Microstructural Studies in Fat Research

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Microstructural studies play an important role in establishing the relation between composition, processing and final properties of many food products. In order to arrive at a full description of microstructure, many visualization- and preparation techniques are needed. A number of fatty products such as shortenings, margarine, butter, and low fat spreads are discussed from a microstructural point of view. Examples of the influence of process parameters on microstructure and rheological properties are given. In particular, attention is paid to the fat crystalline matrix and the emulsion structure.

Further, a new methodology is described making it possible to study interactions of emulsifiers at interfaces between oil and water. In this context, the displacement, at a planar interface, of sodium caseinate by low-molecular mass emulsifiers such as monoacylglycerols and phospholipids has been studied. It appears that saturated monoacylglycerols are more active in displacing the protein than unsaturated monoacylglycerols. With phospholipids, complicated phenomena such as spontaneous emulsification, occur at the oil/water interface. Phospholipids, in general, appear to be much more surface-active than monoacylglycerols.

This type of work generates ideas to control and manipulate the microstructure and product properties of fatty products.

Key Words: Microstructure, fats, shortening, margarine, low fat spreads, rheology, emulsion structure, competitive adsorption, interface, monoacylglycerols, phospholipids.
Figure 1. Relation between composition, processing, structure and function of fat spreads.

Figure 2. Fat crystal network structure in a shortening (from Juriaanse and Heertje, 1988).

Figure 3. Microstructure of shortenings with the same composition: a. Complete crystallization in processing line; b. Partial crystallization in rest (from Heertje et al., 1988).

Figure 4. Stress strain curves obtained from parallel plate compression. \( h_0 \) and \( h \): height of sample before and after compression, respectively. Products shown in Fig. 3 (\( T = 20^\circ C \)). a. Complete crystallization in processing line. b. Partial crystallization in rest.
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Conventional LM, a disturbance-free observation of specimens, which is very important in the study of shear-sensitive samples such as fat spreads in general and low-fat spreads in particular. The confocal technique has also been used in the study of the competitive adsorption of emulsifiers.

Specific EM preparation techniques and their characteristics have been amply discussed (Brooker, 1990; Buchheim, 1982; deMan 1982; Heertje et al., 1987a; Kalab, 1983; Sargent, 1988). In the investigations described in the present paper, most of those EM techniques have been applied. They comprise:

- Transmission electron microscopy (TEM) of thin sections;
- Transmission electron microscopy of freeze-fractured and freeze-etched material (FFEM);
- Cryo-Scanning electron microscopy (Cryo-SEM) of freeze-fractured, freeze-etched, and de-oiled material.

The latter option has been developed in order to arrive at a proper observation of the product structure, in particular, the fat crystalline network, of fat spreads (Heertje et al., 1987a). Otherwise, the liquid oil in fat spread compositions would completely obscure proper observation of the solid fat crystalline matrix.

Shortenings

Shortenings have the simplest product structure of all fat spreads, because they are only composed of liquid oil and solid fat crystals. Depending on the application, the solid/liquid ratio can be varied. Appropriate preparation techniques, extracting the oil from the product, revealed that the fat crystals form a three-dimensional crystalline network (Heertje et al., 1987a). An example of such a network structure is shown in Fig. 2, which indicates the presence of sintered crystals while sheet-like aggregates and crystal bridges are formed. Depending on processing, also other types of aggregation can be discerned. When a fat blend is completely crystallized in the processing equipment (Heertje et al., 1988), a homogeneous structure of small connected plate-like aggregates is formed (Fig. 3a), whereas partial crystallization in rest leads to a non-homogeneous structure of large clusters interconnected by crystal bridges (Fig. 3b). Both products have also been rheologically characterized by parallel plate compression. From the resulting stress-strain curves, three characteristic parameters can be deduced: the maximum stress $\sigma_{\text{max}}$, the deformation at maximum stress $\varepsilon_{\text{max}}$, and the ratio $(\sigma_{\infty}/\sigma_{\text{max}})$, where $\sigma_{\infty}$ is the stress at large deformation. The ratio $\sigma_{\infty}/\sigma_{\text{max}} = \sigma_{\text{rat}}$ is a measure of the work softening: a small ratio indicates a large work softening.

