

## MEAT EMULSIONS - FINE STRUCTURE RELATIONSHIPS AND STABILITY

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Abstract

Fine structure relationships to the thermal stability of meat emulsions were examined by light, scanning and transmission electron microscopy. Modifications of the procedures for fixation, dehydration, and embedding improved the preservation of lipid structure. Structural changes, as a result of increasing chopping temperatures, were evaluated by examining emulsions prepared at 16, 21, and 26°C and after being cooked. These changes were related to fat and water retention, shear and compression values. The lipid organization distinctly changed from oval globules to irregularly shaped structures accompanied with the formation of channels within the protein matrix.

Introduction

Emulsion type meat products make up a significant share of processed meats for the consumer. Approximately one third of all sausage products are produced as frankfurters and in 1976 amounted to approximately 1.5 billion pounds (Agricultural Statistics 1977). Thus additional knowledge of the factors contributing to the stability and shelf life of these products would be advantageous for both the processor and the consumer.

The stability of meat emulsions has been evaluated by determining the extent of fat and water released when heated at 75°C (Swift et al. 1961). Factors known to affect the fat stabilization in the meat emulsion products include: protein solubility (Bard 1965), fat dispersion particle size (Borchet et al. 1967, Ackerman et al. 1971), chopping temperature (Ackerman et al. 1971, Schut 1976, Townsend et al. 1968), chopping temperature-time relationships (Townsend et al. 1968), melting properties of fat (Ackerman et al. 1971), consistency of protein gel (Townsend et al. 1971 and Bard 1965), and water binding (Hamm and Grabowski 1978).

The structure of emulsions in relation to stability was examined first by Hanson (1960) using light microscopy (LM) and later by Borchet et al., (1967), employing transmission electron microscopy (TEM). They observed a dense membrane-like layer surrounding the fat globule, but it did not resemble a true bilayer membrane.

Recently, scanning electron microscopy (SEM) has been used to elucidate emulsion structure. Theno and Schmidt (1978) found differences in the protein matrix and fat globule distribution in commercial frankfurters. Ray et al., (1979) also combined light and scanning electron microscopy to study emulsion type products.

We investigated the structure of all-beef emulsions using LM, SEM, and TEM observations correlated with measurements of physical properties of compression, shear, and water retention as a function of three different processing temperatures (16, 21, and 26°C). In addition, we improved preservation of the lipids by modifying existing techniques of fixation, dehydration and by use of a low viscosity embedding medium.

KEY WORDS: Meat, emulsion, frankfurter, protein matrix, fat, stability, temperature, microscopy, structure, sample preparation

The application of electron microscopy to elucidate emulsion microstructure has probably been held back by lack of suitable sample preparation procedures. Hopefully, the techniques presented in this paper will aid in the use of electron microscopy to elucidate lipid-protein structures in food products.

#### Materials and Methods

Beef emulsions obtained either commercially or prepared in our laboratory were used in this study. Laboratory emulsions were prepared in a Fleetwood\* food chopper. No vacuum was used. Ground lean beef (600 g) was blended with 262 ml of prechilled 10% brine and 35 ml of 50% corn syrup for 15 min to solubilize the protein. Each batter was chopped for 15 min after the addition of ground beef trimming fat (300 g). Fat content of the batter was 26% on the average. Temperatures of the batter were varied to 16, 21, and 26°C by altering either the ingredient or ambient temperature. The ingredients for the batter were not frozen. The prepared batter was stuffed into cellulose casings (23 mm diameter) and cooked in a smokehouse so that the internal temperature rose to 66°C within 30 min and held there for another 30 min.

#### Light Microscopy

For light microscopy, specimens were frozen in liquid nitrogen, sectioned at 16 µm using a microtome-cryostat held at -30°C, fixed in vapors of 25% glutaraldehyde, and stained with oil Red-O for the lipids followed with hematoxylin for the protein matrix, and examined in a Leitz microscope.

