Physical Characteristics and Microstructure of Reduced-fat Frankfurters as Affected by Salt and Emulsified Fats Stabilized with Nonmeat Proteins

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ABSTRACT: Functional properties and microstructure of frankfurters containing 1.5% or 2.5% salt and 15% pre-emulsified fat (PEF) stabilized with 2% pea protein, soy protein, or sodium caseinate were studied. With the exception of frankfurters with pea protein and 1.5% NaCl, all the others made with PEF had greater (p < 0.01) thermal stability than all-meat frankfurters. Frankfurters containing soy protein or sodium caseinate had greater (p < 0.01) shear force than those with pea protein. Reducing NaCl in the frankfurters containing PEF did not influence the shear force. Microstructure examination revealed that many fat globules were entrapped physically within soy protein or sodium caseinate, which stabilized the meat emulsions and contributed to a firmer texture.

Key Words: emulsified fat, reduced-fat, low-salt, microstructure, frankfurters

Introduction

Reducing fat in emulsion-type meat products without a simultaneous addition of water not only increases cost because of higher lean meat content, but also results in a firmer and less juicy texture. Replacing fat with water has been reported to increase cooking and purge losses (Claus and others 1989, 1990; Gregg and others 1993). Furthermore, using less sodium chloride in a formulation will affect the water-holding capacity and emulsifying properties of meat protein (Hamm 1960; Schut 1976; Sofos 1983). Hand and others (1987) reported that low-fat frankfurters containing 1.5% NaCl had softer texture than those containing 2.0 or 2.5% NaCl. Another challenge facing meat processors is how to formulate low sodium meat products while maintaining the flavor of original salted products (Gillette 1985). Matulis and others (1995) studied sensory characteristics of frankfurters as affected by fat, salt, and pH and suggested that acceptable frankfurters could be manufactured with a minimum of 11.25% fat and 1.5% salt at pH 6.0.

Addition of nonmeat proteins in frankfurter formulations is believed to partially compensate for the potential loss of some water-binding properties with high water addition and salt reduction. On a commercial scale, it is easier for meat processors to use dry addition of nonmeat ingredients. However, in order to extend the functionality of nonmeat proteins in comminuted meat products, pre-emulsified fat (PEF) with nonmeat proteins as stabilizers has been employed for several years, especially in Europe (Schut 1978; Tyutyundzhiev and others 1979). When PEF is used, more salt-soluble meat proteins are saved for fat and water binding because part of the fat and water is stabilized completely by the nonmeat proteins. Zayas (1985) reported that addition of PEF with sodium caseinate, nonfat dry milk, and gelatin as stabilizers in sausages increased cooking yield 6% to 7%. Incorporation of PEF with corn germ protein used as a stabilizer in PEF formed a more uniform distribution of fat globules in frankfurters than when added as powder. Scanning electron microscopy revealed that reducing the NaCl level from 2.5% to 1.5% in meat batters with extended chopping time resulted in fat globules with rough surfaces (Barbut 1988). No published papers have reported about the utilization of PEF in reduced-fat and low-salt frankfurters. This research was conducted to study the effects of salt level and PEF with nonmeat proteins on physical characteristics and microstructure of fat-reduced frankfurters.

Results and Discussion

The all-meat frankfurter had the lowest moisture and protein content and highest fat content (p < 0.01) (Table 1). No differences in composition were found among the treatments with the nonmeat proteins.

Thermal stability

Except for pea protein in the treatment with 1.5% NaCl, addition of the nonmeat proteins as PEF in frankfurters increased (p < 0.01) the thermal stability (Table 2). The thermal stability reflects the ability of a sample to retain water and fat during heating. Pea protein isolate had a much lower water hydration capacity (3.21 g H2O/g protein) than soy protein isolate (5.85 g H2O/g protein) (Naczk and others 1986). The water retention property is very critical, especially in the high water added and NaCl reduced frankfurters. NaCl reduction was detrimental to the meat batter stability if the nonmeat protein used had poor water retention. Similar thermal stability was found for the frankfurters containing soy protein or sodium caseinate with 1.5% or 2.5% NaCl and pea protein with 2.5% NaCl.

