml of the assay buffer solution (50 mM Tris buffer, pH 8.2). This mixture was then shaken with a vortex mixer (Scientific Products Deluxe Mixer, setting at 3.0) for 2 min before filtering through Whatman No. 2 filter paper. Assuming that 0.5 g would cause complete inhibition, the filtrate (extract) was further diluted with distilled water to the point where 1 ml would cause 30–70% trypsin inhibition (TI) and this volume was recorded. A suitable final concentration for raw soybean samples was 0.1 mg of dry sample per ml, while the final concentration for processed samples was 1.5 mg of dry sample per ml. The filtrate was then assayed for TIA as outlined in Table 3, which shows the sequence by which the BAPA, the sample filtrate, the trypsin enzyme solution and acetic acid were combined at 37C and the concentration and volume of each. Exactly 10 min after adding the trypsin solution, the reaction was stopped by injecting 0.5 ml of 30% acetic acid with a 1-ml syringe. Sample absorbance (A^s410) was read in a Bausch and Lomb Spectronic 20 spectrophotometer (Rochester, NY) at 410 nm. A reference reading at 410 nm (A^r410) was obtained after reacting 1 ml of distilled water with BAPA, trypsin and acetic acid.

Trypsin inhibitory activity (TIA) was calculated as follows. A trypsin unit is defined as an A_{410} increase of 0.01 under the conditions of the assay. TIA is expressed in trypsin units inhibited (TUI) per mg of dry sample.

$$\text{TUI/mg sample} = \frac{[(A^r410 - A^s410) \times 100] \times \text{ml diluted extract}}{(\text{mg sample/ml diluted extract})}$$

TABLE 3.
PROCEDURE FOR ASSAYING TRYPSIN INHIBITOR ACTIVITY

Mixing Sequence	Reactant	Concentration in working solution	Volume (ml)
1st	BAPA	0.92 mM	2.0
2nd	Sample filtrate	30-70% TI	1.0
3rd	Trypsin	16 μg/ml	0.5
4th	Acetic acid	30%	0.5
Total assay volume			4.0

Liu and Markakis, 1989.

Functionality

Protein Dispersibility Index (PDI). PDI analysis (Ba 10-65, AOCS 1990) was conducted by dispersing duplicate 20-g portions of each sample in 300 ml of distilled water at 25 ± 1C. The dispersions were blended in a Waring Blendor cup by a Hamilton Beach Drinkmaster blender (Hamilton Beach/Proctor Silex, Inc., Glen Allen, VA) for 10 min at 8,500 rpm, poured into a 600-ml beaker and allowed to settle for 5 min. The upper layer of liquid was decanted into a 50-ml glass centrifuge tube and centrifuged for 10 min at 2900 rpm. The protein content was determined in 250 mg of supernatant (% water dispersible protein) and 250 mg of the original soy flour (% total protein) using a LECO FP-228 combustion analyzer (St. Joseph, MI). Two quadruplicate determinations were run for water dispersible protein and total protein for each product. The oxygen flow rate was 6 L/min. Two separate reference standards, EDTA (9.59% nitrogen) and a 49% protein defatted soy flour, were analyzed after every 10 samples. The LECO instrument releases nitrogen from the sample by combustion in pure oxygen at high temperature. The freed nitrogen is measured by a thermal conductivity detector and converted to percent protein by the factor 6.25 (Sweeney 1989). The % PDI was calculated as follows:

$$\% \text{ PDI} = \frac{\% \text{ water dispersible protein}}{\% \text{ total protein}} \times 100$$

Water Absorption. Water absorption was measured by a modification of the method of Wang and Kinsella (1976). Water (10 ml) was added to 0.5 g of protein (ca 1.05–1.36 g flour) in a 13-ml Sarstedt graduated plastic test tube. The mixture was sonicated using a Branson Sonfier Model S110 (Branson Ultrasonics Corp., Danbury, CT) for 30 s at an output setting of 5 to disperse the sample. The mixture was held at 24C for 30 min, and then centrifuged at 2000 rpm for 25 min in a IEC International Centrifuge Model UV (International

Equipment Co., Needham Heights, MA). The volume of free water was measured and the retained water was computed and reported as ml of water (± 0.1 ml) absorbed per g of soy flour.

Fat Absorption. Fat (oil) absorption was measured by a modification combining the methods of Wang and Kinsella (1976) and Lin *et al.* (1974). A 3-ml portion of peanut oil (Planters) was added to 0.5 g of protein (ca 1.05–1.36 g powder) in a 13-ml Sarstedt graduated plastic test tube. The contents were sonicated for 1 min using a Branson Sonifier (model S110) at an output setting of 5 to disperse the sample. After holding at 24C for 30 min, the tube was centrifuged at 2000 rpm for 25 min using an IEC International Centrifuge Model UV. The volume of free oil was measured and the oil retained in the flour pellet was expressed as ml absorbed (± 0.1 ml) per g of flour.

Emulsifying Activity. Triplicate weighed portions of soy flour containing 0.12 g protein were each dispersed and hydrated in 3 ml of 0.1M (pH 8.0) potassium phosphate monobasic buffer in a 15-ml glass vial for 30 min. After the addition of 3 ml peanut oil (Planters), the dispersion was emulsified by a Branson Sonifier, Model S110 (output control setting of 5) for 30 s. The vial was then cooled by immersion in a 15C water bath for 5 min. The emulsion was diluted with 10 ml of the same buffer containing 0.1% sodium dodecyl sulfate (SDS) and mixed well by inverting the bottle gently 3 times; then, 0.05 ml of the diluted emulsion was further diluted with 10 ml of the same buffer containing 0.1% SDS. With the buffer containing 0.1% SDS as the reference blank, the absorbance of duplicate aliquots of each of the triplicate emulsions was measured at 500 nm in a Bausch and Lomb Spectronic 20 spectrophotometer. The emulsifying capacity was expressed as turbidity/g flour (dry basis). The equation for converting absorbance to turbidity is:

$$T = 2.303 \times A/1$$

where T is turbidity, A is absorbance, and 1 is the pathlength of the cuvette in cm (Pearce and Kinsella 1978).

Foaming Properties. Foaming capacity and stability were each determined in duplicate. The method of Coffman and Garcia (1977) was modified by increasing the sample size from 1 to 2 g to compensate for the lower protein content of these full-fat flours. A 2-g sample in 100 ml of distilled water was whipped for 5 min in a 10-speed Osterizer blender and poured into a 250-ml graduated cylinder. Foaming capacity was reported as the percent increase in volume due to whipping:

$$\% \text{ Volume increase} = \frac{\text{whipped volume} - \text{original volume} \times 100}{\text{original volume}}$$

The total volume (ml) was noted at intervals of 1, 20, 40, 60, 80, 100 and 120 min to determine foam stability.

Apparent Viscosity. Triplicate soy flour suspensions (45.0 ± 0.05 g/255 ml distilled water) were made up in 500-ml pyrex beakers and mixed by a Servodyne mixer head (2-in. diameter system, with 3 circular blades at 45° angles, Model 50,000-30) at 800 rpm using a Servodyne mix controller (Model 50,000-00) for 20 s followed by a 5 min hydration period. Each mixture was poured into a 400-ml tall beaker and the viscosity was determined at 23C and 60 rpm with a Brookfield Model LV digital viscometer (Brookfield Engineering Laboratories, Inc., Stoughton, MA) equipped with a number 1 or number 2 disc spindle. Viscosity readings were taken after the spindle had spun for exactly 10 s.