The stress-strain curves (Fig. 4) are in good agreement with the observed microstructure, considering that on deformation, more bonds will be broken in the product with the homogeneous microstructure (Fig. 2a, and curve a in Fig. 4) than in the other product (Fig. 2b, and curve b in Fig. 4), where only crystal bridges between the clusters will be broken.

The product with the homogeneous microstructure has the highest value of $\sigma_{\text{max}}$ (greatest hardness) and shows the greatest work softening $(\sigma_{\text{rat}} = 0.08)$ whereas the other product has a $\sigma_{\text{rat}}$ of 0.41.

Further, how parallel plate compression affects the microstructure of the product which has been partially crystallized in rest, has also been investigated. Before deformation (Fig. 5a), the structure is composed of crystalline clusters interconnected by a fat crystalline network. After deformation (Fig. 5b), the structure between the clusters is more open. Crystal bridges have apparently been broken and the interconnecting network has, at least partly, been removed. This leads to a stress-strain behavior as given in Fig. 4b.
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Figure 6. Water droplets in a margarine. The freeze-fracturing technique gives rise to two different images of water droplets depending on whether the sample breaks over the surface of the droplet (S) or whether cross-fracturing occurs "through" the droplet (C) (from Juriaanse and Heertje, 1988).

Figure 7. Structure of a margarine obtained by thin-sectioning. W: water droplets, F: fat continuous matrix. In the matrix, linear light structures can be discerned (C), indicative of fat crystals.

Figures 8. Examples of fat crystalline network structure (F) and water droplet structure (W) in margarines. Crystalline shell structure around water droplets is clearly discernable.

Figures 9. Examples of butter structure: a. At a low magnification, many globules are noted; b. At a high magnification, the hollow sphere structure is noticeable.

Eighty Percent Fat Spreads

Margarine and butter

Products like margarine and butter contain, apart from oil and fat, about 20% water. This water is present as finely dispersed droplets which have a size of a few micrometers and are covered by a shell of fat crystals as can be shown by freeze fracture electron microscopy (FFEM) (Fig. 6). A similar coverage of the dispersed water phase by fat crystals is found in butter (Precht and Buchheim, 1980; Buchheim and Dejmek, 1990). So, by FFEM, it is possible to get a good impression about the water distribution in a fat spread. However, as can be observed in Fig. 6, this is not the case with the fat phase. Similar arguments apply to other image-forming techniques as well as classical EM preparation techniques. For example, Fig. 7 shows an image of a margarine obtained by thin-sectioning.

As 80% fat spreads derive their consistency from the continuous fat phase rather than from the dispersed water phase, insight into the three-dimensional fat structure is very important for understanding macroscopic behavior. This has been achieved by the aforementioned technique of de-oiling the sample. Typical margarine structures are shown in Figs. 8a, b. Shells of water droplets showing the crystalline nature of the interface, are clearly discernable, and so is the continuous fat matrix which appears to be an interconnected network structure composed of single crystals and sheet-like crystal aggregates.

Butter shows a completely different microstructure; it has a discontinuous structure of fat globules (Fig. 9). This represents an extreme case, in which many milkfat globules of the original cream have survived the churning process. In other cases, depending on the ripening procedure of the cream and on working conditions (Precht and Peters, 1981a, 1981b; Buchheim and Demjek, 1990), fewer globules and more interglobular fat phase are observed (Heertje, 1987a; Juriaanse and Heertje, 1988). The butter globules consist of an outer crystalline layer composed of high melting fat, enclosing liquid oil inside (Buchheim, 1970; Buchheim and Precht, 1979). This crystalline shell structure is, after de-oiling the sample, demonstrated by a frequent occurrence of hollow spheres (Fig. 9a). A more detailed view of this structure is given in Fig. 9b.

