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MICROSTRUCTURE AND TEXTURE OF MEAT EMULSIONS SUPPLEMENTED WITH PLANT PROTEINS

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**Abstract**

A model meat emulsion system was used to evaluate the effects of cooking time and temperature on texture, microstructure and cook stability of meat emulsions containing soy or canola protein isolate. The plant proteins were incorporated either dry or rehydrated at replacement levels of 33.3% and 66.7% of the meat protein, and the emulsions were cooked at 70 or 95°C for 25 or 50 min. Texture of the cooked emulsions was assessed by an instrumental texture profile analysis (TPA) using an Instron tester. Analysis of TPA and stability data revealed several complex interactions between experimental variables; however, level of protein replacement was the predominant factor, with decreased firmness and increased yield resulting from increased replacement of meat protein.

Microstructure of the cooked emulsions was examined by light microscopy and scanning electron microscopy. Although there were slight differences in the fat particle distributions of emulsions containing plant proteins, the distributions were similar, where particles with diameters larger than 50 µm approximated a normal distribution and were thought to be relatively intact fat cells, while the number of particles with diameters of 10-50 µm increased in an essentially logarithmic manner as size decreased. Microstructure of the proteinaceous matrix was affected primarily by protein source, replacement level and cooking conditions.

**Introduction**

Finely comminuted meat products such as frankfurters or bologna are commonly prepared by chopping lean meat in a brine solution to form a proteinaceous slurry in which animal fat is then finely divided and dispersed. The resulting batter, resembling an oil in water emulsion (Hansen, 1960), is subsequently cooked to form a product in which fat globules are entrapped within a rigid protein matrix. Previous work has documented the role of the salt-soluble meat proteins myosin and actomyosin in emulsion formation and stabilization through coating the fat droplets with a stable membrane (Hansen, 1960; Hegarty et al., 1963; Swift et al., 1961). Reviews by Saffle (1968) and Webb (1974) considered emulsification to be the primary factor responsible for stability of such products. Other workers have suggested that too much emphasis has been placed on the importance of emulsification (van den Oord and Visser, 1973). Theno and Schmidt (1978) examined the microstructure of three commercially acceptable frankfurters and found that only one could be called a true meat emulsion. While these products may not be emulsions in the strictest sense, the term "meat emulsion" has been in common use for many years and is retained in the present study.

Attention has shifted from the emulsification properties of meat proteins to their involvement in matrix formation through thermally induced gelation, with the entrapment of fat and development of the characteristic product texture. Several recent reviews have examined this aspect (Schmidt et al., 1981; Acton et al., 1983; Schmidt, 1984). Ziegler and Acton (1984) and Asghar et al. (1985) detailed the denaturation, aggregation and gelation reactions of muscle proteins. Lee (1985) surveyed the microstructural aspects of meat emulsion formation and stabilization. He reviewed the evidence for emulsion and nonemulsion theories of fat stabilization and concluded that although both theories should be considered, from photomicrographic data and physical analysis, the nonemulsion theory should receive more consideration.

The use of plant proteins as extenders or replacements for meat protein in frankfurter-type comminuted meat products has been the subject of

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**Key Words:** Meat emulsions, soy protein, canola protein, microstructure, texture, profile analysis, protein matrix, food lipids.

Table 1. Instrumental texture profile and cook stability of protein-replaced meat emulsions\*

Protein	Level(%)	Pre-treatment**	Cook Temp. (°C)	Cook Time(min)	Rupture Force(N)	First Bite Hardness(N)	Second Bite Hardness(N)	Springiness(%)	Cohesiveness	Cook Stab.(%)	
Soy	66.7	r	70	25	15.2	27.4	19.0	46.9	0.138	99.0	
	66.7	d	70	50	13.3	25.5	19.0	38.9	0.148	98.2	
	66.7	d	95	25	17.0	30.1	22.2	60.8	0.167	98.6	
	66.7	r	95	50	17.5	30.9	22.5	49.0	0.152	97.5	
	33.3	d	70	25	40.2	44.5	32.8	48.7	0.153	98.8	
	33.3	r	70	50	39.7	49.5	38.2	45.6	0.147	97.8	
	33.3	r	95	25	34.4	45.6	34.8	50.2	0.134	96.3	
	33.3	d	95	50	35.1	48.2	36.1	60.1	0.149	95.9	
	Canola	66.7	d	70	25	15.8	29.6	21.7	38.9	0.126	98.7
		66.7	r	70	50	17.2	35.4	26.2	40.3	0.142	99.5
		66.7	r	95	25	18.2	34.4	25.4	45.9	0.133	98.8
		66.7	d	95	50	19.4	37.7	27.2	46.1	0.120	98.1
33.3		r	70	25	36.1	49.4	36.6	47.1	0.141	98.8	
33.3		d	70	50	38.4	49.5	36.9	52.5	0.147	98.6	
33.3		d	95	25	37.0	53.1	41.6	58.7	0.147	96.1	
33.3		r	95	50	34.5	46.8	35.5	49.2	0.143	96.1	
All-meat	-	-	70	25	59.9	60.9	46.8	53.6	0.136	98.4	
	-	-	70	50	62.6	65.6	50.3	64.6	0.135	95.5	
	-	-	95	25	58.6	64.1	52.2	52.2	0.135	87.3	
	-	-	95	50	66.5	64.9	52.3	57.8	0.141	82.0	

