Microscopy in the Study of Fats and Emulsions

J. M. deMan
Abstract

Plastic fats consist of a three-dimensional network structure of crystals in which liquid oil is trapped. This crystal network is held together by weak attractive forces, the nature of which is not definitely established. Crystal size is dependent on temperature history and is subject to polymorphic transitions which greatly affect the microstructure of the system. The microstructure of fats has been investigated by using polarized light microscopy, electron microscopy and X-ray diffraction analysis. Recently, a permeametric method has been developed which enables the determination of the specific surface area of the crystals in a fat. This method is a useful complement of the microscopic techniques. Scanning electron microscopy has not been widely used in studying fat crystal structures. The use of microscopy in the study of microstructure of emulsions presents even greater problems than in the fat field. Emulsifiers may form liquid crystalline mesophases which may be studied by polarized light microscopy and X-ray diffraction analysis.

Introduction

It is becoming increasingly apparent that our understanding of what is now being described as the "functional properties" of many foods is dependent on a knowledge of their fine structure. This can be expressed in the following inter-relationship:

chemical composition → physical structure → physical properties

This indicates that microstructure is dependent on the chemical components of the food, and in turn, the nature of the microstructure determines the physical properties which include a number of functional properties. Fats and fat containing foods are no exceptions to this rule and much research has been devoted to the microstructure of fats. There are a number of factors which make these products unusual and the study of their microstructure difficult. Probably the most important of these is the influence of temperature. Not only is the microstructure of fats temperature dependent, it also depends on temperature history so that two samples of a fat examined at the same temperature may have different properties depending on their temperature history. The physical structure of fats may also change with time at constant temperature because of polymorphic transformations. These factors make it necessary for research on fats and fatty foods to be conducted in or with temperature controlled facilities. The study of emulsions also involves unusual problems since the membrane structures around emulsified droplets and their properties require specialized techniques for their observation and measurement. A good general source of information is the book "Food Microscopy" (Vaughan, 1979).
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Microstructure of fats

Fats differ from oils in that they contain solid triglycerides at room temperature. This mixture of solid and liquid components exhibits the property of plasticity. Triglycerides are long chain molecules with molecular weights in the neighbourhood of 900. The crystallization behaviour of such compounds responds to the normal conditions of nucleation and crystal growth (Figure 1), and these factors are determined by the degree of supercooling. If the extent of supercooling is low, only few nuclei will form and crystals will grow to a relatively large size. If heat is rapidly removed, i.e. high level of supercooling, many nuclei will form and many small crystals will be formed. The situation is complicated by the phenomenon of mixed crystal formation (also called solid solutions). Fats contain many different glycerides which closely resemble each other but have slightly different melting points. Rapid cooling (high supercooling) will result in inclusion of different types of triglycerides in the crystal lattice. The result is higher solid fat content at low temperature. Tempering of such rapidly cooled fats at temperatures below the melting point will lead to recrystallization and reduction in solid fat content. An example taken from the work of Mertens and deMan (1972) illustrates this phenomenon (Table 1).

Fats are subject to some additional phenomena when crystallization occurs. Long chain compounds including the triglycerides show polymorphism, i.e. they can occur in several crystal modifications. These different crystal forms are distinguishable by X-ray diffraction, by infra-red spectrophotometry, by melting point determination and by differential thermal analysis. It is generally agreed that fats occur in three major polymorphic forms, named alpha, beta-prime and beta in order of increasing stability (Lutton, 1972). The packing of the triglyceride molecules in the crystal lattice determines the spacings between adjoining molecules. The cross-sectional structures determine the short spacings which can be determined by X-ray diffraction. The arrangement of long chain compounds in a cross-sectional view of the crystal lattice has been given by Lutton (1950) and is represented in Figure 2. The alpha form is hexagonal and is the least organized of the three forms. It has a low density structure with a cross section of about 0.2 nm² and the chains are packed in an untitled or perpendicular fashion. This form is usually obtained on rapid cooling of the melt. The beta-prime structure is orthorhombic and is in a tilted chain

