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## STRUCTURE AND FUNCTION OF FOOD PRODUCTS: A REVIEW

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### Abstract

A proper understanding of the behavior of food products requires knowledge of its structure, i.e., the spatial arrangement of the various structural elements and their interactions. The structure can properly be studied by visual observation techniques. In products such as fat spreads, creams, dressings, cheese, bread, milk, yoghurt, whipped cream, and ice cream, different structural elements can be distinguished. A number of those elements are discussed, viz., water droplets, oil droplets, gas cells, particles, fat crystals and strands. In addition examples of interactions between structural elements are presented, viz., oil droplets/matrix, protein/protein, protein carbohydrate, and fat crystal/fat crystal interactions. Finally, it is indicated how these elements cooperate in the formation of structure and contribute to function and macroscopic behavior of food products. Particular attention is given to fat spreads, processed cheese, protein gelation, and examples of the mutual interaction of milk proteins and of carbohydrates with milk proteins. It is expected that a proper understanding of the relation between structure and function will help us to design new ways of structuring in our continuing efforts to manufacture high quality, healthy and tasty food products.

**Key Words:** Structure, function, food products, structural elements, interaction, fat spreads, processed cheese, protein gelation,  $\beta$ -lactoglobulin,  $\kappa$ -carrageenan, milk proteins.

### Introduction

Most food products are composed of rather a limited variety of structural elements, such as droplets, air cells, granules, crystals, strands, micelles and interfaces. A proper understanding of the behavior of a food product requires knowledge of its structure, i.e., the spatial arrangement of the structural elements and their interactions. Interaction forces determine the consistency and the physical stability of food products.

The spatial arrangement of structural elements can be studied by visual observation techniques, such as light- and electron microscopy (EM). Also, the result of the interaction between the various components in a system can be studied in this manner. Visual observation techniques are therefore an important aid in the analysis of food structure.

A variety of techniques are available for the determination of functional properties. Rheological measurements give insight into mechanical properties and consistency. Consumer panels, and rheological characterizations are used to measure sensory properties. The stability of a foam can be followed by visual inspection.

It is the aim of this review to illustrate how various structural elements cooperate in the formation of structure and contribute to functional properties and macroscopic behavior of food products. To this end, a number of structural elements, as observed by microstructural techniques, will be described as well as examples of their interaction. Finally, a number of food systems will be discussed.

### Structural Elements

#### Water droplets

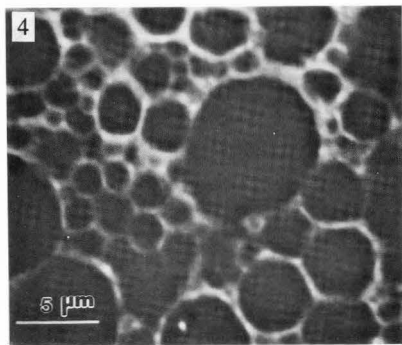
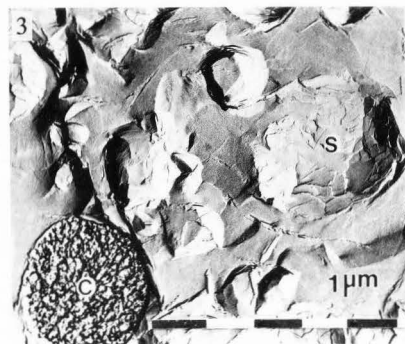
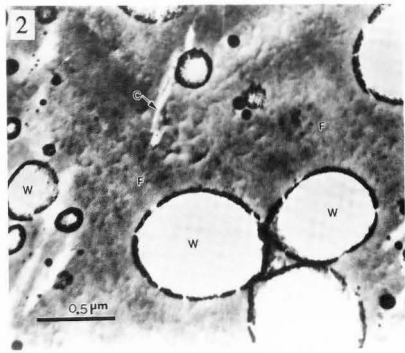
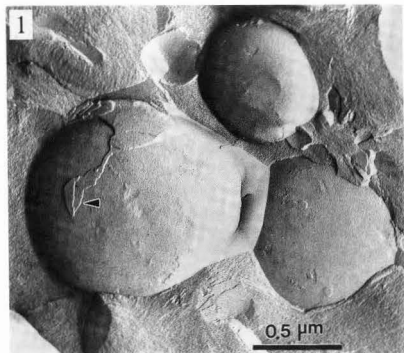
Water droplets are important structural elements in emulsion type food products such as margarine, butter and low-fat spreads. The droplets are stabilized by soluble emulsifiers, such as monoacylglycerols and lecithins (Madsen, 1987) and/or by solid particles, such as fat crystals (Lucassen-Reynders and van den Tempel, 1963).

An example of water droplets stabilized by a soluble emulsifier in a water-in-oil emulsion, observed by

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**Figure 1.** Water droplets in a water in oil emulsion stabilized by DKF 10, observed by freeze-fracture EM. Arrow points to multilayered shells.

**Figure 2.** Water droplets (W) in a margarine, observed by thin-sectioning EM. Linear structures (C), indicative of fat crystals, can be observed in the continuous fat matrix (F). Dark structures at the W/F interface indicate emulsifier.

**Figure 3.** Water droplets in margarine. The freeze fracture technique gives rise to two different images of water droplets depending on whether the sample breaks above the surface (S) of the droplet or whether the cross fracture (C) runs through the droplet.

**Figure 4.** Water droplets in a 25% fat spread, observed by fluorescence confocal scanning laser microscopy. Protein fluorescer: fluorescein isothiocyanate (FITC). The protein is located at the oil/water interface.

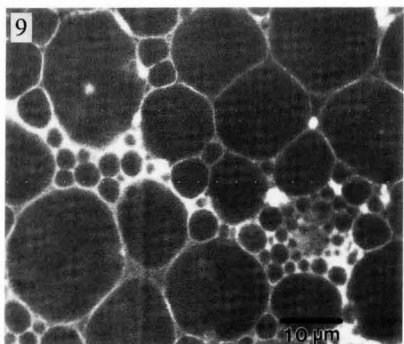
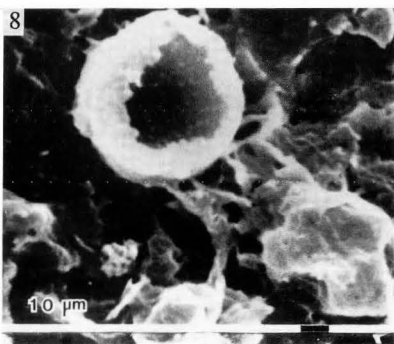
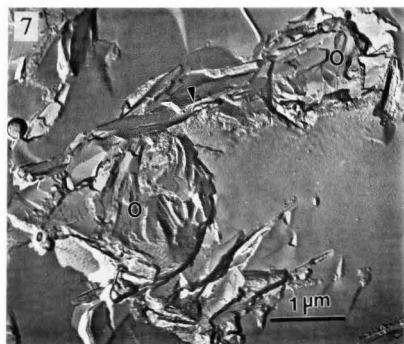
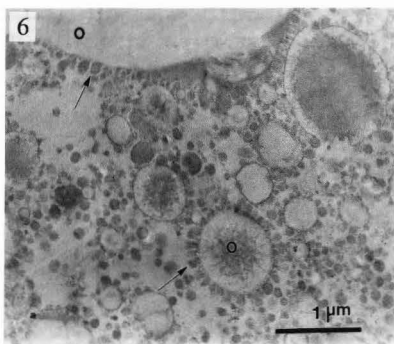
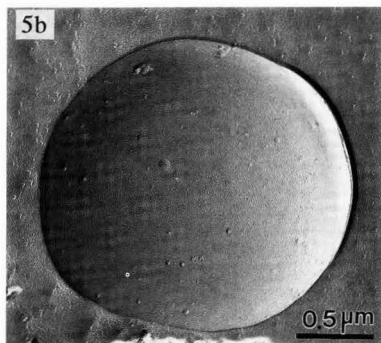
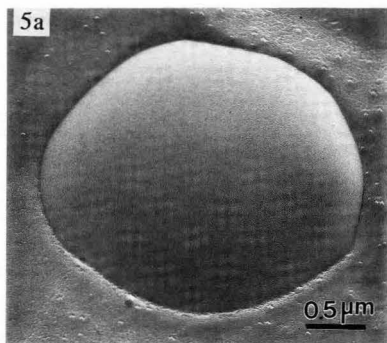
**Figure 5.** Oil droplets in oil-in-water emulsions stabilized by proteins: (a) with sodium caseinate a smooth and homogeneous interfacial layer is obtained. (b) with whey protein a smooth interfacial layer with some particles is observed. Freeze-fracture EM. (Courtesy W. Buchheim).

**Figure 6.** Oil droplets (O) in homogenized milk, covered with casein micelles (arrow). Thin-sectioning EM.

**Figure 7.** Bridge formation (arrow) between oil droplets (O) caused by fat crystallization. Freeze-fracture EM.

**Figure 8.** Butter globule in butter, indicating an outer crystalline shell composed of high-melting fat, surrounding liquid oil inside. Cryo SEM after de-oiling.

**Figure 9.** Distorted oil droplets in mayonnaise. CSLM. Fluorescent staining of the continuous water phase and the interface by Nile Blue.



freeze fracture EM is given in Fig. 1. The emulsifier used is DK-F10, which is a mixture of di-, tri-, and polyesters of hydrogenated tallow fatty acids with sucrose (Van Voorst Vader and Groeneweg, 1989).