Shelf Stability

Peroxide Values. Six of the flours (A, B-2, C-1, C-2, D-2, E) were stored in duplicate in sealed Kerr 2-qt jars with a 2-in. headspace at 37C and 23C. The samples were exposed to air once a month and peroxides were determined at 0, 2, 4, 6, 8, 10 and 12 months of storage using the official method Cd 8-53 (AOCS 1973). These samples were also subjected to sensory evaluation at each of these intervals.

Sensory Evaluation. Three paired comparison tests of the six flours were performed by 20 untrained panelists to determine the group recognition level of flavor change over a 10-month period. A fresh sample of each flour was paired with the aged version held for 6, 8 or 10 months at 37C. An obviously oxidized and a fresh flour served as references. All flours were dispersed at 10% solids concentration in bottled spring water (Glenwood Inglewood, Minneapolis, MN). The order of test sample presentation to panelists was randomized. The samples were presented in plastic cups in 20-ml aliquots. The 3 pairs of samples for each flour were presented in order of their increasing age from 6 to 10 months. Samples were presented at room temperature and evaluated in individual sensory booths under fluorescent lighting in a private room.

The observed percentage of correct answers for samples stored at 6, 8 and 10 months was recorded (c) from the paired comparison test. This value (c) was then transformed into the value (p) indicating the percentage of correct answers without guessing. The formula used was: $p = [c - 100\ (0.5)] / 1 - 0.5$.

Physical Methods

Light Microscopy. Soy flours were dispersed in mineral oil and observed with a Zeiss polarizing light microscope (Karl Zeiss, Inc., Thornwood, NJ) at 102×. Photomicrographs were taken with a Polariod instant camera (Polaroid Corp., Cambridge, MA) using Polariod 553 film.

Laser Particle Sizing. Particle size was determined in duplicate using 100 g of soy flour, dry fed into a Model 2600 Malvern laser particle sizer (Malvern Instruments, Inc., Southborough, MA). Lens size was 300 mm and obscuration was between 20 and 30%.

Bulk Density. A tared 25-ml graduated cylinder was filled to the 25-ml mark and the weight recorded for each flour. Unpacked bulk density was expressed as g/ml. For packed bulk density, also in g/ml, the same cylinder was subjected to shaking for 15 s by holding the cylinder firmly on the base of a Cenco-Meinzer sieve shaker at a setting of 3. Results are means of three determinations.

Statistical Analysis. Analysis of variance was performed using the SYSTAT program. Tukey's HSD was used for multiple comparisons of means.

RESULTS AND DISCUSSION

Composition of the products (Table 4) shows moisture to be in an acceptable range at 2.8 to 5.6%. Protein and fat contents reflect normal differences due to variety and other factors and the extent to which fiber was removed or retained. Wet process product A was lowest in dietary fiber and the wet process, high-fiber product B-1 was highest. The dry process, high fiber product D-1 was second highest at 17.9%. Dry process products C-1 and C-2, whose manufacture differed only in heat treatment, were about the same. These data are comparable to those reported for full-fat flours (Snyder and Kwon 1987; Smith and Circle 1972; Piper and Morse 1943).

Contents of six major minerals, i.e., Ca, Mg, Na, K, P, and Fe (Table 5) varied somewhat due to such differences in raw product as variety, growing location, etc. However, the higher calcium contents of wet process products B-1 and B-2 probably reflect hardness of the water used in the process. These products were also high in sodium as a result of the pH adjustment with sodium hydroxide. Wet process product A was also high in sodium, possibly due to pH adjustment or perhaps the emulsifier used contained sodium. The slightly higher

TABLE 4.
PERCENTAGE MOISTURE AND COMPOSITION (DRY BASIS) OF 8 FULL-FAT SOY FLOURS*

Product	Moisture	Crude Protein	Crude Fat	Ash	Dietary Fiber
	%(WB)		%(DB)		
A	2.8	49.1	19.5	5.1	5.8
B-1	4.1	40.3	16.3	5.7	21.0
B-2	3.2	46.8	16.9	5.7	17.0
C-1	4.9	41.4	21.3	5.4	12.9
C-2	4.6	43.5	21.0	5.4	12.7
D-1	3.7	38.0	20.0	5.2	17.9
D-2	5.5	40.2	21.6	5.1	11.5
E	5.6	47.3	17.6	5.7	10.8
HSD ($p<0.05$)	0.2	3.6	1.4	0.2	3.5

*Means of triplicate determinations.

sodium and phosphorus levels in dry process product E might indicate use of a sodium phosphate additive. The minor minerals showed little variation, although some differences were statistically significant.

Fatty acid profiles (Table 6) were similar among products in that linoleic acid was highest followed in order by oleic, palmitic, linolenic and stearic. Snyder and Kwon (1987) reported comparable data for full-fat flours. The Japanese dry process product E was unique in being significantly higher in oleic acid and lower in linoleic acid than the others which might impart better stability to autoxidation (Sonntag 1979; Park *et al.* 1981). This difference was possibly due to genetic/environmental influences on soybean composition. Inherent varietal differences in fatty acid composition do exist. Relatively low mean growing temperatures result in increased 18:2 and 18:3 fatty acid levels, whereas relatively high mean temperatures result in increased levels of the 18:1 fatty acid (Canvin 1965; Cherry *et al.* 1985; Lusas 1983).

Residual trypsin inhibitor activities (Table 7) in all products were lower than the 24 TUI/mg reported by Liu and Markakis (1989) for cooked soybeans. Rackis and McGhee (1975) observed maximum weight gains in rats fed dehulled, defatted soy flakes containing 13–21% of original TIA and absence of pancreatic hypertrophy at residual TIA levels up to 45%. Based on the raw reference sample and literature values for raw soybeans, all were safely below recommended residual levels. The dry heating processes (products C and D) appeared adequate. As expected, product C-2, which was heated to 165C to produce the enzyme inactive flour, retained significantly less TIA than its enzyme active sister product, C-1, although both appeared to be at safe levels.