Shear force

Shear forces for all the treatments are shown in Table 2. No significant differences in shear forces occurred between the all-meat frankfurters and the frankfurters containing soy protein or sodium caseinate with 1.5 or 2.5% NaCl. However, the addition of pea protein in PEF decreased (p < 0.01) the shear force regardless of NaCl content. As mentioned in the paragraph above for thermal stability, pea protein isolate showed poor water retention...
Salt and Emulsified Fat Effects on Franks . . .

Color

Incorporation of the nonmeat protein as PEF in frankfurters had no effect (p > 0.01) on a and b values (Table 2). With the exception of frankfurters with soy protein and 1.5% NaCl and those with pea protein and 2.5% NaCl, all frankfurters with PEF had lower (p < 0.01) L values than all-meat frankfurters. NaCl reduction of frankfurters containing PEF with nonmeat proteins did not have a significant effect on color.

Microstructure

Transmission electron microscopy (TEM) micrograph of a raw meat batter without the nonmeat proteins is shown in Fig. 1. Because the micrographs of batters for all the treatments were very similar, only one of them is presented. The micrographs were characterized by a continuous protein matrix (dark stain) in which many small fat globules with 0.1 to 5 µm in diameter were evenly distributed. No obvious differences were noted among the treatments. Many fat globules were coated with a very thin protein membrane, which was also observed by Swasdee and others (1982) and Hermansson (1986) for raw meat batters using TEM and light microscopy, respectively. Before cooking, all the treatments had a homogeneous protein matrix containing evenly dispersed fat globules indicating that fine and stable emulsions were formed.

Results of the cooked meat batters are shown in Fig. 2 to 5. The micrographs of the all-meat frankfurters revealed that most of the fat globules, which were oval and varied in size (0.2 to 5 µm), were coated with a layer of protein membrane and dispersed in a dense protein matrix (Fig. 2). The protein membranes surrounding the fat globules were thicker than those observed in the raw meat batters (Fig. 1). Jones (1984) suggested that free myosin accounts for the initial development of a protein membrane by orienting the heavy meromyosin (HMM) head toward the hydrophobic phase and leaving the light meromyosin (LMM) tail protruding into the aqueous phase to form a surface monolayer. The random protein-protein interactions among myosin, actomyosin, and other proteins that occur after the monolayer forms then thicken and strengthen the protein membrane. Swelling of the protein membranes during cooking may contribute to the observed thicker protein membranes in the cooked meat batters.

Micrographs of the meat batters containing pea protein in PEF with 1.5% or 2.5% NaCl are shown in Fig. 3. Some irregularly shaped and elongated fat globules were embedded in the protein matrix, which was not as dense as the one observed for the all-meat frankfurter. This indicated that less stable emulsions

and water absorption properties when compared with soy protein isolate (Naczk and others 1986). This may explain the lower shear force obtained for the frankfurters containing pea protein.

Table 1—Proximate compositions of cooked frankfurters

Table 2—Color measurements, thermal stability (Ts), and shear force of frankfurters

![Fig. 1—TEM micrograph of a raw all-meat frankfurter. Me = protein membrane; FG = fat globule; PM = protein matrix.](image1)

![Fig. 2—TEM micrograph of a cooked all-meat frankfurter. Me = protein membrane; FG = fat globule; PM = protein matrix.](image2)

![Table 2—Color measurements, thermal stability (Ts), and shear force of frankfurters](table2)
were formed during cooking. More large fat globules were also observed. The fat globules ranging from 0.5 to 15 μm in diameter were larger than those in the all-meat frankfurters, particularly for the meat batters with 1.5% NaCl (Fig. 3B). The denser areas within the protein matrix (DPM) and the protein layer attached on part of some irregularly shaped fat globule surfaces (Fig. 3B) appeared to be the pea protein. Fat agglomeration must have occurred for the meat batters containing pea protein during heat processing, because a higher frequency of large fat globules was seen thereafter in cooked samples. Voids also were found in the protein matrix of the meat batters with pea protein. These could have resulted from the release of fat, water, or gelatin during cooking.

