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FUNCTIONAL AND MICROSTRUCTURAL EFFECTS OF FILLERS IN COMMINUTED MEAT PRODUCTS

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Abstract

Fillers are used in comminuted meat products such as wieners to increase yield, improve stability, and modify textural properties. Light microscopy, scanning electron microscopy and transmission electron microscopy show that comminuted meat products are mechanical mixtures in which the microstructural features of starch and insoluble protein ingredients are largely retained. The water absorption and gelation properties of these ingredients contribute to the stability and textural firmness. Soluble proteins may improve stability through emulsion formation but the role of emulsion formation is clearly secondary to that of gelation. The characteristic springy gel structure of wieners is determined by the gelation of myofibrillar meat proteins. Provided the structure of the meat protein gel is not disrupted, fillers will generally increase both textural firmness and stability. Starch and protein fillers have been shown to increase the stability of wiener homogenates prepared at a higher (26°C) temperature than that which is normally used (16°C). Light microscopy revealed that the "all-meat" wieners had a higher degree of fat agglomeration than did the more stable wieners containing added starch fillers. Electron microscopy revealed that the starch granules participated in the process of physically entrapping the fat globules. Fat globules varied in size and shape, and were observed in environments ranging from low to high protein densities. In summary, comminuted meat products are shown to have a complex heterogeneous microstructure.

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Introduction

Fillers are used in a wide range of processed meat products. Comminuted meat products such as wieners, bologna, and luncheon meats are the largest and most complex class of such products. These products are often referred to as "fine emulsion products" to differentiate them from coarsely chopped sausage and patty products. A food emulsion is a two-phase system, *e.g.* oil in water, in which the dispersed droplets have diameters between 0.1 and 10 µm (Powrie and Tung, 1976). When applied to comminuted meat products, therefore, the term "emulsion" is a misnomer which unfortunately has encouraged food scientists to perform seemingly endless model system and functionality tests based on the emulsion concept. One reason why the concept has endured, and to some extent continues to endure, may be because the emulsifying capacity of a protein can be quantified accurately (Carpenter and Saffle, 1964).

Saffle (1964, 1966) established a bind value scale for meat ingredients based on emulsifying capacity data which is widely used in least-cost computer programs for formulating comminuted meat products. For undegraded skeletal muscle proteins, salt solubility and emulsifying capacity may correlate with textural and stability performance (Saffle and Galbreath, 1964; Schut, 1978). However, for other meat proteins and for non-meat proteins, these correlations do not hold as shown by Comer (1979) and Comer and Dempster (1981). The effective use of least-cost formulation programs involves refinement of ingredient bind values and the use of both analytical and ingredient constraints.

Comminuted meat products are complex food systems in which water absorption, gelation phenomena, and emulsion formation influence the stability and texture of the cooked product. The functional effects of fillers are best understood by taking each of these mechanisms into consideration.

The microstructure of comminuted meat products and its relationship to stability and texture have previously been examined and reviewed (Lee, 1985). Most of the previous work has involved "all-meat" systems. The purpose of this review is to re-examine the microstructural and functional data for "all-meat" systems, and to critically examine the role fillers play in comminuted meat product stability, texture, and microstructure. Data from our previous work are presented as well as new data

which examine the effects which starch and caseinate fillers have upon the stability, texture, and microstructure of wieners which have been homogenized at a normal temperature (16°C) and at a higher temperature (26°C).

"All Meat" Systems

Function of ingredients

To understand the effects of fillers, comparisons must be made with "all meat" systems. In the preparation of comminuted meat products, several non-meat ingredients are used which are not generally considered as fillers (Paquette, 1986). Salt is added at a 2-3% level to improve the functional performance of the meat ingredients and contribute to flavour. A curing system ("cure") of sodium nitrite (120 ppm) and sodium erythorbate or ascorbate (550 ppm) is used to produce the cured meat colour, contribute to flavour, prevent fat oxidation, and retard microbial growth. Spices, seasonings, and smoke flavouring are added at levels of 1 to 3% to provide flavour and colour. Water is added at levels of 20% to 40% to increase yield, improve the functional performance of the meat ingredients, and to modify the textural properties of the finished product. Like fillers, all of these non-meat ingredients increase yield, and, in the use of some seasonings and added water, this is clearly one of their designed functions. Mustard flour and yeast products are two seasonings which are also functional fillers. The major components of these products are proteins and polysaccharides which influence the stability and textural properties of meat systems principally through water binding mechanisms.

In "all meat" systems, water and salt contribute to the stability and textural properties through interactions with the myofibrillar proteins. In experiments which involved using both sodium chloride and alkaline phosphates, Trout and Schmidt (1983) showed that low ionic strength and low pH resulted in large losses of fat and water during cooking, and a soft product texture. Recent attempts to reduce the salt levels in comminuted meat products have also resulted in reduced stability and textural firmness at salt levels below 2% (Sofos, 1983a, 1983b; Whiting, 1984a, 1984b).

Trout and Schmidt (1986) found that high salt concentrations are required to maintain the water binding ability of comminuted meat products at a normal minimum cook temperature of 68°C. In the presence of salt and phosphates, myofibrillar proteins bind water and swell. During heat treatment at temperatures of 50°C to 70°C these proteins form an irreversible gel which provides the matrix that holds both water and fat in the meat system. Water released during heating by the shrinkage of collagen fibres and, to a lesser degree, by shrinkage of the myofibrillar proteins, affects the thermal stability. In the process of comminution, some of the proteins are solubilized. These proteins may interact with the fat particles, which have been reduced in size, to form protective membranes. Hansen (1960) presented photomicrographs which showed the reduction in fat globule size during chopping and the formation of fat globule membranes. The emulsion theory was born and soon given support by model system experiments which showed that salt-soluble meat proteins can form emulsions, (Hegarty *et al.*, 1963; Swift *et al.*, 1961; Swift and Sulzbacher, 1963).

Stability and microstructure

The emulsion theory focussed attention on the size of the fat globules and the protein-fat interface. Emulsion stability, in particular, the effect of chopping temperature on stability, has been investigated by a number of workers (Ambrosiadis and Wirth, 1984; Brown and Toledo, 1975; Carroll and Lee, 1981; Girard *et al.*, 1985; Hansen, 1960; Helmer and Saffle, 1963; Jones and Mandigo, 1982; Lee *et al.*, 1981; Townsend *et al.*, 1968, 1971). Chopping temperatures of 10° to 16°C produce the most stable products. Stability decreases rapidly in the temperature range of 18° to 30°C. Light microscopy has been used to show that fat agglomeration occurs at higher chopping temperatures (Hansen, 1960; Helmer and Saffle, 1963). Using differential thermal analysis (DTA), Townsend *et al.* (1968) showed that fat melting in the range of 18° to 30°C is the most likely cause of reduced cook stability at higher chopping temperatures. Myosin protein transitions are revealed by DTA at 42.0°, 49.5° and 60.5°C (Siegel and Schmidt, 1979).

Carroll and Lee (1981), Lee *et al.* (1981), and Jones and Mandigo (1982) showed that losses in stability at high chopping temperatures are accompanied by decreases in textural firmness. Both light microscopy (LM) and scanning electron microscopy (SEM) were used to reveal a protein matrix disrupted by fat channels.

A critical element of the emulsion theory is the presence of protein membranes around fat globules. In model systems, true emulsions of salt soluble meat proteins and oil are formed. Both light microscopy (Carpenter and Saffle, 1964; Hansen, 1960; Swift *et al.*, 1961; Tsai *et al.*, 1972) and transmission electron microscopy (TEM) (Acton *et al.*, 1982) have clearly shown complete membranes. Evidence presented in micrographs of meat products is less convincing. TEM and SEM evidence has been presented to show protein membranes in comminuted meat products (Borchert *et al.*, 1967; Jones and Mandigo, 1982; Theno and Schmidt, 1978). However, Swasdee *et al.* (1982) using TEM showed that even after extended chopping times, not all fat globules were uniformly surrounded with protein membranes. In a recent review, Lee (1985) has questioned the significance of protein membranes in meat product stability. Meyer *et al.* (1964) showed that emulsifiers such as mono- and diglycerides decrease the stability of sausage emulsions.

Light microscopy was used to support the explanation that the emulsifiers affected the matrix and prevented protein film formation. Based on light microscopy observations, van den Oord and Visser (1973) and Evans and Ranken (1975) concluded that the fatty tissue in comminuted meat products was not emulsified. Hamm (1973) also de-emphasized the role of emulsification by drawing attention to the importance of the water-holding capacity of meat proteins in producing stable meat products. Brown and Toledo (1975) concluded that mechanical entrapment of fat particles is responsible for the stability of comminuted meat batters. Hermansson (1986) reported that differential interference contrast light microscopy revealed some kind of

protein film around all fat particles in raw meat batters. However, after cooking, structural changes such as gel shrinkage may reduce the rate of film formation in determining fat stability.

Using SEM, Theno and Schmidt (1978) showed that commercial frankfurters vary widely in microstructure from a fine protein matrix structure to a very coarse matrix structure containing large fat globules and intact muscle pieces. It is clear that for many comminuted meat products, emulsion formation plays a minor role in determining product stability and texture. Based on SEM and TEM observations, Katsaras and Stenzel (1984) described frankfurter-type sausages as mechanical mixtures. This term seems appropriate to describe the mixture of fat particles and various meat proteins which have been produced by mechanical chopping and mixing action in the preparation of meat homogenates. Microstructural observations by Ray *et al*. (1979, 1981), Lewis (1979), Swasdee *et al*. (1982), and Comer *et al*. (1986) support this description. In a recent publication, Oelker (1988) described frankfurters, on the basis of TEM observations, as a structural multiphase system, or microflake structure, which emphasized the water binding properties of myofilaments.