#### Electron Microscopy

Conventional sample preparation procedures for electron microscopy usually employ a primary fixation with glutaraldehyde, post fixation in OsO<sub>4</sub>, dehydration through increasing ethyl alcohol series, and embedding in epon 812 resin. Results obtained from emulsions prepared by these procedures were poor; the fat globules gave a nonuniform appearance and many holes in the sections were found. An improved method of sample preparation was required. The method which gives better uniform preservation of the fat globule is as follows.

Samples (1 x 1½ mm) from the frankfurters (pH = ~5.9) were fixed in 1% OsO<sub>4</sub> in 0.07 M phosphate buffer at pH 5.9 for 4 hr. The samples were washed four times in the phosphate buffer at pH 5.9. The secondary fixation was carried out overnight in 3% glutaraldehyde - 1% paraformaldehyde in the same buffer. The samples were washed four times in water and rapidly dehydrated (5 min each) in increasing concentrations of acetone - H<sub>2</sub>O mixture [50, 70, 80, 95, and 100% (3 times)]. The samples were prepared both for the SEM and the TEM.

For TEM, the samples were placed in 50% acetone - Spurr resin (Spurr 1969), followed by two changes of fresh resin, and placed in capsules to be cured overnight at 65°C. Sections were cut on an LKB-8800 ultramicrotome and stained 5 min each

with uranyl acetate and lead citrate. A Zeiss 10-B TEM operating at 60 kV was used to examine the sections.

The samples for the SEM were cryofractured (Humphreys et al. 1974) in acetone at liquid nitrogen temperature and critical-point dried from carbon dioxide in a Denton DCP-1 unit. The samples were sputter-coated with gold in a Denton DSM-5 unit and examined with a JEOL 50-A SEM operating at 15 kV.

The amount of fat and water released from heated emulsions in a water bath at 60°C for 30 min was used as a stability index. This test was a modification of the method reported by Townsend et al., 1968. The internal temperature of batter at which such release commenced was measured by monitoring internal temperature changes of the batter which had been placed in a water jacketed funnel attached to a graduated cylinder. The temperature of the water jacketed funnel was maintained at 60°C by circulating water.

The mechanical strength of the cooked emulsion products was determined by compression and shear test using a straight blade (1 mm thick) with an Instron Universal testing machine (Model 1122). Cylindrical specimens (five replications) of uniform geometry (20 mm height and 20 mm diameter) were used for both tests at a deformation rate of 50 mm/min and at 1:2 ratio of chart speed to crosshead speed. The data were analyzed by an analysis of variance including emulsion treatment contrast.

#### Results

Frankfurters made from the emulsion prepared at 26°C showed visual evidence of liquids on the surface of the casings. In addition, these frankfurters had a mushy texture and were difficult to cut precisely with a razor blade.

LM photomicrographs of meat emulsions processed at 16, 21, and 26°C are shown in Fig. 1. At 16°C, Fig. 1A, a homogeneous mixture of lipid droplets surrounded by the protein matrix was observed. The lipid droplets ranged in size from > 100 µm down to the limits of resolution of the light microscope. As processing temperature was increased to 21°C, Fig. 1B, the fat droplets tended to be irregularly shaped. Some voids or air pockets were seen and the protein matrix was less homogeneous. At 26°C, Fig. 1C, the air pockets increased in size, some forming channels through the protein matrix; the lipid droplets tended to coalesce, indicating melting of the fat. The channels which have developed may have allowed egress of the lipids to the outside casing with release to the exterior of the frankfurter.

SEM photomicrographs of the same emulsions, fixed in osmium tetroxide and glutaraldehyde-formaldehyde are presented in Fig. 2. Emulsion at 16°C, Fig. 2A, showed the fractured interior surface of the protein matrix surrounding various size fat droplets, some of which have been lost during sample preparation. At 21°C, Fig. 2B, evidence for the beginning of lipid coalescence was observed. Irregular shaped fat droplets of various sizes were found. The protein matrix in this emulsion was less homogeneous than that in

\*Reference to brand or firm name does not constitute endorsement by the U. S. Department of Agriculture over others of a similar nature.