Figure 4 shows the micrographs of the meat batters containing soy protein in PEF with 1.5% or 2.5% NaCl. Many fat globules with smooth surfaces were entrapped by the denser protein matrix that was thought to be the soy protein (Fig. 4A). Most fat globules were smaller than those in the meat batters containing pea protein but similar to those in the all-meat frankfurters and the raw meat batters, indicating less fat agglomeration during cooking.

A relatively large and elongated fat globule with a rough surface was observed on the micrograph of the meat batter with soy protein and 1.5% NaCl (Fig. 4B). This globule had a very light and negligible protein membrane. The two small round fat globules coated with a thin protein membrane above the large fat globule may have resulted from fat exudation through the weak spots on the protein membrane of the large fat globule. Exudation of fat through pores in the protein membrane is thought to contribute to fat stabilization during cooking (Gordon and Burbut 1992).

Micrographs of the meat batters containing sodium caseinate in PEF with 1.5% or 2.5% NaCl are shown in Fig. 5. The fat globule in the center of Fig. 5B seems to be well stabilized by a layer of the hydrated sodium caseinate. Many small fat globules also

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**Fig. 3—TEM micrographs of cooked frankfurters containing pea protein in PEF with 2.5% NaCl (A) and 1.5% NaCl (B).** Me = protein membrane; IFG = irregularly shaped fat globule; PM = protein matrix; DPM = dense protein matrix; V = void.

**Fig. 4—TEM micrographs of cooked frankfurters containing soy protein in PEF with 2.5% NaCl (A) and 1.5% NaCl (B).** FG = fat globule; EFG = elongated fat globule; PM = protein matrix; DPM = dense protein matrix; IM = intact muscle.
were embedded in a denser protein matrix composed of the sodium caseinate in the treatment with 1.5% NaCl (Fig. 5B). This is similar to the treatment containing soy protein (Fig. 4). When the fat globules are confined locally within the denser nonmeat protein matrix, the chances for fat coalescence during cooking may be reduced so that emulsions with high fat and water binding properties are formed. Thus, the products with firmer texture are expected. One fat globule with small-diameter pores on the protein membrane was found in the protein matrix (Fig. 5A). A similar phenomenon was also observed in previous studies (Borchert and others 1967; Jones and Mandigo 1982; Barbut 1988). These pores are believed to serve as fat exit routes and play an important role in a possible pressure-releasing mechanism for the thermally induced fat expansion within the globules during cooking (Jones and Mandigo 1982).

Reduction of NaCl in the formulations with the nonmeat proteins in PEF did not affect the microstructures of the cooked meat batters except the one with added pea protein, which showed larger irregularly shaped fat globules (Fig. 3B). Fat globules with wrinkled surfaces observed by Barbut (1988) for the meat batter with 1.5% NaCl was not evident in this study for the meat batters containing the soy protein or sodium caseinate as PEF with 1.5% NaCl. Addition of soy protein or sodium caseinate as PEF in the meat batters may have compensated for the lack of salt-soluble proteins with NaCl reduction or excess water addition and stabilized both fat and water in the meat emulsions.