Gelation

During cooking, meat proteins aggregate and form irreversible gels. Yasui *et al*. (1979); Ishioroshi *et al*. (1979); and Siegel and Schmidt (1979) examined myosin gels using SEM, and showed that myosin transitions occur stepwise in the temperature range of 30° to 60°C. Optimal gel strength for myosin gels occurs at 60° to 70°C (Foegeding *et al*., 1986a; Ishioroshi *et al*., 1979). Replacement of part of the lean meat with blood plasma in wiener formulations results in softer texture, whereas in retorted products (120°C cook) an increase in textural firmness may occur (Comer and Dempster, 1981). Foegeding *et al*. (1986a) have shown that albumin gels do not form until 95°C, although the gelling temperature could be lowered to 80°C by interactions with myosin (Foegeding *et al*., 1986b). Using SEM, Siegel and Schmidt (1979) showed that in the absence of salt, myosin forms a spongy network which has a weak gel strength. Hermansson (1986) used SEM to show that salt caused a disintegration of the myofibrils and formation of an aggregated protein network. It is now clear that the characteristic textural properties of comminuted meat products are largely determined by the gelation of myosin. Other meat proteins and nonmeat ingredients may modify the textural properties, but if an insufficient amount of myosin is present in the formulation, "mushy" or "cakey" texture is the result.

Investigations on the gelation of protein ingredients is an active research area (Asghar *et al*., 1985; Beveridge *et al*., 1983, 1984, 1985; Foegeding and Lanier, 1987; Hamann, 1987; Hermansson and Larsson, 1986; King, 1977; Montejano *et al*., 1984a, 1984b; Sadowska and Sikorski, 1976). Unlike emulsifying capacity, gelation properties are difficult to quantify and are significantly influenced by protein interactions.

Effects of Fillers

Functional fillers

Milk-derived and plant-derived food ingredients are used as functional fillers in comminuted meat products. The milk ingredients include skim milk powder, buttermilk powder, sodium caseinate, calcium caseinate, whey protein concentrates, calcium-reduced milk powder, and whey powder. For each milk product it is the protein component which provides the functionality. The carbohydrate component, lactose, has a sweetening effect upon flavour but at the levels normally used appears to have little effect on either the texture or stability. The plant-derived ingredients include soy isolate, wheat gluten, soy concentrate, textured soy flour, inactive yeasts, mustard flour, cereal flours, legume flours, baked cereals, starches, and modified starches. In plant products, both protein and carbohydrate components provide functionality when incorporated into comminuted meat products.

Functional effects of adding fillers

The economic incentive for using fillers in meat products is to reduce ingredient costs. Yields for comminuted meat products are commonly expressed in terms of 100 parts of meat. For "all-meat" products, the addition of water, salt, cure, spices, and seasonings may increase the yield 10-30% depending on the strength of the meat block, product textural quality standards, and government regulations. The functional effects of adding protein and starch-based fillers to "all-meat" systems have been shown by Comer *et al*. (1986) to be increased yield, textural firmness, and stability (reduced processing "shrinks" and cooking losses). Table 1 summarizes previously published data (Comer *et al*., 1986) showing the analytical and functional effects of adding various fillers to a comminuted meat formulation.

The results for the five fillers are compared with the "all-meat" control in row 1 which is labelled "Nil". Water and fat are lower with added filler simply because of dilution. The protein content is lower for the wieners containing starch, but is higher for the samples containing other fillers which have more than 11% protein found in the control. The yields are higher principally because of the direct effects of adding 7% filler; however a little more moisture was also retained than in the control. The stability data is a composite value determined by subtracting moisture losses in the smokehouse, and fat and moisture losses during cooking in boiling water (to simulate home preparation) from 100%. In every case, the added fillers reduced both processing and cooking losses for a 4% to 7% increase in stability. All fillers also increased textural firmness. These positive effects of fillers in meat products have often been obscured by comparisons with lean meat ingredients and by attempts to extend meat products beyond yields of 150%.

Standardized comminuted meat products in Canada and in the United States are not "extended" products in the sense that meat ingredients are the principal sources of protein. The meat protein content is generally in the range of 9.5% to 11%, which is sufficient to ensure a good gel structure. The major factors controlling the textural firmness and stability are the proportion of lean meat ingredients and the water/fat ratio. When fillers are added to this system, they bind some of the water which allows the meat proteins to form a firmer gel structure. This function of fillers is especially important for low fat products which have correspondingly higher moisture contents.

Functional effects of replacing meat proteins

Heat induced gelation of the myofibrillar muscle proteins, especially myosin, is responsible for the springy, chewy gel structure of comminuted meat products (Comer, 1979; Roberts, 1974b; Schut, 1978; Webb, 1974). Attempts to replace myofibrillar proteins with non-meat proteins have resulted in softer, less chewy products (Decker *et al.*, 1986; Comer and Dempster, 1981; Terrell, *et al.*, 1979a, 1979b). Although replacement of lean meat proteins with non-meat proteins results in loss of textural firmness, stability may be maintained or even enhanced by replacement (Comer, 1979; Randall *et al.*, 1976; Van Eerd, 1971). Table 2 summarizes previously published data (Comer, 1979; Comer and Dempster, 1981). In these experiments, 22% of the lean beef in the formula was replaced with 4.4% of filler solids (equivalent to the protein solids of the lean beef replaced). Moisture and fat were adjusted to be equivalent to the lean beef control. The skeletal muscle protein contents of the strong, medium, and weak meat systems were 8.0%, 4.5% and 1.8%, respectively, which is almost in direct proportion to the textural firmness values for the lean beef control. These data show that the springy gel structure is determined by the skeletal muscle proteins.

The fillers being compared are soy concentrate, sodium caseinate, textured soy flour, potato starch, wheat flour, and skim milk powder. With the exception of the skim milk powder, the fillers are composed principally of proteins and polysaccharides. The milk powder contains only 35% protein and the rest is non-binding lactose. This is a disadvantage from the stability viewpoint, as shown in Table 2 in the strong meat system, where only the skim milk powder performed more poorly than the lean beef. For the medium and weak meat blocks, the beef outperformed all fillers except caseinate. Caseinate is the only filler in this table which has a higher emulsifying capacity than lean beef (Comer, 1979). Commercial wieners contain medium to strong meat blocks and, therefore, based on the data in Table 2, replacement of meat protein with fillers has minimal effects upon yield and stability. Even in the weak system, wieners containing fillers were stable in the sense that they did not render out fat during processing. The lower stabilities reflect predominantly higher moisture losses.

The texture results in Table 2 reveal a significant effect of meat block strength upon the relative functional performance of fillers. In the medium and weak systems, the soy concentrate and textured soy flour clearly produced the firmest texture. In the strong system, however, starch and skim milk powder performed better. Both ingredients absorb less water than the soy proteins. This is desirable in a strong system, since the meat proteins require the water to form a strong gel. In the weak system, there is too much water available to the lean meat proteins to form a firm gel and, therefore, stronger absorbing fillers are desired.

Sodium caseinate is unique in that it does not gel but holds the moisture in an emulsion (Van den Hoven, 1987). Since the caseinate is soluble, there is a greater potential for interaction with the soluble meat proteins. This may be one of the causes of the softer gel structure revealed by the low texture values for the wieners containing caseinate (Table 2). The adverse effects of caseinate upon texture have also been reported by other workers (Oelker, 1988; Schut, 1976).

Several studies have shown that up to 50% of the meat protein in a comminuted meat system can be replaced by non-meat proteins (Randall *et al.*, 1976; Roberts, 1974a; Rongey and Bratzler, 1966); and simulated non-meat sausage products have been prepared from soy isolate (Frank and Circle, 1959). Non-meat protein and starch gels are much softer than myosin gels (Circle *et al.*, 1964), and, therefore, replacement of lean meat with fillers results in products having a softer and a less chewy texture. If the gel structure is weakened too severely, then the stability of the system is adversely affected as shown for the weak system in Table 2.

Correlation of functional properties and functional effects

The effects of specific fillers have been described in a recent review by Mittal and Usborne (1985), and in a comprehensive review of meat emulsions by Schut (1976). Most of the research done to date has involved protein ingredients. Initially, the emphasis was directed towards the emulsifying properties of non-meat proteins (Acton and Saffle, 1971; Crenwelge *et al.*, 1974; Pearson *et al.*, 1965; Puski, 1976; Saffle, 1968). However, Smith *et al.*, (1973) demonstrated that higher nitrogen solubility index (NSI) values for non-meat proteins did not correlate with stability performance in frankfurters. Many of the papers compare the effects of soy proteins with plant (Keeton *et al.*, 1984; Lin *et al.*, 1975; Patana-Anake and Foegeding, 1985; Rakosky, 1970; Terrell and Staniec, 1975; Thompson *et al.*, 1984) and milk proteins (Casella, 1983; Hermansson, 1975; Hermansson and Akesson, 1975a, 1975b; Lauck, 1975; Parks and Carpenter, 1987; Porteous and Quinn, 1979; Thomas *et al.*, 1973, 1976). It is difficult to deduce general conclusions on efficacy of filler ingredients from these papers because of differences in experimental design and purpose. Torgersen and Toledo (1977) concluded that fillers which have high water binding capacities and which form firm gels have better functionality in meat systems than those with high solubility. Comer (1979) proposed that the effects of fillers were best understood by considering comminuted meat products as gel systems rather than emulsions.

The stability and textural properties of the gels are influenced by competition for moisture between proteins and carbohydrates and by protein interactions with water, fat, and other proteins. It is not surprising that attempts to develop correlations between functional properties of meat and filler ingredients and functional effects in comminuted meat products have been of limited success (Comer and Dempster, 1981; Li-Chan *et al.*, 1987; Mittal and Usborne, 1986; Parks *et al.*, 1985; Porteous and Quinn, 1979). Nevertheless, we have found that the functional effects of ingredients can be rationalized and predicted by considering their water binding and gelation properties. Relative functional performance of ingredients is dependent upon the composition of the total system, *i.e.*, ingredients do not have absolute bind values.