\*mean of duplicate samples; \*\* r = rehydrated, d = dry

much investigation. At high levels of replacement, the texture of these products usually becomes soft and mushy. Frankfurter processing schedules do not usually employ temperatures greater than approximately 75°C but higher temperatures are often required for denaturation and gelation of many globular proteins (Catsimpoilas and Meyer, 1970; Hermansson, 1979). The objectives of this research were to investigate the effects of processing conditions on texture, cook stability and microstructure of a model meat emulsion system in which large amounts of meat protein have been replaced with soy or canola protein isolate. Soy protein is used in a wide variety of foods including bakery products, cereals, dairy foods and comminuted meats (Kinsella, 1979). Canola is the major oilseed crop grown in Canada and northern Europe, and canola protein possesses numerous attractive functional properties (Sosluski et al., 1976; Thompson et al., 1982; Gill and Tung, 1978).

#### Materials and Methods

##### Experimental design

A model meat emulsion formulation containing 10.5% beef protein, 29% pork fat, 57.5% water, and 2.5% sodium chloride was used as a control. Beef protein was replaced with either soy or canola protein isolate at either 33.3 or 66.7% (w/w). Lauck (1975) found that the hydration state of a whey protein product influenced the stability of frankfurters. To test this effect, the isolates were added either dry or rehydrated overnight in distilled water [3:1 (w/w), H<sub>2</sub>O:isolate]. The emulsions were then cooked at 70 or 95°C for 25 or 50 min. The effects of (i) replacement, (ii) protein source, (iii) pretreat-

ment (i.e., state of hydration), (iv) cook temperature and (v) cook time on texture and cook stability of the finished products were tested using a 2<sup>5</sup> fractional factorial experimental design and analysis of variance (Taguchi, 1957; Tables 1 and 2). Product microstructure was examined using light microscopy (LM) and scanning electron microscopy (SEM).

##### Emulsion preparation

Boneless beef chuck and pork backfat were purchased from a local abattoir, trimmed of visible fat and meat traces, respectively, minced and vacuum packaged separately in 450 g lots, then frozen at -35°C. Prior to use, beef and backfat were allowed to thaw at 4°C, then kept on ice when taken from the coldroom. Soy protein isolate [N(d.b.)=14.56] and canola protein isolate [N(d.b.)=14.42] were purchased from the POS Pilot Plant Corp. (Saskatoon, SK). The isolates were prepared by alkaline extraction followed by acid precipitation, neutralization, and recovery of the protein by spray drying (POS Corp., personal communication). Differential scanning calorimetry of the isolates indicated little protein denaturation.

Laboratory scale emulsion batches were prepared with a Sorvall Omnimixer (Ivan Sorvall, Inc., Norwalk, CT). The Omnimixer was modified to allow the jar to be moved up and down relative to the blades to give improved chopping of the entire sample (Morrison et al., 1971). Ground beef, salt, distilled water and plant protein were blended for 25 s at half speed, pork backfat was added, and the emulsion formed by chopping at full speed for 2 x 30 s with intermediate scraping and hand mixing. Final emulsion temperatures after chopping ranged from 16-18°C. The emul-

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Table 2. Instrumental texture profile analysis of protein-replaced meat emulsions; significant experimental factors

Texture Profile Component		Significant Experimental Factors	
Rupture Force (N)	(i) Replacement Level**	33.3%	66.7%
		Control: 61.9 ± 3.5	37.0 ± 2.3
	(ii) Level x Cook Temp.*	33.3%	66.7%
		70°C: 38.6 ± 1.9	15.4 ± 1.6
		95°C: 35.3 ± 1.2	18.0 ± 1.1
First Bite Hardness (N)	(i) Replacement Level**	33.3%	66.7%
		Control: 63.8 ± 2.2	48.3 ± 2.7
	(ii) Protein Source**	Soy	Canola
		37.8 ± 10.1	42.0 ± 8.7
(iii) Pretreatment x Cook Temperature*	Dry	Rehydrated	
	70°C: 37.3 ± 11.6	40.5 ± 10.9	
	95°C: 42.4 ± 10.4	39.4 ± 7.9	
Second Bite Hardness (N)	(i) Replacement Level**	33.3%	66.7%
		Control: 50.4 ± 2.5	36.6 ± 8.1
	(ii) Protein Source**	Soy	Canola
		28.1 ± 8.1	31.4 ± 7.1
(iii) Pretreatment x Cook Temperature*	Dry	Rehydrated	
	70°C: 27.7 ± 8.6	30.0 ± 9.0	
	95°C: 31.8 ± 8.6	29.5 ± 6.6	
Springiness (%)	(i) Replacement Level**	33.3%	66.7%
		Control: 57.1 ± 5.6	51.5 ± 5.3
	(ii) Cook Temperature**	70°C	95°C
	44.9 ± 5.0	52.5 ± 6.3	

\* p&lt;0.05; \*\* p&lt;0.01

sions were stuffed into stainless steel tubes (2.54 cm diam. x 10 cm long, internal dimensions), capped, then cooked in a water bath at 70 or 95°C for 25 or 50 min.