TABLE 5.
CONTENTS OF 15 MINERAL ELEMENTS IN FULL-FAT SOY FLOURS

	Mineral Content of Soy Flours (mg/100g)														
Product	Ca	Mg	Na	K	P	Fe	Al	Mn	Cu	Zn	B	Ni	Pb	Cr	Cd
A	163	273	144	1840	668	5.7	0.5	2.7	1.5	4.4	2.8	0.5	0.1	0.06	0.02
B-1	353	279	356	1754	587	8.2	1.5	2.9	1.2	4.4	3.6	0.8	0.1	0.10	0.02
B-2	345	309	251	1916	747	9.8	1.0	3.2	1.2	6.0	3.3	0.8	0.2	0.09	0.02
C-1	170	237	1.5	2084	732	5.3	0.8	2.8	1.4	4.3	3.2	0.6	0.1	0.06	0.01
C-2	183	240	0.9	2045	732	5.3	0.4	2.7	1.4	4.4	3.2	0.6	0.1	0.06	0.01
D-1	187	250	0.5	1912	583	7.7	0.4	2.6	1.1	4.2	4.0	0.6	0.1	0.07	0.01
D-2	186	311	0.4	1813	635	6.0	0.6	2.6	1.5	4.2	3.0	0.8	0.1	0.06	0.03
E	182	277	4.9	2084	819	6.6	0.6	3.0	1.5	4.9	3.1	0.6	0.6	0.07	0.02
HSD($p<0.05$)	8.0	4.0	2.1	5.0	5.0	0.3	1.0	0.3	0.2	0.2	0.2	0.2	0.2	0.03	0.05

*Means of triplicate determinations

TABLE 6.
FATTY ACID PROFILE OF 8 FULL-FAT SOY FLOURS*

Product	Fatty Acid					
	Myristic 14:0	Palmitic 16:0	Stearic 18:0	Oleic 18:1	Linoleic 18:2	Linolenic 18:3
A	0.1	10.7	5.4	19.1	52.9	8.6
B-1	0.1	9.7	4.1	21.1	54.3	7.1
B-2	0.1	9.7	4.3	24.6	51.8	7.7
C-1	0.1	10.7	3.8	19.3	55.9	8.7
C-2	0.1	10.6	3.8	19.5	56.0	8.7
D-1	0.1	11.0	4.1	20.2	55.4	8.3
D-2	0.1	11.0	4.0	18.9	55.4	9.2
E	0.1	10.5	3.0	31.7	46.1	6.9
HSD ($p<0.05$)	0.2	0.8	0.3	1.1	3.6	0.5

*Means of duplicate determinations.

TABLE 7.
TRYPSIN INHIBITOR ACTIVITY OF 8 FULL-FAT SOY FLOURS
COMPARED TO A RAW REFERENCE SAMPLE*

Samples	TUI/mg	(±SD)
A	4.7	0.7
B-1	3.7	0.3
B-2	21.7	0.0
C-1	23.5	0.2
C-2	7.6	0.4
D-1	20.6	0.0
D-2	12.4	0.2
E	20.7	0.0
Raw reference cv Hardin	176.7	2.9

*Means of triplicate determinations.

Although many researchers have recommended moist (steam) heat treatment to adequately reduce TIA (Snyder and Kwon 1987; Rackis and McGhee 1975; Smith 1988), Phillips *et al.* (1983) and Jansen *et al.* (1978) reported reduction of TIA to safe levels by application of dry heat to cowpeas and full-fat soy flours, respectively. The effectiveness of dry heat is likely due to actual production of steam as residual moisture in these products is converted to vapor.

PDI values (Fig. 1) were highest, and equivalent, in wet process product A and dry process product E. Products B-1 and B2 were relatively high in PDI

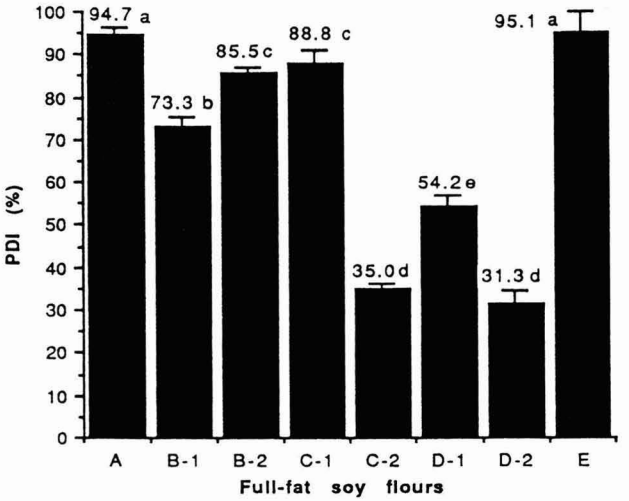

FIG. 1. PDI OF 8 FULL-FAT SOY FLOURS
Means of eight determinations. Error bars represent standard deviations. Means with different letters are signficantly different ($p < 0.05$).

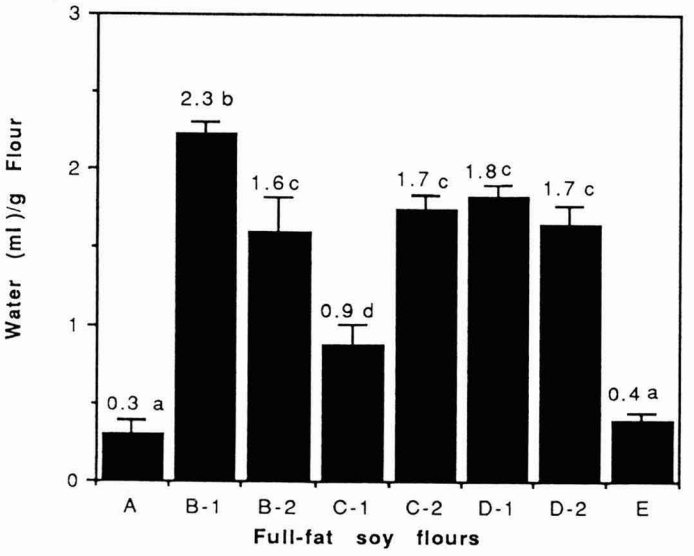

FIG. 2. WATER ABSORPTION OF 8 FULL-FAT SOY FLOURS
Means of three determinations. Error bars represent standard deviations. Means with different letters are significantly different ($P < 0.05$).

as was the enzyme active product C1. The lowest PDI were in the enzyme inactive C-2 product and the low fiber D-2 product. Apparently, the highly proprietary process for producing product E inactivates trypsin inhibitor activity without seriously affecting PDI.

Water absorption values (Fig. 2) were lowest and equivalent in the two products with the highest PDI, A and E. Water absorption generally varied inversely with PDI, particularly in the high PDI products. This relationship between PDI and water absorption capacity was noted by Kinsella *et al.* (1985), who also observed that at very low PDI, e.g., below 15%, water absorption is also very low, probably due to excessive protein denaturation. The role of fiber in water absorption was somewhat unclear; high-fiber product B-1 absorbed more water than its low-fiber sister product (B-2). There was no difference in water absorption between products D-1 and D-2 despite evidence that soy hull fiber is capable of holding 4 times its weight of water (Ashraf and Lee 1988).

Fat absorption (Fig. 3) did not differ among the three wet process products and was significantly higher than in the five dry process products, where it also did not differ. Better fat absorption by the spray-dried products may have been due to encapsulation of the soy oil by a carbohydrate/protein matrix, making the particle surfaces more lipophilic. Possibly, the higher contents of calcium in B-1 and B-2 and sodium in all three wet process products played a role.