Thin-sectioning EM of a water droplet in a margarine reveals the presence of emulsifier at the interface between oil and water (Fig. 2).

Freeze-fracture EM of an 80% fat spread shows (Fig. 3) that fracture occurs either over the surface of the water droplets or through the droplet. Fat crystals on the surface of the droplets are clearly perceptible, indicating stabilization by fat crystals. Similar observations have been reported (Precht and Buchheim, 1980b).

Water droplets can also be properly observed by light microscopical techniques. Fig. 4 reveals the structure for a 25% fat spread. In this case, the droplets are stabilized by protein, which is located at the oil/water interface.

An important aspect of water droplets is their size which influences both rheological and sensorial properties. Water droplets in spreads should be kept small (preferably  $< 5 \mu\text{m}$ ) to reduce microbial risks (Verrips and Zaalberg, 1980; Verrips *et al.*, 1980). Small droplets induce a greasy taste (Larsson 1986, p. 221).

#### Oil droplets

Oil or fat droplets occur as structural elements in such diverse products as milk, cream, cheese, mayonnaise, dressings, meat products and ice cream. Oil droplets can be stabilized by high-molecular mass surfactants, such as proteins or by low-molecular mass surfactants such as Tweens and lecithins or combinations thereof. Crystals in the oil phase may induce aggregation of the oil droplets, resulting in partial coalescence (Boode, 1992; Boode and Walstra, 1989). The stabilization of emulsions has been amply discussed (Larsson and Friberg, 1991).

Examples of oil droplets covered with a smooth interfacial protein layer (Larsson and Friberg, 1991, p. 216), observed by freeze fracture EM, are given in Fig. 5. Fig. 6 shows milk fat droplets covered with casein micelles in a homogenized milk, observed by thin-sectioning EM.

Bridge formation between oil droplets in an oil-in-water emulsion, due to fat crystallization, is indicated in Fig. 7 (Heertje, 1993). Such crystallization phenomena are also important in the production of butter by churning (Precht and Buchheim, 1979, 1980a). Milk fat globules of the original cream may survive the churning process. A butter globule, observed by scanning EM (SEM, Fig. 8) after deoiling (Heertje, *et al.*, 1987), shows an outer crystalline layer composed of high-melting fat, surrounding liquid oil inside.

Mayonnaise is an oil-in-water emulsion containing a high percentage of oil (80% or more). As observed with confocal scanning laser microscopy (CSLM) (Fig. 9), this high volume of oil causes the formation of a honeycomb structure of closely packed and often distorted oil droplets. In this case, emulsion stabilization is achieved mainly by egg yolk lecithin.

**Figure 10.** Air cells in a shortening. Cryo SEM.

**Figure 11.** Air cell in a churned product. The air interface is covered with fat globules. (g) fat globules; (w) water. Cryo SEM.

**Figure 12.** Air cells in a whipped cream at low (a) and (b) high magnification, showing interface stabilized by fat globules and protein film. Cryo SEM.

**Figure 13.** Air cell in whipped cream. The air cell is stabilized by fat globules (fg) adsorbed at the interface and remnants of protein film (i). Thin-sectioning EM. (Courtesy B. Brooker).

**Figure 14.** Deformed gas cells in dough, stabilized by a solid matrix of starch and protein. Cryo SEM.

An important aspect of oil droplets is their size and the homogeneity of the size distribution. It may affect both rheological and sensorial properties.

#### Gas cells

In many food products, such as bread, cake, whipped cream, ice cream, beer, and chocolate mousse, gas cells play an important role. Foam stability depends on many factors. Gas cells can be immobilized in a solid matrix, whereas in other cases, stabilization is achieved by protein films around the air cells. In the latter case, solid particles can easily rupture the fragile foam lamellae. Also interactions at the interface (e.g., between protein and fat) may enhance foam stability (Buchheim, 1991; Brooker, 1993).

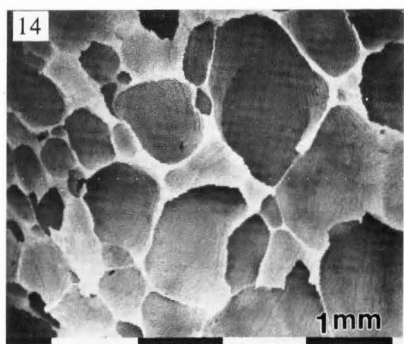
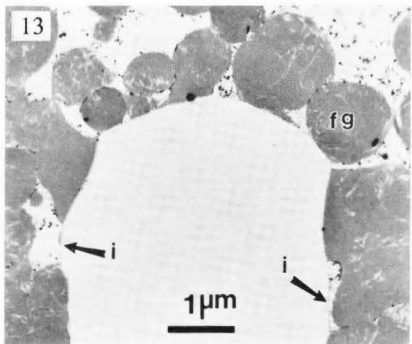
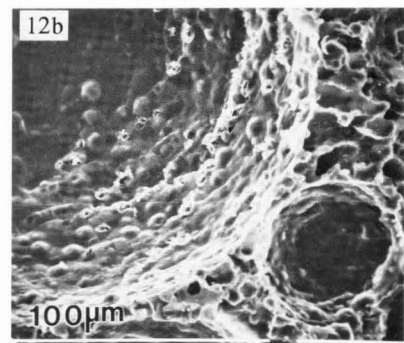
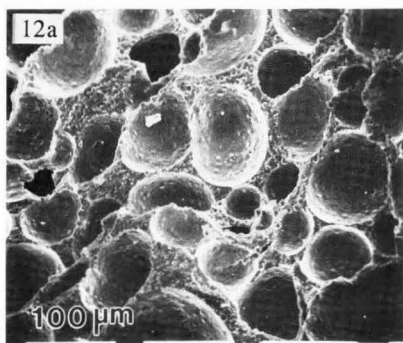
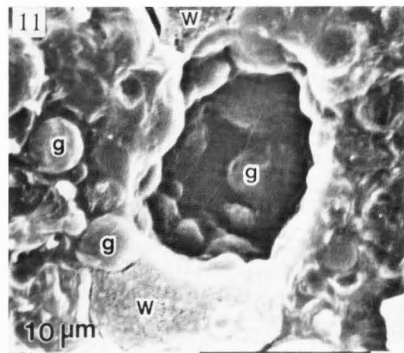
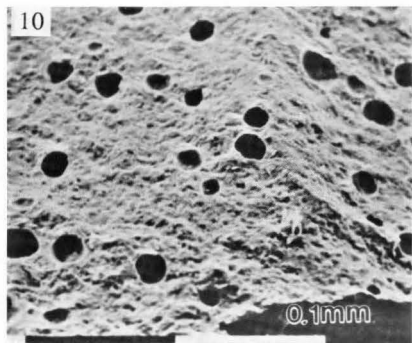
An example of air cells immobilized in a solid matrix is given in Fig. 10. Air was dosed at an early stage of margarine processing at low solid levels, giving rise to smooth round air bubbles. Deformed air bubbles are found when high solid contents are present at the moment of air introduction, e.g., during churning (Fig. 11).

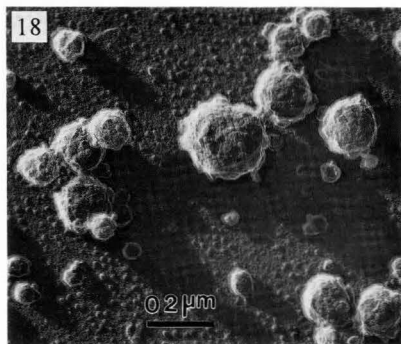
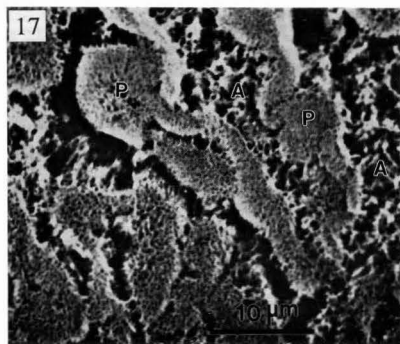
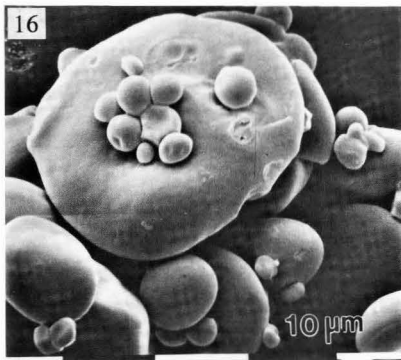
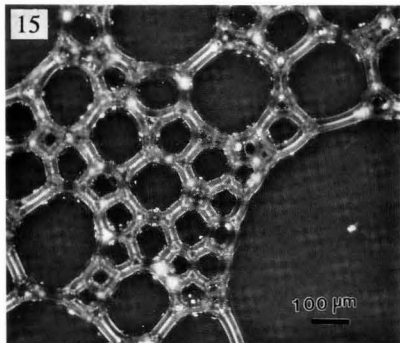
Air cells in a whipped cream, observed by cryo SEM, are presented in Figs. 12a,b. Stabilization in this case is achieved by the combined action of protein and fat globules (Brooker *et al.*, 1986). Thin sectioning EM (Fig. 13) clearly demonstrates the localization of fat globules at the interface (Brooker, *et al.*, 1986).

Gas cells (Fig. 14) play an important role also in dough to obtain proper bread volume. In this case, the lamellae between the gas cells consist of a solid matrix of starch and protein.

