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Physical properties of fats in food¹

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1.1 Introduction

Oils and fats are important ingredients in a wide variety of manufactured foods, and constitute a significant part of food recipes. The major foods in which they are used are all discussed in detail in this volume. However, it is important to note that the forms in which oils and fats are made available to food manufacturers have changed significantly over the years, particularly since the 1960s, largely because of the major shifts that have taken place in consumer lifestyles and the increasing concerns with health, food safety and a balanced diet. Many of the food products that are now available to consumers reflect this new direction. Important examples arising out of the lipid research that has followed are *trans-free* fatty acids, reduced high-melting, in particular saturated, fats, very-low-yellow fat emulsions, spreadable butter, aerated fats, structured oils, molecularly designed structured fats with new nutritional advantages, and so on. All these initiatives have required an in-depth understanding of the behaviour of the fats concerned so that they can be used effectively as ingredients in food. Consequently, the study of their physical properties is of major interest and is covered in this chapter.

In general, fats form networks of crystal particles, maintaining specific polymorphic forms, crystal morphology and particle–particle interactions (Marangoni, 2005). The control of the physical properties of food fats has therefore been of importance in research efforts and can be considered under five headings:

- clarification of molecular and crystal structures of triacylglycerols (TAGs) with different fatty-acid moieties (Kaneko *et al.*, 1998; Kaneko, 2001);
- crystallisation and transformation mechanisms of TAG crystals (Sato, 1996, 1999; Sato and Koyano, 2001; Sato and Ueno, 2005);

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- clarification of formation mechanisms of mesoscale and macroscale fat crystal network starting from nanoscale primary fat crystals (Acevedo *et al.*, 2011);
- rheological and texture properties that are dominated mainly by fat crystal networks (Boode *et al.*, 1991; Marangoni and Hartel, 1998; Marangoni *et al.*, 2012; Walstra *et al.*, 2001);
- influences of external factors such as shear, ultrasound irradiation, minor lipids on fat crystallisation kinetics (Martini *et al.*, 2008; Mazzanti *et al.*, 2011; Smith *et al.*, 2011; Wright *et al.*, 2000).

The first topic is of an introductory nature and so will not be elaborated in this chapter (for more details, see the cited references). The remaining four topics are related to observed systems of food fats, with which this chapter is mainly concerned.

The chapter begins with a brief review of the three basic physical properties of fats by collecting together recent work on the crystallisation and transformation of the fats in bulk and in emulsion states. We will then focus on fundamental aspects of the crystallisation and transformation of fats employed in real food systems, through describing the use of important examples, such as cocoa butter, palm oil and palm mid-fractions. Since these natural fats are multi-TAG systems, knowledge of the fundamental properties of pure TAGs composing the natural fats may be necessary, as will be argued. Those who wish to compare real fats with pure fats are directed to the literature (Himawan *et al.*, 2006; Sato, 1996; Sato and Koyano, 2001; Sato and Ueno, 2001; Sato *et al.*, 1999).

1.2 Basic physical properties of fat crystals

The physical properties of the food fats are influenced primarily by three factors: (1) polymorphism (structural, crystallisation and transformation behaviour); (2) the phase behaviour of fat mixtures; and (3) the rheological and textural properties exhibited by fat crystal networks. In this section we cover the fundamentals and look at recent research work on these three properties.

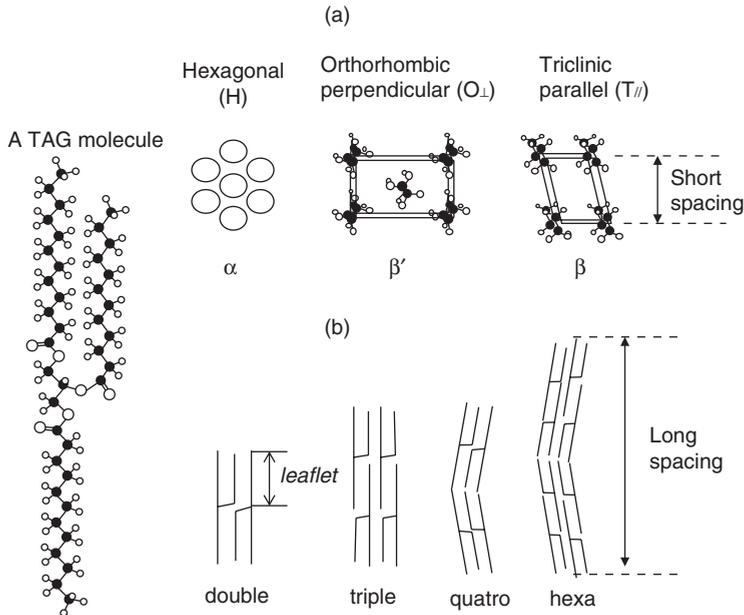
1.2.1 Polymorphic structures of fats

Polymorphism is defined as the ability of a chemical compound to form different crystalline or liquid crystalline structures. The melting and crystallisation behaviour will differ from one polymorph to another.