Microstructural effects

There have been only a few papers in which the microstructural effects of fillers in meat products

Table 1. Effects of adding fillers (Wieners contain 7% filler[a])

Filler	Water (%)	Fat (%)	Protein (%)	Yield[b] (%)	Stability (%)	Texture[c] (kg)
Nil	59.4	25.9	11.0	125	79	1.1
Modified corn starch	55.5	23.4	10.4	135	86	2.0
Hard wheat flour	55.4	23.7	11.3	136	86	1.4
Skim milk powder	56.7	23.7	12.5	137	84	1.2
Soy concentrate	56.3	23.2	15.2	136	84	1.6
Vital wheat gluten	56.4	23.3	15.3	137	83	1.5

[a] Comer *et al.*, 1986.
[b] Yield is based on meat ingredients equal to 100%.
[c] Texture is determined by the comparison force required to rupture 2 cm segments of cooked wieners (Comer *et al.*, 1986).

Table 2. Effects of fillers relative to lean beef in strong, medium, and weak meat systems (Wieners contain 4.4% filler)[a]

Ingredient	Total stability (%) Strong	Medium	Weak	Texture[b] (kg) Strong	Medium	Weak
Lean beef	84	83	78	3.6	2.1	1.1
Soy concentrate	86	82	70	2.7	1.4	0.9
Sodium caseinate	88	85	81	1.9	1.0	0.4
Textured soy flour	84	81	62	2.2	1.3	0.8
Potato starch	84	80	74	2.9	0.8	0.4
Hard wheat flour	84	80	76	1.9	1.1	0.4
Skim milk powder	82	78	67	2.8	0.9	0.4

[a] Comer, 1979, Comer and Dempster, 1981.
[b] See footnote *c* in Table 1.

have been investigated. Flint and Pickering (1984) and Flint and Firth (1981) developed staining procedures which differentiate between a variety of ingredients found in comminuted meat products, including muscle cells, collagen, starch granules, wheat gluten, and soy protein. Cassens *et al.* (1975) used light microscopy to show the physical distribution of textured soy flour particles in frankfurters. Textured soy flour absorbs moisture but does not disperse. Irregularly shaped fat globules were sometimes observed next to particles of textured soy flour but there were no significant effects upon stability at usage levels of 3-4% filler. Lee (1985) used light microscopy to show that similar levels of soy protein isolate also had minor effects upon fat distribution. The soy isolate increased the viscosity of the raw meat batter. This may have been responsible for the observed slight increase in average fat globule size. Soy protein isolates are dispersible and form heat-set gels. Siegel *et al.* (1979a, 1979b) used SEM to study the gel structure of soy isolates and other non-meat proteins in model systems alone and in the presence of myosin. The non-meat protein gels were shown to have structures different from those of myosin gels and in several cases interactions with myosin were evident. Comer *et al.* (1986) used SEM and TEM to show that the gel features of wheat gluten and soy proteins were observable in comminuted meat products containing 6% filler. Both wheat gluten and soy proteins have limited dispersibility at the 5% salt concentration found in frankfurters. Therefore, it is difficult to observe interactions of these fillers with meat proteins. Skim milk powder is highly dispersible at this salt concentration, and it was shown that the micelle structure is lost and replaced by a distinctive granular protein density pattern possibly due to interactions of casein micelles and salt-soluble meat proteins (Comer *et al.*, 1986).

Kempton *et al.* (1982, 1983) applied a statistical evaluation procedure in analyzing photomicrographs to describe the microstructural effects of textured soy flour, soy protein concentrate and yeast fillers. They observed increased clumping of fat and protein ingredients in some fields of some slices. These observations are consistent with increased densities reported by Lee *et al.*, (1981) and Cassens *et al.*, (1975). However, whereas Lee *et al.*, (1981) reported a larger number of smaller fat globules, Kempton *et al.* (1982, 1983) reported a greater frequency of fat agglomerates due to vegetable proteins. The different effects may be due to differences in either chopping times or viscosities. Kempton (1983) observed that neither stability nor textural firmness were correlated with the degree of clumping. Similar observations were made by Comer *et al.* (1986). Schmidt *et al.*

(1982) used light microscopy to show that soy concentrate formed an integral part of the matrix to stabilize a high fat, canned luncheon meat formula.

Comer *et al.* (1986) used light microscopy, SEM, and TEM to show corn starch and wheat starch granules in wieners. In water, the starch granules lose their birefringence at about 60°C and start merging in the temperature range 60° to 70°C. However, in wieners cooked to 72°C (internal temperature), most of the starch granules were discrete and several possessed birefringence. It was shown that this is the result of the limited moisture environment in the comminuted meat products.

Lin and Zayas (1987a) used TEM to show that pre-emulsification of fat with corn germ protein produced a finer dispersion of fat globules in the meat batter than if the filler was added directly to the batter. Zayas (1985) prepared pre-emulsified fat with sodium caseinate and skim milk powder and also showed a more uniform dispersion of fat particles using TEM. Soy proteins and wheat gluten have also been used in preparing fat emulsions for use in comminuted products (Hand *et al.*, 1983). The pre-emulsions bind both fat and added water which may result in higher fat stability (Lin and Zayas, 1987b), and increased yield and softer texture because of higher water binding capacity (Zayas, 1985). Pre-emulsions are being used in Germany (Wirth, 1985). However, in Canadian comminuted meat formulations, fat stability is rarely a concern and the water holding capacity is generally sufficient to nullify the beneficial yield effects of pre-emulsion addition.

Summary of effects

In general, meat systems have the capacity to tolerate substantial amounts of filler ingredients, especially starches and flours where levels of 5% to 10% are currently used in Canadian formulations. Ingredients which interfere with the meat gel structure such as gums and soluble proteins must be used at lower levels. Beyond a certain point, more filler can be added to form a dry texture but the "springy" gel structure is transformed into a "cakey" structure such as is found in vegetable protein extended products.

Experimental design of present study

To further our knowledge on the effects of fillers, we have carried out experiments to determine the functional and microstructural effects of fillers in wieners at different homogenizing temperatures. Previous studies of the effects of chopping temperatures have involved "all-meat" systems. For comparison with "all-meat" systems, we selected two fillers: modified corn starch at a level of 7% to bind excess moisture and produce a firm texture, and sodium caseinate at a 1% level to increase the emulsifying capacity of the system. The homogenizing temperatures selected were 16°C, which is known to produce good stability and textural firmness, and 26°C, which has previously been shown to result in lower stability and textural firmness (Carroll and Lee, 1981). Yield, stability, and textural firmness of the wieners were determined and the microstructural features of the wieners were examined by light microscopy, SEM, and TEM. The meat formulation was selected to be typical of that used in manufacturing wieners in Canada.

Materials and Methods

Preparation and analysis of wieners and fresh homogenates

The meat ingredients were obtained from local meat processors and stored frozen. The modified corn starch was a crosslinked, derivatized waxy maize starch supplied by National Starch and Chemical Corp, Bridgewater, New Jersey. The sodium caseinate was supplied by Rovianda EMBH & Co., Engelsberg/Wiesmühl, Germany.

With the exception of the backfat, the meat ingredients were tempered overnight in a cooler at 3°C, ground twice through a 0.48 cm (3/16 inch) plate, and then preblended in a Hobart mixer for 5 min. The formulations are shown in Table 3. The preblended meat was divided into two parts. One-half was stored at ambient temperature (23°C) for 4 h prior to use in the preparation of batches 5 to 8 at the higher homogenizing temperature of 26°C. The other half was used directly from the cooler to prepare batches 1 to 4 at the homogenizing temperature of 16°C. In each case, all of the meat for the four batches was placed in a Hobart mixer, and salt, cure, seasoning, and one-half of the water (40°C) were added. After 5 min of mixing, the fat was added and mixing continued for 3 min. The mixture was divided into four parts, and for each batch, the remaining ingredients (one-half of water and filler) were added in a Hobart mixer. Mixing was continued for 3 min and then the mixture was homogenized through a laboratory Mincemaster™ colloid mill (The Griffith Laboratories, Ltd.). The batch size was 3 kg. The homogenizing temperature was controlled by varying the temperature of the water added. For batches 1 to 4, the meat temperature prior to addition of the second half of the water was 9°C. Water was added at 10°C and the temperature rise was about 3°C during mixing and 3°C during homogenizing.

For batches 5 to 8, water at 40°C was added to meat at 20°C to give a homogenate temperature of 26°C. The homogenates were stuffed into 22 mm diameter cellulose casings, linked in 12.7 cm sections and cooked in a smokehouse to an internal temperature of 72°C in a 1 hour cycle with 40% relative humidity for the last 30 minutes. The wieners were hung in a cooler for 16 hours, smokehouse losses were determined, and the wieners were vacuum packaged and stored in the cooler. The fresh homogenates remaining in the stuffer were used to determine the cook stability at 75°C.

The textural firmness and stability of the wieners were determined according to the methods reported by Comer *et al.* (1986). The textural value is a compression rupture force (kg); yield is the product weight (parts) per 100 parts meat block; stability takes into account both processing and home preparation cooking losses. These data are shown in Table 4. The presence of fat caps, indicative of partial homogenate breakdown during preparation of the wieners, is also shown in Table 4.

The cook stabilities of the fresh homogenates were determined by a modification of the method reported by Comer (1979). Samples (35 g) of homogenate were centrifuged at 1600 xg in 50 mL glass tubes. The tubes were placed in a 50°C water bath for 10 min and the bath temperature was then increased to 75°C over 45 min to complete the cook cycle. The released fat and moisture were weighed

Table 3. Formulation data (w/w) for experimental wieners
(Expressed in units of parts by weight)

Component	Formula number[a] 1	2	3	4	5	6	7	8
Meat block[b]	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Salt/seasoning/cure[c]	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5
Added water[d]	41.0	39.9	39.8	40.9	41.0	39.9	39.8	40.9
Modified corn starch	0	10.3	10.3	0	0	10.3	10.3	0
Sodium caseinate	0	0	1.4	1.4	0	0	1.4	1.4

[a] Formulae 1 to 4 homogenized at 16°C and formulae 5 to 8 homogenized at 26°C. The experimental design features two sets of the same four formulae, each set being homogenized at a different temperature.
[b] Lean beef chuck 20, lean pork trim 20, mechanically separated chicken meat 25, pork hearts 14.2, pork backfat 20.8.
[c] Salt 3.6, spice oleoresin blend 0.8, sodium erythorbate 0.075, sodium nitrite 0.03.
[d] Added water adjusted to take into account the moisture contribution of the fillers.