A foam prepared from saturated  $C_{16}$  and  $C_{18}$  monoacylglycerols shows a polyhedral structure, indicative of a close packing of air cells (Fig. 15).

It is evident that there is an enormous difference in shape, size and phase volume of gas cells as well as in the composition of foams. Such differences explain the wide variety of properties of foamed food products, such as beer and bread. Recently, a new method to determine bubble size distributions in foamed food products has been described (Bisperink *et al.*, 1992; Akkerman *et al.*, 1992).



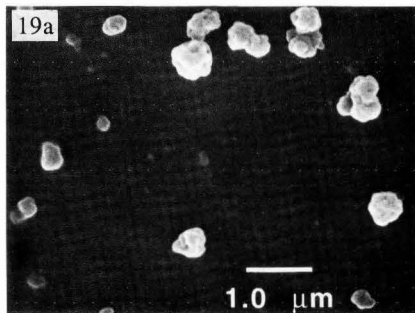


#### Starch granules, casein micelles and other particles

Different varieties of unmodified starch exhibit wide variations in granule appearance (Fitt and Snyder, 1984). Large differences in shape and size are observed in starch granules from corn, maize, wheat, rice and potato.

Fig. 16 gives an example of a native wheat starch. Large lenticular granules and small spherical granules are observed. However, in most finished food products, such as bread, soups, sauces and dressings, starch is not present in its native form, but gelatinized by heating in the presence of water. Upon heating above the so-called gelatinization temperature, irreversible swelling takes place. Even after extensive swelling, the starch granules can still be recognized.

Microstructural changes in wheat starch dispersions during heating and cooling have been described (Langton and Hermansson, 1989). An example of a 5% maize starch suspension, heated to 95°C and cooled to 20°C is shown in Fig. 17. The more compact regions



represent the irreversibly swollen granules which have been shown to be largely amylopectin, whereas the less dense, leached out amylose surrounds the compact regions.

There are a number of serious disadvantages related to the use of native starches as ingredients for food products. In recent years, this has led to commercial production of a whole range of particulated modified starches. Maltodextrins from National Starch (N-oil), Grain Processing Corporation (Maltrin), Cerestar (Snowflakes) and Avebe (Paselli SA2), should be mentioned in this regard. It has been claimed that in the latter case, a high yield of uniform particles (1-2  $\mu\text{m}$ ) is responsible for the fat mimetic properties of the resulting gel.

In other, non-starch, carbohydrate-based products, particles have also been suggested to be responsible for fat-like properties. For example, in Slendid (Copenhagen Pectin Company) soft particles of Ca-pectinate are approximately 40  $\mu\text{m}$  in diameter whereas particles of microcrystalline cellulose (Avicel, FMC corporation) are approximately 0.2  $\mu\text{m}$  long.

Also protein particles play an important role in food structuring. Casein micelles are an important component of milk. In fresh milk, the micelles are present in the form of colloidal particles of about 0.1  $\mu\text{m}$  in diameter (Fig. 18, Kalab, 1993). Their aggregation behavior is responsible for such important products as cheese and yoghurt.

Other protein-based particles have been developed, the most noteworthy example being Simplese (NutraSweet Company; Singer and Dunn, 1990), prepared from egg white and whey or milk proteins in a size of about 2  $\mu\text{m}$  (Fig. 19). It is claimed, that particles of this size roll easily over one another yielding a

creamy perception and a rich texture. This is in contrast with particles equal or smaller than 0.1  $\mu\text{m}$  (e.g., casein micelles) which are perceived as gelatinous and slippery. On the other hand, particles larger than 3.0  $\mu\text{m}$  are perceived as powdery or gritty (Singer and Dunn, 1990).

#### Fat crystals

The formation of texture in fat spreads is the result of crystallization of triacylglycerols with high melting points. In general, the crystals in food products do not occur as single crystals, but show different types of aggregation. Triacylglycerols crystallize in four different modifications: sub- $\alpha$ ,  $\alpha$ ,  $\beta'$  and  $\beta$  (Larsson, 1986). The sub- $\alpha$  and  $\alpha$  modifications are unstable and therefore do not exist in spreads. The  $\beta'$  modification is stable but its crystal lattice is less well ordered than that of the  $\beta$  modification. The  $\beta$  modification has the highest ordering and consequently the highest melting point. As a consequence of the rather complicated triacylglycerol composition, the  $\beta'$  modification is the predominant one in commercial fat blends. Fat crystals in the  $\beta'$  modification can be either needles or platelets (Fig. 20). Sometimes, a defect called sandiness or graininess is observed in margarines and shortenings (Heertje, 1993). This phenomenon appears to be caused by the formation of large, and often spherulitic, crystal aggregates in the  $\beta$  modification (Fig. 21).

#### Strands

Biopolymers such as proteins and polysaccharides are present in many food systems, often in the form of gels. The gels are formed by networks of polymer strands. Several types of strand formation can be distinguished (Clark, 1987). Strands may be formed by aggregation of macromolecules or macromolecular assemblies in globular form (particularly proteins). Such strands usually produce particle gels (Dickinson, 1980).

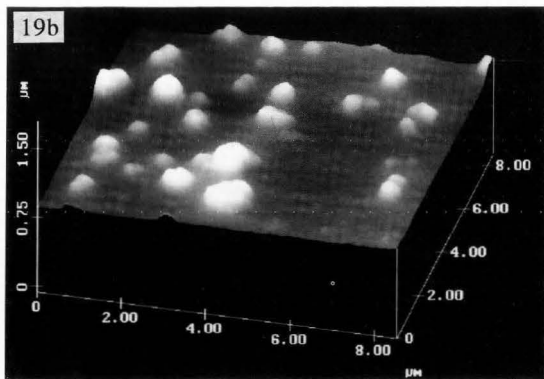
**Figure 15.** Air cells in a foam, prepared from monoglycerides, showing a polyhedral structure. Light microscopy.

**Figure 16.** Large lenticular and small spherical granules of wheat starch. Cryo SEM.

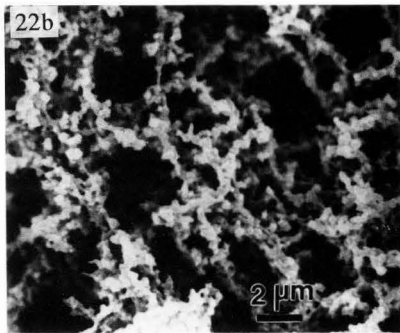
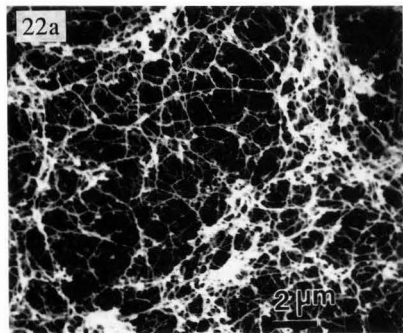
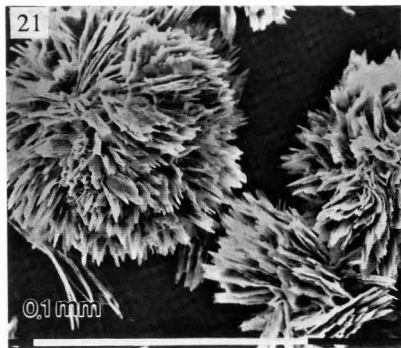
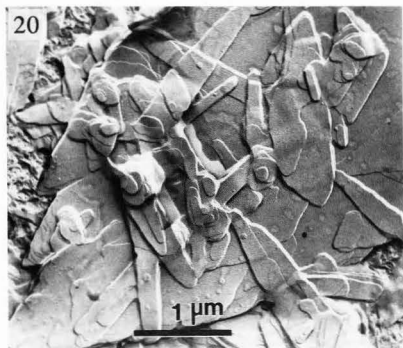
**Figure 17.** Swelling of starch granules in a 5% maize starch suspension on heating to 95°C. (P) dense amylopectin-rich zone; (A) leached out amylose. Cryo SEM. Note: the apparent network structure is induced by the freezing process during sample preparation and is not a representation of the true structure.

**Figure 18.** Casein micelles from cows' milk. Replica TEM. (Courtesy M. Kalab).

**Figure 19.** (a) Simplese microparticles. SEM after air drying (Courtesy N. Singer & C.J. Pellegrin, Monsanto Company). (b) 3-D view of Simplese microparticles. Atomic force microscopy (Courtesy N. Singer, S. Chastain, N. Desai, the NutraSweet Company).