## Meat Emulsion Structure and Stability

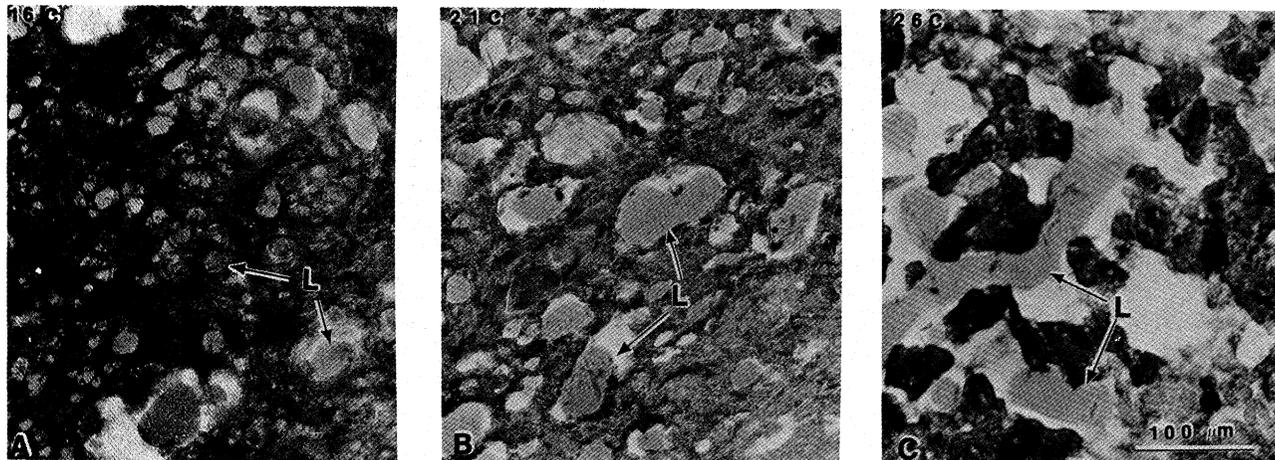


Fig. 1 LM micrographs of emulsions prepared at (A) 16°C, (B) 21°C, and (C) 26°C. L = lipid.  
Note: Increased open space in (C) where channels have formed.

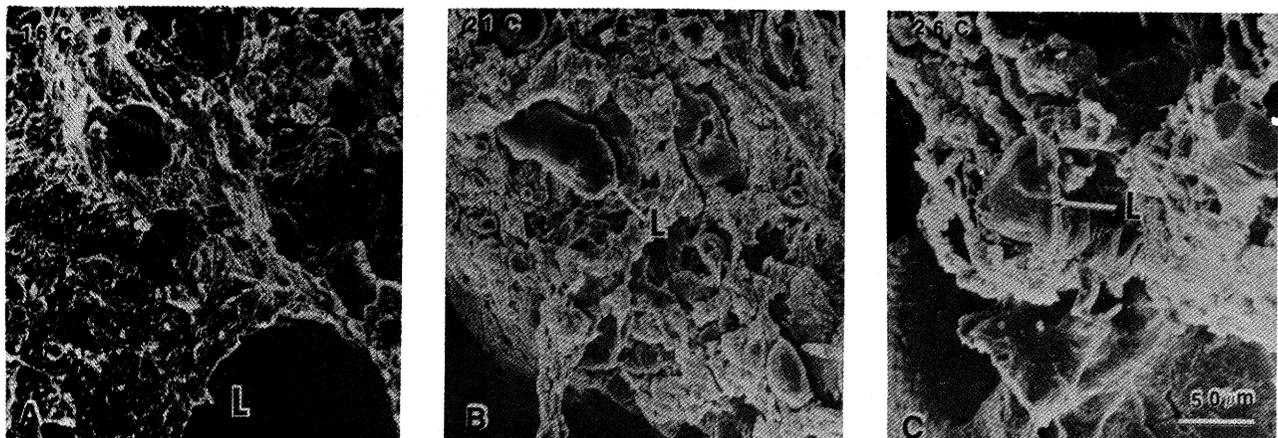


Fig. 2 SEM micrographs of emulsions prepared at (A) 16°C, (B) 21°C, and (C) 26°C. L = lipid.  
Note: In (A) lower right a void results from lipid removal during fracture.