Table 4. Effects of fillers and homogenizing temperatures
(Wieners contain 7% starch, 1% caseinate, or both)[a]

No.[b]	Filler	Temp. (°C)	Water (%)	Fat (%)	Protein (%)	Yield (%)	Stab. (%)	Text.[c] (kg)	Fat caps
1	Nil	16	63.5	22.4	10.9	132	88	1.1	Nil
5	Nil	26	64.0	21.8	11.1	133	83	1.0	+++
2	Starch	16	59.8	19.3	10.1	141	90	2.0	Nil
6	Starch	26	59.5	19.4	10.3	141	90	1.7	Nil
3	Caseinate	16	59.1	18.8	11.4	134	89	1.6	Nil
7	Caseinate	26	58.3	19.5	11.2	135	88	1.3	+
4	Starch+cas.	16	63.0	21.7	11.6	143	90	2.1	Nil
8	Starch+cas.	26	62.9	21.7	11.8	143	90	2.1	Nil

[a] Wieners were cooked to 72°C internal temperature in a 1-hour smokehouse cycle.
[b] Formulae numbers grouped in pairs, *e.g.*, 1 and 5, have the same composition but were homogenized at a different temperature.
[c] Honest significant difference (Tukey's test, Steel and Torrie, 1960) at the 95% confidence level is 0.2 kg; see footnote *c* in Table 1.

and are reported in Table 5 as percentage values of the total fat and moisture in the sampler.

Fat, protein and moisture were determined by AOAC procedures as reported by Comer *et al*. (1986).

Microscopic Analysis of Wieners

Light microscopy

The methods previously described by Comer *et al*. (1986) were used. Wiener slices (2x2x0.4 cm) were soaked for 2 h in a gum sugar solution (water: sucrose:gum acacia, 100:100:35), frozen using liquid carbon dioxide, and 30 µm sections were obtained and fixed on glass slides by immersion for 6 h in a mixture of a saturated aqueous mercuric chloride solution and ethanol (1:1, v/v). Two staining procedures were used.

Fat/protein stain: Fat was stained orange to red by immersion in a 0.06% Sudan IV (Aldrich, C.I. 26105) solution (isopropanol:water, 3:2) for 30 min. After rinsing with water for 10 min, the protein was counterstained green by immersion in a 0.5% aqueous solution of Light Green (BDH., C.I. 42095) for 20 min.

Table 5.
Effects of fillers and homogenizing temperatures on 75°C cook stability of fresh homogenates
(Wieners contain 7% starch, 1% caseinate, or both)[a]

No.[b]	Filler	Temp. (°C)	Fat stability (%)	Moisture stab. (%)
1	Nil	16	98	81
5	Nil	26	77	56
2	Starch	16	100	94
6	Starch	26	88	77
3	Caseinate	16	99	90
7	Caseinate	26	82	60
4	Starch + caseinate	16	100	95
8	Starch + caseinate	26	91	86

[a] The homogenates were cooked in a water bath in a 55 min cycle at bath temperature starting at 50°C and finishing at 75°C.
[b] Formulae numbers grouped in pairs, *e.g.*, 1 and 5, have the same composition but were homogenized at a different temperature.

Carbohydrate/protein stain: Carbohydrate was stained red by first immersion in a 2% periodic acid solution for 5 min, followed by running water (5 min), then immersion in Schiff's reagent for 5 min, as described by Disbrey and Rack (1970). The protein was counterstained with Light Green, as described above.

All photomicrographs were taken at 100 X magnification using 35 mm colour slide film (100 ASA), and the field size of the photomicrographs was 540 µm x 812 µm.

Scanning and transmission electron microscopy

The basic methods previously described by Comer *et al*. (1986) were used. All samples were fixed in a 3.5% aqueous glutaraldehyde solution. For SEM, the fixed samples were dehydrated in a graded ethanol series, defatted with chloroform, freeze-fractured after freezing in Freon 12 cooled to its freezing point with liquid nitrogen, thawed in absolute ethanol, and critical point dried from carbon dioxide. For TEM, the fixed samples were postfixed in a veronal-acetate buffered (pH 6.75) 2% osmium tetroxide solution for 18 to 24 h at 22°C, dehydrated in a graded ethanol series for 30 min per step at 22°C, and embedded in Spurr's resin (Spurr, 1969).

For comparison purposes, two modifications of the basic sample preparation method were evaluated. The first modification was done in an effort to promote optimal retention of fat in the sample. For samples destined for SEM, the preliminary glutaraldehyde fixation was followed by postfixation in a buffered osmium tetroxide solution. The samples were then dehydrated through an acetone series at 6°C as described by Carroll and Lee (1981). The defatting step using chloroform was eliminated. Samples destined for TEM were treated as in the basic method except that acetone dehydration in the cold replaced the ethanol dehydration at 22°C.

The second modification was employed to see whether a significant improvement in the quality of fixation could be achieved with a more complicated fixative. A Karnovsky-type fixative (Karnovsky, 1965), containing glutaraldehyde and paraformaldehyde and, in our case, buffered with cacodylate, was used similar to the fixatives used by other researchers in studies on muscle and meat (Carroll and Lee, 1981; Jones *et al*. 1976; Katsaras and Stenzel 1984; Katsaras *et al*., 1984). This fixation was followed by dehydration in an ethanol series at 22°C, or by acetone dehydration as described by Carroll and Lee (1981).

Discussion of Results

The data in Table 4, obtained from samples prepared at a homogenizing temperature of 16°C are in agreement with the data from our previous study (Comer *et al*., 1986) and show that fillers increase yield, stability, and textural firmness. A direct comparison of the data for the "all meat", *i.e.*, labelled "Nil", and starch filler batches with the data in Table 1 from our previous study shows that the same texture values, but significantly higher yields and stabilities, were obtained in the current test. Based on a comparison of the analytical data, the meat block used in this test had a higher protein content which may explain the differences. Variations in the water binding capacities of the meat ingredients may also contribute to the differences.

Homogenizing at a higher temperature (26°C) did not have a significant effect upon yield, even in the case of the control which rendered, *i.e.*, produced fat caps. We have determined yield with the casings on and, therefore, the fat caps are included. However, it is important to note that the homogenates processed at 26°C were able to retain as much water as those processed at 16°C. In addition, the textural quality of the wieners was not altered by the higher homogenizing temperature. In only two cases were the textural firmness values significantly lower, but not low enough to change the eating quality.

The expected loss in stability as shown by the presence of fat caps and lower stability value was observed for the "all meat", "Nil" control. The value of 83% stability for formula 5 is probably higher than the actual value since some fat was retained by the casing during peeling and was not included in the weight prior to cooking. The batches containing fillers, on the other hand, showed a negligible loss in stability with the exception of the one containing caseinate alone which had a few fat caps. The data in Table 4 show that the effects of higher homogenizing temperatures upon wiener yield, stability and texture are less severe for formulae containing filler ingredients.

Although we observed some decreases in wiener stability and textural firmness at the higher homogenizing temperature, some workers have previously reported considerably more severe changes. Possible reasons for this discrepancy may be that we have used a Mincemaster™ colloid mill which generally gives a more uniform homogenate than a bowl chopper; and secondly, most of the previous studies have used a model system cook test to determine stability. We have shown previously (Comer and Dempster, 1981) that model system cook tests are unreliable indicators of actual performance in wieners. For comparison with the wiener stability data, samples of the fresh homogenates were subjected to a cook stability test which is analogous to a model system cook test.

The data in Table 5 show that the model system cook test does give lower stability values than those obtained for wieners, and differences between homogenates prepared at 16°C and 26°C are much more pronounced. As for the wieners, the fillers improved the stability with the starch and the starch and caseinate combination being the most effective. Caseinate alone was able to maintain high fat stability but could not bind the water as effectively as did the starch. These data emphasize that one must be cautious in extrapolating stability data from model system cook tests to performance in wieners. In some cases, such as for the starch plus caseinate binder in this study, the stability differences shown in Table 5 were not statistically significant. The moisture losses and surface drying which occur in the smokehouse play an important role in the stability of the wieners.

To the naked eye wieners are homogenous products. The major source of nonhomogeneity is the air pockets which are more prevalent in laboratory prepared wieners than in commercial products. Even low-powered light microscopy reveals that the cooked "homogenate" has a heterogeneous microstructure. It is not possible to obtain a photomicrograph which characterizes the microstructure even at a magnification of only 30X (Kempton *et al*.,

1982). Therefore, the procedure generally employed is to scan many fields and select a "representative field". Figures 1 and 2 are examples of representative fields to compare the microstructure of wieners prepared in a silent cutter (Figure 1) with those prepared using a Mincemaster™ colloid mill (Figure 2). Colloid mills have previously been shown to produce finer homogenates than do cutters (Danchev *et al.*, 1984).

Figures 1 and 2 (Comer, unpublished) are good examples which show the range of fat globule size that is found in commercial wieners. Although Figure 1 shows a higher frequency of larger fat globules, there were no significant differences in yield, stability, or textural firmness between the two samples. Provided the fat is broken down into globules of an approximate diameter of 100 µm or less, the texture and stability are largely determined by the nonfat matrix which consists principally of meat proteins. In Canadian wieners, fillers, especially starches and flours, are generally present in the amounts of 30% to 50% of the nonfat, nonsalt solids, and therefore contribute significantly to the matrix.