**Figure 20.** Fat crystals (platelets and needles) in the  $\beta'$ -modification. Replica TEM.

**Figure 21.** Spherulitic fat crystal aggregate in the  $\beta$ -modification. Cryo SEM.

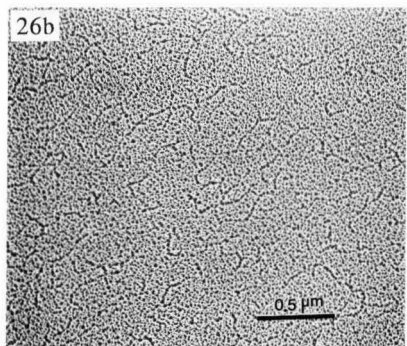
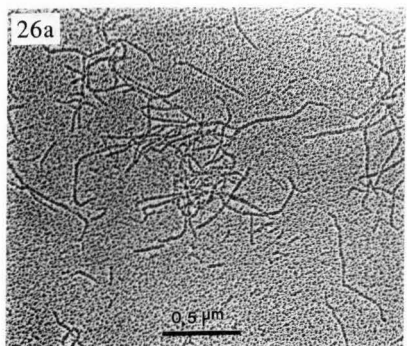
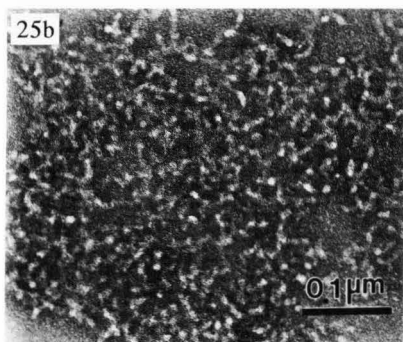
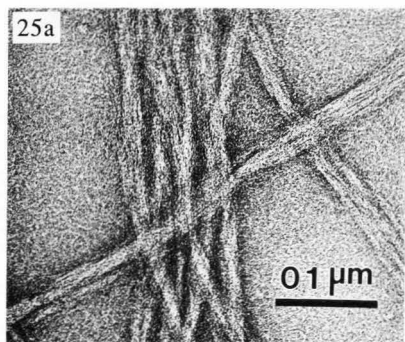
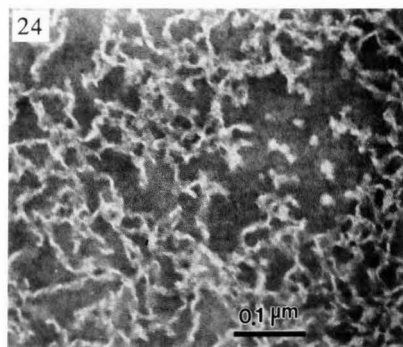
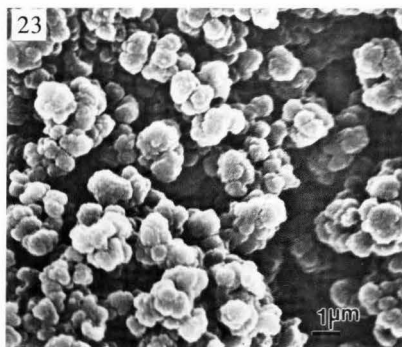
**Figure 22.** Fine strands (a) and particle strands (b) in gels of myosin, induced by differences in ion concentration. SEM. (Courtesy Anne Marie Hermansson).

**Figure 23.** Particle strands in ovalbumin gel close to its isoelectric point. SEM.

**Figure 24.** Fine strands in a  $\beta$ -lactoglobulin gel at pH 8.0. Negative staining.

**Figure 25.** Strands of polysaccharides: (a) stiff strands in curdlan gels, and (b) flexible strands in gelatinized potato starch. Negative staining. (Courtesy T. Harada).

**Figure 26.** Strands in  $\kappa$ -carrageenan: (a) strands in 0.1 M KCl have a large persistence length, while (b) strands in 0.01 M NaCl have a small persistence length. Replica from glycerol-containing aqueous solutions after vacuum-drying on mica.



Alternatively, strands may consist of extended random coil molecules. These latter strands form association networks with entanglements or junction zones.

Numerous publications deal with the formation of strands from proteins and carbohydrates. Strands from bovine myosin show different morphologies, depending on ionic strength (Hermansson *et al.*, 1986; Hermansson and Langton, 1988). Fine strands (Fig. 22a) were formed at low ionic strength (0.25 M KCl), whereas coarse aggregated particle strands (Fig. 22b) were formed at high ionic strength (0.6 M KCl). Similar differences are reported for other, heated, proteins as a function of pH. Aggregated particle strands are found close to the isoelectric point (IEP) of the protein, whereas fine strands are found both at low and high pH, away from the IEP (Stading and Hermansson, 1991; Heertje and van Kleef, 1986; Harwalkar and Kalab, 1985). Fig. 23 gives an example for ovalbumin, close to its IEP and Fig. 24 an example for  $\beta$ -lactoglobulin at pH 8.0.

Also the morphology of strands of polysaccharides has been amply investigated (Stokke and Elgsaeter, 1987; Stokke *et al.*, 1989; Hermansson, 1989; Harada *et al.*, 1990). Strands largely varying in size, shape, flexibility and branching can be observed. An example taken from the work of Harada *et al.* (1990) is provided in Figs. 25a,b.

Figs. 26a,b present micrographs of heated (80°C) solutions of  $\kappa$ -carrageenan in 0.1 M KCl and 0.01 M NaCl, respectively. A strong influence of the electrolyte type and its concentration on the size, thickness and flexibility (persistence length) of the strands is indicated (Hermansson *et al.*, 1991). This is in agreement with the well-known influence of potassium ions on the gelation behavior of  $\kappa$ -carrageenan (Morris *et al.*, 1980). Firm cohesive gels of  $\kappa$ -carrageenan are obtained in the presence of potassium, whereas under identical conditions of concentration and ionic strength no gels are formed in the presence of sodium.

#### Interactions

By combining the various structural elements discussed in the preceding section, a large number of possible interaction types can be distinguished, such as droplet/matrix, droplet/air cell, strand/strand, strand/particle, particle/droplet, crystal/crystal, crystal/droplet, and droplet/droplet interactions. In this section a number of examples will be presented.

#### Oil droplets/matrix interaction

Various authors have discussed the importance of the interaction between the dispersed phase and the continuous phase in relation to rheological and sensorial properties (Van Vliet and Dentener-Kikkert, 1982; Masson and Jost, 1986; Jost *et al.*, 1986; Langley and Green, 1989; Aguilera and Kessler, 1989; Aguilera and Kinsella, 1991; Xiong and Kinsella, 1991).

Milk gels, containing fat in acid medium, have been studied as a function of fat globule membrane composition (Van Vliet and Dentener-Kikkert, 1982; Van Vliet 1988). In natural (not homogenized) milk, the

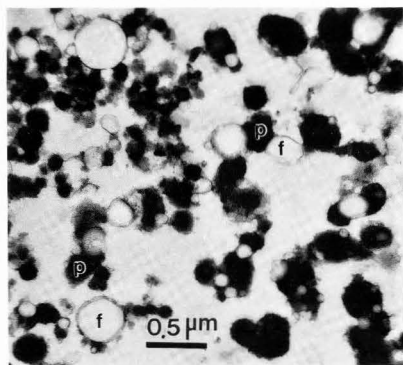
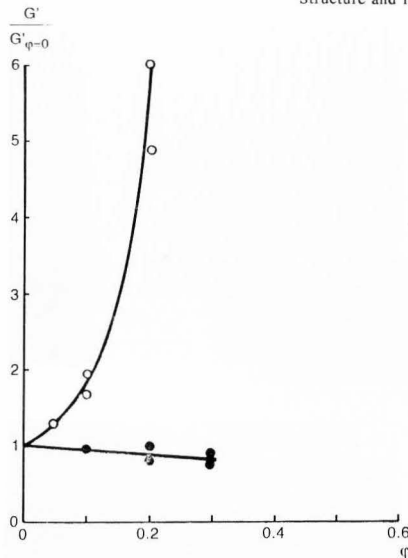


Figure 27. A strong interaction between the interface of the fat globules (f) in homogenized milk and the continuous matrix (p) composed of casein particles. Thin-sectioning EM. (Courtesy M. Kalab).

membrane consists mainly of specific proteins and lipids which do not interact with the continuous network of casein strands in the acid milk gels. When the milk is homogenized fat globules are transformed into markedly smaller globules, covered with milk proteins such as whey proteins and casein micelles. In that case, a strong interaction occurs between the species (in particular casein micelles) located at the interface between oil and water and the continuous matrix as is indicated in Fig. 27 (Kalab, 1993). This is also reflected in a strong difference in rheological behaviour of the two types of acid milk gels (Fig. 28).

Similar observations are reported for oil-in-water emulsions stabilized by heat-treated whey protein (Masson and Jost, 1986; Jost *et al.*, 1986; Langley and Green, 1989). The size of the dispersed oil droplets appears to be a very important parameter for proper gel formation. Emulsions with a high gelation capacity are characterized by a single droplet family of relatively narrow size distribution and a mean droplet diameter ranging from 0.3-0.7  $\mu\text{m}$ . Extensive coating of the oil droplets with coagulated protein lead to a continuous gel structure in which the oil droplets are strongly embedded (Fig. 29, Jost *et al.*, 1989). This coating does not occur when the oil droplets are first stabilized by lecithin. In the latter case, very weak gels are obtained with smooth oil droplets, devoid of any adsorbed protein (Jost *et al.*, 1989).

Also, gels filled with fat globules in a mixed matrix of casein and whey protein have been studied (Aguilera and Kessler, 1989; Xiong and Kinsella, 1991; Aguilera and Kinsella, 1991). By combined microstructural and rheological studies, proper insight into the structure formation of these mixed and filled gels could



**Figure 28.** Storage moduli  $G'$  of acid milk gels with particles divided by  $G'$  of gels without dispersed particles as a function of the volume fraction of dispersed particles  $\Phi$ . Open symbols: interacting particles; filled symbols: non-interacting particles. (Courtesy T. van Vliet).