Summarizing this information on the microstructure of butter and margarine, it appears that margarine is composed of a continuous network structure of fat crystals or fat crystal aggregates. In contrast, butter is a much more discontinuous structure, containing fat globules which do not interact with the rest of the matrix or only to a limited extent. This is reflected in the rheological behavior. Both products have been analyzed by parallel plate compression (Fig. 10). The product softening, which again is determined by the ratio of the stress at infinity and the stress of the maximum, appears to be much higher for margarine than for butter. Apparently, on deformation, many more bonds are broken in the connected margarine structure than in the discontinuous butter structure.

Further aspects of 80% fat spreads

Graininess: Sometimes a product defect called sandiness (Madsen, 1971) or graininess (deMan, 1982) is observed in margarines and shortenings. This phenomenon appears to be caused by the formation of large, often spherulitic, crystal aggregates in the β-crystal modification. In particular, fat blends with a uniform composition in triacylglycerols, such as sunflower seed- and canola oils are prone to this effect. Different types of aggregates have been described (Meara et al., 1974; Berger et al., 1979).
The development of graininess as a function of composition has been followed by microstructural observations. Starting from a fat blend, mainly composed of an interesterified palm oil fraction, how the addition of other triacylglycerols influence graininess development was investigated. The starting material contains appreciable amounts of 1,2-dipalmitoyl 3-oleylglycerol (PPO) and 1,3-dipalmitoyl 2-oleylglycerol (POP). By crystallization of a 1:1 mixture, PPO and POP can form a molecular compound in the $\beta$-modification (Birker and Padley, 1987) which may give rise to graininess.
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Figure 13. Freeze-fracture EM of a margarine stabilized by saturated monoacylglycerol (a) showing frequent surface fracturing (S); and by unsaturated monoacylglycerol (b), showing frequent cross-fracturing (C).

Figure 14. Influence of shear in a stirred vessel (C-unit) on the microstructure of margarines. a. Without shear, a fine emulsion, no shells around the interface. b. With shear, a coarser emulsion, shells around the interface (from Heertje et al., 1988).

The various products have been analyzed by LM and SEM. In addition, the grains have been separated from the products by a detergent washing technique and observed by SEM. Triacylglycerols were added to the starting material at a 5% level. Structures of fat blends which contained additives which were ineffective and effective in the prevention of graininess are shown in Fig. 11 and Fig. 12, respectively. Highly crystalline grains showing spherulitic growth were observed in the starting material and in case of an ineffective additive (Fig. 11), whereas fewer, more amorphous, and smaller grains were observed in case of an effective additive (Fig. 12).

The large crystalline grains are mainly in the $\beta$-crystal modification with only up to a few % in the $\beta'$-modification, whereas the mostly amorphous grains contain about 25% $\beta'$-crystals.

It may be concluded that certain triacylglycerols show an inhibitory effect on grain formation. The grains are less crystalline and smaller as a result of the formation of many small $\beta'$-crystals, which disturb the unlimited growth of $\beta$-crystals from the POP/PPO compound.

Emulsion structure and fracture behavior: The freeze-fracture behavior has contributed to a better insight into the emulsion structure of fat spreads as a function of the nature of the emulsifier. It is generally assumed that by microscopical studies only morphological information can be obtained. However, when studying the fracture behavior, unique information about the forces active in the system can be obtained. Fracture will occur through the weakest points of a system. In an emulsion, fracture may occur through (cross fracture) or over (surface fracture) the dispersed emulsion droplets.
This is determined by the nature of the internal phase, the nature of the interface, and the possible interaction of the interface with the continuous phase. Similar studies have been reported earlier (Simonds, 1974; Buchheim and Precht, 1979; Buchheim, 1982). Simonds’ study is concerned with the fracture behavior of starch granules in hard and soft wheats. In hard wheat, with a strong interaction between the protein matrix and the starch granules, frequent cross fractures occur, whereas in soft wheat, without interaction between the protein matrix and the starch granules, fractures over the surface of the granules are observed frequently. In the work of Buchheim on oil in water emulsions, it is shown that peripheral fractures of oil droplets occur when crystalline shells are present, whereas cross-fractures are more frequent in oil droplets without crystalline shells (Buchheim, 1982). It indicates that the fracture behavior in the frozen solid state can provide proper information about forces acting at ambient conditions.