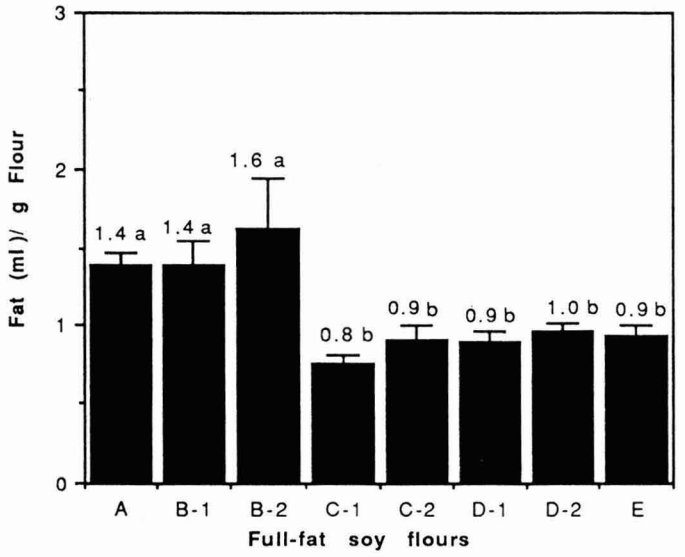

FIG. 3. FAT ABSORPTION OF 8 FULL-FAT SOY FLOURS
Means of three determinations. Error bars represent standard deviations. Means with different letters are significantly different ($p < 0.05$).

Emulsifying activity (Fig. 4) was highest in products A, C-1 and E, which had the highest PDI. This is in agreement with findings of Yasumatsu *et al.* (1972) and Holm and Eriksen (1980) that solubility tends to parallel emulsifying activity. This pattern followed through among the other products except for wet process products B-1 and B-2, which exhibited fairly high PDI but low emulsifying activity. Product B-1 (high-fiber) was lower in PDI than its low-fiber counterpart (B-2) and exhibited greater emulsifying activity. The same relationship did not exist between products D-1 and D-2 where the high-fiber product was considerably higher in PDI, but the two were not different in emulsifying activity. Obviously, functionality of full-fat soy flours is influenced by both composition and method of manufacture.

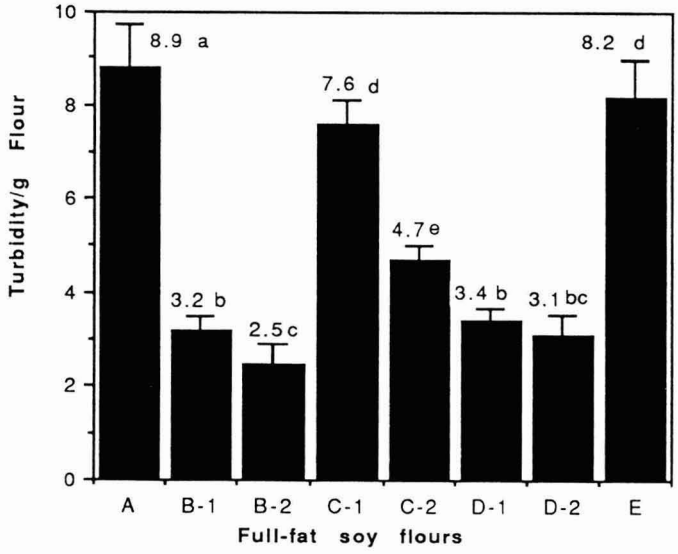

FIG. 4. EMULSIFYING ACTIVITY OF EIGHT FULL-FAT SOY FLOURS
Means of three determinations. Error bars represent standard deviations. Means with different letters are significantly different ($p < 0.05$).

Foaming capacity (Table 8) was highest in the enzyme active, dry process flour C-1 and lowest in its enzyme-inactive sister product C-2, suggesting that heat denaturation of protein played a major role. These two products differed similarly in PDI. The three products with lowest PDI, C-2, D-1 and D-2, showed the poorest foam stability, dropping to 2–4% after 2 h. Yasumatsu *et al.* (1972) and Narayana and Narasinga Rao (1982) reported similar relationships among foaming capacity and stability and heat denaturation of proteins in soybean and winged bean flours, respectively. The generally low foaming

capacity in the eight flours (16–32%) was likely due to their relatively high fat contents (Yasumatsu *et al.* 1972).

TABLE 8.
FOAM CAPACITY AND ITS STABILITY OVER TIME OF 8 FULL-FAT SOY FLOURS*

Sample	Foam capacity %							
	minutes							
	0	1	20	40	60	80	100	120
A	20	18	16	15	14	13	11	10
B-1	21	20	18	18	18	18	17	14
B-2	29	29	20	20	16	15	15	14
C-1	32	28	26	20	18	16	16	13
C-2	16	15	9	8	7	6	5	4
D-1	26	20	14	10	9	6	4	3
D-2	18	16	6	4	3	3	2	2
E	28	26	20	18	10	10	8	8
HSD($p<0.05$)	1							

*Means of duplicate determinations

TABLE 9.
APPARENT VISCOSITIES OF 15% AQUEOUS DISPERSIONS OF 8 FULL-FAT SOY FLOURS*

Sample	Spindle	Viscosity (cP)
A	2	118
B-1	2	348
B-2	2	339
C-1	1	47
C-2	1	37
D-1	1	21
D-2	1	14
E	1	20

*Means of triplicate determinations.

Viscosities (Table 9) were significantly higher for dispersions of the three wet process products than for the dry process ones. In addition, the higher calcium (B-1, B-2) and sodium contents (A, B-1, B-2) may have caused bridging of protein and other macromolecules. Circle *et al.* (1964) observed increased viscosity in 5% soy isolate dispersions upon addition of calcium ion. Effects of fiber were not significant. Possibly, the wet processing and high shear imparted by spray drying opened up and uncoiled protein chains.

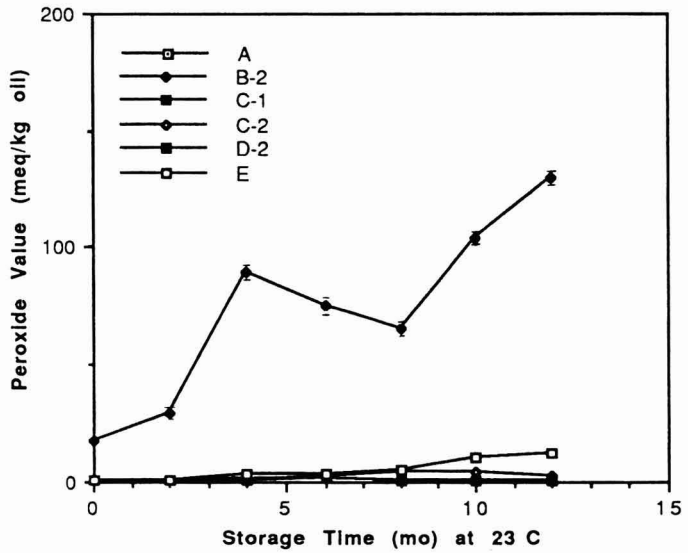

FIG. 5. INFLUENCE OF STORAGE DURATION AT 23C ON 6 FULL-FAT SOY FLOURS
Means of duplicate determinations on replicate samples. Error bars represent standard deviations.