Whiting and Miller (1984) evaluated a laboratory blender and a food processor for making model frankfurter emulsions and concluded that, although the frankfurters were not identical to those produced by large-scale commercial machines, good quality batters could be produced by each machine tested. Smaller scale machines have the advantages of being inexpensive, economical with ingredients, and time-saving.

#### Cook stability

The cooked emulsions were cooled in ice water for 10 min, removed from the tubes and weighed after drainage of syneresed liquid. Cook stability was determined as the mass ratio of the cooked to uncooked emulsions, expressed as a percentage.

#### Texture

Texture of the cooked emulsions was evaluated at room temperature by an instrumental

texture profile analysis (TPA) using a Model 1122 Instron Universal Testing Machine (Instron Corp., Canton, MA) (Bourne, 1968). Bite-sized cylinders of cooked emulsions (12 mm long, 23 mm diam.) were subjected to two consecutive compressive strokes between large-diameter flat plate fixtures to 75% of their original height at a crosshead speed of 100 mm/min and a chart speed of 1000 mm/min. The resulting force-deformation curves were analyzed for rupture force, first bite hardness, second bite hardness, springiness and cohesiveness (Fig. 1). Areas under the force-deformation curves were measured with an electronic digitizer. Duplicate measurements were used for statistical analysis of texture and stability data.

#### Light microscopy

Unfixed pieces of sample were frozen in liquid nitrogen, allowed to equilibrate to -25°C in a cryostat microtome, then sectioned to a thickness of 14 micrometers. The sections were affixed to glass slides with Mayer's glycerol albumen adhesive prior to staining. For lipids, sections were immersed in 100% propylene glycol

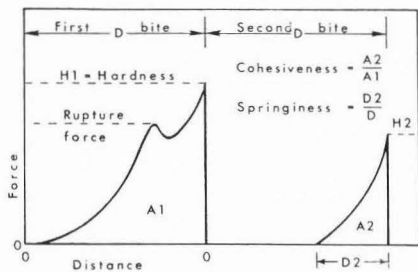


Fig. 1. Typical force/deformation curves for texture profile analysis of meat emulsion samples.

for 6 min, stained in 0.5% Sudan Black B in propylene glycol for 10 min, then differentiated in 85% propylene glycol for 6 min and 50% propylene glycol for 2 min. These sections were not counterstained for protein as fine detail tended to be obscured. To examine the protein matrix independently, separate sections were stained with 0.17% Light Green in 0.33% acetic acid for 1 min, rinsed in distilled water, dehydrated with 90%, then 100% ethanol, then delipidated with xylene. The samples were examined and photographed under brightfield illumination with 5X and 10X objectives, using a Wild M20 microscope and a Pentax 35 mm camera. Micrographs representative of those from each treatment are shown in Figures 3 to 7. Although there have been several reports where differential staining has been used to successfully identify soy proteins (e.g., Coomaraswamy and Flint, 1973) or wheat gluten (Flint and Johnson, 1979) in meat products, in this study attempts to distinguish between canola and meat proteins by differential staining with periodic acid - Schiff reagent and Luidine Blue (Coomaraswamy and Flint, 1973) or Toluidine Blue (Flint and Johnson, 1979) were unsuccessful.

Fat particle distributions of the emulsions containing plant proteins heated at 95°C were obtained from 18 cm x 24 cm light micrographs of sections stained for lipid (actual specimen area =  $1.89 \times 10^6$  square micrometers). One micrograph was used for each treatment. As not all fat particles were spherical, the equivalent area diameter of the particles [the diameter of a circle having the same area as the particle (Davies, 1962; Silverman et al., 1971) was obtained using a circle template, and these were classed into 32 size categories between 10 and 126  $\mu\text{m}$  in diameter.