the emulsion processed at 16°C. The emulsion processed at 26°C, in Fig. 2C, showed a change in the protein matrix as well as larger fat droplets. Little or nothing can be discerned in the SEM regarding the protein-lipid interface even at higher magnifications.

Alternate embedding procedures explored to improve retention of the fat globule structures involved water miscible resins, bovine serum albumin, and gelatin. All of these procedures resulted in either poor penetration of embedding mediums or the inability to obtain good uniform sections on the ultramicrotome. Conventional fixation, dehydration and epon embedding of commercial frankfurters resulted in the extraction of the lipid droplets and development of large holes as seen in Fig. 3A. Fig. 3B shows the improved lipid stabilization obtained with the revised procedure; the lipid is in close contact with the electron dense peripheral layer indicating minimal lipid extraction. In order to preserve the lipid moiety, the initial fixation was performed in osmium tetroxide, followed by

aldehyde fixation, in phosphate buffers at the pH of the specific emulsion sample. Rapid acetone dehydration at 0°C was followed by embedding in Spurr low viscosity resin.

TEM micrographs of thin sections of the frankfurter emulsions prepared at 16, 21, and 26°C are shown in Fig. 4. The protein matrix, Fig. 4A, consisted of disrupted muscle tissue components around the lipid droplets. The lipid droplets were limited by an electron dense membrane similar to that reported by Borchet et al. 1967. This membrane surrounded the lipids and was in partial contact with the protein matrix of the emulsion. Fat globule sizes varied with some being as small as 0.1 - 0.3 μm.

When the processing temperature was raised to 21°C, the fat globules tended to change to irregular shapes (Fig. 4B). The protein matrix was similar to that of the 16°C processed emulsion. In contrast, emulsions prepared at 26°C showed a drastic change in fat globule size and shape as seen in Fig. 4C. The fat globules increased in size, formed irregular shapes and