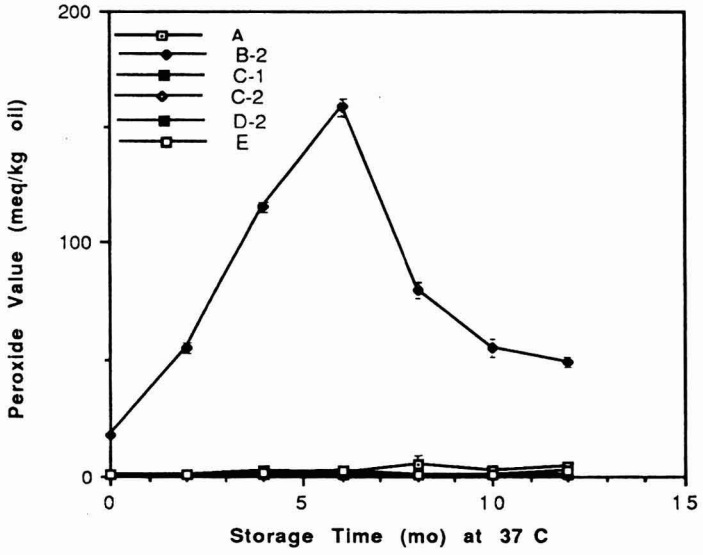

FIG. 6. INFLUENCE OF STORAGE DURATION AT 37C ON 6 FULL-FAT SOY FLOURS
Means of duplicate determinations on replicate samples. Error bars represent standard deviations.

TABLE 10.
MEAN PARTICLE DIAMETER SIZE DISTRIBUTION OF 8 FULL-FAT SOY FLOURS BY LASER SIZE ANALYSIS*

Sample	10 Percentile (μm)		90 Percentile (μm)	
		Range		Range
A	35.5	0.1	190.9	0.1
B-1	22.4	0.1	198.8	7.4
B-2	15.2	0.1	113.4	3.7
C-1	23.5	2.9	172.4	6.9
C-2	21.3	1.1	172.5	0.9
D-1	22.2	2.4	323.1	23.1
D-2	21.8	3.9	262.7	22.8
E	23.2	0.9	230.3	17.3

*Means of duplicate determinations.

Only one flour, wet process product B-2, developed a peroxide value greater than 10 meq/kg of oil over 12 months storage, reaching maxima of 140 meq/kg at 23C in 12 months (Fig. 5) and 159 meq/kg at 37C in 6 months (Fig. 6). However, this product may have been several months old when it was acquired. The sensory tests could not detect any flavor changes in products A, C-2 or D-2 at 10 months. These dry process flours were also lowest in PDI, indicating high heat treatment. Off-flavors were not detected in the other flours until 8 and 10 months; that is, no changes could be discerned at 6 months. More importantly, the "catch throat" phenomenon was detected in all products, but to the least degree in product A, which was lowest in fiber.

Laser particle size data (Table 10) show relatively little difference among the products, with the wet process, spray-dried product B-2 having the smallest particle size. The data do not support manufacturers' claims that product E had 80% of the particles smaller than 30 μm nor that products C-1 and C-2 had 99% of the particles smaller than 100 μm. Laser particle sizing is based on the theory that small particles scatter light over larger angles than do large particles. However, factors other than size may influence the angle of light scattering in highly heterogeneous biological systems such as full-fat soy flours. This could produce erroneous results. Photomicrographs (Fig. 7) tended to support the laser particle size data, with all samples having a relatively large proportion of large particles. There was no attempt to quantitate particle size distribution by this procedure.

Finally, bulk densities (Fig. 8) varied over a rather narrow range. Wet process flour B-2 was least dense, with respective unpacked and packed densities of 0.26 and 0.36 g/ml. Dry process flour D-1 was most dense with respective unpacked and packed densities of 0.36 and 0.45 g/ml.