In our own work, effects of the nature of the emulsifier on the emulsion structure of margarines were investigated. A saturated (SMG) or an unsaturated monoacylglycerol (UMG) was added to a margarine in a concentration of 2%. In the product containing UMG, much more cross fracture, through the water droplets, occurs than in the product containing SMG, which shows much more surface fracture (Fig. 13a, b). This is ascribed to the presence of SMG crystals in the interface between water and oil under the formation of a concentric layered shell structure. Forces between the layers are weak and, consequently, fracture is likely to occur between these layers. In the case of UMG the stabilization is obtained by triacylglycerol crystals forming a network having a random orientation around the droplets. The droplets become the weakest points in the system and fracturing occurs through them. This information on the structure of the interface between water and oil is important for the consistency and oral perception (e.g., salt release) in fat products.

Influence of shear on water droplet structure: In a study on the influence of processing conditions in
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Figure 15. Influence of shear in a scraped-surface tube cooler (rotational speed of A-unit) on microstructure of margarines. a. Rotational speed 600 rpm, fine emulsion; b. Rotational speed 100 rpm, coarse emulsion. From Heertje et al. (1988).

Figure 16. A fat crystal piercing through the fat crystalline shell of a water droplet.

Figure 17. Bridge formation between droplets (D) in an oil-in-water emulsion. Arrow indicates bridge.

Figure 18. Microstructure of a cream slowly cooled down to room temperature. Before evaporation of water. a: low magnification; b: high magnification. Arrows indicate position of water. F: globular fat phase.

Figure 19. Microstructure of a cream slowly cooled down to room temperature. After evaporation of water. a: low magnification; b: high magnification. Arrows indicate the original position of the water. F: globular fat phase.