Table 1.1 summarises the basic physical properties of the three typical polymorphic modifications of α , β' and β . Polymorph α is least stable, easily transforming to either the β' form or the β form, depending on the thermal treatment. Polymorph β' , the meta-stable form, is used in margarine and shortening because of its optimal crystal morphology and fat crystal networks, which give rise to optimal rheological and texture properties. The most stable β form tends to form large and plate-like crystal shapes, resulting in poor macroscopic properties in shortening and margarine.

Table 1.1 Three typical polymorphic forms of fats and their main physical properties.

Form	Stability	Density	Melting point	Morphology
α	Least stable	Lowest	Lowest	Amorphous-like
β'	Metastable	Intermediate	Intermediate	Rectangular
β	Most stable	Highest	Highest	Needle-shaped

**Figure 1.1** Polymorphic structures of three typical forms of triacylglycerol (TAG). (a) Subcell structures and (b) chain length structures.

The three main polymorphs, α , β' and β of fats, are defined in accordance with subcell structure: α polymorphs have a hexagonal subcell (H); β' polymorphs have an orthorhombic–perpendicular subcell (O_{\perp}); and β polymorphs have a triclinic–parallel subcell ($T_{//}$) (Larsson, 1966; see Figure 1.1 (a)). The subcell structures can be determined most clearly by measuring X-ray diffraction (XRD) short spacing patterns of poly-crystalline samples.

Figure 1.1 (b) shows the chain-length structure, illustrating the repetitive sequence of the acyl chains involved in a unit cell lamella along the long-chain axis (Larsson, 1972). A double chain-length structure (DCL) is formed when the chemical properties of the three acid moieties are the same or very similar. In contrast, when the chemical properties of one or two of the three chain moieties are largely different from those of the moieties, a triple chain-length (TCL) structure is formed because of chain sorting. The relevance of the chain-length structure is revealed in the mixing phase behaviour of the different types of the TAGs in the solid phase: when the DCL fats are mixed

with the TCL fats, phase separation readily occurs. The chain length structures can be determined solely by measuring the XRD long spacing patterns of the poly-crystalline samples.

In food fats, transformation from polymorph β' to polymorph β often causes deterioration of the end product, mostly because of changes in the crystal morphology and network, as indicated in Table 1.1. The β -type polymorph is found in confectionery fats made of cocoa butter (Timms, 2003). There are two β -type crystals: a meta-stable β_2 form is more useful than the more stable β_1 form (Sato and Koyano, 2001; Van Mechelen *et al.*, 2006a, 2006b). Atomic-level structure analyses of the TAGs have been attempted to resolve the microscopic mechanism of the polymorphic β' – β transformation. Results were reported first for the β forms (as reviewed for the β forms in Kaneko, 2001), and have been reported for the β' form (Sato *et al.*, 2001; van Langevelde *et al.*, 2000). Mechanistic processes of solid-state transformation from β' to β forms in tri-lauroyl-glycerol crystals were observed by a cutting-edge method with synchrotron radiation microbeam XRD (SR- μ -XRD) (Ueno *et al.*, 2008), as will be presented below.

As the physical properties of food fats are greatly influenced by fat polymorphism, it is a prerequisite for those who are engaged in the material production of oils and fats to know how the fatty-acid composition influences the fat polymorphism. Two categories of fatty-acid composition may be considered: (1) mono-acid TAGs in which the three fatty-acid moieties of the TAG are of the same type; and (2) mixed-acid TAGs in which different fatty-acid components are connected to three different glycerol carbons on the TAG. The following diversity in fatty-acid composition of TAGs can be found:

- Mono-acid TAGs:
 - the acids may be saturated;
 - the number of carbon atoms in the fatty-acid chain, N_c , may be odd or even;
 - the acids may be unsaturated.
 - the number of carbon atoms in the fatty-acid chain, N_c , may be odd or even;
 - there may be a *cis* or a *trans* conformation around the double bond;
 - the number of double bonds may vary;
 - the position of the double bonds may vary.
- Mixed-acid TAGs:
 - there may be three saturated acids with different chemical species;
 - there may be three unsaturated acids with different chemical species;
 - there may be three acids containing saturated and unsaturated species;
 - the different fatty acids may be connected to carbon atoms of different stereo-specific number (*sn*).

In 1988, Hagemann summarised the melting behaviour of TAGs with different combinations of fatty-acid moieties with different chemical species (Hagemann, 1988).

Hagemann showed a general tendency in the melting behaviour of mono-acid TAGs to be as follows:

- In saturated mono-acid TAGs, the melting points of the α , β' and β forms increase when N_c is increased from 8 to 30. With respect to the quantitative dependence of the melting point of the polymorphs on N_c , the melting points of the α form increase smoothly with N_c , whereas the melting points of the β' and β forms increase in a 'zig-zag' manner with N_c odd or even.
- In the mono-unsaturated mono-acid TAGs, the melting points of the β forms are available, showing specific dependence on double-bond conformation and on the position of the double bond. For example, *trans* unsaturated TAGs showed higher melting points than those of *cis* unsaturated TAGs at every double-bond position.