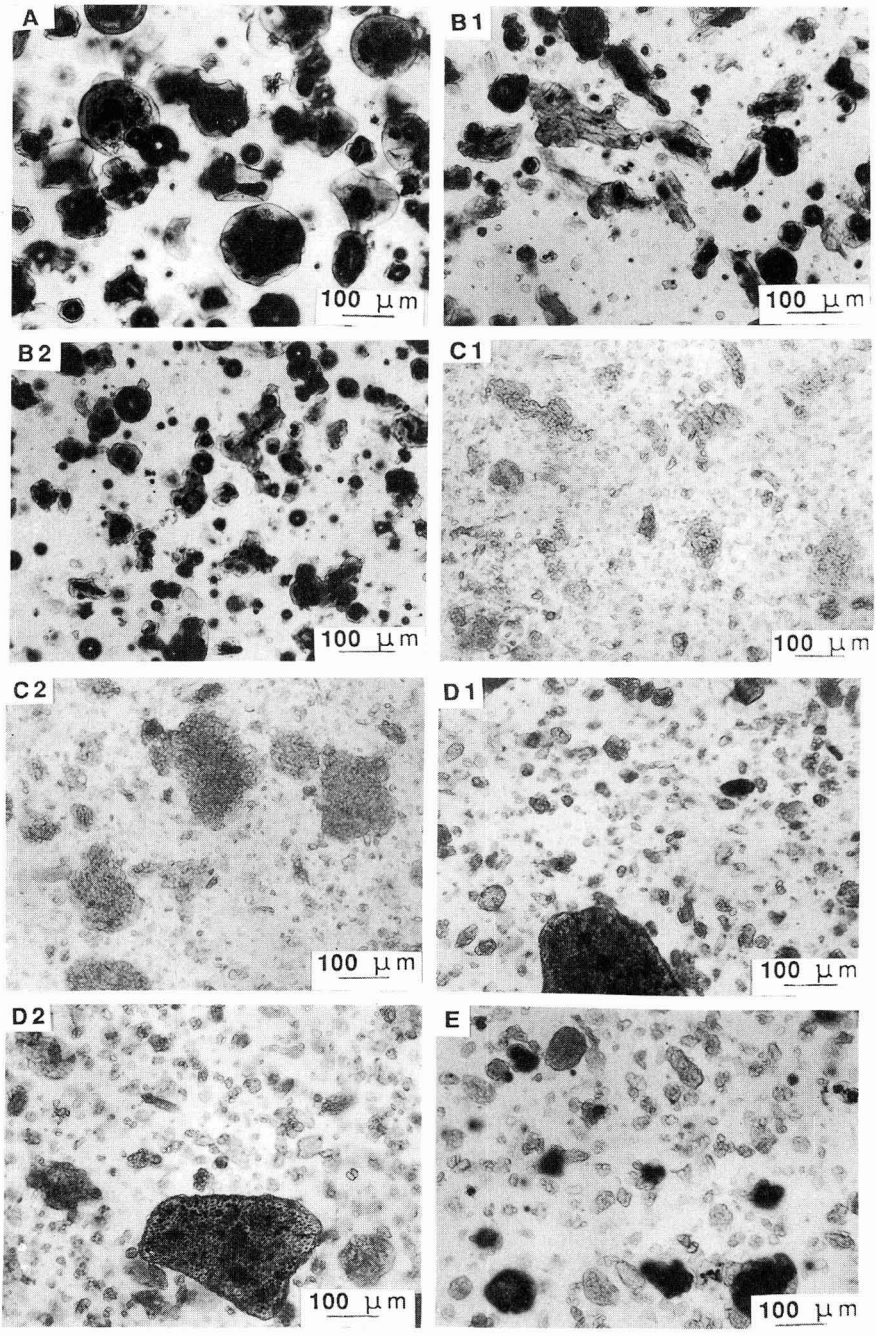

FIG. 7. PHOTOMICROGRAPHS (102X) OF 8 FULL-FAT SOY FLOURS

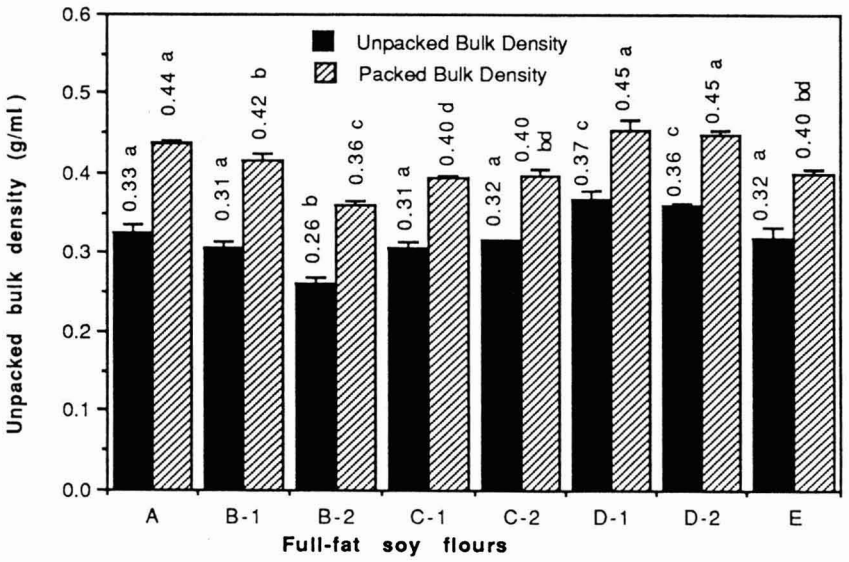

FIG. 8. UNPACKED AND PACKED BULK DENSITIES OF 8 FULL-FAT SOY FLOURS
Means of three determinations. Error bars represent standard deviations. Means (bars) with a similar pattern but different letters are significantly different ($p < 0.05$).

CONCLUSIONS

It would be tempting to make statistical correlations among the properties studied, but since raw products as well as processes differed considerably, they would have little real meaning. However, the chemical, physical and functional properties do provide useful information as to the effects of processing variables and suggest possible food ingredient applications.

ACKNOWLEDGMENTS

Published as paper No. 20,758 of the contribution series of the Minnesota Agricultural Experiment Station on research conducted under Project 18-43 with additional support from The Minnesota Soybean Research and Promotion Council. We thank Dr. Frank Martin of the University of Minnesota Department of Applied Statistics for his assistance in experimental design and data analysis.

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THIAMIN DEGRADATION KINETICS IN PUREED RESTRUCTURED BEEF[1]

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Accepted for Publication January 14, 1994

ABSTRACT

The kinetics of thiamin degradation in pureed restructured beef with 70% moisture (w/w) were determined at 110, 120, and 130C. As expected, the degradation kinetics followed a first-order reaction. The rate constant at 121C was 0. 01881 min^{-1}. The temperature dependence of the rate constant obeyed an Arrhenius relationship. The activation energy was 26.5 kcal/mol. The activation energy compared very well to those obtained from the literature for a variety of foods including beef puree containing 85% moisture. Thiamin degradation kinetics in beef were independent of the form (free or combined) of the thiamin.

INTRODUCTION

It is well known that beef always has been and remains to be a major food in the human diet. In addition to its taste, beef is a nutrient-dense food, that provides a high quantity and quality of nutrients (particularly protein, the B vitamins, iron and zinc) relative to its caloric content (Hansen and Wyse 1990). The thermal processing of beef has a negative effect on the vitamin content. Therefore, it is necessary to measure the loss of vitamins that occurs when beef is exposed to high temperatures.

Thiamin is very heat labile, thus it is commonly used as an index of quality retention resulting from thermal treatment (Feliciotti and Esselen 1957). In addition to being a good index of quality, thiamin is an important nutrient for humans. The disease that results from a deficiency of thiamin in humans is

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Journal of Food Processing and Preservation **18** (1994) 253–262.

known as beriberi. The three clinical signs of this disease are (1) loss of appetite, nausea, and associated weight loss; (2) cardiovascular involvement; and (3) neurological symptoms (Windham *et al.* 1990). It is also known that the thermal destruction of thiamin leads to the formation of a characteristic odor, which is involved in the development of meaty flavors in cooked foods (Tannenbaum *et al.* 1985).

In order to predict thiamin loss during cooking and thermal processing, kinetic parameters, rate constants and activation energy have to be known. It has been known from literature that many factors such as pH, trace quantities of heavy metals, moisture content, and the presence of oxygen (Villota and Hawkes 1986) influence the kinetics for the thermal destruction of thiamin. It was noted by Mulley *et al.* (1975) that proteins also provided protection against thiamin degradation.

Thiamin occurs in natural foods in the free form or in a combined form as a protein complex, as a phosphorus-protein complex or as the pyrophosphoric acid ester, cocarboxylase (thiamin pyrophosphate) (Mulley *et al.* 1975). More than 50% of the thiamin in meat products is in the combined form (Gubler 1991). Feliciottti and Esselen (1957) have reported that the combined thiamin contents in beef heart and beef liver are 58% and 55%, respectively. Greenwood *et al.* (1943) stated that free thiamin is less stable than cocarboxylase. On the other hand, many researchers agreed that the cocarboxylase was less stable than free thiamin (Farrer 1955). Mulley *et al.* (1975) concluded that when both forms of thiamin were present together, the increased lability of cocarboxylase became apparent only when its concentration in the mixture exceeded 35%.

Thiamin degradation kinetics have previously been measured in beef (Mulley *et al.* 1975). Their studies were carried out in 15% beef puree (15% beef, 85% water, w/w). However, the moisture content of raw beef is generally in the range of 70–73% (Hultin 1985). Difference in water content can alter the ionic strength and affect the pH. In thermal processing, the pH of the food material can be changed due to various chemical reactions. The rate of thiamin degradation has been well known to be strongly affected by pH (Feliciotti and Esselen 1957). Therefore, thiamin degradation kinetics in raw beef (70% water) could be different than in beef puree with 85% water.

Restructured beef is homogeneous and has a moisture content similar to that of raw beef. The process of meat restructuring enables lower-priced meat cuts to be transformed into higher quality products. This has led to its increasing popularity and use by the military, as well as many fast-food chains and institutional outlets (Secrist 1987).

The objective of this study was to determine the thiamin degradation kinetics in pureed restructured beef containing 70% water. In addition, experiments were performed to determine the effect of vitamin form on the thiamin degradation kinetics.

MATERIALS AND METHODS

Sample Preparation

Restructured beef obtained from E. Huttenbauer & Son Inc. (Cincinnati, Ohio) was cut into 1 cm cubes and freeze dried (Labconco Freeze Dry-12 system, Fisher Scientific, Springfield, NJ). The restructured beef was made using USDA utility grade beef with a maximum fat content of 15%. The restructured beef contained 1% (weight basis) NaCl and 0.25% sodium tripolyphosphate. The moisture content of the restructured beef was determined by the AOAC (1984) vacuum oven method. The average moisture content was found to be 69.71 $\pm$ 1.13% for three measurements. The dried beef was ground into a fine powder with a Fitzmill Model D comminuting machine (W.J. Fitzpatrick Co., Chicago, IL) using the 1A screen. The beef powder was sealed in Ball home canning jars and stored at -20C until use.