<table>
<thead>
<tr>
<th>Sample</th>
<th>Treatment</th>
<th>10°</th>
<th>20°</th>
<th>30°</th>
<th>40°</th>
</tr>
</thead>
<tbody>
<tr>
<td>Margarine</td>
<td>Tempered</td>
<td>37.6</td>
<td>24.2</td>
<td>11.7</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>Non tempered</td>
<td>47.3</td>
<td>29.8</td>
<td>11.9</td>
<td>0.3</td>
</tr>
<tr>
<td>Frying fat</td>
<td>Tempered</td>
<td>63.7</td>
<td>45.7</td>
<td>25.8</td>
<td>3.4</td>
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<tr>
<td></td>
<td>Non tempered</td>
<td>75.8</td>
<td>55.1</td>
<td>26.2</td>
<td>4.2</td>
</tr>
</tbody>
</table>

Table 1. Solid fat content of a margarine oil and a frying fat with and without tempering at 25° C for 30 min. Initial cooling at 0° C for 60 min.
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Row 1: Alpha, Beta, Beta Prime
Row 2: Alpha, Beta, Beta Prime
Row 3: Alpha, Beta, Beta Prime

Fig. 2. Cross-sectional structures of long chain compounds. (Source: E. S. Lutton, 1972. Reproduced with permission).

The arrangement. This is the more common form for many natural fats. It is a more closely packed structure than the alpha form. The beta form has all of the chain axes oriented in one way as shown in Figure 2, and is triclinic. Both beta prime and beta forms have an approximate cross-sectional area of about 0.185 nm². These three cross-sectional structures give rise to short spacings as determined by X-ray diffraction and indicated in Figure 3.

The development of the knowledge about polymorphism has not been without controversy. This mainly involved the idea of the existence of a glassy state as proposed by Malin (1954), but this is now generally accepted as erroneous. A form intermediate between beta prime and beta has been proposed by Hoerr (1960). A more unified and rigorous treatment of polymorphism has emerged in the recent literature (Larsson, 1964, 1966, 1972). Examples of the application of this knowledge to problems in fat crystallization are studies on the phase behavior of hydrogenated fats by Kliner (1971) and studies on cocoa butter and confectionery fats using programmed temperature X-ray diffraction and differential scanning calorimetry by Chapman et al. (1971).

X-ray diffraction patterns can be recorded with a Debye-Scherrer camera or

Fig. 3. Cross-sectional structures of long chain polymorphic modifications. (Source: E. S. Lutton, 1950. Reproduced with permission). d spacings expressed in Angstrom units (Å).

with a diffractometer, the equipment commonly used by crystallographers. The Debye-Scherrer patterns appear as concentric circles on the X-ray film. This type of equipment has the drawback of being usable only at room temperature which does not permit the study of polymorphic transitions as a function of temperature. Special cameras for this purpose have been developed such as the DFT camera used by Aleby (1969) and a particularly useful instrument is the Guinier-Simon camera with integral temperature programming capability (Figure 4). The different polymorphic forms have different melting points and different crystal habits. Another useful X-ray camera is the triple focussing Guinier camera.

Polymorphism and the structure of triglyceride crystals can also be studied by using the electron microscope for electron diffraction of single crystals. This procedure has been demonstrated by Buchheim (1970a) for the study of trilaurin crystals. Methods were developed for preparing suitable crystals of trilaurin, but this procedure may not be suitable for crystals of natural fats. Using this procedure, Buchheim (1970a) found evidence for two beta-prime modifications of trilaurin, one a vertical and the other a tilted form. Two beta modifications of trilaurin were described.

Fig. 4. Guinier-Simon X-ray diffraction camera with temperature programming capability. A = X-ray tube, B = camera, C = control console.

Fat crystals can be observed with the aid of polarized light microscopy. According to Hoerr (1960), crystals of the alpha form appear as platelets of about 5 μm size. Since the alpha form is unstable, it is hard to obtain photographs of these crystals. The beta prime crystals are described as small needles not exceeding one μm in length. Beta
crystals are large, ranging from 20 to 100 um in size and often growing in clumps. Such clumps of large crystals can lead to visually noticeable graininess in fats. The packing of triglyceride molecules in the crystal lattice is influenced by the variety of molecular sizes present in natural fats. The greater the non-uniformity in size, the more difficult it is for the beta form to occur, and the beta-prime form will then be the predominant one. There are natural fats which have relatively uniform fatty acid compositions and these will tend to recrystallize in the beta form. Canola and sunflower oil are examples of this behaviour. Figure 5 shows polarized light micrographs of hydrogenated Canola oil. When this fat was rapidly cooled, the crystal structure included aggregates of up to 10 um (Figure 5A). After tempering for a day at 25 C, there were more small crystals in evidence of up to about 2 um in size (Figure 5B). Ascribing definite polymorphic modifications to fat crystals of specific morphology is probably not justified for many natural fats. This might be applicable in special cases only, such as for highly purified simple triglycerides. The recrystallization of fats into the beta form is a disastrous occurrence when taken place in a consumer product. The product becomes visibly grainy with a mottled color appearance and acquires a crumbly texture (Figure 6) and the crystal structure shows a mass of large needle shaped crystals (Figure 7). This is a good example of how the arrangement of molecules in the crystal lattice directly affects appearance factors such as smoothness and texture. Some selected surface active agents have been found helpful in preventing or delaying the beta-prime to beta transformation in fats. This phenomenon is not well understood and studies are under way to elucidate the mechanism of this action. Recently, Garti et al. (1981, 1982) described the effect of food emulsifiers on crystal structure and habit of