When the Light Green protein stain is used, it is difficult to see microstructural changes in the matrix caused by fillers. Protein fillers tend to show up as an overall darker green matrix, whereas ungelatinized starch fillers appear as lighter coloured oval-shaped particles. We have used a carbohydrate stain to reveal the hydrated starch granules (Figures 3 and 4). Representative fields were not selected but rather two regions were selected to show high starch density in contrasting environments. In Figure 3, the starch granules form the continuous matrix as there is very little protein. Many of the voids have a globular shape and are believed to be fat particles immobilized in the starch matrix. Lewis (1981) reported a similar microstructure for British meat pastes. Hermansson (1986) also showed an example of fat droplets in a meat batter surrounded by starch particles. Figure 4 shows the starch granules embedded in a continuous protein matrix with only a few voids which may contain fat or air. During smokehouse cooking, the starch granules absorb any excess moisture not bound by the protein. The hydrated starch granules have an average diameter of about 15 µm which is one-half the thickness of the section examined. This increased diameter is achieved by the granules absorbing 2 to 4-times their weight of water (Comer, unpublished results). The granules are largely discrete and what appears to be clumping may, in part, be due to overlapping.

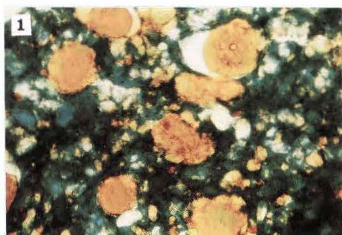
From the photomicrographs we conclude that the starch filler, like most protein fillers, contributes to stability by absorbing water, and by becoming a part of the continuous matrix which immobilizes the fat globules. The positive effects upon textural firmness cannot be directly deduced from the photomicrographs. From related studies (Comer, unpublished results) we observed that various starches and gums produce wieners with significantly different textural firmness values. Pregelatinized starches and nongelling gums produce a softer texture than hydrated but intact starch granules. The latter have an inherently firmer, nonflowing gel structure which reinforces the meat protein gel structure rather than weakening it. Therefore, we believe that gelation properties, rather than simply water binding capacity, are the primary determinant of firmness.

To examine the effects of homogenizing temperatures upon the microstructure, we found it most useful to use fat/protein staining and to select fields which have a high density of fat globules or agglomerates. Our microscopic analysis tends to support earlier conclusions that a homogenizing temperature of 26°C produces a greater incidence of fat agglomeration, and in extreme cases, the formation of fat channels (Carroll and Lee, 1981).

To illustrate the different degrees of fat agglomeration we have designated them as Types 1 to 4 as shown in Figures 5 to 8. We observed that both the nonfat solids level and the homogenizing temperature had significant effects upon the degree of fat agglomeration in the wieners. The "all meat" system homogenized at 16°C had many regions with a Type 2 pattern but some regions with both Type 1 and Type 3. The same system homogenized at 26°C had many regions with a Type 3 pattern with the occasional evidence of severe channelling, i.e. merging of fat agglomerates into continuous bands, as revealed by the Type 4 pattern in Figure 8.

The use of caseinate alone as a filler did not have a significant effect upon the fat agglomeration pattern at the 16°C treatment. However, there was no evidence of severe channelling at the 26°C treatment. The use of starch or the starch and caseinate combination had the greatest effect upon agglomeration. At the 16°C treatment, the wieners containing starch were almost free from fat agglomeration with the pattern shown in Figure 1. At the 26°C treatment regions of Type 1 and Type 2 agglomeration were observed but no evidence of channelling as shown in Figure 7 (Type 3) was observed. The effects of solids is in agreement with the observation by Lee (1985) that fat particle size increased with increased moisture content. The "all meat" control has too high a moisture content to produce a stable product with firm texture. The addition of filler solids physically stiffens the homogenate, which prevents fat agglomeration, and by binding excess moisture permits the meat proteins to form a firm gel during the smokehouse cooking.

Previous microstructural observations using electron microscopy have clearly shown that comminuted meat products such as wieners are mixtures in which the microstructural features of the individual meat protein and filler ingredients are still recognizable. Neither muscle and collagen fibers (Swasdee *et al.*, 1982) nor starch and hydrated nonmeat protein particles (Comer *et al.*, 1986) are completely destroyed by the comminuting processes. Meat fatty tissue is normally described as consisting of cells with connective tissue cell walls. The microstructure has been shown to vary from a highly regular, honeycomb pattern of cells found in soft fats (Katsaras *et al.*, 1984) to a pattern of irregular shaped cells with poorly organized collagen fibers found in highly crystalline hard fats (Lewis, 1979, 1981). During the comminution process the fat cells are separated and then broken down to release free fat which will form large amorphous regions unless emulsified or restrained in a matrix. Since the normal, regular shaped fat cell has a diameter of 70-80 µm, and since fat globules in wieners range in size from less than 20 µm to greater than 100 µm (van den Oord and Visser,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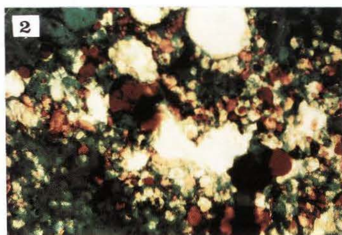
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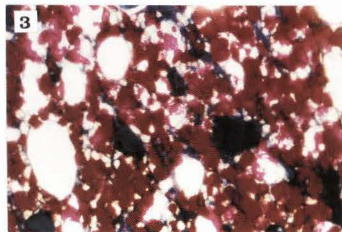
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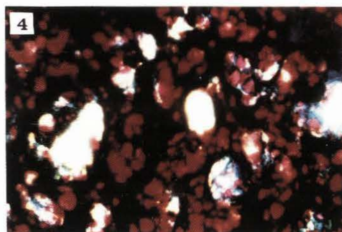
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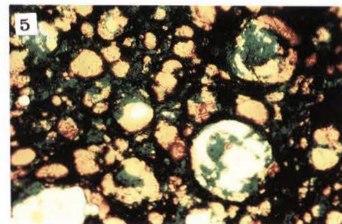
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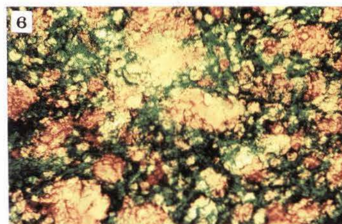
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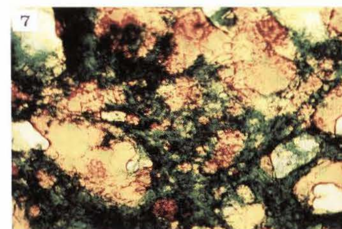
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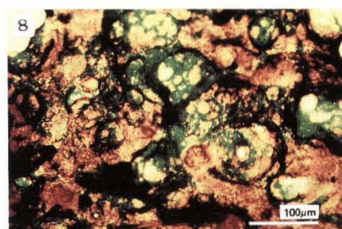
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Figure 1. *Wiener from homogenate prepared in a laboratory silent cutter. Fat stained orange-red with Sudan IV; protein counterstained with Light Green. Representative field. Bar in Figure 8 represents 100 μm for Figures 1 to 8 (original magnification 100 X).*

Figure 2. *Commercial wiener from homogenate prepared in a Mincemaster™ colloid mill; staining as Figure 1. Representative field.*

Figure 3. *Wiener containing starch binder. Carbohydrate stained red with periodic acid-Schiff's reagent; protein counterstained with Light Green. Region of high starch density.*

Figure 4. *Wiener containing starch binder. Same staining as Figure 3. Region of high starch and high protein densities.*

Figure 5. *Type 1 fat agglomeration. Wiener section from formula 6. Same staining as Figure 1.*

Figure 6. *Type 2 fat agglomeration. Wiener section from formula 1. Same staining as Figure 1.*

Figure 7. *Type 3 fat agglomeration. Wiener section from formula 5. Same staining as Figure 1.*

Figure 8. *Type 4 fat agglomeration. Wiener section from formula 5. Same staining as Figure 1.*

1973), it appears that few intact fat cells remain in the wiener. The fat cell membranes have been shown to have a lattice fibre structure by SEM (Katsaras *et al.*, 1984). However, in comminuted meat products the protein coverings may be from salt soluble myofibrillar proteins, non-meat proteins or cell membranes. It has not yet been possible to characterize the origins of the protein membranes using electron microscopy. Carroll and Lee (1981) concluded that nothing could be discerned about the protein-lipid interface in wieners using SEM. We used an acetone dehydration technique similar to the one used by Carroll and Lee (1981) to retain fat globules and obtained topographically low contrast SEM images in which the relationship between the fat globules and the protein matrix could not be easily seen.

Ethanol dehydration and chloroform defatting (without post fixation with osmium tetroxide) produced a much clearer view of the relationship between the fat and the protein matrix. The voids in the matrix left behind by the intentional extraction of fat using chloroform are believed to represent the original positions of fat globules, so a relationship between the fat globules and protein matrix could be seen.

In our comparison of sample preparation methods for electron microscopy we found no significant differences in the quality of specimen preservation between using either a glutaraldehyde solution or a Karnovsky-type fixative. Replacing ethanol with acetone in the dehydration step of the TEM sample preparation procedure produced some cases of poor embedding and poor staining. Ethanol dehydration followed by chloroform defatting was used in all the SEM micrographs shown.

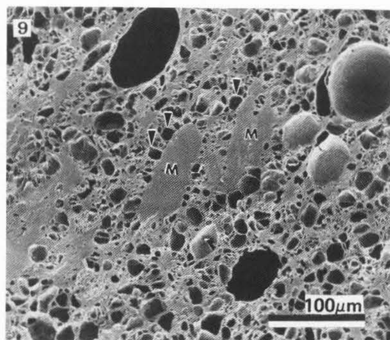
Electron microscopy of the wiener samples showed heterogeneity in the density of the protein matrix, and in the size, shape and environment of the fat globules. The protein matrix is best shown by low magnification (150-200X) SEM. Figure 9 is an example of a strong matrix where the majority of the fat globules are finely dispersed. Some large elongated pieces of muscle tissue are revealed in Figure 9. Most of the voids are believed to have contained fat prior to defatting, although some voids may be due to air or water pockets. The larger voids may be due to the presence of individual fat cells.