The micrographs of this study indicated that comminuted meat batters probably were complexes of a true emulsion and a mechanical matrix. Both the protein membrane surrounding fat globules and the continuous protein matrix played important roles in meat batter stability during cooking. Most fat globules seen in the all-meat frankfurters were coated with a thicker protein membrane (Fig. 2) and remained the same size as before cooking (Fig. 1). Also, some small fat globules of similar size observed in the meat batters containing the nonmeat proteins were surrounded with a protein membrane even though the membrane was thinner. Protein membranes appear to successfully immobilize the fat globules and prevent fat agglomeration during cooking. On the other hand, a lot of fat globules (about 50% as observed) that were entrapped physically within the hydrated soy protein or sodium caseinate in the meat batters with the 2 nonmeat proteins were well stabilized. This suggests that immobilization by a protein membrane as well as physical restriction by a protein matrix is responsible for the fat stabilization of a meat batter during cooking. However, in meat batters with extremely low fat, gelling ability and water retention capacity of nonmeat ingredients may play a greater role rather than emulsion formation in determining the stability of the products. Chin and others (1998a) reported that low-fat bologna (<3% fat) containing konjac blend (0.5% to 1.5%) as a fat replacement increased most texture profile analysis (TPA) parameters over those of a control with 25% fat through moisture retention and increased stability of gel matrix. The increased TPA values declined with incremental increases in moisture : protein (M:P) ratio from 5% to 6%, with the exception of cohesiveness (Chin and others 1998b). Scanning electron microscopy revealed that unlike the relatively large fat globules coated with layers of salt-soluble meat proteins in a control bologna, the fat globules in the low-fat bologna appeared as small “dot-like” structures dispersed in the protein matrix in which konjac blend was embedded.

The results of microstructure examination for all the treatments appeared to be consistent with shear force data (Table 2). The meat batters containing pea protein found to have lowest shear forces had a higher frequency of irregularly shaped and elongated fat globules and a less dense protein matrix (Fig. 2).

**Conclusion**

TEM MICROSTRUCTURE STUDY SUPPORTED THE RESULTS OBTAINED FROM THE SHEAR FORCE TEST SHOWING THAT PEAS PROTEIN HAD A WEAKENING EFFECT ON THE TEXTURE OF THE PRODUCT WITH PEF. Nonmeat protein functionality, such as water retention, played an important role in meat batter stability during cooking. Based on the micrographs, the comminuted meat batters were likely to be a complex of a true emulsion and a mechanical matrix. Utilization of PEF using soy protein isolate or sodium caseinate as stabilizers in reduced-fat frankfurters may help sustain the desired texture that is lost because of salt reduction and/or high water addition.
Materials and Methods

Three nonmeat proteins used in this study were: (1) soy protein isolate (PRO-FAM 974, ADM Co., Decatur, Ill., U.S.A.), (2) pea protein isolate (PISANE, COSUCRA SA, Belgium), and (3) sodium caseinate (ERIE Foods International Inc., Rochelle, Ill., U.S.A.). Proximate compositions of the nonmeat proteins are shown in Table 3.

Emulsified fat preparation

Emulsified fats were prepared using 1 part nonmeat protein (pea protein isolate, soy protein isolate, or sodium caseinate), 4 parts water, and 4 parts pork backfat (Meat Laboratory, Dept. of Animal Sciences and Industry, Kansas State University, Manhattan, Kan., U.S.A.). The nonmeat proteins were hydrated with water in a Braun food processor (UK 280, Kromberg, Germany) with a speed of 3000 to 4000 rpm until a sheen surface developed (3 min). Premixed pork backfat (4.69 mm plate, 0 °C) then was added to the food processor, and the mixture was warmed to a 3-4 °C until a smooth emulsion was formed. The emulsified fats were stored at 4 °C for 24 h until frankfurter preparation.

Frankfurter preparation

Beef (18% fat) was formulated to make all-meat batters with 15% fat and 2.5% NaCl. Beef (10% fat) and pork backfat (90% fat) were used to make meat batters containing 2% nonmeat proteins with 15% fat and 1.5% or 2.5% NaCl. Based on a total batch weight, 12% more water was added to the experimental treatments because of the addition of the nonmeat proteins. Three replicates were conducted for each treatment. Each lean and fat sample was ground through a 9.38 mm plate, and reground through a 4.69 mm plate. The raw materials were then vacuum-packaged (Super-Vac, Smith Equipment Co., Clifton, N.J., U.S.A.) and frozen (-20 °C) for subsequent use. The lean and fat meats were allowed to temper at 4 °C for 48 h prior to making frankfurters.