As the number of fat particles within each category increased rapidly as size decreased and a manual counting method was employed, the following procedure was used to obtain an unbiased estimation of the size distributions while reducing counting time. Each micrograph was partitioned into 48 squares (3 cm x 3 cm) to facilitate counting. Fat particles with diameters of 34-126  $\mu\text{m}$  (23 size categories) were counted in

all 48 squares of each micrograph. Particles with diameters of 24-34  $\mu\text{m}$  (4 size categories) were counted in 36 squares selected at random, and the counts were projected to an estimated count for the entire field. Similarly, fat particles with diameters of 18-24  $\mu\text{m}$  (2 size categories) were counted in 14 randomly selected squares, while particles of 10-18  $\mu\text{m}$  in diameter (3 size categories) were counted in 5 squares. The individual fat particle distributions were then combined and averaged to give the following contrasts: (1) 66.7% soy vs. 66.7% canola protein substitution, (2) 33.3% soy vs. 33.3% canola, (3) 33.3% soy vs. 66.7% soy, (4) 33.3% canola vs. 66.7% canola, (5) soy vs. canola, and (6) 33.3% vs. 66.7% substitution. Each fat particle distribution in the contrasts was the mean of either 2 (contrasts 1, 2, 3 and 4) or 4 (contrasts 5 and 6) fields. As the distributions could not be represented by a single mathematical relationship, no statistical comparative procedures were performed.

#### Scanning electron microscopy

Small cubes of cooked emulsions approximately (4 mm)<sup>3</sup> were cryofractured in liquid nitrogen by placing the cubes between 2 flat surfaces and administering a sharp blow (Theno et al., 1978). Small fragments approximately (1-1.5 mm)<sup>3</sup> were fixed in 4% glutaraldehyde in 0.07 M phosphate buffer (pH 7.0) for 12-24 h at 4°C. After rinsing three times in phosphate buffer, secondary fixation was accomplished with 1% osmium tetroxide in phosphate buffer for 4 h. After a second set of phosphate buffer rinses, the samples were dehydrated through a graded ethanol series followed by exchange of ethanol with a graded series of amyl acetate in 100% ethanol, then 100% amyl acetate (to more easily detect completion of drying) for 1 h. The samples were dried in a Parr critical point drying bomb (Parr Instrument Co., Moline, IL) using liquid CO<sub>2</sub>, mounted on aluminum stubs, gold coated in a Technics sputter coating unit (Technics Inc., Alexandria, VA), and observed with a Cambridge Stereoscan 250 SEM (Cambridge Instruments (Canada) Inc., Montreal, PQ) at an accelerating voltage of 20 kV.

#### Results and Discussion

##### Texture profile analysis

Analysis of variance of TPA data revealed a complex interaction between experimental factors and texture profile components, although replacement level was most predominant (Tables 1 & 2). For rupture force, replacement level was the only significant main effect ( $p < 0.01$ ) where rupture force values decreased as replacement level increased. An interaction was also found between replacement level and cook temperature ( $p < 0.05$ ); at 66.7% replacement of meat protein, the 95°C cook produced higher rupture force values than at 70°C, whereas at 33.3% replacement the opposite was true.

For first bite hardness and second bite hardness, replacement level was again significant ( $p < 0.01$ ) with decreased hardness values at increased substitution levels. Canola protein isolate produced significantly greater hardness values

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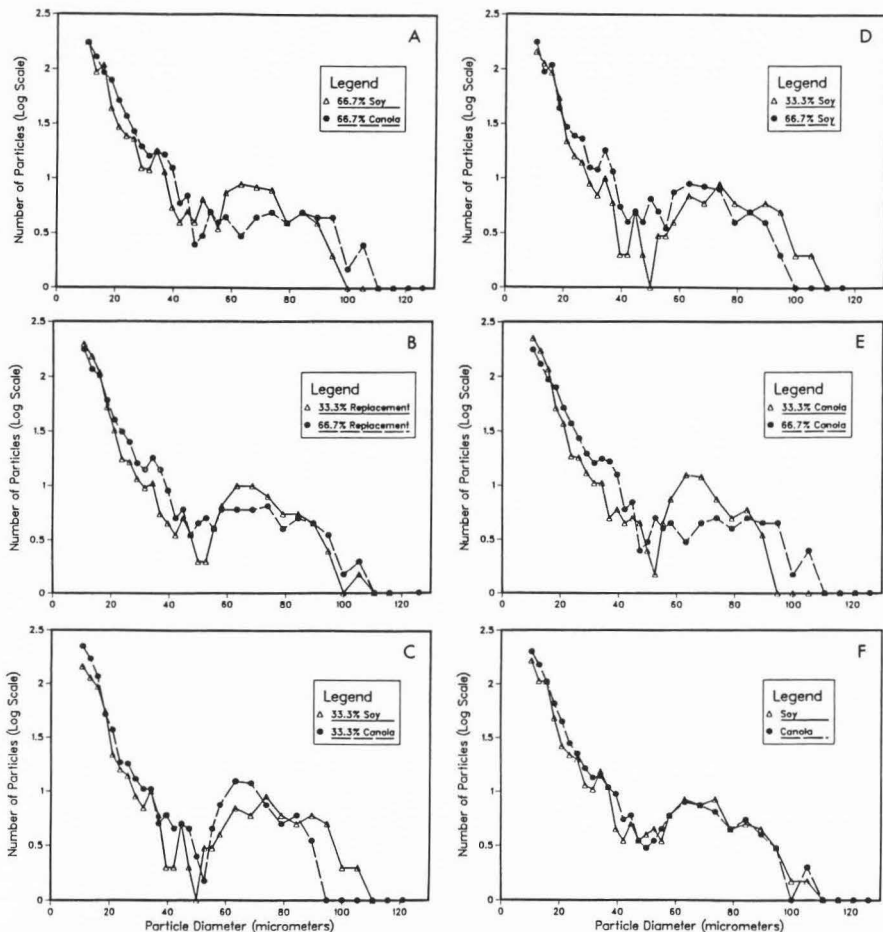