The beef puree was prepared by mixing 70 g of water with 30 g of dry beef powder. The puree was enriched with 50 mg of thiamin hydrochloride (Fisher Scientific, Springfield, NJ) per 100 g of puree. To ensure that the thiamin was evenly distributed throughout the puree, the thiamin was first dissolved in the water and then added to the beef powder. The sample was then mixed well. Since the pH of the 30% beef puree was too dry to be measured, the pH of beef samples (without added thiamin) with a moisture content of 90%, 85%, 80% and 75% were measured using a pH meter (Accumet Model 915, Fisher Scientific, Pittsburgh, PA).

Small glass screw cap vials (Supelco, Bellefonte, PA) were filled with approximately 6 g of the beef puree and sealed. The glass tube was 5 cm long and had an internal diameter of 1.3 cm. An additional Teflon-faced silicone septa was placed in each screw cap for added protection against leaking. The vials were immersed into a constant temperature oil bath (Model TU-16D, Techne Tempunit Co., Princeton, NJ) programmed at 110, 120, and 130C. The oil bath was continuously mixed using a DC motor (Model 5X412, Dayton Electric Mfg. Co., Chicago, IL). One of the vials had an opening in the top into which a type T thermocouple was placed to measure the come-up time at the center. When the cold point (center temperature) reached the desired temperature, the first sample was taken, and used to determine the initial thiamin concentration (C_o). The average come-up time for all three temperatures was approximately 8.5 min. Samples were taken at various time intervals during the course of the heating study, and were quickly cooled via agitation in an ice bath. The samples were refrigerated and protected from light until assayed for free thiamin concentration. The degradation reactions were studied in duplicate at 110, 120, and 130C.

Following the same sample preparation procedures, the experiments were repeated using 30% beef puree without the addition of free thiamin HCl. The samples were heated at 100, 110, and 120C for periods of time that corresponded to a 50% reduction in thiamin concentration. The heating times were calculated using kinetic parameters determined based on free thiamin HCl. The total thiamin in beef was extracted and quantified. At least two replicates were conducted.

Thiamin Assay

To measure free thiamin HCl, 5 g of beef puree was diluted with 20 g of HPLC grade water (Fisher Scientific, Springfield, NJ). The dilution was mixed well and then filtered first through Whatman No. 1 filter paper, Whatman No. 42 filter paper, and then through 0.22 μm filter paper (Fisher Scientific, Springfield, NJ). The thiamin assay was performed in accordance with the high performance liquid chromatography procedure described by Arabshahi and Lund (1988). The HPLC system consisted of the following components, all manufactured by Waters Associates (Milford, MA): a C18 μBondapak column, a Model U6K injector, a Model 510 pump and a Model 484 tunable UV detector set at 254 nm. The HPLC system was interfaced to an IBM PS/2 computer by the Waters system interface module.

The mobile phase used for thiamin detection was an isocratic solution consisting of 100 ml HPLC grade methanol, 10 ml glacial acetic acid, 25 ml of buffered pentane sulfonic acid, brought to 2000 ml with HPLC grade water (all solvents purchased from Fisher Scientific, Springfield, NJ). The flow rate was set at 1.5 ml/min. An aliquot of 30 μl of the extracted solution was injected into the reversed-phase column. Each sample was injected in duplicate. The chromatographic data were analyzed using the Baseline 810 software package manufactured by Waters Associates. The area under the thiamin peak was recorded for each injection and compared to a standard thiamin calibration curve. The calibration curve was constructed by injecting known concentrations of solutions of pure thiamin hydrochloride in HPLC grade water and recording the resulting peak areas. Peak area versus thiamin concentration was linear throughout the concentration range studied.

The quantification of total thiamin (free thiamin and its esters) in beef, without the addition of thiamin HCl followed the method of Kambhampati *et al.* (1993), which was a modified version of the thiochrome method (Anon. 1985). The HPLC system consisted of the following components: a Varian Vista 5500 Liquid Chromatograph (Varian Associates, Walnut Creek, CA), an injector (Model 7125, Rheodyne, Cotati, CA), a polychrome diode array detector (Model 9065, Varian Associates, Walnut Creek, CA) set at 367 nm and a C8/cation exchange mixed phase (150mm $\times$ 4.6mm) column (Alltech,

Deerfield, IL). The mobile phase consisted of 90% 0.05 M ammonium acetate, and 10% acetonitrile adjusted to pH 4.5. The flow rate was 1 ml/min. The chromatographic data were analyzed through a personal computer using Varian Star Workstation Revision C software (Varian Associates, Walnut Creek, CA).

RESULTS AND DISCUSSION

Normalized concentration curves for the degradation of thiamin HCl in beef puree at 110, 120, and 130C are shown in Fig. 1. C_o was measured from beef samples taken immediately after the cold point (center) reached the desired temperature. The degradation of thiamin in 30% beef puree followed a first-order reaction as indicated by the linearity of the data on a semi-log plot for two replicates, which was consistent with that reported by Mulley *et al.* (1975).

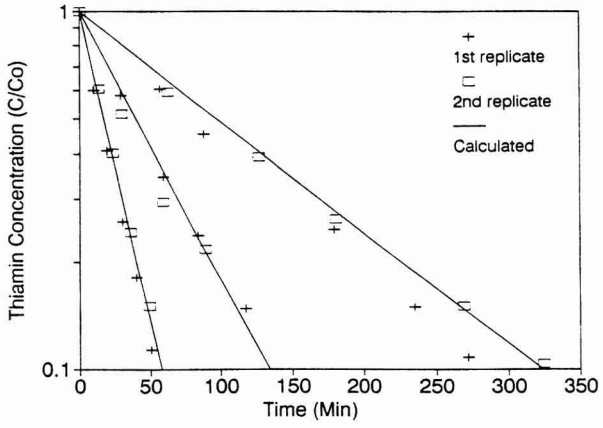

FIG. 1. NORMALIZED CONCENTRATIONS OF THIAMIN HYDROCHLORIDE IN 30% BEEF PUREE AS A FUNCTION OF TIME AT 110, 120 AND 130C

The reaction rate constants, k, were determined from the slopes of the normalized curves and are tabulated in Table 1. The r^2 values obtained from the linear regression performed on each replicate were in the range of 0.98–0.99. Figure 2 shows that the rate of degradation of thiamin in beef puree obeyed an Arrhenius relationship for temperature dependence. Therefore, the reaction rate constant, k, for the degradation of thiamin in restructured beef can be related to temperature through the following equation:

$$\ln(k) = 29.83 - \frac{13321.6}{T} \quad (1)$$

where k is the rate constant (min^{-1}) and T is the temperature in Kelvin. Linear regression performed on the Arrhenius plot provided the numerical values in Eq. 1. The slope of the curve is $-E_a/R$, where E_a is the activation energy in cal/mol, and R is the ideal gas law constant (1.987 cal/mol·K), and the intercept is the preexponential factor. The activation energy was 26.5 kcal/mol and the r^2 of the linear regression was 0.99.