margarine manufacturing on microstructure, how shear affected the water droplet size distribution was investigated (Heertje et al., 1988). Shear applied during processing influences the size distribution in a complicated manner. On strong working in a stirred vessel (C-unit), after partial crystallization of the fat blend in a scraped surface cooler (A-unit), water droplets become larger and shell formation around water droplets is more pronounced (Fig. 14). The stronger shells around the water globules on working may be associated with the enhanced possibility of transport of fat crystals to the oil-water interface. Several authors (Lucassen-Reynders, 1962; Lucassen-Reynders and van den Tempel, 1963; Overbeek, 1952) noticed that stirring strongly accelerates the rate of adsorption of crystals onto the emulsion droplets. A similar effect is induced by the presence of surfactants (Lucassen-Reynders and van den Tempel, 1963). When no stirring or working is applied, or in the absence of an emulsifier, free diffusion of crystals is hindered due to the rapid formation of a solid crystalline network. Consequently, the main part of the crystals
sticks together to form a network and is not available for
the so called Pickering stabilization of the emulsion
droplets. When the emulsion is worked in a C-unit,
however, crystals adhere to the emulsion droplets and
form a crystalline shell around the water droplets during
the crystallization stage. In addition, smaller crystals,
induced by working, can better adjust and consequently
better adhere to the droplet surface.
The coarsening of the emulsion droplets after C­
unit processing may also be attributed to the influence of
working. As a result of the crystallization process in the
C-unit, viscosity increases, and this increase may lead to
deformation of the droplets and their subsequent coales-
cence.
On the other hand, when the fat blend is still
largely in its liquid state in the A-unit, a much finer
emulsion is observed under the conditions of high shear
(Fig. 15a) than under the conditions of low shear (Fig.
15b). This difference in behavior should be ascribed to
the more liquid-like character of the emulsion, which is
still in a supercooled α-crystalline state in the A-unit
(Haighton, 1976). In this case, higher shear leads to a
stronger emulsification. Coalescence of droplets does
not take place. This in contrast with the situation,
described above, in which the high viscosity of the con-
tinuous phase after C-unit processing induces coales-
cence.
Crystalline growth in the water phase: In the
course of our studies on the morphology of margarines
a peculiar phenomenon has been observed: fat crystals in
the water phase. A typical example is shown in Fig. 16,
where a fat crystal pierces, through the interfacial crys-
talline shell, into the dispersed water phase. Although
this may sound quite remarkable, similar observations
have been reported in O/W emulsions (Boode, 1991). A
study on the coalescence of oil droplets in an oil in
water emulsion showed that large crystals can grow out
of the emulsion droplets and lead to bridge formation be-
tween droplets. An example of this type of coalescence
is presented in Fig. 17. When such phenomena occur,
a little shear on a "stable" emulsion can very easily
induce coalescence. This type of cream emulsion desta-
bilization may play an important role in churning and
other types of inversion processing. However, it is not
yet clear how crystal growth in the dispersed aqueous
phase (Fig. 16) can usefully be applied.
Phase inversion during processing: Different
routes are available in the processing of fat spreads. In
preparing W/O systems, one either starts from a W/O
emulsion as in conventional margarine processing
(Haighton, 1976), or from an O/W emulsion, the most
noticeable example being the churning of butter. In the
latter case, an O/W cream is transformed into a system
having a dispersed water phase by inversion of the emul-
sion system. Many detailed studies have been published
regarding the factors affecting the churning process
(Walstra and Jenness, 1984). However, phase inversion
or partial phase inversion may be applied also in the
processing of other products. We investigated the in-
fluence of the cooling regimen on phase inversion of an
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Figure 20. Microstructure of three products made from a cream, observed after de-oiling the samples. a. Product slowly cooled down to room temperature. b. Product rapidly cooled down to 10°C. c. Product rapidly cooled down to 15°C.

Figure 21. Microstructure of a product made from a cream and rapidly cooled down to 15°C. After evaporation of water. a. Low magnification; b. high magnification. Arrows indicate position of enclosed water areas.

Figure 22. Structure of a 40% fat spread, without proteins or carbohydrates in the aqueous phase observed by: a. CSLM; b. FFEM; and c. SEM after de-oiling the sample. The fat is stained by Nile Blue.
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Figure 23. Structure of a 40% fat spread, containing 5% gelatin and 2% milk proteins: a. observed by CSLM, the protein was stained by FITC, arrow indicates milk protein, asterisk indicates gelatin particles; b. Cryo-SEM after some etching; and c. SEM after de-oiling the sample. Note: differences in staining intensity of the protein particles in the aqueous phase by observed by CSLM; particles in the aqueous phase shrunken by de-oiling.

Figure 24. Structure of a 40% fat spread, containing 16% sodium caseinate and 5% buttermilk powder in the aqueous phase. a. At a high magnification, observed by cryo-SEM. b. At a low magnification, observed after de-oiling the sample. Note the irregularly shaped protein phase (p).

Figure 25. Structure of a 25% fat spread, with 4% protein and 1% carbohydrates in the aqueous phase, observed by CSLM. a. the fat was stained by Nile Blue. b. The protein was stained by FITC.

Figure 26. Structure of a 25% fat spread, with 11% milk proteins and 7% carbohydrates in the aqueous phase, observed by CSLM. The fat was stained by Nile Blue. a. At a low magnification, showing the bicontinuous character of this spread. b. At a high magnification, showing large water areas (L) and small water droplets dispersed in the fat phase.