Fig. 2. Fat particle distributions of meat emulsions containing soy or canola protein: (A) 66.7% soy vs. 66.7% canola; (B) 33.3% vs. 66.7% substitution; (C) 33.3% soy vs. 33.3% canola; (D) 33.3% vs. 66.7% soy; (E) 33.3% vs. 66.7% canola; (F) soy vs. canola substitution.

than soy protein isolate ( $p < 0.01$ ). An interaction was found between cook temperature and state of hydration ( $p < 0.05$ ) with greater hardness at 70°C if the isolates were rehydrated prior to addition, whereas the 95°C cook produced the opposite effect. As replacement level increased, rupture force decreased at a faster rate than hardness.

Springiness, expressed as percent recovery from the original deformation, was influenced by

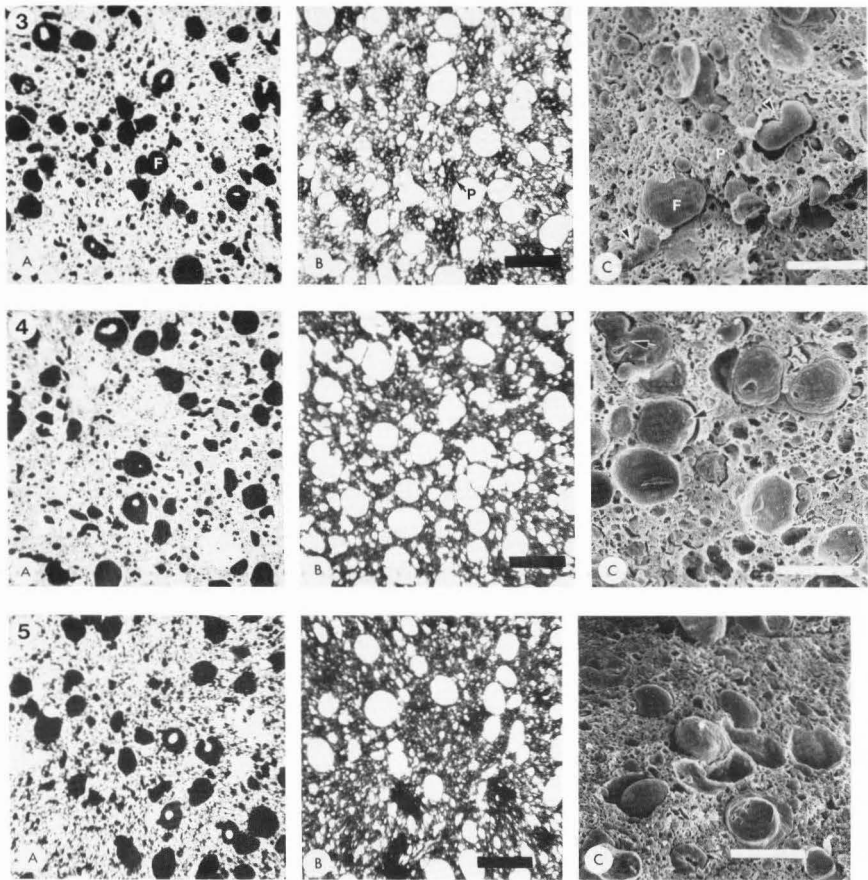


Fig. 3. 66.7% soy protein meat emulsion cooked at 95°C for 50 min: (A) and (B), light micrographs of lipid and protein staining, respectively. (bar=200µm); (C) SEM micrograph (bar=100µm). F and P indicate fat and protein, respectively. Arrows indicate deformed fat particles (Fig. 3C).

Fig. 4. 66.7% canola protein meat emulsion cooked at 95°C for 50 min: (A) and (B), light micrographs of lipid and protein staining, respectively. (bar=200µm); (C) SEM micrograph (bar=100µm). Arrows (Fig. 4C) indicate pores.