Fig. 5. Polarized light photomicrographs of hydrogenated Canola oil.  
A. After rapid cooling at -15 C.  
B. After rapid cooling at -15 C and tempering for one day at 25 C.  
S.M. = 5 um.  
(S.M. = Scale Marker)

Fig. 6. Mottled appearance of recrystallized Canola margarine. One pound print broken in two pieces.

Fig. 7. Crystal structure of Canola oil margarine as seen in the polarizing microscope. This margarine is recrystallized in the beta modification.  
S.M. = 5 um.
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stearic acid. The crystal habit was examined by using scanning electron microscopy. It was assumed that the stearic acid could serve as a model for the more complex triglyceride mixtures but this is by no means certain. Hernqvist et al. (1981) have described the strong stabilizing effect of 1,2-di-glycerides on the #triglyceride crystal form.

The polymorphism of fats can also be studied by infra-red spectroscopy, a technique extensively investigated by Chapman (1965) and by deRuig (1977). The major area of interest in the infra-red region is between 690 and 770 cm\(^{-1}\). An example from Woodrow and deMan (1968) (Figure 8) shows the formation of the alpha form on cooling milkfat from 40 to 0 C and the formation of the beta prime form after holding at 5 C for ten hours.

Short spacings observed by X-ray diffraction methods are in the order of 0.1 nm. Polarized light microscopy makes it possible to view crystals in the range of 0.5 - 100 \(\mu\)m. For details in the area below 0.1 \(\mu\)m electron microscopy is required. Since fine structures of fats are delicate and temperature sensitive, special techniques are required. Initial electron microscopy studies involved ultra-thin preparations and carbon replica techniques. An example is an electron micrograph of trilaurin crystals produced by Buchheim (1970a) (Figure 9). This figure presents a clear view of the layered architecture of the trilaurin crystal. Much of the work concerned with electron microscopy of fats has been carried out at the German Federal Dairy Research Institute at Kiel and has been mainly focussed on milkfat structure in butter. The work involving fixation, thin sectioning and carbon replica formation produced micrographs showing little more than the outlines of fat globules (Figure 10) (Knoop and Schulz, 1960).

More recently the work of this group has involved freeze fracturing, followed by platinum-carbon replica preparation (Buchheim and Precht, 1979; Precht and Buchheim, 1979; 1980; Precht and Peters, 1981). Based on this work, the fat globules in cooled cream have been divided into 4 types based on how much of the fat is in the solid form. Figure 11 represents a fat globule which is stated...
to be mostly liquid. However, the highly magnified section appears as a definitely layered structure. Figure 12 is a micrograph of a fat globule with more irregularly formed crystal aggregates. The conclusion of this work is that the amount of solid fat in different fat globules in cream varies widely. This would mean that the fatty acid composition of the fat in the globules would be different, and this is not supported by any previous evidence. Walstra (1976) has discussed the effect of the state of dispersion of fat on its crystallization behavior and on the physical properties of the products. He indicates that emulsified fat needs a much higher degree of supercooling to initiate nucleation, and, therefore, the amount of solid fat in emulsion droplets may be considerably less than in the same fat in bulk form. The microstructure of fat in butter is described by Precht and Buchheim (1980). Two figures from this paper show one area of liquid and crystalline fat (Figure 13) and crystal lamellae of butter after 10 days' storage (Figure 14). The layered structure of this crystalline fat is clearly visible at different magnifications. These authors have attempted to relate the results of the electron microscopy studies to the known rheological behavior of butter. This is a difficult task, especially since the fine structures observed by electron microscopy bear little apparent relation to observations made by polarized light microscopy. The question arises as to whether the rheological properties of fats are more

Fig. 10. Electron micrograph of the fat globule structure in butter. (Source: Knoop and Schulz, 1960. Reproduced with permission). S.M. = 1 µm.