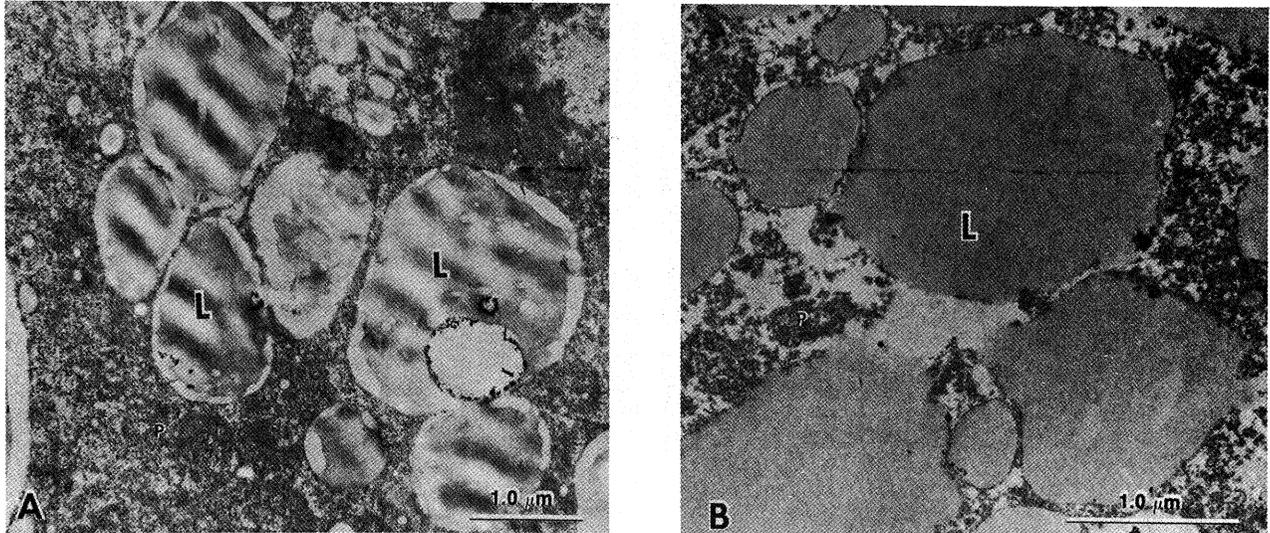


Fig. 3 TEM micrographs of commercial frankfurters: (A) prepared in buffered glutaraldehyde-paraformaldehyde, post fixed in  $\text{OsO}_4$ , dehydrated in ethyl alcohol and embedded in epon 812; and (B) fixed in buffered  $\text{OsO}_4$  - post fixed in glutaraldehyde-paraformaldehyde, dehydrated in cold acetone, embedded in Spurr resin. L = lipid. P = protein.  
Note: Much better preservation of lipid structure in (B) than in (A).

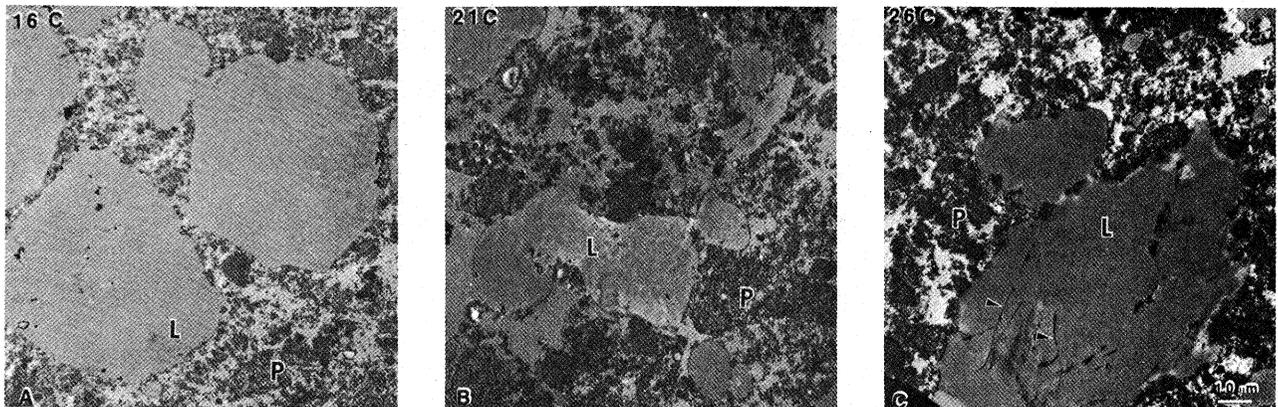


Fig. 4 TEM micrographs of emulsion prepared at (A) 16°C, (B) 21°C, and (C) 26°C. Coalescence of lipids is evident in (B) and (C). L = lipid. P = protein. Arrowheads = electron dense material.

incorporated electron dense membrane-like material in the interior of the fat globule (arrowheads). Not all the fat globules were affected, since some small globules were still observed.

These observations correlated well with results of physical tests of shear force, compression and water retention of the emulsions prepared at increasing processing temperatures. Shear force, Table 1, significantly ( $p < 0.05$ ) decreased with increasing processing temperature. The shear force required to shear emulsions prepared at 26°C was 65% of that required for those prepared at 16°C. Compression force required to compress emulsions was 76% less (significant,  $p < 0.05$ ) for those prepared at 26°C than for those prepared at 16°C, indicating a soft and mushy texture.