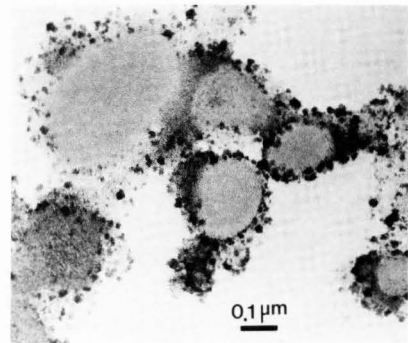
be obtained. Stabilization of the globules by whey protein resulted in the strongest gels, followed by stabilization by sodium caseinate and skim milk powder. Stabilization by Tween 80 gave relatively weak gels (Xiong and Kinsella, 1991). These results are once more explained considering the interaction forces between the interface of the fat globules and the continuous protein matrix.

These examples show the important effect of particle/matrix interactions on the behavior of food systems.

#### Protein/protein interaction

As a typical example of protein/protein interaction, the heat induced association between  $\beta$ -lactoglobulin and  $\kappa$ -casein and casein micelles will be discussed.

The properties of dairy products are influenced by heat treatment of the milk. The influence of pre-heating at 85–90°C on texture, firmness and syneresis of yoghurt and cheese is well known (Kalab, 1993). Many studies attribute this effect to associations between  $\beta$ -lactoglobulin and  $\kappa$ -casein (Wheelock and Kirk, 1974; Davies *et al.*, 1978; Creamer *et al.*, 1978). A possible mechanism



**Figure 29.** Extensive coating of oil droplets with coagulated whey protein, cross-linking of the oil droplets. Thin-sectioning EM). (Courtesy R. Jost).

of the interaction has been described (Haque, 1987). Minor variations in pH were shown to have severe effects on the adherence of protein aggregates to casein micelles (Creamer *et al.*, 1978). In this context, model experiments with  $\beta$ -lactoglobulin and casein micelles have been performed as a function of pH. Figs. 30 and 31 show micrographs of the heat-induced association at pH 6.5 and 7.0, respectively:

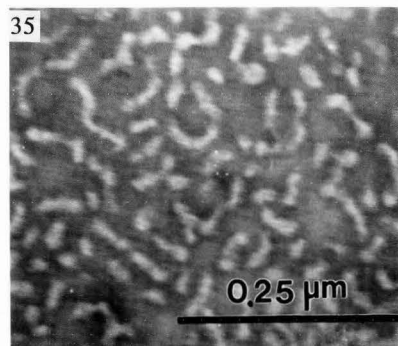
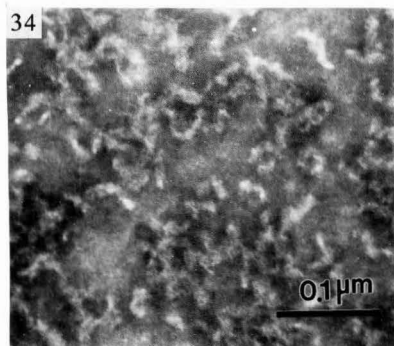
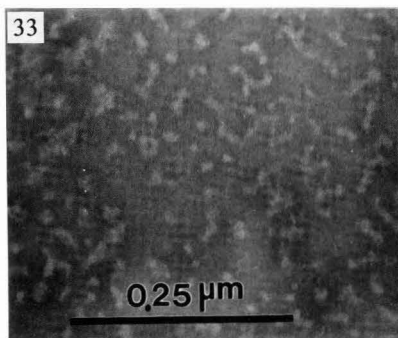
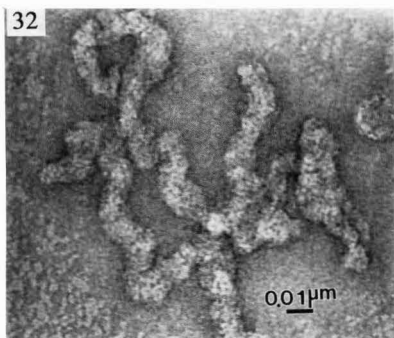
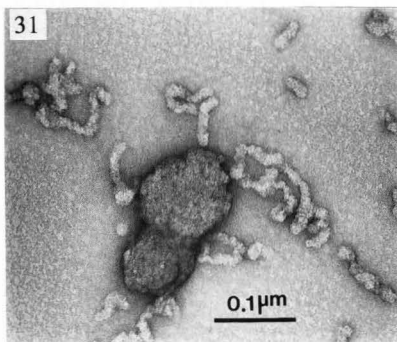
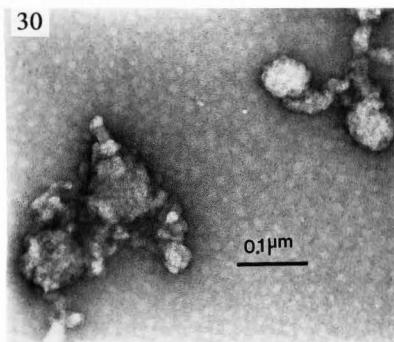
- At pH 7.0, separate micelles and thread-like protein structures are visible. At pH 6.5,  $\beta$ -lactoglobulin is concentrated on the micelles;

- at pH 7.0, the granular micelle structure is clearly visible. At the lower pH, the micelles have a woolly, fuzzy appearance, apparently due to a reaction of the outside protein with the micelle surface;

- the separate protein particles at pH 7.0 are in the form of threads with a limited length and sometimes a few cross-links (Fig. 32).

Apparently, a minor change in pH has a severe effect on the reactions taking place at the micellar surface. This may be related to the properties of the  $\beta$ -lactoglobulin. It has been shown (deWit, 1981) that both the heat stability in milk and the thermal behavior of  $\beta$ -lactoglobulin change quickly in a very narrow pH range between pH 6.7–7.0.

To get a better insight into the nature of the free protein particles observed at pH 7.0, micrographs of heated solutions of  $\kappa$ -casein,  $\beta$ -lactoglobulin and of a 1:1 mixture of both proteins have been prepared. Heated  $\kappa$ -casein, often exhibits spherical particles (Fig. 33). Heated  $\beta$ -lactoglobulin shows long, irregular thread-like particles, which appear to be connected into a network (Fig. 34). The tested mixture of both proteins exhibits noodle-like truncated particles (Fig. 35), similar to those observed for the interaction between  $\beta$ -lactoglobulin and



**Figure 30.** Heat-induced association of  $\beta$ -lactoglobulin and casein micelles at pH 6.5. Strong interaction. Negative staining.

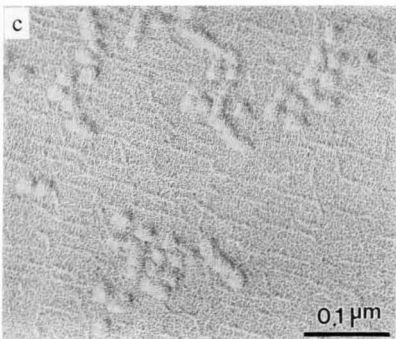
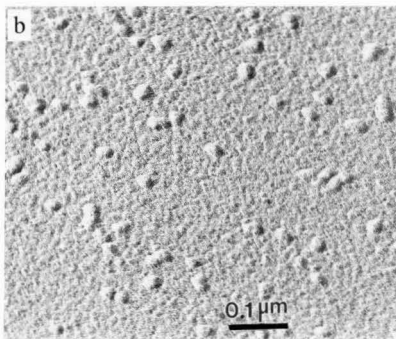
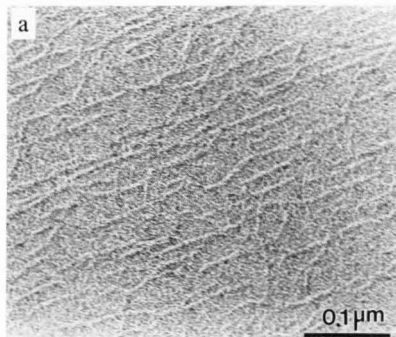
**Figure 31.** Heat-induced association of  $\beta$ -lactoglobulin and casein micelles at pH 7.0. No interaction. Negative staining.

**Figure 32.** Free protein particles from the heat-induced association of  $\beta$ -lactoglobulin and casein micelles at pH 7.0. Negative staining.

**Figure 33.** Heated  $\kappa$ -casein, showing mostly spherical and some extended particles. Negative staining.

**Figure 34.** Heated  $\beta$ -lactoglobulin, showing thread-like particles connected into a network. Negative staining.

**Figure 35.** Heated mixture of  $\kappa$ -casein and  $\beta$ -lactoglobulin, showing noodle-like particles. Negative staining.



casein micelles at pH 7.0 (Fig. 31). Apparently, the free protein particles represent the product of interaction between  $\beta$ -lactoglobulin and  $\kappa$ -casein. This has been further substantiated by ultracentrifugation and electrophoretic analysis (Smits and van Brouwershaven, 1980). These results indicate that  $\kappa$ -casein exerts an aggregation-limiting influence, similar to its role as a protective colloid in limiting the size of casein micelles.