The ground meat components were mixed in a Hobart bowl mixer (model 403) with tripolyphosphate for 30 to 45 s and then with NaCl (1.5% or 2.5%) and Prague powder (6.25% sodium nitrite, Griffith Lab., Alsip, Ill., U.S.A.) for 1 min. Ice water was added during mixing to keep the temperature low. Preemulsified fat (experimental treatments only), 0.5% spice mix, 0.05% sodium erythorbate, and 1% dextrose (Fisher Scientific Co., Pittsburgh, Pa., U.S.A.) were added one by one, and the meat batter was mixed for an additional 3 min. After mixing, the meat slurries were processed twice through the Minicemaster Emulsion Mill (Griffith Design and Equipment Co., Chicago, Ill., U.S.A.) with a 1.7-mm-diameter orifice plate. The batter temperature was around 12 to 13 °C after the emulsification. The meat batters were transferred to a vacuum stuffer (VEMAG, Robert Reiser Co., Canton, Mass., U.S.A.) and stuffed into 25-mm dia cellulose casings. The frankfurters were cooked in a commercial smokehouse (Maurer and Sohne, Reichenau, Germany) using the following schedules: 10 min at 48 °C; 30 min at 55 °C; 5 min at 55 °C, smoke on; at 80 °C (85% RH) to an internal temperature of 70 °C; and 15 min cold show until frankfurter preparation.

The internal color of the frankfurters. The chopped frankfurters were scanned to determine L, a, and b values with illuminate A and 10 degree observer angle.

Microstructure evaluation

Transmission electron microscopy (TEM) was used to view the microstructural changes of the raw and cooked meat batters. Methods of Schiff and Gennaro (1979) and Mollenhauer (1964) were followed in the sample preparation. For cooked meat batters, 1-mm³ cubes from 3 different portions (2 ends and middle) of the samples were cut in a pool of fixing solution of 2% glutaraldehyde buffer with 0.1 M PIPES (piperazine-N-N bis20 ethanol sulfonic acid) pH 7.4 and 4% solution of tannic acid. For raw meat batters, a whole link of the sample with casing was soaked in the same fixing solution for 24 h and then 1 mm³ cubes were cut from 3 different portions (2 ends and middle) of the fixed samples. An ice bath was used for the following processes through the acetone dehydration steps. The samples were washed 3 times in sucrose PIPES buffer (0.1 M/0.1 M) for 30 min. The post fixation was done with 1% osmium tetroxide (OsO₄)-PIPES buffer for 2 h to improve protein resolution. The samples were washed 4 times for 5 min each, using distilled water at 4 °C. Uranyl acetate (1% aqueous) was used to stain the samples for 2 h, and then the samples were rapidly dehydrated for 10 min in each increasing concentration of an acetone series (30%, 50%, 70%, 85%, 95%, and 100%). The dehydrated samples were then vacuum-packaged (Super-Vac, Smith Equipment Co., Clifton, N.J., U.S.A.) and frozen (-20 °C) for subsequent evaluations.

Chemical analysis

Moisture content was determined by drying 10-g samples in a conventional oven (Thermotainer Franklin Products Corp., Chicago, Ill., U.S.A.) at 105 °C for 18 h. Fat analysis was performed with a Foss-Let fat analyzer (Foss Electric, Denmark) using 22.5 g samples. Protein content was determined with a Leco FP 2000 nitrogen analyzer using 0.5 g sample for each measurement.

Thermal stability

The method of Haq and others (1973) was used to measure the thermal stability of the samples. A 30-g sample was placed in a centrifuge tube (27.5 mm × 110 mm) with a screw cap and heated for 30 min in a 75 °C water bath. The tube was centrifuged at 1,200 × g for 1 min at room temperature. After 5 min standing, separated fat and water were measured. The cooking yield percentage was expressed as thermal stability.