Emulsions prepared at increasing temperatures decreased in the ability to hold water and fat in suspension at 60°C, Table 2. Emulsions prepared at 26°C retained 22% less water and 37% less fat than emulsions prepared at 16°C ( $p < 0.05$ ).

These physical measurements reflect a decrease in emulsion stability with increasing processing temperature, and correlate with the formation of channels observed in the LM and the changes in the appearance of the fat globules found by TEM. Formation of the channels observed in the emulsion prepared at 26°C suggests a possible mechanism for release of water and fat from the protein matrix.

### Summary

Observations with light microscopy and transmission electron microscopy showed definite changes in emulsion structure with increasing processing temperature. The formation of fat channels, accompanied by changes in size and shape of the fat globules, is observed. Decreases in shear strength, compression, and water and fat retention ability are correlated with structure changes in emulsions prepared at increasing temperatures. This study reflects definitive changes occurring in emulsion morphology with decreases in emulsion stability as a function of processing temperature.

An improved procedure for preparing emulsion samples for TEM observations is presented. This procedure preserves the fat globules in the emulsion with minimum extraction of the lipids.

### Acknowledgment

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Table 1

Mechanical Properties of Emulsions		
Emulsion temp °(C)	Shear force (kg)	Compression force (kg)
16	0.60 ± 0.07	4.6 ± 0.25
21	0.57 ± 0.04	2.3 ± 0.10*
26	0.39 ± 0.04*	1.1 ± 0.21*

\*Significantly different from emulsion prepared at 16°C (p < 0.05).

Table 2

Retention by Emulsion at 60°C		
Emulsion temp °(C)	Water %	Fat %
16	97.63 ± 3.76	98.75 ± 2.65
21	86.04 ± 5.27*	72.50 ± 6.21*
26	75.93 ± 6.11*	62.50 ± 8.17*

\*Significantly different from emulsion prepared at 16°C (p < 0.05).

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### Discussion with Reviewers

F.K.Ray: Do the authors have an opinion what the open space or clear areas not containing fat or proteins are in the micrograph, Figure 1?

Authors: The irregular clear areas represent voids in the emulsion resulting from trapped air or water. Some oval areas result from loss of fat during sectioning or staining. When the stained sections are viewed under the light microscope, it is much easier to discern the empty fat globules from the water or air voids in the emulsion.

D.N.Holcomb: Did you find uniform osmium staining on the meat emulsions?

F.K.Ray: Did you find any penetration problems with OsO<sub>4</sub>?

Authors: Yes, problems with osmium tetroxide penetration of the specimen blocks were encountered initially. This was overcome by trimming the blocks as small as possible, no more than 1-1.5 mm on a side. Usually four hours fixation time resulted in complete penetration of the osmium tetroxide.

D.N.Holcomb: In many liquid to semi-solid oil-in-water emulsions, the oil droplets assume spherical shapes. Are the fats spherical in the batter, then elongate after stuffing and working?

Authors: The oil droplets in oil-in-water type emulsions are bound by absorbed proteins forming an interface which stabilizes the spherical shapes. In contrast, the fat globules in a meat emulsion are confined within a continuous protein matrix. When uncooked emulsions prepared at increasing temperatures were observed in the microscope, the fat globules tended to change from spherical to irregular and coalesced shapes prior to stuffing the emulsion in the casing. The change in shape of the fat globule results from a weakening of the protein matrix with increasing processing temperature.

M.A.Christman: What causes the striations across the lipid droplets in Figures 3A and 4A? Is this just the difference in density and/or hardness such that the fat is softer and the knife chatters when it contacts the fat?

Authors: The striations are not the result of knife chatter. The knife passes through the resin embedded tissue and meets the much softer lipid which tends to flow upon impact, causing a build-up of thick and thin areas within the fat globule. Chatter would extend across the entire section perpendicular to the direction of the block travel.