O/W cream consisting of 70% fat from a commercial margarine fat blend and 30% water as the continuous phase. The cream was prepared at 60°C and slowly cooled to room temperature or alternatively rapidly cooled with vigorous stirring to 10° or 15°C.

The three samples have been examined by SEM using three procedures:
(a) direct observation in the frozen state;
(b) observation after sublimation of the ice from the still frozen sample (at about -80°C); by comparing the morphologies before and after this freeze-etching process, the original location of the water was determined;
(c) observation of the solid crystalline phase after de-oiling the product using a suitable solvent (Heertje et al., 1987a).

The product that was slowly cooled down from 60°C to room temperature showed, by direct observation in the frozen state, a uniform structure of globular elements (Figs. 18a, b). A continuous water structure was observed between the globules (see arrows Fig. 18b). After evaporating the water, a similar uniform structure of the globules was seen (Figs. 19a, b). However, void spaces were observed between the globules indicating that water originally occupied this space (see arrows in Fig. 19b). Apparently, the structure was, for the greater part, a cream with water as the continuous phase, although inclusion of water between agglomerated fat globules was also observed. This agglomeration was further substantiated by a cellular structure of hollow spheres, observed after de-oiling the product (Fig. 20a).

The exclusive preservation of the outside contours of the
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24a

24b

25a

25b

26a

26b
fat globules suggests that the outer crystalline shells were composed of fat melting at a higher temperature than the interior of the globules. Similar observations have been reported for butter (see section on Margarine and butter above).

The sample that was rapidly cooled to 10°C showed, after de-oiling, a close network of small crystals without any indication of fat globules (Fig. 20b). The working and deep cooling had resulted in a complete phase inversion of the original O/W emulsion structure into a margarine-like structure.

The sample that was rapidly cooled to 15°C had some of the characteristics of both the above samples. This is clearly indicated after de-oiling (Fig. 20c), revealing globular structures as well as crystalline margarine-like regions. This inversion was also noticeable in other SEM observations. The original distribution of the aqueous phase was established from the void spaces which developed after its evaporation (Fig. 21a and b). These images give a good impression of the partial phase inversion from an O/W into a W/O system.

Apparently, subtle changes in the process variables during inversion processing influence the microstructure and the related product characteristics of fat spreads.

Low Fat Spreads

In addition to 80% fat spreads, spreads with a lower fat content have also become commercially available. Spreads with 60, 40, and even 20% have appeared on the market. With the decreasing fat content, the composition of the aqueous phase becomes gradually more important for obtaining a good fat spread. Various proteins (gelatin, casein, whey) and carbohydrates (carrageenan, starch, alginate, gums) are used in low-calorie fat spread formulations. In microstructural analysis of these products LM techniques play an important role.

Fat spreads containing 40% fat

Figs. 22a-c show the structure of a spread without proteins or carbohydrates present in the water phase. Confocal scanning laser microscopy (CSLM) shows the dispersive character of the water phase in the continuous fat phase (Fig. 22a). The fat phase has been revealed using the fluorescent Nile Blue dye (Heertje et al., 1987b). A similar observation was made using FFEM (Fig. 22b). In addition, crystalline fat shells similar to those present in margarines (see section on Margarine and butter above) were found. A cellular structure of water droplets as shown in Fig. 22c was obtained after de-oiling the product (Heertje et al., 1987a). As expected, no material was found in the aqueous phase.

Figs. 23a-c show the structure of a spread containing 5% gelatin and 2% milk protein in the water phase. The protein can be made visible by CSLM using a fluorescent dye (FITC) (Fig. 23a). In the dispersed water phase, small milk protein particles and large gelatin particles can be distinguished. The milk proteins are more intensely stained than gelatin. Further, the interface layer surrounding the droplets can be discerned by its fluorescence. This layer is most likely composed of milk proteins, because they are much more surface-active than gelatin (Dickinson et al., 1987). This sample was also observed by cryo-SEM after some freeze-etching of the frozen aqueous phase (Fig. 23b). The aqueous phase structured by the protein mixture can also be seen using this technique but a distinction between the proteins is not possible. A proper three-dimensional image of the structure is obtained after de-oiling the product (Fig. 23c). Although it is evident that some material is present in the aqueous phase, it should be realized that the particles in the water phase have shrunk under the influence of the organic solvent during de-oiling.