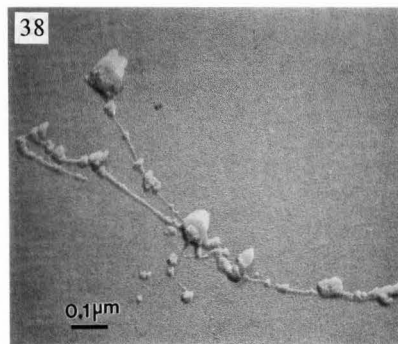
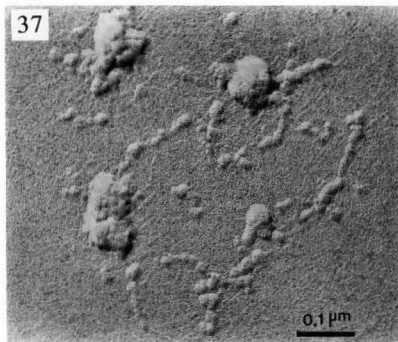
#### Protein/carbohydrate interaction

Protein/carbohydrate interaction can be illustrated by the reaction between  $\kappa$ -carrageenan and milk proteins. Carrageenans are widely used as thickening agents and stabilizers in the food industry, in particular, in neutral dairy products such as cocoa milk, puddings, creams, ice-creams and mousses. Carrageenans are particularly suited in neutral dairy applications due to their milk reactivity. The milk reactivity in the pH range 6-7 is ascribed to an electrostatic interaction between the negative sulphate groups in carrageenan and positive charges in the  $\kappa$ -casein (Snoeren, 1976). In this context, the influence of heat treatment on mixtures of  $\kappa$ -carrageenan and  $\kappa$ -casein as well as mixtures of  $\kappa$ -carrageenan and casein micelles was studied.

#### $\kappa$ -carrageenan/ $\kappa$ -casein

Micrographs of  $\kappa$ -carrageenan,  $\kappa$ -casein and a mixture of both components, heated at 60°C for 10 minutes are presented in Fig. 36. It shows the very fine carrageenan threads with a diameter of about 3 nm (Fig. 36a), spherical  $\kappa$ -casein particles with a diameter of about 15 nm (Fig. 36b) and the structure of the mixture (Fig. 36c). Some particles of  $\kappa$ -casein appear to be adhering to the carrageenan threads, sometimes accompanied by chain formation. Similar observations have been reported by Snoeren *et al.* (1976) and Snoeren (1976) who ascribe the reactivity of  $\kappa$ -carrageenan to an electrostatic interaction between the negative sulphate groups in  $\kappa$ -carrageenan and positively charged areas in the  $\kappa$ -casein.

**Figure 36.** (a)  $\kappa$ -carrageenan, (b)  $\kappa$ -casein, (c) a mixture of both components; heated to 60°C for 10 minutes. Replica.

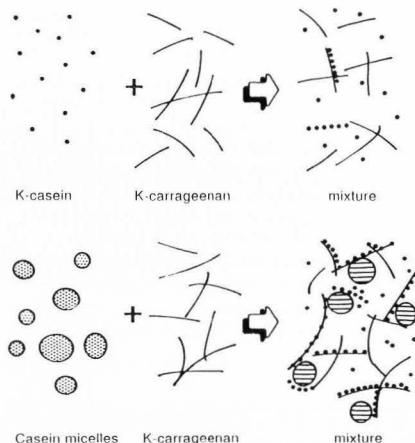


**Figure 37.** A mixture of  $\kappa$ -carrageenan and casein micelles heated to 80°C for 10 minutes. Note the partial break-up of casein micelles and adherence of their remnants to carrageenan threads accompanied by chain formation. Replica.

**Figure 38.** A mixture of  $\kappa$ -carrageenan and casein micelles heated to 90°C for 15 minutes. Note the adherence of casein particles of varying size to the carrageenan threads. Replica.

#### K-carrageenan/casein micelles

Mixtures of  $\kappa$ -carrageenan and casein micelles at pH 6.6 heated for 10 minutes to temperatures of 60, 80 and 90°C have been studied. The structure of isolated micelles has been amply described and discussed (this paper, Fig. 18). A typical micrograph showing the



**Figure 39.** Schematic drawing of the interactions between  $\kappa$ -carrageenan and  $\kappa$ -casein and casein micelles, respectively. Interaction between  $\kappa$ -carrageenan and  $\kappa$ -casein at 60°C. At low temperatures (20 and 60°C) no appreciable disintegration of casein micelles occurs whereas at high temperatures (90°C) almost complete disintegration occurs, accompanied by the appearance of loose casein particles and the aggregation of casein particles in threads.

interaction, obtained after heating a mixture for 10 minutes at 80°C, is presented in Fig. 37. It shows that under the influence of carrageenan, the micelles are partly disintegrated into smaller units that partly adhere to the carrageenan threads and form chains.

It further appears that the extent of disintegration of the casein micelles depends on the heating regimen. At low temperatures (20 and 60°C), no appreciable disintegration of casein micelles occurs and consequently, only a very limited degree of protein particle aggregation into threads is observed. At high temperature (90°C), almost complete disintegration of casein micelles occurs, accompanied by the appearance of loose casein particles as well as the aggregation of casein particles into threads. An example showing the irregular build-up of different size casein particles on the carrageenan strands is presented in Fig. 38. A schematic drawing of these observations is shown in Fig. 39.

The mechanism of interaction between carrageenan and proteins (milk and plant proteins) has been the subject of a number of investigations (Hansen, 1968; Lin and Hansen, 1970; Chakraborty and Randolph, 1972; Hansen, 1982; Dea *et al.*, 1991).  $\alpha_s$ - and  $\beta$ -casein react with carrageenan only in the presence of Ca-ions, whereas the reaction with  $\kappa$ -casein does not require Ca and is

shown to be electrostatic in nature (Snoeren *et al.*, 1976; Snoeren, 1976). In this view, the stabilization of  $\alpha_s$ - and  $\beta$ -casein and also of plant proteins against precipitation by Ca-ions is achieved by entrapping small calcium aggregated-protein bodies in the carrageenan structure before such bodies can further agglomerate into large, colloidal unstable particles. The protein aggregates are considered to interact to a greater extent in some areas of the polysaccharide strand and not in other areas, but in no case is the protein distribution uniform and continuous over the entire carrageenan structure. This behavior is ascribed to the presence of a double helix structure in the carrageenan, which is considered to be a region of low protein reactivity. The double helix junction zones may provide effective separation of the protein particles from each other, thus imparting physical stability to the system. This alternating structure of protein-free zones (where carrageenan strands are seen) and of protein-rich zones (Hansen, 1982) is presented in Fig. 37, and in particular, in Fig. 38.

Stronger gels are obtained by heating carrageenan with skim milk or casein micelles than by heating carrageenan alone. It is tempting to assume that this phenomenon is related to the present microstructural observations. Under the influence of heat, calcium-sensitive proteins such as  $\alpha_s$ -casein in casein micelles become exposed (P. Smits, unpublished results). Subsequently, a reaction occurs between these calcium-sensitive proteins and  $\kappa$ -carrageenan accompanied by partial disruption of the casein micelles. Protein bodies, formed by aggregation under the influence of calcium become part of the carrageenan network. The stronger gel is the result of the more homogeneous distribution of proteinaceous material along the polysaccharide chains and the interaction between the carrageenan network and the protein bodies. This effect on rheological properties is comparable to the influence of particle/matrix interactions on rheological properties discussed before.

#### Protein/lipid surfactant interaction

Interactions of surfactants with proteins are of importance in a wide variety of systems, such as biomembranes and pharmaceutical preparations. Also, in the foods area, this type of interaction plays an important role (Larsson, 1986). Reactions between polar lipids (monoacylglycerols and phospholipids) and proteins are important in the stabilization of emulsions such as margarine and ice-cream (Barford, 1991).

Also, in bakery doughs, the interaction with the gluten proteins is the major function of lipidic surfactant molecules (Krog, 1977).

The interaction between milk proteins and naturally occurring fatty acids is the basis of a new product called Lipro (Lipophilized protein) (Haque, 1992). Lipro is claimed to be a fat-like perception enhancer and functions by decreasing particle/particle interactions thus causing a slippery or oily feeling.

#### Fat crystal/fat crystal interaction

Fat spreads derive their consistency from

interactions between fat crystals which may form a three dimensional network (Figs. 40a,b). The nature of the interactions between the fat crystals determines the network structure and the rheology of the final product. Two types of bonds are assumed for crystal-crystal interactions (Haighton, 1963):

- primary bonds, which result from crystals growing together at some points. These bonds are regarded as "irreversible", i.e., they do not reform after rupture;
- secondary bonds, which are weak London-van der Waals forces, are "reversible", i.e., they do reform after rupture.

According to others (Shama and Sherman, 1970), such a distinction between primary and secondary bonds is considered to be arbitrary and it was suggested that a true characterization should be based on the concept of a spectrum of bond strengths.

Many aspects are related to the amount of fat crystals and the nature of the interaction between fat crystals (Haighton, 1976; deMan *et al.*, 1990). Among these are:

- the hardness of a spread depends on the amount of fat crystals;
- blend composition influences the molecular arrangement and modification of fat crystals and consequently the strength of interactions between crystals; e.g., the presence of  $\beta$ -crystals prevents the formation of a continuous fat crystalline network of  $\beta'$ -crystals (Juriaanse and Heertje, unpublished results);
- slowly crystallizing blends continue to crystallize after packaging, which favors the formation of a strong network;
- high crystallization speeds give rise to many small crystals and soft and overworked products.

An example of the influence of processing on the microstructure and rheology of a shortening is presented in Figs. 41a,b and 42 (Heertje *et al.*, 1988). In product (a), fully crystallized in the processing line, strong (primary) bonds form the major part of the bond strength spectrum. In product (b), partially crystallized during storage at rest, weak (secondary) bonds dominate. The rheology (Fig. 42) is in agreement with the observed microstructure.