Fig. 5. 33.3% soy protein meat emulsion cooked at 95°C for 25 min: (A) and (B), light micrographs of lipid and protein staining, respectively. (bar=200µm); (C) SEM micrograph (bar=100µm).

two factors; replacement level ( $p < 0.01$ ), where greater springiness was found with 33.3% than 66.7% replacement, and cook temperature ( $p < 0.01$ ), where 95°C produced springier products than 70°C. For the control emulsions, cook time appeared to be more important for springiness than temperature, with a 50 min cook producing

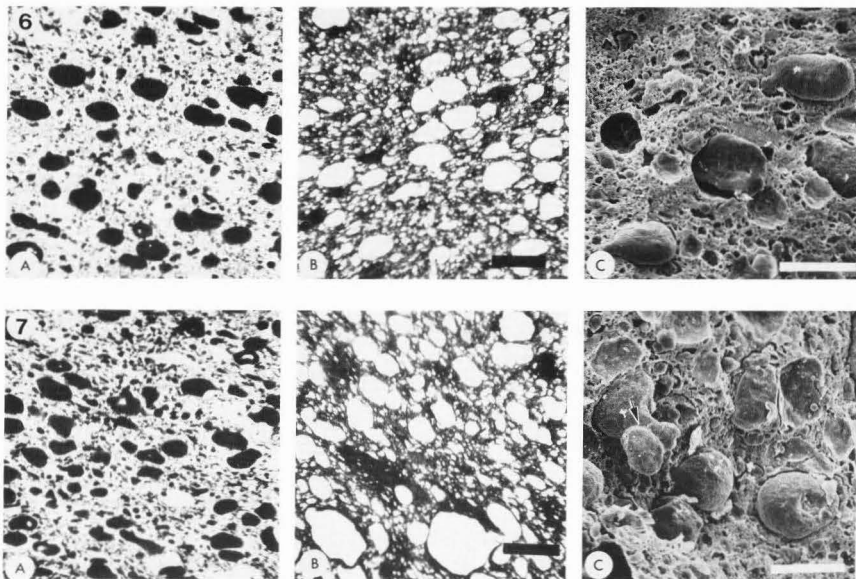


Fig. 6. All-meat emulsion cooked at 70°C for 25 min: (A) and (B), light micrographs of lipid and protein staining, respectively, (bar=200 $\mu$ m); (C) SEM micrograph (bar=100 $\mu$ m).

Fig. 7. All-meat emulsion cooked at 95°C for 50 min: (A) and (B), light micrographs of lipid and protein staining, respectively, (bar=200 $\mu$ m); (C) SEM micrograph (bar=100 $\mu$ m). Arrow indicates possible coalescence (Fig. 7C).

greater springiness than 25 min, although slightly greater springiness was produced at 70°C than 95°C. Cohesiveness was not affected significantly by any of the factors under investigation.

Patana-Anake and Foegeding (1985) also found significant interactions between heating temperature and time of cooking for stability and textural characteristics of meat batters containing soy protein or wheat gluten.

#### Cook stability

The treatments had small but statistically significant effects on cook stability. Stability was affected by replacement level (98.5% yield at 66.7% replacement vs. 97.2% at 33.3% replacement;  $p < 0.01$ ), cooking temperature (98.6% at 70°C vs. 97.1% at 95°C;  $p < 0.01$ ), cooking time (98.1% at 25 min vs. 97.7% at 50 min;  $p < 0.05$ ) and protein source (canola, 98.1% vs. soy, 97.8%,  $p < 0.05$ ), as well as interactions between protein source and pretreatment ( $p < 0.05$ ), protein source and cooking time ( $p < 0.05$ ), and cook temperature and replacement level ( $p < 0.01$ ). These small variations are probably unimportant from a practical standpoint when compared to the all-meat control emulsions, in which yield varied from 82.0% with a 95°C, 50 min cook to 98.4% with a 70°C, 25 min cook. These data are consistent with the work of Randall et al. (1976) and Sosulski et al. (1977)



who attributed improved cook stability of frankfurters containing plant proteins to increased water holding capacity, and Schut (1976) who described decreased water holding capacity of meat proteins with increased severity of thermal treatment as being due in part to protein denaturation, coagulation and shrinkage.

#### Microstructure

Fat particles ranged in size from less than 1  $\mu\text{m}$  to 130  $\mu\text{m}$  in diameter. The distributions of fat particles with diameters of 10-126  $\mu\text{m}$  were obtained for emulsions containing plant proteins (Fig. 2).

Fig. 3A shows the fat particles in a 66.7% soy-substituted emulsion cooked at 95°C for 50 min. The proteinaceous matrix (Fig. 3B) had an open, lacey appearance with regularly spaced areas of more densely staining protein material. An SEM micrograph (Fig. 3C) showed fat particles embedded in the proteinaceous matrix, several of which appeared to be deformed perhaps during comminution and stuffing or by coalescence during cooking. The 66.7% canola-substituted emulsions had a greater number of fat particles with diameters of 10-50  $\mu\text{m}$  and fewer particles with diameters greater than 50  $\mu\text{m}$  (Fig. 2A and 4A) and the protein matrix had a more compact and less lacey appearance than the 66.7% soy emulsion (Fig. 4B and C). Also seen were a number of pores and openings in the fat particles (Fig. 4C, arrows) which are similar to those reported by Jones and Mandigo (1982) who suggested that the pores may serve as a pressure release mechanism for the fat globules during cooking. These were seen in other samples as well. At this replacement level the canola emulsions had firmer texture but were less springy than those containing soy protein. It was noted that in addition to having poorer textural attributes, emulsions containing plant protein that were cooked at 70°C were very difficult to section for LM and tore easily during staining so were not used for determination of fat particle distributions. These effects may be attributable to greater thermal denaturation and enhanced gelation of plant proteins under the more severe cooking conditions.