Fig. 11. Electron micrograph of a fat globule in cream with liquid milkfat. b represents an enlarged section (dotted rectangle) of a. (Source: Buchheim and Precht, 1979. Reproduced with permission). S.M.a = 1 µm, b = 0.2 µm.
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Fig. 12. Electron micrograph of a fat globule in cream with solid milkfat. b represents an enlarged section (dotted rectangle) of a. (Source: Buchheim and Precht, 1979. Reproduced with permission). S.M. a = 1 um, b = 0.2 um.

Fig. 13. Electron micrograph of liquid (A) and solid (B) fat in 10 day old butter. (Source: Precht and Buchheim, 1980. Reproduced with permission). S.M. = 0.2 um.

Influenced by the internal structure of the crystals than by their ultimate size and pattern of interaction. It is generally agreed that the solid fat content of plastic fats is the major factor determining their rheological behavior. Solid fat content can be measured quantitatively by dilatometry or nuclear magnetic resonance (both wide line and pulsed NMR). It is possible to obtain an idea of the shape and size distribution of crystals by polarized light and electron microscopy. However, these latter techniques are not quantitative. Recently, a method has been developed for determination of the specific surface area of the crystals in a plastic fat by permeametry (deJager et al., 1963, Riiner, 1971). The method is based on the subsidence of the liquid phase of a disperse system under the influence of compression. The compressive force is delivered by a constant vacuum applied to a fat sample. This method promises to be a useful tool in the study of fat microstructure and will hopefully complement the results obtained by microscopic methods.

Although the equipment itself is relatively simple, the associated vacuum control instrumentation is not (Figure 15). Riiner (1971) has applied this method to relate polymorphic transitions to crystal sizes in hydrogenated fats. Transition of the beta-prime to beta modification in hydrogenated sunflower oil reduced the number of crystals by a factor of fifty as determined by the permeametric method.

The objective of studies of the microstructure of fats is to obtain a better understanding of the physical properties, especially rheology, with the aim of better control of such properties.
Excellent methods for this purpose are now available which should lead to a rapid advancement in our knowledge. However, the usefulness of electron microscopy in this field at this time is limited. It is to be hoped that improved preparation and handling techniques can expand the application of electron microscopy of fats in the future.

Microstructure of emulsions

Emulsions are disperse systems of two immiscible liquids stabilized by an emulsifier or combination of emulsifiers and stabilizers. The importance of associative structures of emulsifiers has been given increasing emphasis and liquid crystalline structures are an important aspect of many emulsions. The International Union of Pure and Applied Chemistry defines an emulsion as follows: "In an emulsion, liquid droplets and/or liquid crystals are dispersed in a liquid" (Frisberg, 1976). Food emulsions contain natural emulsifiers and/or synthetic ones. Some food emulsions, especially those based on dairy products have a fat phase which is not liquid but partly solid and this results in special types of microstructure and properties. Proteins are often the main emulsifiers in food emulsions in many cases in combination with phospholipids and other substances. The original milkfat globule membrane in milk as it leaves the udder has been extensively studied and consists of a complex of several proteins and phospholipids as well as several minor components including enzymes and metals. It is possible to emulsify oils or fats in milk serum (skimmilk) and a stable emulsifier layer is formed almost instantaneously. This also happens when milk is homogenized. The newly formed globule membranes in homogenized milk consist of protein. The conformation of proteins at interfaces and the role of these proteins in stabilizing emulsions has been described by Graham and Phillips (1974). They have visualized the structure of two proteins at the interface (Figure 16). One of
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Fig. 16. Schematic representation of the structures of absorbed films of β-casein and lysozyme at interfaces at different surface concentration. β-casein (disordered, flexible) left column, lysozyme (globular, rigid) right column. Surface concentration top row < \( \theta \) sat, middle row = \( \theta \) sat, bottom row > \( \theta \) sat. (Source: Graham and Phillips, 1974. Reproduced with permission).