A product containing a very high concentration of sodium caseinate (16%) and buttermilk powder (5%) in the water phase showed a peculiar water phase structure (Figs. 24a, b). Apart from many small water droplets (Fig. 24a) having diameters between 0.5 and 13 µm, large water areas having sizes of up to 50 µm were observed (Fig. 24b). These areas are irregular in shape and are filled with the structured aqueous protein phase. Apparently, emulsification of the aqueous phase during processing was hampered, which may be caused by the high viscoelasticity of the sodium caseinate solution.

Fat spreads containing 25% fat

Fig. 25 was obtained by fluorescence CSLM. It shows the aqueous phase, containing 4% proteins and 1% carbohydrate, to be densely and uniformly distributed in the product. The protein, stained with FITC, was found to be located at the oil/water interface (Fig. 25b). The continuous fat phase, stained with Nile Blue, shows a fragmented structure (Fig. 25a).

In contrast, a product containing of 11% milk proteins and 7% carbohydrates in the aqueous phase (Figs. 26a, b) shows a bicontinuous character, in which both the fat phase and the aqueous phase are continuous. A coarse water structure is revealed at low magnification (Fig. 26a). At high magnification (Fig. 26b) many small water droplets dispersed in the fat phase can be distinguished. This type of microstructural information may help in understanding the properties of low- and very low-fat spreads, such as loose moisture, stability, and dispersibility in the mouth.
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Interactions of Emulsifiers at Interfaces

Emulsifiers play an important role in the processing, the shelf life, and the oral melt behavior of fat spreads. In order to control processes such as churning, coalescence of emulsion droplets, or the manufacture of fat spreads, detailed knowledge of the role of emulsifiers is required. Interactions of different emulsifiers at the interface are of particular interest, because in many food systems low-molecular-mass surfactants such as monoacylglycerols and lecithins are present in addition to high-molecular-mass surfactants such as proteins. Under the influence of experimental conditions complete displacement of proteins by low-molecular-mass surfactants may occur. Various methods are used to measure the displacement from interfaces. Heertje et al. (1990) observed a vertical planar interface between oil and water using CSLM. This type of microscopy allows a disturbance-free observation of the interface without interference by scattered radiation from off-focus levels of the specimen, encountered in conventional LM (Heertje et al., 1987b).

The displacement studies are performed by using fluorescent labeled proteins. The displacement can be quantified by measuring the change in the fluorescence signal of the interface upon addition of other emulsifiers. Using this methodology, the displacement of fluorescent labeled sodium caseinate by monoacylglycerols (MAG) and phospholipids has been measured. Results on the displacement of a 0.08% solution of sodium caseinate by monoacylglycerol (MOG) are presented in Fig. 27. Progressive desorption of the caseinate with increasing amounts of MAG in the oil phase is clearly noticeable. At a MOG concentration of 1%, complete desorption has taken place. For monostearoylglycerol (MSG) complete desorption of the caseinate has already occurred at 0.3% of the emulsifier in the oil phase (Fig. 28). On the other hand, at 0.2% of MSG most of the caseinate is still present at the interface. Apparently, in this concentration range, the desorption critically depends on the concentration of the low-molecular-mass emulsifier. Data for the two compounds are summarized in Fig. 29. It appears that the shape of the displacement curve for MSG differs considerably from that for the MOG. The fact that MSG is more effective at high concentrations in displacing the protein than MOG can be attributed to the packing of the hydrophobic chains at the interface. The straight MSG chains are more tightly packed than the bent chains of MOG. Therefore, MSG shows appreciable deviations from ideal behavior because of the more pronounced interactions between the tight-packed chains; the tight packing favors displacement of the protein at high concentration of MSG in comparison with MOG. At low concentrations, on the other hand, the order is reversed, because MOG has a higher standard free energy of adsorption.