TABLE 1.
REACTION RATE CONSTANTS FOR THE THERMAL DEGRADATION OF THIAMIN HYDROCHLORIDE IN 30% BEEF PUREE AT 110, 120 AND 130C

Replicate	Temperature (C)	Rate Constant (1/min)	R^2
1	110	.007511	.99
2	110	.006900	.99
1	120	.017244	.98
2	120	.016316	.99
1	130	.042288	.99
2	130	.038719	.99

Thiamin concentration as a function of heating time and temperature can be calculated from the following equation:

$$\ln\frac{C}{C_o} = -kt \tag{2}$$

where C_O is the initial thiamin concentration, k is the temperature dependent reaction rate constant determined from Eq. 1 and C is the thiamin concentration at any time, t. Thiamin concentration as a function of heating time at 110, 120, and 130C as calculated by Eqs. 1 and 2 are also shown by the solid lines in Fig. 1.

In order to compare our data with those reported in literature, the rate constant at 121C, the most frequently used reference temperature in thermal processing, was calculated to be 0.01881 min^{-1} using Eq. 1. Table 2 summarizes rate constants at 121C and activation energies in 30% beef puree and in other food materials. It can be observed that the activation energy is independent of food material and is in the neighborhood of 27–28 kcal/mol. The agreement between our data and the literature values in terms of activation energy is very

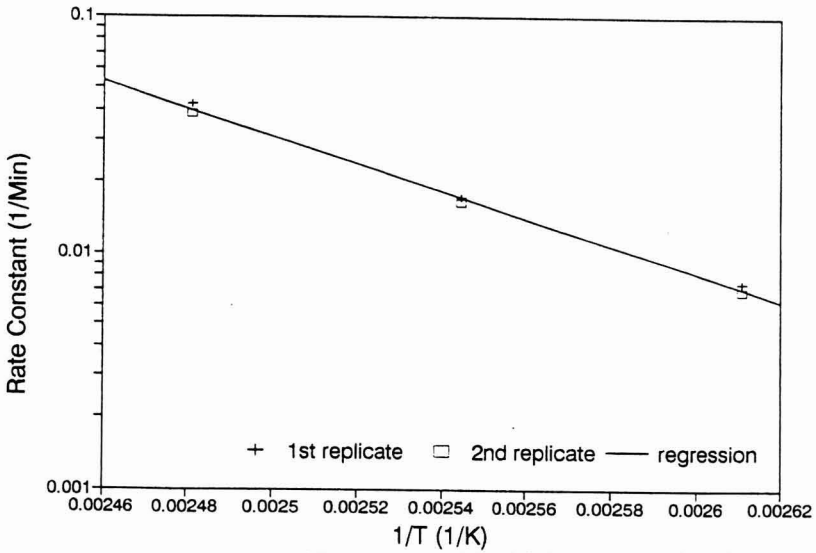

FIG. 2. ARRHENIUS PLOT OF THIAMIN HYDROCHLORIDE DEGRADATION IN 30% BEEF PUREE

good. On the other hand, the thiamin degradation rate is material dependent. In general, the degradation rate obtained from this study agreed well with most of the meat products listed in Table 2, except for the 15% beef puree. It is interesting to observe that thiamin degradation in 30% beef puree was twice as high as that for 15% beef puree at 121C.

TABLE 2.
ACTIVATION ENERGIES FOR THIAMIN DEGRADATION IN VARIOUS FOODS

	E_a (kcal/mol)	k_{121} (1/min)
Beef (70% moisture content) (This work)	26.5*	0.01881*
Beef (85% moisture content)	27.5	0.00897
Beef heart	27.9	0.01982
Meat loaf	27.1	0.02643
Pork	27.4	0.01565
Lamb	27.7	0.01794
Peas	27.1	0.01017
Spinach	28.2	0.01839
Green beans	28.6	0.01564
Carrots	28.3	0.01467

*Note: Besides 30% beef puree, all other data were taken from Villota and Hawkes (1986)

The difference in degradation rate can be explained by differences in pH, ionic strength, and moisture content of the food material. Feliciotti and Esselen (1957) demonstrated that pH significantly affected the stability of thiamin, but it had no effect on the activation energy. The most pronounced change in rate occurred between pH 6.0–6.5. A small variation in pH strongly changed the degradation rate. The pH of different food materials varies; therefore, it is not surprising to find that the rate constants are not the same for different food materials. For meat products, the pH depends upon physiological state, postmortem biochemical processes, additives, and handling variables. Therefore, it is important to characterize the samples before kinetic studies are performed. The pH of beef puree samples containing 90%–75% moisture, without the addition of free thiamin HCl are given in Table 3. It is reasonable to state that the pH of 30% beef puree is approximately 6.14, even though its pH cannot be measured directly with a glass electrode. It was also found that the addition of free thiamin HCl at the level of 50 mg/100 g puree did not change the pH of the beef puree samples. The pH of various meat products have also been reported to be in the range of 6.07–6.18 (Feliciotti and Esselen 1957). Since the moisture content (70%) and the pH of the restructured beef are similar to regular beef in spite of the presence of a small percent of NaCl and sodium tripolyphosphate, it is believed that the kinetic parameters determined from this study can be used to predict thiamin degradation for both restructured and regular beef.

TABLE 3.
THE PH OF BEEF PUREE SAMPLES
AS A FUNCTION OF MOISTURE CONTENT

Moisture content of beef puree (g H_2O/g sample)	pH
0.90	6.19
0.85	6.17
0.80	6.15
0.75	6.14

The kinetic parameters were determined based on the addition of free thiamin HCl, and it was not clear whether these parameters could be used for predicting thiamin degradation in beef during thermal processing because more than 50% of the thiamin in beef was in the combined form (Gubler 1991). Beef puree samples without the addition of thiamin were heated at 100, 110, and

120C for periods of time shown in Table 4. These times corresponded to a 50% reduction in free thiamin concentration in beef as predicted by Eq. 1 and 2. Table 4 also shows the measured total thiamin retention. There was an excellent agreement ($\leq 2\%$) between the calculated and measured values suggesting that thiamin degradation kinetics in beef were independent of the form of the thiamin. Although the kinetic parameters were originally determined at 110, 120, and 130C, the good agreement at 100C also indicates that rate constants outside the temperature range studied can be extrapolated by the Arrhenius equation.

TABLE 4.
PREDICTED AND MEASURED THIAMIN RETENTION IN 30% BEEF PUREE AT 100, 110 AND 120C WITH AND WITHOUT THE ADDITION OF FREE THIAMIN HCL

Temperature (C)	Time (min)	Calculated C/C_o by eq. 1 and eq. 2	Measured $C/C_o \pm$ S. D.
100	249.3	0.50	0.51±0.030
110	98.1	0.50	0.49±0.059
120	40.5	0.50	0.52±0.033

ACKNOWLEDGMENT

This is publication No. D-10575-1-93 of the New Jersey Agricultural Experiment Station supported by State Funds and the Center for Advanced Food Technology (CAFT). The Center for Advanced Food Technology is a New Jersey Commission on Science and Technology Center. This research was supported by contract #DAAK60-92-C-0087 of the U.S. Army Natick Research Development & Engineering Center. The authors also greatly acknowledge Miss I. Kambhampati and Dr. K.S. Roinestead for analyzing the total thiamin content in beef puree.

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AND PRESERVATION VOL. 18, NO. 3

CONTENTS