these is β-casein which has a disordered structure and the other is a globular protein, lysozyme. Protein membranes formed in emulsions containing milk proteins do not have the liquid crystalline structure demonstrated with certain emulsifiers. As a consequence, the globules show no birefringence as is indicated in the polarized light photomicrograph of an emulsion of cottonseed oil in skim milk (Figure 17). The birefringence seen in cream when examined under polarized light at 5 C results from the layers of crystallized milkfat which are formed when the milkfat inside the globules crystallizes (Figure 18). The churning of cream to produce butter involves the destabilization of a limited number of these globules and the expelled liquid fat acts as a cementing material to bind the remaining globules together. For this reason, the microstructure of butter as seen with polarized light is remarkably similar to that of cream (Figure 19). The nature of the birefringent layer of fat globules in milk or cream was clarified by Buchheim (1970b). He used electron microscopy to demonstrate the presence of crystallized fat in the peripheral layers of the globules.

It is now recognized that many emulsifiers interact in a number of ways with both the aqueous and lipid phases and these interactions take the form of liquid crystalline structures or mesomorphic phases. The nature of these liquid crystalline structures has been found to influence viscosity and elasticity of interfacial films with a resultant effect on emulsion stability (Krog,

Fig. 17. Emulsion of cottonseed oil in skim milk as seen in the polarizing microscope at 5 C. S.M. = 5 μm.

Fig. 18. Whipping cream as seen in the polarizing microscope at 5 C. S.M. = 5 μm.

Fig. 19. Crystal structure of butter as seen in the polarizing microscope at 5 C. S.M. = 5 μm.
The transformation of a crystallized emulsifier such as a monoglyceride into a liquid crystalline structure has been represented schematically by Krog and Lauridsen (1976) as shown in Figure 20. The major mesomorphic structures are lamellar, hexagonal and cubic. The lamellar structure (Figure 20b) is formed when the emulsifier is heated in the presence of water and alternating bimolecular layers of lipid are separated by layers of water. This structure when examined in the polarizing microscope appears as threadlike striated networks (Figure 20a) and is also known as the neat phase. In addition to polarized light microscopy, the mesomorphic phases can be characterized by their long spacings as determined by X-ray diffraction. The hexagonal phase consists of cylindrical structures of two types, hexagonal I with the lipophilic hydrocarbon chains occupying the interior core of the cylinders and hexagonal II where the hydrocarbon chains are arranged on the outside of the cylinders (Figure 22). The hexagonal phase when examined in the polarizing microscope presents a characteristic appearance (Figure 21b). Sometimes monoglycerides may form micellar aggregates which have an inner structure of the lamellar type and appear in polarized light as rod shaped "batonnets" (Figure 21c).

The layer structure of liquid crystal formations between flocculated emulsion droplets has been demonstrated by Friberg et al. (1976) by using the technique of freeze etching electron microscopy (Figure 23).

Liquid crystalline structures occur in food emulsions formulated with a...
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Fig. 23. Electron micrograph obtained by freeze etching of emulsion, demonstrating the layer structure of a liquid crystal between flocculated droplets. (Source: Friberg et al., 1976. Reproduced with permission).

A variety of emulsifiers including monoglycerides, emulsifiers of the Span and Tween type and as shown by Hemker (1981) by tri- and octaglycerol esters. The latter discussed the relationship between the liquid crystalline structures and the functional properties of the emulsifiers in food emulsions.

Food processing often involves formation, destruction, or modification of emulsions, and various forms of microscopy may be used to elucidate the microstructure of the products. An example of the useful combination of scanning and transmission electron microscopy is the study of process cheese microstructure by Rayan et al. (1980). In this case, the protein matrix was visualized by SEM and the nature of the fat globules by TEM. In this way, valuable information about microstructural changes was obtained (Figure 24).

The examples presented in this paper are intended to demonstrate the usefulness of light and electron microscopy in the study of fat and emulsion microstructures, especially when used in conjunction with other techniques such as X-ray diffraction.

Acknowledgement

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References


Fig. 24. Development of microstructure in process cheese in the presence of sodium aluminum phosphate (SALP). a = SEM at 0 time. Large fat particles (F) started to be degraded into smaller particles, (G) fragment of a calcium phosphate crystal. b = SEM after 10 min in the cooker. Fat is still in the form of large particles, many of which are being emulsified (F) into smaller particles. c = SEM after 40 min in the cooker. Some fat particles (F) are still undergoing emulsification. d = TEM at 0 time. Dark areas are the cheese protein matrix, light areas indicate fat. e = TEM after 40 min in the cooker. The emulsification process has not been completed and fat particles (F) are still undergoing emulsification. f = TEM detail of one of the added SALP crystals found in abundance during the initial 10 min in the cooker. (Source: Rayan et al., 1980. Reproduced with permission).
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Riiner U, Phase behaviour of hydrogenated fats. III. Phase equilibria and crystal sizes, Lebensm. Wiss. Technol. 4, 1971, 139-144.