Figure 9 is at too low a magnification to identify starch granules in the matrix. Figures 10 and 11, however, show starch granules clearly. In Figure 10, one granule has been sliced and rests in a cavity. It has probably been shrunken during the dehydration step. The intact granule in Figure 10 is partly enclosed in muscle fibre and membrane material adjacent to a void. In Figure 11, the starch granule is in a similar environment except the membrane has taken the shape of the granule. The voids are believed to be due to fat globules.

The environments of the "fat globule" voids are more clearly shown through SEM at a higher magnification (Figures 10-12). Most of the voids are embedded in a protein matrix which varies from the spongy gel form of the salt-soluble proteins (Comer *et al.*, 1986) to pure muscle fiber. Some voids are, in part, adjacent to other voids with a membrane separating them. SEM reveals that the fat globules are not uniformly covered in membrane material but rather are dispersed in a matrix of variable protein density.

Figures 13 and 14 were taken at low magnification and show less homogeneous environments than does Figure 9, with a cluster of voids which may be due to regions of undegraded clumps of adipose tissue. The protein membrane pattern is very similar to the honeycomb patterns for adipose tissue shown by Katsaras *et al.* (1984), and is in agreement with similar observations by van den Oord and Visser (1971). We did not detect areas like this using light microscopy; instead we observed what appeared to be agglomerated fat globules. It is possible that our sample preparation methods could cause this discrepancy in observations. Figures 13 and 14 were obtained from low viscosity batters (batches 1 and 8 respectively). Perhaps one of the reasons for the observed effect of viscosity upon fat agglomeration is that the adipose tissue is not broken down as much in batters having a lower level of solids.

TEM, like LM, did reveal a greater incidence of larger, irregularly-shaped fat globules in the wieners homogenated at the higher temperature (Figures 15-17). This was especially evident in the wieners not containing starch filler. Figure 15 shows a dispersion of smaller fat globules found in batch 7 (starch and caseinate) which was the most stable of the high temperature homogenates. Figure 16, from batch 5 (no filler, 26°C), shows a large, irregularly-shaped fat agglomerate indicative of instability. In Figure 17, from batch 8 (caseinate, 26°C) the amorphous fat globule contains darkly staining material The source of this material is unknown but it could be broken protein membrane fragments. These materials were more prevalent in the batches homogenized at the higher temperatures. Like SEM, TEM revealed a diverse protein environment for the fat globules. Figures 18 and 19 show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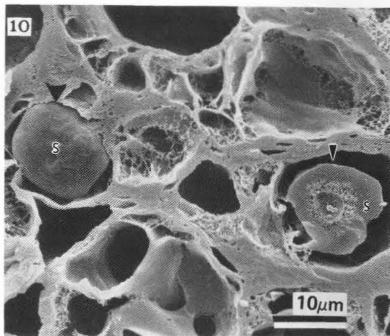
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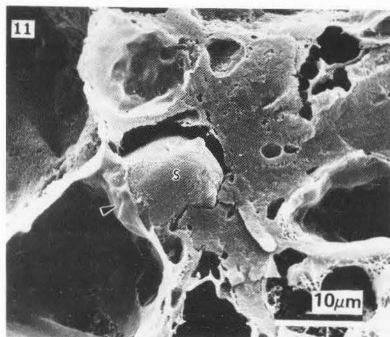
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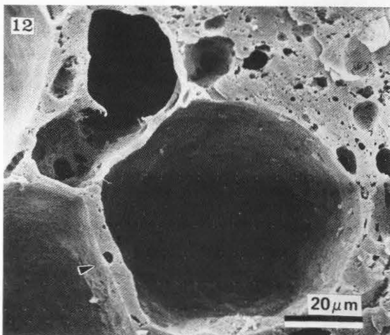
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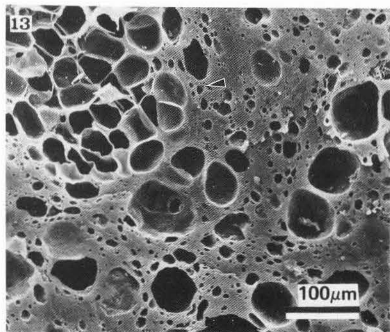
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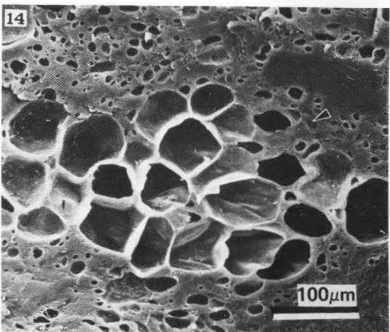
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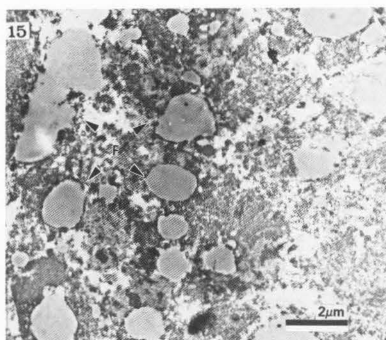
Figure 9. A protein matrix in a starch-containing wiener with fat globules finely dispersed throughout (arrows). Pieces of intact muscle (M) are also seen. (SEM).
Figure 10. Wiener sample containing starch (S). The shrunken granule (small arrow) is broken and sits in a cavity; the intact granule (large arrow) is enclosed in muscle fibre and membrane material. (SEM).
Figure 11. Wiener sample containing starch (S). In this micrograph the membrane (arrow) has taken the shape of the granule. (SEM).
Figure 12. Wiener sample showing a fat globule "void" adjacent to other voids and separated by a membrane (arrow). (SEM).
Figure 13. Sample of a low-viscosity batter wiener showing a cluster of voids (arrows) thought to be undergraded clumps of adipose tissue. (SEM).
Figure 14. Sample of a low-viscosity batter wiener homogenized at 26°C, showing a cluster of voids (arrow). (SEM).

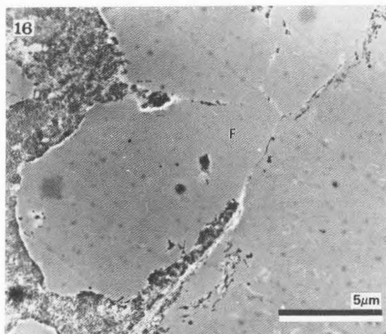
fat globules which are respectively embedded in a complete and partial protein covering. Figure 20 shows a fat globule, almost entirely coated by a membrane of variable thickness positioned in a region of low protein density.

Starch granules were also found in different environments. A characteristic feature of the starch granules is that their shape is distorted as shown in Figure 21. It is likely that the granules are misshapen during the dehydration step of the sample preparation procedure for TEM (Comer *et al.*, 1986). In Figure 21, a small fat droplet is shown adjacent to the starch granule. This illustrates how the starch granules contribute to wiener stability by physically constraining the movement of the fat droplets.

Figure 22 brings us, so to speak, full circle. At first glance, Figure 22 appears to have the same structure as undegraded adipose tissue; however, the fat droplets in Figure 22 are at least two orders of magnitude smaller than are the fat cells of adipose tissue. Figure 22 shows a region of a true emulsion with a fine dispersion of fat globules surrounded by meat protein membranes, as first described by Hansen (1960), using photomicrographs of model meat systems. However, in our observations it was a rare occurrence to find regions of emulsion as shown in Figure 22.

As mentioned previously, we can only speculate on the origins of the protein membranes surrounding the fat globules since the protein staining is





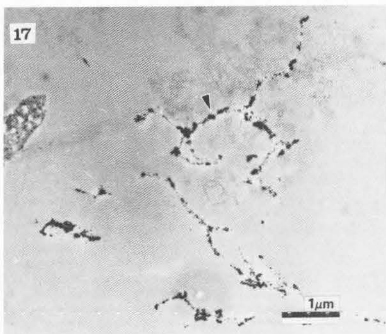


Figure 15. Wiener sample containing both starch and caseinate, which has been homogenized at 26°C shows a dispersion of small fat globules (F). (TEM).
Figures 16. Wiener containing no fillers, homogenized at 26°C. A large, irregularly shaped fat agglomeration (F) is seen, which is indicative of instability. (TEM).
Figure 17. Wiener made with caseinate and homogenized at 26°C. A region of amorphous fat which contains darkly-staining material (arrow) is shown. (TEM).

nonspecific. Both SEM and TEM evidence suggests, however, that the role of membranes in homogenate stability is secondary to that of the continuous matrix. A strong matrix is required for high stability.

In our experiments to evaluate the effects of fillers, we have shown that both starch and protein fillers do strengthen the matrix leading to increased stability and firmness. Homogenizing the batters at higher temperatures (26°C) does not produce a drastic change in stability or texture when fillers are present. From our examination of the microstructure, we conclude that these effects of the fillers are the result of reduced fat agglomeration and a more dense matrix.

Concluding Remarks

As meat prices continue to rise, the economic incentive to use fillers in comminuted meat products increases. Canadian regulations (Agriculture Canada, 1985) offer no restrictions on the use of ingredients as fillers in comminuted meat products, provided minimum meat protein (9.5%) and total protein (11%) requirements are met. Regulatory and economic factors encourage the development of cost effective "binders", i.e., blends of fillers. Experiments to evaluate the functional effects of "binders" are carried out in production size batches by manufacturers of comminuted meat products. Ultimately, this is the only way that the effectiveness of a binder can be determined.

Research experiments, particularly those involving the preparation of small scale batches of wieners, have put a great deal more science into the "art of sausage making" than existed a few decades ago. The model of comminuted meat products as mechanical mixture of ingredients held in a gel matrix, which has resulted from experiments on the functional effects of meat and filler ingredients is proving to be more useful than the simpler emulsion model. Research emphasis is now shifting to the interactions of ingredients, particularly as they relate to gelation properties. The simplicity of model systems and functional property tests is often irresistible, but the data obtained may be of limited value. The functional effects of fillers in comminuted meat products relate to stability and textural improvements. Because these effects are dependent upon the composition of the meat system, it is necessary to design experiments which involve evaluation of ingredients in the actual meat products.