All fat droplet distributions (Fig. 2) had similar shapes; the large droplets (greater than approximately 50  $\mu\text{m}$  in diameter) approximated a normal distribution, while below 50  $\mu\text{m}$  the particle number increased in an essentially logarithmic manner. The size range of the large droplets was very similar to that reported by van den Oord and Visser (1973) and Lee (1985) for the cell diameter of adipose tissue. Thus it appears as though the fat particle distributions were the result of relatively intact fat cells as well as a wide range of finely dispersed particles that resulted from fat disintegration during the comminution process. The microstructure of meat emulsions is influenced by such factors as the types of meat and fat, the levels of fat, moisture and salt, the comminution process (e.g., chopping speed), emulsion viscosity, and cooking conditions (Ackerman et al., 1971; Lee, 1985).

The microstructure of a 33.3% soy-substituted emulsion is shown in Fig. 5. At this replacement level, the protein matrix of both soy

and canola-substituted emulsions had a tighter, less lacey appearance than at 66.7% replacement, whereas the matrix of the canola emulsion (not shown) again appeared somewhat more compact than the soy emulsion. The fat particle distribution of the 33.3% substituted emulsions showed fewer particles with diameters greater than 15  $\mu\text{m}$  than the 66.7% substituted emulsions (Fig. 2B), but a greater number of particles with smaller diameters. As with the 66.7% replacement level, the 33.3% canola emulsions had a greater number of fat particles with diameters less than 50  $\mu\text{m}$  as compared to the 33.3% soy emulsions, while the 33.3% soy emulsions had more particles larger than 50  $\mu\text{m}$  in diameter (Fig. 2C). The 66.7% soy emulsions had more fat particles between 10 and 100  $\mu\text{m}$  in diameter than were found in the 33% soy emulsions (Fig. 2D) while 66.7% canola emulsions had more particles between 20 and 55  $\mu\text{m}$  and greater than 90  $\mu\text{m}$  than 33.3% canola emulsions (Fig. 2E). Overall, both soy and canola emulsions had similar numbers of large particles (Fig. 2F) but canola emulsions had more particles smaller than 50  $\mu\text{m}$  in diameter. As the fat particle distributions were estimated by a manual counting technique, only a small number of fields could be examined, which limited the reliability of the data. Recent developments in image analysis, which allow for rapid collection and processing of this type of data, should greatly increase the efficacy of analyzing not only the fat particle distribution but also the matrix structure of meat emulsions (Kempton et al., 1982; Kempton and Trupp, 1983). The significance of fat particle size distribution on texture is unclear; Cassens and Schmidt (1979) observed that commercial processed meats tended to have an inverse relationship between fat particle mean diameter and resistance to penetration, but in the present investigation the effect of particle size would appear to be secondary to factors influencing strength of the protein matrix.

It is interesting to note that greater rupture force was obtained with a 95°C cook at 66.7% replacement, whereas the opposite tendency was found at 33.3% replacement, especially with soy protein. It would appear that at 66.7% replacement the functional behavior of the non-meat proteins predominated, while at 33.3% replacement, the meat protein predominated. Siegel et al. (1979) suggested that isolated soy protein interferes with gel-forming interactions between myosin molecules. King (1977) found an interaction between the 7S fraction of soy protein and myosin when exposed to temperatures in the 75-100°C range, while Peng et al. (1982a,b) reported an interaction between the 11S fraction of soy protein and myosin at temperatures greater than 85°C. Since these interactions take place at temperatures which are greater than those generally used in comminuted meat products, high levels of non-meat proteins probably act only as a diluent, decreasing meat protein interactions and gelation, and resulting in softer product texture when cooked at normal processing temperatures.

Microstructure of all-meat control emulsions varied with processing conditions. With a 70°C, 25 min cook (Fig. 6) the distribution of fat particles appeared fairly similar to that of the

33.3% replacement emulsions, but with a slightly more open matrix. The large lipid droplets were also more oblong than in the samples containing plant protein, perhaps reflecting the increased viscosity of the system and an orientation effect during stuffing. In the sample cooked at 95°C for 50 min (Fig. 7) there were more large and intermediate size droplets, perhaps as a result of shrinkage of the matrix and droplet coalescence (Fig. 7C, arrow). This sample had greater firmness and springiness than that shown in Fig. 6 but also had much lower yield, which was probably a contributing factor to the textural and microstructural differences.