Discussion with Reviewers

W. Buchheim: The electron micrographs of cream after different thermal treat­ments clearly show the great variation of fat crystallization and morphology in individual fat globules. This must not necessarily mean that the composition of the butterfat in the globules has to be different, although such evidence has been described by Walstra and Borggreve (Netherlands Milk Diary J. 20, 140, 1966). The other reason could be that this great variation reflects differ­ences in the degree of supercooling of individual globules due to the absence of crystal nuclei. Please comment.

Author: If in fact this phenomenon will be proven to exist (and not be an arti­fact of the preparation technique), I would find the latter explanation more plausible than the former.
H. Buchheim: It has been clearly demonstrated that the rheological behaviour of butter made from a given cream is dependent on how crystallized and liquid fat is spatially distributed within the final product. Please comment.

Author: I fully agree with this. However, we still have to explain how the amount of solid fat within a fat globule affects the texture. In butter, we have also fat crystals in the continuous liquid phase. Both types of solid particles are important in determining rheological properties.

D.N. Holcomb: Are the liquid crystal structures shown in Figure 22 consistent with rheological properties of these phases? In general, how important are liquid crystals in determining rheological properties of fat containing foods?

Author: The liquid crystal structures in bulk are definitely consistent with rheological properties. These structures are most important in emulsions and much of the research in this area has been related to emulsion stability.

D.N. Holcomb: Will the author give his opinions as to the future course of studies of the microstructure of fats and emulsions? Can we expect advances in our understanding of these systems through techniques such as low angle X-ray scattering, Fourier transform IR, etc.? Are there any staining techniques that might prove useful in lipid microscopy?

Author: I have tried to emphasize in my paper that this is an extremely difficult area of study. No single technique is adequate and various complimentary techniques should be used. The problem with much of microscopy is that it is not quantitative. More quantitative information is desirable. Low angle X-ray techniques are required for long spacings. Unfortunately, cameras such as the Guinier-Simon are not suitable for this. There is a possibility that suitable modifications to this equipment can be made. Differential staining, e.g. of solid and liquid fat, may be useful in certain cases.

N. Krog: Can you explain in more detail what the Guinier-Simon camera can do? Indicate advantages and limitations in using this camera against others.

Author: The Guinier-Simon camera can provide sharp diffraction patterns of fat samples being temperature programmed in as many as 8 steps over the range of -100 to 100 C. It is not suitable for low angle measurements.

N. Krog: Have you studied the influence of crystal modifiers like sorbitan tri-stearate on the recrystallization problems in Canola margarine?

Author: Yes, we are just concluding the first phase of our study. Sorbitan tri-stearate lowers the melting point, does not affect solid fat content and is effective in delaying the 'S' to 'S' transition. It also seems to be present in higher concentration in the solid than in the liquid phase of the fat.

N. Krog: It is of interest to know if the fat globules represented in Figs. 11 and 12 have been exposed to different temperature treatment? Can the temperature vary during sample preparation and give cause to the difference between Figs. 11 and 12 rather than the proposed difference in fatty acid compositions?

Author: The possibility of artifacts is an ever present concern. It is more likely, however, that the state of dispersion of the fat influences the rate of crystallization.

N. Krog: Referring to Fig. 19, it would be of interest to know where the milk proteins are located in butter? Are all the proteins in the water droplets or are some of the proteins present on the surface of the fat globules in butter?

Author: Yes, butter contains a proportion of the original fat globules of the cream in an undisturbed condition. The protein membrane of these globules still contain a protein layer. Additional protein is present in the aqueous phase.

N. Krog: Have you done any work with phase contrast or fluorescence microscopy? Or can you give any reference to such studies in relation to food texture?

Author: No, we have not used phase contrast or fluorescence microscopy for work with fats. There appears to be no particular advantage for these techniques in the area of fats, and I know of no recent published work.

F.R. Paulicka: Please comment on the influence of mechanical working (shear, compression) on fat crystal morphology.

Author: The effect of mechanical working on fat rheology is well documented. However, to my knowledge, no body has been able to demonstrate what happens by using microscopy.