The principal functional fillers are polysaccharides, especially starches, and proteins, i.e. ingredients which have the ability to hold moisture and/or fat in a gel structure after the heat treatment used in processing. The tolerance of a meat system for a filler ingredient is limited by either competition for moisture or a disruption of the meat gel structure. Provided the tolerance limit is not exceeded, then fillers added to a comminuted meat system will generally increase both stability and textural firmness.

Microscopy has had a relatively small influence upon the development of "binders" for comminuted meat products. The contribution of microscopy can best be described as substantiative, since it has often been used in a secondary role to support one theory or another. The data, when taken collectively, however, are unambiguous. Comminuted meat products are mechanical mixtures of fat globules and filler components held in a heat-set gel composed principally of hydrated meat proteins.

The value of microscopy in detecting filler ingredients and in determining their effects in comminuted meat systems will be increased by developing methods which eliminate artefacts and which reveal the fine structure of component interfaces more clearly. The protein and fat staining procedures used in light microscopy obscure fine structural features such as protein membranes. Nonstaining procedures, such as using polarized light, which has been used to reveal collagen membranes in adipose tissue (Lewis, 1979), and selective staining procedures may reveal more detail than previously reported. Comminuted meat products are complex heterogeneous products. The amount of structural detail which has been obtained from SEM and TEM work done to date is overwhelming and may be confused by artefacts.

Time should be spent in documenting these artefacts. Another approach, which may prove to be worthwhile, is to "map" microscopic regions of about 2 mm^2 rather than to randomly scan a particle to obtain "characteristic features". The collection and interpretation of electron microscopy data is a time-consuming process; the practical benefits are not always apparent. However, only through the scrutiny of electron microscopic data can we understand the functional effects of filers in comminuted meat products.

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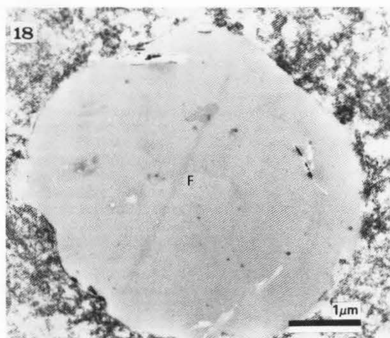

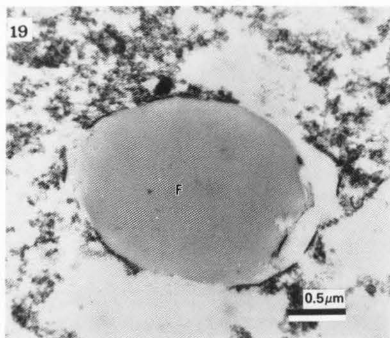

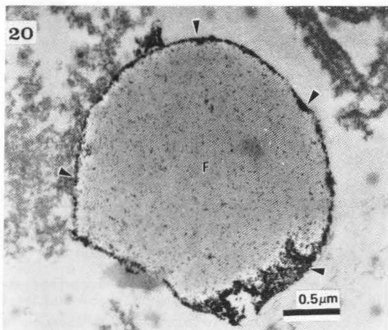

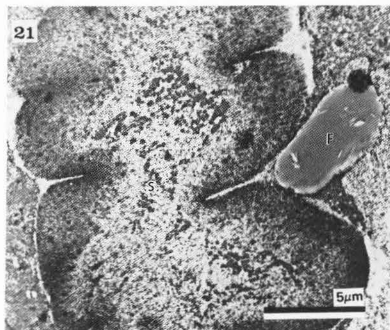

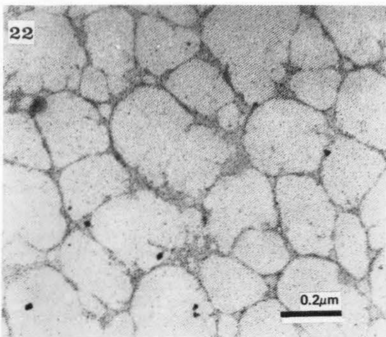

Figure 18. A fat globule (F) is shown, which is completely surrounded by protein. (TEM)

Figure 19. A fat globule (F) which has only a partial protein covering is shown. (TEM).

Figure 20. A membrane (arrows) of variable thickness almost completely coats a fat globule (F) in a region of low protein density. The dark "spots" on the globule result from difficulties encountered at the staining step in this particular sample. (TEM).

Figure 21. A small fat globule (F) is shown adjacent to a starch granule (S), illustrating how starch granules may physically restrain the fat. (TEM).

Figure 22. A rare "true emulsion" area, which has a fine dispersion of fat globules surrounded by protein membranes. (TEM).

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Discussion with Reviewers

G. R. Schmidt: Would it have been useful to measure the pH's of the raw batter and finished products in treatments 1 through 8 in the experiments reported in tables 3 through 5? The various fillers may have modified the pH or, as the authors suggest, the storage of the meat block may have modified the pH. Do the authors feel that this could have happened?
Authors: pH values were not determined. However, we do not believe that the fillers would have changed the pH significantly. The modified starch used has pH 6.0 and the caseinate has a pH of 6.5. The pH of the batters should be in the range of 6.0 to 6.4.

G. R. Schmidt: Do the authors feel that the reason that they did not get as great an effect due to increasing chopping temperature was due to the fact that they are at a much lower fat percentage, *i.e.*, 20% rather than the 30% often used by other researchers? Or perhaps, did the authors use a smokehouse schedule that is much less conducive to cook loss than the model system?
C. Lee: Could the differences in cook stabilities between the wieners and cooked homogenates be due to differences in heating rate?
Authors: We selected a heating cycle for our "model system" which approximates the heating rate achieved in our smokehouse schedule for cooking wieners. The results convince us that our smokehouse schedule is indeed much less conducive to cook loss than "model systems". Since fat agglomeration is a major cause of instability, it is also likely that higher fat percentages would magnify the differences.

C. Lee: How do you rationalize using a water temperature of 40°C for preparation of the meat emulsion? Would this temperature be too high to keep the functionality of myosin?
Authors: The thermal transitions for myosin are above 40°C. Immediately after the water was added the mixture was comminuted to the temperatures of 16°C or 26°C. Warm water is often used in industry to raise the temperature of cold meat blends. Based on the results, we feel there is a negligible effect upon myosin functionality from using 40°C water.

A. M. Hermansson: It is not generally agreed upon that gelation of myosin alone determines the textural properties of comminuted meat products. The solubility of myosin is less than one percent by weight in comminuted meat systems before heating. Why do you stress the importance of gelation?
C. Lee: Have you developed quantitative data on gelation properties?
Authors: We have not tried to develop functional property tests based on gelation phenomena. Gelation mechanisms involve not only the heat setting of soluble proteins and gums, but also the heat-induced swelling of proteins and starch granules. The available moisture in the system has a tremendous effect on the firmness of gels and, indeed, whether a gel will be formed. We believe gelation tests involving excess moisture may have limited value in predicting functional effects.

A. M. Hermansson: You state that the muscle fibers are not destroyed. How can this be if protein gelation is responsible for the network structure and the texture as you claim? The statement is also contradictory to the micrographs shown.
Authors: During the comminution process, the muscle fibers are broken down but not completely destroyed. Figure 9 shows muscle fibers. We were also able to detect heart muscle. Comments on these observations were made to support the description of wieners as mechanical mixtures rather than as emulsions. Complete destruction of the muscle fibers is obviously not required for the formation of a gel. However, a high degree of comminution is required for the solubilization and swelling mechanisms mentioned in our previous comments on gelation.

C. Lee: How do you justify the statements "...good dispersion may lead to interactions"; "...interaction with soluble proteins may result in soft texture"; and "...the incorporation of non-meat proteins may result in a soft texture because their gels are softer than myosin gel"?
Authors: Protein-protein interactions in food systems need to be studied further. However, the fact that soluble proteins interact has been well-established in model systems. We have not carried out model system experiments, but since we did observe a change in the micelle structure of milk proteins in a meat system, we have further speculated that caseinate may interact with soluble meat proteins in the system. We are not aware of gel systems involving non-meat proteins, or blends of non-meat proteins and myosin, which produce the springy gel structure of myosin. Our comments perhaps form the basis of an hypothesis which should stimulate further experimentation.

D. F. Lewis: Soy protein isolates often do not disperse very well when present at the high concentrations and salt contents found in meat products. How do you think that this affects their performance?
Authors: One of the authors (Comer, unpublished) has shown that wieners containing native, dispersible pea protein are much softer than wieners containing heat-denatured pea proteins. Low dispersibility is probably advantageous to the performance of soy protein isolates in wieners, since the dispersible soy proteins are not completely gelled at the cooking temperatures used.

D. F. Lewis: Have you considered the possibility of more than one continuous matrix within a product?
Authors: At high levels of filler, there may be regions where the filler forms a continuous matrix, *e.g.*, Figure 3 shows a "starch matrix". However, once the matrix of meat proteins is severely disrupted by fat, carbohydrates, or non-meat proteins, then textural firmness decreases rapidly. Wieners have essentially one major continuous matrix, but for extended meat pastes it is possible that non-meat matrices may have a larger presence.

C. Lee: You state that starch granules were discrete and several possessed birefringence because of the limited moisture environment in the comminuted meat products. I agree with the effect upon birefringence but how do you justify the effect upon the discrete nature of the granules? Starch granules can merge in water but I believe in the meat system the protein gel would restrict their movement and prevent merging.
Authors: The protein matrix does not prevent the merging of granules as shown in our previous publication (Comer *et al*., 1986). If there is sufficient moisture available the granules will rupture and the starch will flow. At a 7% starch level, there are many starch granules within close proximity as shown in Figures 3 and 4. The merging of starch granules is analogous to the process of fat agglomeration; but as we have shown, it does not generally occur in meat products because of limited moisture availability.