Several authors have attempted to relate functional properties of non-meat proteins to their performance as ingredients in comminuted meat products. Thomas et al. (1973) and Lauck (1975) suggested a relationship between apparent viscosity of the formulation and cooking loss. Hermansson (1975) and Hermansson and Akeson (1975a,b) related moisture loss in a heated lean meat system containing non-meat proteins to salt concentration, protein type and amount, swelling, viscosity and gelation of the added proteins. Torgersen and Toledo (1977), working with novel proteins in a comminuted meat system, found a significant negative correlation between protein solubility and fat binding, as well as solubility at 100°C and textural mechanical properties. Comer (1979) stated that the performance of fillers in comminuted meat products was better indicated by gelation and water binding than emulsifying ability. Comer et al. (1986) found that a number of different fillers improved stability and firmness of wieners when added at levels of 6.8% solids but also appeared to increase fat agglomeration. Cassens et al. (1975) observed that textured soy flour in a frankfurter emulsion retained its morphology after processing and suggested that it merely took up space within the matrix. Paulson et al. (1984) reported that the stability and firmness of model frankfurters containing modified plant proteins were influenced mainly by variables relating protein-lipid interactions such as fat absorption, hydrophobicity, and oil emulsification properties of the added proteins.

In the present study, differences in texture and cook stability due to protein source, pretreatment, and cooking conditions were small compared to the effects of replacement level. These results may be due not only to superior gelation properties of the rod-like salt-soluble meat proteins but also as a result of coagulation and gelation of meat proteins at temperatures lower than those required for denaturation of plant proteins. Therefore, by the time the plant proteins were able to contribute to structure formation, the matrix had already set. The observed differences in fat droplet distribution and matrix appearance may have resulted from the thermal processing conditions as well as decreased apparent viscosity of the emulsions as replacement levels increased (Voisey and Randall, 1977), which would affect the mobility of the fat droplets during comminution and their resistance to the cutting action of the Omnimixer blades. As noted by Froning and Neelakantan (1971), for

chicken frankfurters showing greater tensile strength there was greater uniformity in the appearance of the fat globules and a heavier matrix of protein surrounding the fat globules. This may also be a contributing factor to the textural differences found between protein replacement levels in the present study.

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Discussion with Reviewers

P. Allan-Wojtas: What is the origin of the methods you used for staining fat and protein?

Authors: Staining meat emulsions with Light Green for protein or with Sudan Black B for lipid have been used routinely in a number of laboratories (e.g., Kempton and Trupp, 1983 (Light Green); Ackerman et al., 1971 (Sudan Black B)). The specific methods we used were modifications that we found to be suitable for our studies.

A.-M. Hermansson: To what extent is the fat extracted and recondensed in the structure during the dehydration and critical point procedure and how has the result been checked for artifacts due to redistribution of fat particles during preparation?

Authors: Conventional fixation techniques (glutaraldehyde and osmium tetroxide) were used to preserve protein and lipid structure. If fat is extracted and recondensed or redistributed, this should be readily apparent by the presence of numerous fat globules that appear "out of place", i.e., not associated with a corresponding void in the matrix or just sitting on the surface. We did not observe features such as these in our micrographs.

P. Allan-Wojtas: Why did you analyze the light micrographs by the methods you described?

Authors: As we did not have access to an image analyzer, we had to rely on a manual counting technique to obtain fat particle size data. We decided to determine particle size distribution rather than an arithmetic mean and standard deviation because there appeared to be a lack of distribution data in the meat emulsion literature. We were limited to the number of fields that could be examined, however, because of the laborious nature of manual counting methods. The "equal area diameter" is a rapid and reasonably accurate method for obtaining a single measurement for non-spherical particles, and a circle template was used with photomicrographs rather than direct observation and measurement with an eyepiece graticule because more size categories could be obtained with less fatigue using the template method. Although we would have preferred to examine more than one field per treatment, we felt that since different micrographs within each treatment were very similar and the treatment contrasts were based on mean values from at least two fields, the particle size distributions would provide sufficient complementary information to the textural and stability data and would be superior to data based on mean particle size. In light of the bimodal nature of the distributions, we feel that our choice was justified, as merely obtaining a mean particle size and standard deviation would be misleading and statistically incorrect.

C.J. Randall: Would the meat industry use isolates in the manufacture of meat emulsion type products, given their cost in relation to flours and concentrates?

Authors: Yes, provided that the isolates, because of their greater functionality, provide benefits in product quality (by either enhancing or maintaining quality while replacing other components) that outweigh their cost. Non-meat fillers have been used mainly at low levels to improve water and fat binding, and for this application there is little real advantage in using protein isolates. We are seeing work now, though, where plant proteins are being modified in various ways to improve their functionality, especially the ability to form rigid gels at temperatures used in meat processing. If these improved isolates can be provided at a reasonable cost, we can expect to see their increased use in the future.

P. Allan-Wojtas: Why wasn't transmission microscopy used? Plant and muscle proteins can be distinguished using this technique.

Authors: The high resolution and histochemical opportunities afforded by TEM have indeed been valuable in previous studies on meat emulsions. As our study was in many respects a screening experiment where we examined the effects of a relatively large number of treatments on texture and cook stability, we felt that sufficient complementary microstructural information would be provided by LM and SEM. In future studies where we wish to study fewer variables in greater depth, we will certainly consider using TEM.