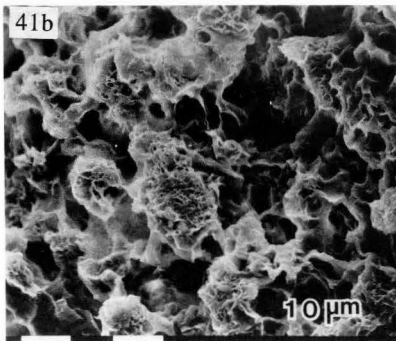
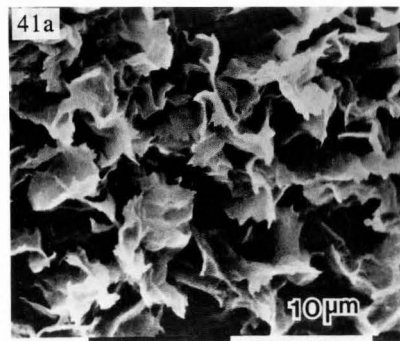
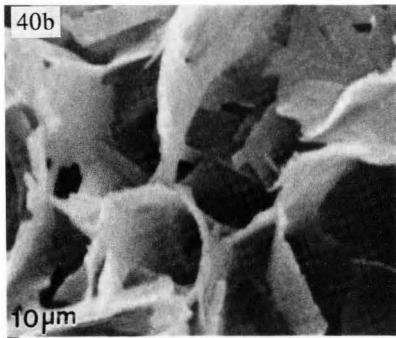
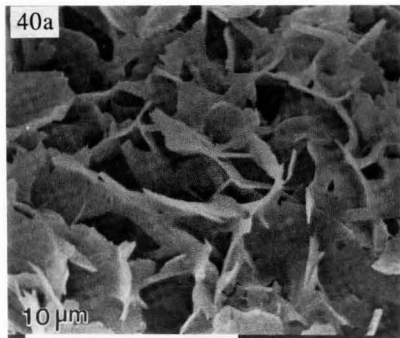
### Examples of Structure and Function

#### Processed cheese

Processed cheese is prepared by mixing natural cheese and melting salts (sodium citrate or a mixture of sodium phosphates) for a period of time, the so called creaming time, at a temperature of 90°C. The influence of creaming time on the microstructure and rheological properties of a pasteurized processed cheese has been investigated.

The torque exerted on the stirrer, which can be considered as a measure of the viscosity of the cheese mass, was recorded as a function of creaming time (Fig. 43). The structure as a function of creaming time was followed by thin sectioning EM.





**Figure 40.** Crystal/crystal interactions lead to the formation of a three-dimensional fat crystalline network: (a) at low and (b) at high magnification. Cryo SEM after deoiling.

**Figure 41.** Influence of processing on the microstructure of a shortening: (a) fully crystallized in processing line, and (b) partially crystallized after processing, during storage. Cryo SEM after deoiling.

**Figure 42.** Influence of processing on the rheology of a shortening: (a) fully crystallized in processing line, and (b) partially crystallized after processing, during storage. Stress strain curves obtained from parallel plate compression after elimination of friction.  $h_0$  and  $h$ : height of the sample before and after compression respectively. Products shown in Fig. 41 ( $T = 20^\circ\text{C}$ ).

**Figure 43.** Torque versus time curve obtained with a torque-measuring device in a processed cheese mass. At the indicated positions, samples were drawn for microstructural observations.

**Figure 44.** Processed cheese after 7 minutes creaming time (sample 1 in Fig. 43). Thin-sectioning TEM.

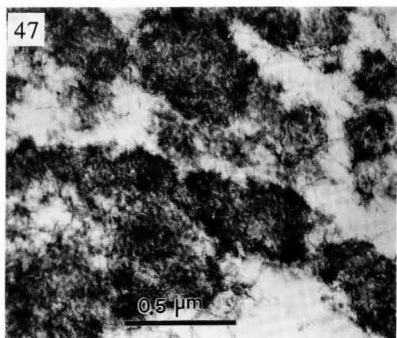
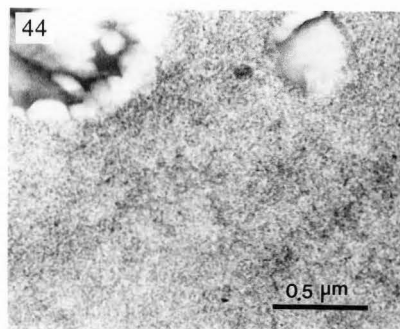
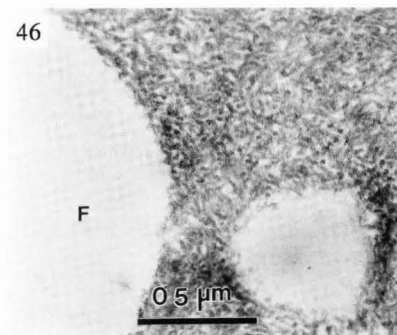
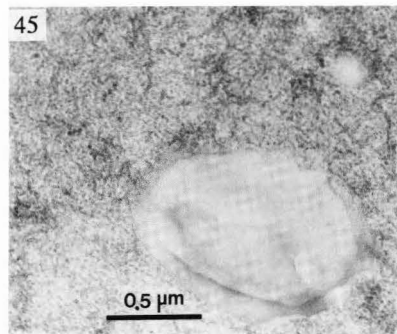
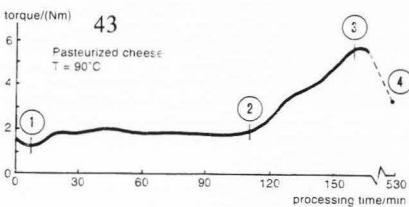
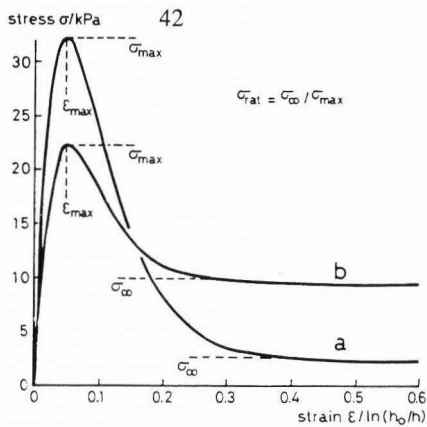
**Figure 45.** Processed cheese after 110 minutes creaming time (sample 2 in Fig. 43). Note beginning strand formation of the protein phase. Thin-sectioning TEM.

**Figure 46.** Processed cheese after 160 minutes creaming time (sample 3 in Fig. 43). Note: strong structuring of the protein phase with parallel alignment of strands. Large fat fields (F) are observed. Thin-sectioning EM.

**Figure 47.** Processed cheese after 530 minutes creaming time (sample 4 in Fig. 43). Note the formation of a coagulated, strongly aggregated, protein phase. Thin-sectioning TEM.

Table 1. Breaking stress (in kPa) of protein gels.

Gel	Particle	Fine stranded
12% $\beta$ -Lactoglobulin	15	4
14% $\beta$ -Lactoglobulin	23	26
20% Ovalbumin	20	600



After 7 minutes creaming time (sample 1), structuring of the protein phase is scarcely observable (Fig. 44). The well-dispersed oil droplets are about 1  $\mu$ m. After 110 minutes (sample 2), a limited structuring in the form of protein strands is observed (Fig. 45). The fat phase is not yet affected. After 160 minutes (sample 3, the maximum of the torque curve), a strong structuring of the protein phase with distinct and aligned protein strands is observed, indicating a strong protein-protein interaction (Fig. 46). Large fat fields are observed, indicating an expulsion of the fat phase from the protein matrix. A discontinuous protein phase can be discerned (Fig. 47) in the strongly overcreamed case (sample 4), after 530 minutes. The protein shows an aggregated structure, a type of internal coagulation, and fat and protein are present as separate phases.

These observations are in good agreement with macroscopic behavior. Sample 1 (Fig. 44) shows typical liquid-like behavior, samples 2 (Fig. 45) and in particular 3 (Fig. 46) have a gel-like character. Moreover, the latter sample is brittle. Sample 4 (Fig. 47) is softer and even more brittle than sample 3.

As is generally assumed, protein gelation involves the thermal denaturation of protein molecules followed by aggregation into a network (Hermansson, 1979). The optimal conditions for gel formation are a delicate balance between chain-chain and chain-solvent interactions. When the chain-chain interaction is too strong (sample 4), the phases may separate and undesirable product properties may develop; when this interaction is too weak, no gel will be obtained at all (sample 1).

#### Protein gels

Understanding the gelation behavior of proteins is of paramount importance in order to manipulate the properties of many food systems in the context of product or process improvement. Many studies on protein gelation have recently been published (Hermansson, 1988; Clark, 1987). Two distinctly different gel types can be distinguished: (i) particle gels composed of more or less spherical protein precipitates which form an irregular fractal structure, and (ii) fine stranded gels composed of extended polymer molecules which form entanglements and junction zones. The occurrence of both gel types critically depends on external conditions, such as pH and ion concentration.

Figs. 48 and 49 show the microstructure of 20% ovalbumin gels at pH 5 and pH 10 respectively (Heertje and van Kleef, 1986). A homogeneous distribution of fine protein strands is observed at pH 10, whereas an inhomogeneous distribution of strongly aggregated protein particles is found at pH 5 (see also Fig. 23). Consequently, the gel at pH 5 has a much more open structure than the gel at pH 10. Similar observations have been reported by others (Clark *et al.*, 1981; Stading and Hermansson, 1990, 1991).