Shear force

The texture of the frankfurters was determined using a TA-XT2 Texture Analyzer (Texture Technologies Corp., Scarsdale, N.Y., U.S.A.). Shear force was measured by shearing a 5.0-cm sample with a simulated molar (Prusa and others 1982) attached to the Texture Analyzer (speed = 3 mm/s; distance = 10 mm).

Color measurement

A Hunterlab Ultrascan reflectance spectrophotometer (Hunter-Lab Corp., Reston, Va., U.S.A.) was used to measure the internal color of the frankfurters. The chopped frankfurters were scanned to determine L, a, and b values with illuminate A and 10 degree observer angle.

Microstructure evaluation

Transmission electron microscopy (TEM) was used to view the microstructural changes of the raw and cooked meat batters. Methods of Schiff and Gennaro (1979) and Mollenhauer (1964) were followed in the sample preparation. For cooked meat batters, 1-mm³ cubes from 3 different portions (2 ends and middle) of the samples were cut in a pool of fixing solution of 2% glutaraldehyde buffer with 0.1 M PIPES (piperazine-N-N bis20 ethanol sulfonic acid) pH 7.4 and 4% solution of tannic acid. For raw meat batters, a whole link of the sample with casing was soaked in the same fixing solution for 24 h and then 1 mm³ cubes were cut from 3 different portions (2 ends and middle) of the fixed samples. An ice bath was used for the following processes through the acetone dehydration steps. The samples were washed 3 times in sucrose PIPES buffer (0.1 M/0.1 M) for 30 min. The post fixation was done with 1% osmium tetroxide (OsO₄)-PIPES buffer for 2 h to improve protein resolution. The samples were washed 4 times for 5 min each, using distilled water at 4 °C. Uranyl acetate (1% aqueous) was used to stain the samples for 2 h, and then the samples were rapidly dehydrated for 10 min in each increasing concentration of an acetone series (30%, 50%, 70%, 85%, 95%, and 100%). The dehydrated samples were then vacuum-packaged (Super-Vac, Smith Equipment Co., Clifton, N.J., U.S.A.) and frozen (-20 °C) for subsequent evaluations.

Table 3—Proximate compositions of nonmeat proteins

<table>
<thead>
<tr>
<th>Protein source</th>
<th>Moisturea %</th>
<th>Proteinb %</th>
<th>Fatb %</th>
<th>Ashb %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pea</td>
<td>6.8</td>
<td>91.0</td>
<td>4.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Soy</td>
<td>6.3</td>
<td>94.8</td>
<td>1.0</td>
<td>4.2</td>
</tr>
<tr>
<td>Caseinate</td>
<td>5.2</td>
<td>91.6</td>
<td>1.1</td>
<td>3.9</td>
</tr>
</tbody>
</table>

a Means are the average of three replicates.
b Data are from the manufacturers.
were embedded through acetone and Klomparens embedding plastic series (2:1; 1:1; 1:2) for a minimum of 2 h each and, then, through 100 plastic series for about 8 h. Vacuum was applied for 30 min (22 mm Hg) at ambient temperature to remove air that may have been entrapped in the tissues. The samples were embedded in a Flat BEEM mold and then cured for at least 48 h. Samples were sectioned with a diamond knife on a Reichert-Ultracuts microtome (American Optical Corp., Buffalo, N.Y., U.S.A.), and then post-stained with uranyl acetate and lead citrate for 10 min. The sections were collected on uncoated copper grids. A Philips 201 transmission electron microscope operating at 60 kV was used to view the prepared samples. Twenty four micrographs were taken for each treatment from different fields on the grids.

Statistical analysis

Three replications of the treatments were performed in a randomized complete block design with replications as blocks. Data were analyzed using General Linear Model procedures of Statistical Analysis System (SAS Institute Inc. 1990) to identify treatment effects. Effects of the different nonmeat proteins and of NaCl level on physical properties were analyzed using a two-way analysis of variance. Least square means procedures were used to determine differences between treatments.

References