Displacement studies have also been performed with two phospholipids, viz. di-myristoyl phosphatidylcholine (DMPC) and di-palmitoyl phosphatidylethanolamine (DPPE). In the latter case, both sodium caseinate and the phospholipid were labeled with a fluorescent markers. This allowed simultaneous observation of adsorbing and desorbing species at the interface.

Results on the displacement of sodium caseinate by a concentration of 0.005% DPPE in the oil phase are presented in Fig. 30. It appears that only the fluorescence signal of the DPPE is present at the interface. This means that sodium caseinate, at this low concentration of DPPE, has already been completely displaced from the interface.

With a DMPC concentration of 0.05% in the oil phase, typical lamellar phase structures appear in the water phase (Fig. 31). The phospholipids migrate from the dark, non-fluorescent oil phase and become visible as dark structures in the fluorescent water phase. Oil is present in some of those structures. DMPC does not dissolve well in the oil phase and the dispersion is opaque even at very low concentrations which indicates the presence of aggregated structures in that phase. The interface did not fluoresce, which implies that the protein had already been displaced at this concentration of the low-molecular-mass surfactant.

It may be concluded that sodium caseinate is displaced from the interface at very low concentrations of phospholipid. It should be realized that a part of DMPC is not active because it is present in an aggregated state. These results indicate that phospholipids are much more surface-active than monoacylglycerols.

The difference in behavior of PE and PC at the interface, can probably be ascribed to the difference in phase behavior. PE is more apt to form hexagonal II phase structures, whereas PC is a bilayer-forming lipid. This behavior is linked to the size of the polar head groups in relation to the size of the apolar acyl-chains (Cullis and Kruijff, 1979). Small polar head groups will preferentially lead to inverted structures enclosing water, with a hydrophobic outer surface (hexagonal II or inverted micellar structures), with a preference for the oil phase. On the other hand, bilayer-forming lipids such as PC, can form mono- or multi-lamellar vesicles, with a hydrophilic outer surface, with a preference for
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the aqueous phase. The occurrence of lamellar phase structures, in the case of PC, is indeed observed in the aqueous phase (Fig. 31). With PE, no structures in the water phase are observed.

Concluding Remarks

Microstructural investigations are an important tool in the study of food products and food components. Electron microscopical techniques give a proper insight into the structure of high-fat spreads. For low calorie products, where the fat function is, at least partly, replaced by proteins and carbohydrates, also light microscopical techniques appear to be of great value. In particular, fluorescence techniques in combination with confocal microscopy prove to be very instrumental in this area. In this sense, the development of low calorie foods has been stimulated by the developments in instrumental techniques, viz. the revival of light microscopical techniques.

It will be a challenge for the future to see whether other new developments, such as acoustic microscopy, X-ray microscopy, environmental SEM, immuno-techniques, nuclear magnetic resonance (NMR) imaging, Infrared (IR) microscopy, scanning tunneling microscopy, atomic force microscopy and photon scanning tunneling microscopy, can be used in our continuing effort to relate the structure of food products and food components to their function.

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References


Figure 30. Displacement of sodium caseinate by fluorescent labelled di-palmitoyl phosphatidylethanolamine (DPPE), showing complete displacement of sodium caseinate. Right: signal from the caseinate fluorescent marker (FITC). Left: signal from the DPPE fluorescent marker (Texas Red).

Figure 31. Displacement of sodium caseinate by dimyristoyl phosphatidylcholine (DMPC), showing complete displacement and lamellar phase structures in the aqueous phase. Note the oil (o) and water (w) inclusions and differences in the thickness of the multilayers.


I. Heertje


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