C. Lee: The authors state that "Figures 10 and 11 show clearly starch granules." How can you prove that they are indeed starch granules? Provided that this sample also contains fat, how can one differentiate starch from fat?
Authors: The starch granules are identified mainly by their shape and size (Swinkles, 1985). They are differentiated from fat through the selective extraction of fat from the samples (using chloroform) that is part of our preparatory process. Simply put, the fat is extracted; the starch is not.

A. M. Hermansson: Figure 22 is said to show the characteristic structure of a starch granule. Are there any other characteristic features of the structure apart from the shape?
Authors: Shape is the first quality that is readily apparent in micrographs. The starch has a characteristic appearance after it has been prepared for TEM (Gallant and Guilbot, 1971). In addition, size is also taken into consideration (Swinkles, 1985). When these factors are considered together, we feel that starch can be identified realiably.

C. Lee: What are the white voids in Figure 2? They appear to be places where fat globules have been removed as a result of preparation error.
Authors: The large voids are believed to be air pockets. We have observed that our laboratory-prepared wieners do have some large air pockets which are clearly visible in the sections.

D. F. Lewis: There is some scope for fat mobility during staining with solubility-based oil dyes. Staining with osmium tetroxide vapour or solution is, in my view, a better fat stain. Please comment.
Authors: Ray *et al*. (1981) have successfully used osmium tetroxide to stain fat in LM sections. It is a method which gives informative results and should be included as a standard LM staining technique for the study of comminuted meat products.

C. Lee: In the photomicrographs, I find it difficult to evaluate the fat dispersion. This is, in part, a resolution problem, but also I believe the field of magnification was not adequate. Please comment on your choice of conditions.
A. M. Hermansson: I believe the quality of the photomicrographs could have been improved by using thinner sections to see finer details. Please comment.
Authors: Our conditions for light microscopy were determined by reviewing the literature and selecting staining techniques which gave good contrast. Sections from 15 µm to 30 µm thickness were evaluated and we did not observe a large enough improvement in detail to cope with the greater difficulties in obtaining and handling the thinner sections. Selecting a magnification level involves compromises. Lower magnification levels provide a more representative view of the microstructure but the fat and starch are more difficult to see. Higher magnification levels provide more detail but the representative view is lost.

A. M. Hermansson: The most serious criticism to this paper is with respect to the SEM micrographs where the fat has been extracted. The discussion of fat globules should be omitted and discussion restricted to features seen in the micrographs. Voids can be due to entrapped air and created during processing, or preparation of the samples for microscopy. Please comment on your approach to the problem.
Authors: The fat has been intentionally removed for the purpose of illustrating specific points about the protein matrix of the samples. Other authors have used a defatting step such as the one we have used to clarify certain structures in their samples (Ray *et al*., 1979; Ray *et al*., 1981; Katsaras and Stenzel, 1983; Katsaras *et al*., 1984). Ray *et al*. (1979, 1981) have discussed the limited use of SEM in the study of comminuted meat products, mainly because of the absence of easily recognizable structures in the micrographs. They have suggested a method where useful information can be obtained from SEM micrographs by doing histology (specific staining and examination in the LM) on the same samples. They were also questioned about identity of the voids shown in their micrographs; they suggested examining meat emulsions of the same composition but mixed using different methods. Katsaras *et al*. (1984) used a defatting step to expose the honeycomb structure of collagenous materials in adipose tissue. Katsaras and Stenzel, (1983) used defatting to illustrate the protein structure of their wiener samples. They also showed techniques to retain fat, as well as cryo-SEM to retain all the components. They discussed how the use of SEM is very limited in the study of comminuted meat products. Sample preparation is not easy, and interpretation of the resulting micrographs is difficult since the structure of muscle and fat tissue is lost. Because of the problems inherent in using SEM as a method of study for meat emulsions, our discussion on fat globules has been kept brief.

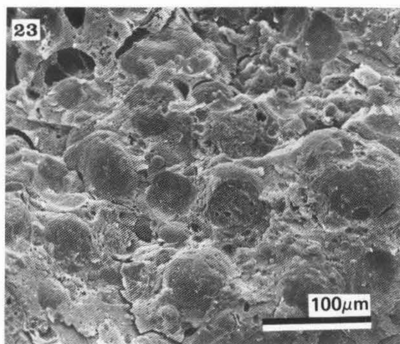
A. M. Hermansson: Was there any shrinkage or deformation of the samples after the preparation for SEM?
Authors: The samples prepared for SEM were dehydrated and critical-point dried according to standard procedures. It is well known that there is a certain amount of shrinkage and deformation which occurs in the sample as a result of these preparatory steps (Cohen, 1979; Boyde, 1978; Boyde and

Maconnachie, 1981). Boyde and Maconnachie (1981) have discussed this problem at length, and have concluded that critical-point drying (and freeze drying) of fixed samples give perfectly acceptable results for SEM. The shrinkage/deformation of our samples was not quantified.

D. F. Lewis: Regarding Figures 18 and 19, is the membrane changed after processing?
Authors: Most likely, processing would cause some changes in the membrane. However, a separate study would be necessary to investigate this phenomenon.

A. M. Hermansson: Is there any evidence that the darkly stained material surrounding fat globules is pure protein?
Authors: The stains that have been used to impart contrast to the sections destined for TEM are the standard stains, uranyl acetate and lead citrate. These are not specific for protein, but are general stains. Carroll and Lee (1981) and Borchert *et al.* (1967) have also observed the electron-dense material surrounding fat globules under conditions of preparation similar to ours. Stanley *et al.* (1977) have also observed material in their bacon samples with apparently the same staining characteristics; they speculate that the material is phospholipid in nature. Dutson (1974) has suggested that specific staining can be accomplished (in muscle tissue) by using specific electron-dense enzyme substrates for enzyme histochemistry, and for specific tissue components such as mucopolysaccharides. This is an interesting approach and should be explored in the area of comminuted meat products.

A. M. Hermansson: Comminuted meat systems are difficult to handle and prepare for microscopy. Conventional techniques for EM may produce artefacts, especially with regard to the structure of fat and starch distributed in the meat structure. What are the authors' opinions about the preparation techniques used in this study?
Authors: Comminuted meat systems have as their main components protein (in the form of muscle), fat, starch, protein additives (such as casein), water and air. Protein can be fixed using aldehydes (glutaraldehyde and/or formaldehyde). Only unsaturated fat can be fixed with osmium tetroxide. Starch cannot be properly fixed with either of the standard methods (Kaláb, 1984) and water is lost through the standard dehydration techniques required to ready the sample for the in vacuo conditions found in the SEM. The work discussed in this paper concentrates on observations of the protein matrix, and so the fat was extracted using chloroform. Information is gained through the use of the preferential extraction step; it was possible to differentiate starch (which was not extracted) from fat (which was extracted). Also, areas of undegraded adipose tissue (see Figures 13 and 14) became obvious after the extraction. Ideally, especially because of the known artefacts which can be associated with different methods of preparation, a number of different types of fixation/preparation conditions should be tried on the samples, since each technique will provide its own type of information. As mentioned in the review, we had done, at the outset, a fixation which allowed fat to be preserved *in situ* (see Figure 23). From analyses of micrographs obtained from samples such as these, very little can be said about the protein matrix, since it is obscured by the retained fat, which cannot be differentiated from starch. Nothing definite can be said about the origin of the voids, which may be due to either water or air in samples prepared in this manner. In cold-stage SEM, where fixation is a physical rather than a chemical phenomenon, and no components of the samples are lost, even less can be said about the microstructure of the sample (see Figure 24). The protein matrix is rather featureless and globules of unknown identity (fat, water, starch) can be seen.



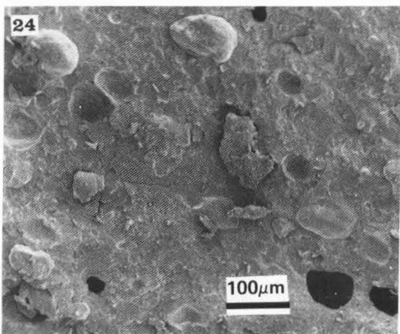


Figure 23. Conventional SEM of a wiener which has been prepared to retain fat in situ. A low-contrast, topographically flat image results in which no positive identification of components can be made. The voids are due to air or removed water.
Figure 24. Cryo-SEM of a wiener in which all components have been retained. The protein matrix appears featureless and globules of unknown identity can be seen. The voids are due to air.

The only information which can be gleaned from a sample prepared in this manner is the identification of the voids due to air. We feel, therefore, that the methods that we have chosen to prepare the samples for conventional SEM are reasonable and provide the illustration of aspects which we have discussed. LM and TEM, which have been done in conjunction with SEM, provide information, respectively, on voids due to air, and the appearance of fat *in situ* and the interface between fat and protein components.

A. M. Hermansson: References are given to a lot of published work without many comments on their quality or expertise with regard to microscopy. Artefact always needs to be taken into account and some analysis of advantages and disadvantages of preparation techniques would improve the paper. Please comment.

Authors: Lee (1985), in his review, has already dealt with this matter, comparing the techniques which have been used for microscopy to study meat emulsions (LM, TEM, SEM). At that time and since then, workers in Germany have carried out microscopy using different preparatory methods for SEM and TEM (Katsaras and Stenzel, 1983; Oelker, 1988, 1987, 1986). There are a number of methods available now which can be selected for different tasks (such as showing different relationships). All, however, have artefacts associated with them. These artefacts arise from the different fixation and fracturing methods which are used, as well as methods which only partially fix or retain fat. These artefacts lead to confusion when one wants to differentiate water from air voids, and other such problems. Because so many techniques are now available for studying comminuted meat products, a thorough review of them is beyond the scope of this paper. Dealing with this problem could provide the substance for a review paper which would discuss only the development and effective use of such techniques in this field.

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