At the same time, the rheological properties of the two gel types show great differences. The fine stranded gels appear to be much more extensible than the aggregated particle gels (Heertje and van Kleef, 1986; Stading

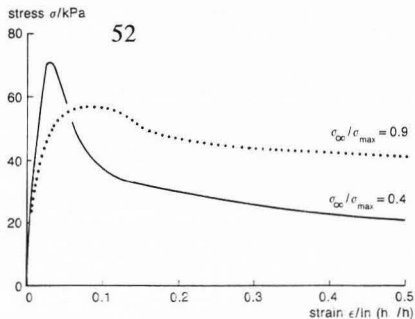
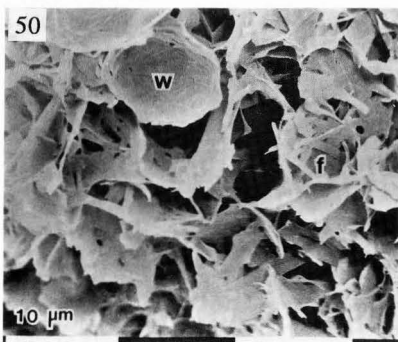
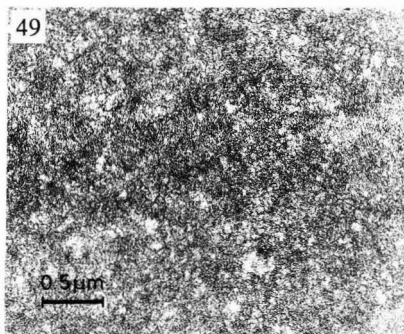
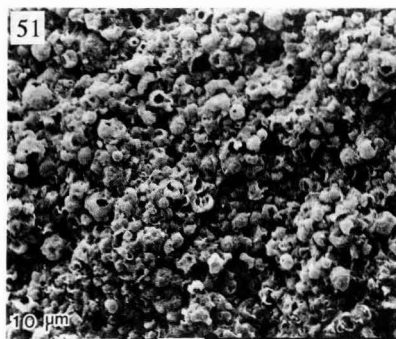
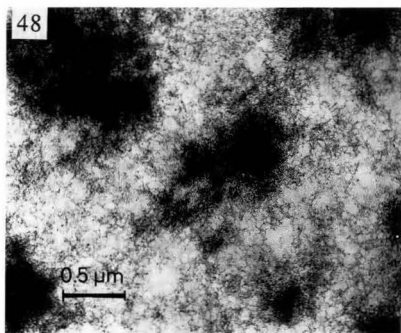
and Hermansson, 1991). However, the breaking stress appears to depend on the protein concentration in a complicated manner. For 12%  $\beta$ -lactoglobulin gels (Stading and Hermansson, 1991), the fine stranded gels at pH 7.5 show a lower breaking stress (4 kPa) than that of the particle gel close to the isoelectric point at pH 6.0 (approximately 15 kPa). The opposite behavior is observed when the protein concentration is increased to 14% (Table 1, Langley *et al.*, 1986). In this case, the particle gel shows a lower breaking stress (23 kPa) than the fine stranded gel (26 kPa). This is ascribed to the large difference in concentrations required for proper gel formation (Stading and Hermansson, 1991). Formation of the open wide-pore particle gel occurs already at a concentration of 1% protein, whereas formation of the fine stranded gel requires a protein concentration of about 10%. Below this concentration, a viscous system is obtained. Consequently, when a fine stranded gel is made close to its critical gelation concentration, it is soft and has a low breaking stress. However, when the protein concentration is well above the critical concentration for gelation, the homogeneous fine stranded gels will be stronger than the inhomogeneous particle gels, because regions of low protein concentration in the inhomogeneous gels will act as weak points, resulting in a low breaking stress.

The same phenomenon is observed with the 20% ovalbumin gels far above the critical gel concentration for both gel types. Under those circumstances, the breaking stress for the fine stranded homogeneous gel at pH 10 (600 kPa) is considerably higher than that of the particle gel at pH 5 (20 kPa). From these data, it is apparent that the increase in protein concentration does not strongly affect the breaking stress of the particle gel, but has a very great influence on the ultimate property of the fine stranded gel. This structural information is highly relevant in understanding such functional properties like water-binding and melting behavior of protein gels.

#### Margarine and butter

Products like margarine and butter contain, apart from oil and fat, about 20% water which is present as finely dispersed droplets which are several micrometers in diameter. Fat spreads containing 80% fat derive their consistency mainly from the continuous fat phase rather than from the dispersed water phase. In a margarine, the continuous fat phase appears to be an interconnected network structure (see the section **Fat crystal/fat crystal interaction**) composed of single crystals and sheet-like crystal aggregates (Fig. 50, Juriaanse and Heertje, 1988).

Butter shows a completely different microstructure: it has a discontinuous structure of fat globules (Fig. 51). This example represents an extreme case, in which many milk fat globules of the original cream persisted during the churning process. In other cases, depending on the ripening procedure of the cream and on working conditions (Precht and Peters, 1981a,b) fewer globules and larger amounts of interglobular fat phase were observed (Heertje *et al.*, 1987; Juriaanse and



**Figure 48.** A 20% heat-set ovalbumin gel at pH 5. Note: an aggregated inhomogeneous protein structure. Thin-sectioning TEM.

**Figure 49.** A 20% heat-set ovalbumin gel at pH 10. Note: a homogeneous structure of fine protein strands. Thin-sectioning TEM.

**Figure 50.** Interconnected fat crystalline network structure (f) and water droplet structure (W) showing a crystalline shell in margarine.

**Figure 51.** Discontinuous structure of fat globules in butter.

**Figure 52.** Stress strain curves for butter and margarine, obtained from parallel plate compression.  $h_0$  and  $h$ : height of sample before and after compression, respectively. The product softening, which is determined by the ratio  $\sigma_{oc}/\sigma_{max}$ , is much more pronounced for margarine than for butter.

Heertje, 1988) (see also the section **Oil droplets under structural elements**).

Summarizing this information on the microstructure of butter and margarine, it appears that margarine is composed of a continuous network of fat crystals or fat crystal aggregates, whereas butter has a much more discontinuous structure, containing fat globules with no interaction or a limited interaction with the rest of the matrix. This is reflected in functional properties, such as hardness, spreadability, mouth-feel, emulsion stability and salt release of both products. By parallel plate compression (Fig. 52), some of these properties can be determined. The maximum stress ( $\sigma_{max}$ ) is a measure of the product hardness. The product softening or plasticity, which is determined by the ratio of the stress at infinity ( $\sigma_{\infty}$ ) and the maximum stress, appears to be much higher for margarine than for butter. It shows that, on deformation, many more bonds are broken in the connected margarine structure than in the discontinuous butter structure, which is in accordance with the observed microstructure.

### Conclusions

Water, air, lipids, proteins and polysaccharides are the main components of all food products. They are often present in a specific aggregation or dispersion state:

- water as dispersed water droplets;
- oil as dispersed oil droplets;
- fat as crystals, crystal aggregates, globules and networks;

- polysaccharides and proteins as particles, strands, strands of particles and networks.

Functional properties are obtained by specific interactions between the various structural elements. Those properties are strongly influenced by interactions such as between:

- the dispersed phase and the continuous phase, e.g., in oil- in-protein gels;
- fat crystals by forming continuous networks (e.g., margarine) or weakly-interacting globular aggregates (e.g., butter);
- protein strands by forming fine stranded gels or aggregated particle gels.

On the basis of this type of information, numerous developments have taken place in the past decade in the design of new food products and materials. In particular, biopolymers have been used in low-calorie applications as fat substitutes. These developments will continue and require a sound knowledge of the relation between structure and function and how structure can be manipulated in order to achieve proper functionality. Also, new ways of structuring may be envisaged in our continuing efforts to manufacture high-quality, healthy and tasty food products.

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#### Discussion with Reviewers

D.F. Lewis: Why are the fat crystals at the interface of the water droplets in Fig. 3 not seen as white outlines in the osmium fixed preparation of Fig. 2?

**Author:** The observation of fat crystals depends on the degree of unsaturation of the triacylglycerols (TAG) in the fat crystals. Only fat crystals composed of more or less fully saturated TAG will show up as white outlines, because those crystals will not be stained by the osmium tetroxide. Only a limited amount of those crystals are observed in Fig. 2. Apart from the oil, also a large number of fat crystals with a more unsaturated composition will be stained by the osmium tetroxide. No proper distinction between those crystals and the oil phase can be made. That type of crystals is mainly present at the interface. However, also some indications for white outlines on the interface can be observed.

D.F. Lewis: Why are protein foams destroyed by the presence of low levels of fat in some cases whilst in other cases fat stabilizes foams? How do cake batters behave in this respect?

**Author:** Various physical aspects of the fat phase are responsible for foam stabilization. In addition, the interaction with proteins at the air/water interface is important in many foamed systems. Fat may occur as fat globules or as fat crystals. In the former case, the solid/liquid ratio of the fat phase determines proper foam stabilization. In the latter case, the size of the crystals and the crystal modification play a decisive role. Large crystals in the  $\beta$ -modification are often detrimental for foam stabilization.

In batters for high calorie cakes (pound cakes), air bubbles are initially stabilized by fat. In a later stage, during baking, air bubbles are transferred from the fat to the aqueous phase. Lowering the amount of fat in those formulations, below a critical concentration, is often detrimental for final cake volume because the initial stabilization of air bubbles by fat is no longer possible. A detailed review on the stabilization of air in foods containing fat has recently been published (Brooker, 1993).