Since 1988, much work has been done on the polymorphic behaviour of mixed-acid TAGs. It is important to understand such behaviour as natural oils and fats contain these mixed-acid TAGs (for reviews, see Larsson *et al.*, 2006; Sato *et al.*, 1999; Sato and Ueno, 2001; Sato and Ueno, 2005).

The fatty-acid compositions of TAGs are closely related to β' -tending properties. TAGs containing different types of fatty acids are more stable in the β' form, as exemplified in pure TAGs (Hagemann, 1988). In natural fats, milk fats having long-chain and short-chain saturated and unsaturated fatty acids are β' -tending; palm oil is also categorised as a β' -tending fat because of the presence of asymmetric mixed-acid TAGs such as POO (1-palmitoyl-2,3-dioleoyl-*rac*-glycerol) and PPO. Recent work on single-crystal structure analyses of the β' form of 1,3-dilauroyl-*sn*-2-caproyl-glycerol (CLC; van Langevelde *et al.*, 2000) and 1,2-dipalmitoyl-*sn*-3-myristoyl-glycerol (PPM; Sato *et al.*, 2001) has indicated that chain-chain interactions through methyl end stacking combined with glycerol group conformations may stabilise the β' structures (Hernqvist and Larsson, 1982).

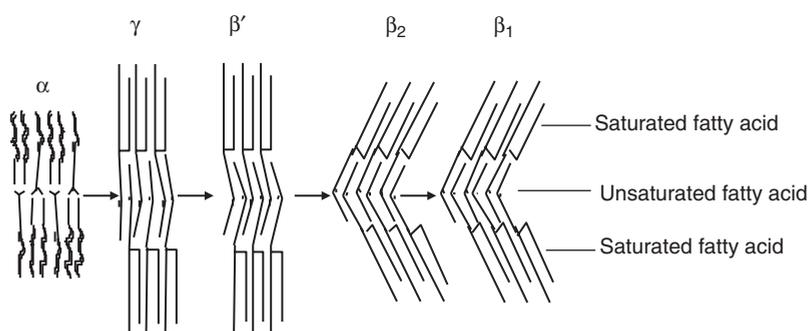
The polymorphic behaviour of mixed-acid TAGs differs greatly from that of mono-acid TAGs. For example, Table 1.2 shows variations in polymorphic occurrence and the melting behaviour of a series of TAGs in which the two fatty-acid chains at the *sn*-1 and *sn*-3 positions are stearic acid, and in which the fatty acid at the *sn*-2 position may be stearic (to give SSS), elaidic (SES), oleic (SOS) (Sato *et al.*, 1989), ricinoleic (SRS) (Boubekri *et al.*, 1999) linoleic (SLiS) (Takeuchi *et al.*, 2000), α -linoleic (SLnS) or eicosapentanoic acid (SEpS) (Sato *et al.*, 2009). Three typical polymorphs of α , β' and β polymorphs are revealed in SSS, all stacked in a DCL structure. Substitution of the *sn*-2 acid with elaidic acid (SES) caused a decrease in the melting point for the three polymorphs, which exhibit basically the same properties as those of SSS. However, large differences are produced when the *sn*-2 acid is replaced by oleic, ricinoleic or linoleic acid, revealing a new polymorph, γ , and variation in chain-length structure from double (α form) to triple (the other, more stable, forms). In addition, the β form is absent in SRS and SLS, and the β' form does not occur in SLiS. Quite recent work

Table 1.2 Polymorphic occurrence and melting points ($^{\circ}\text{C}$) of SSS, SES, SOS, SRS, SLiS, SLnS, and SEpS.

Polymorph ^a	SSS	SES	SOS	SRS	SLni	SLnS	SEpS
α -2	55.0	46.0	23.5	25.8	21.6	–	–
β' -2	61.6	58.0	–	–	–	–	–
γ -3	–	–	35.4	40.6	34.5	35.9	32.5
β -2	73.0	61.0	–	–	–	–	–
β' -3	–	–	36.5	–	–	–	–
β'_2 -3	–	–	–	44.3	–	–	–
β_2 -3	–	–	41.0	–	–	–	–
β'_1 -3	–	–	–	48.0	–	–	–
β_1 -3	–	–	43.0	–	–	40.1	–

Note: SSS, tristearoylglycerol; SES, 1,3-distearoyl-2-elaidoyl-*sn*-glycerol; SOS, 1,3-distearoyl-2-oleoyl-*sn*-glycerol; SRS, 1,3-distearoyl-2-ricinoleyl-*sn*-glycerol; SLiS, 1,3-distearoyl-2-linoleoyl-*sn*-glycerol; SLnS, 1,3-distearoyl-2- α -linolenoyl-*sn*-glycerol; SEpS, 1,3-distearoyl-2-eicosapentanoyl-*sn*-glycerol.

^aSuffixes 2 and 3 refer to double and triple chain-length structures, respectively.

**Figure 1.2** Polymorphic structure of 1, 3-stearic-2-unsaturated mixed-acid TAGs.

on SLnS and SEpS (Sato *et al.*, 2009) unveiled the following properties of the TAGs exhibited in Table 1.2, and as illustrated in Figure 1.2.

- In the mixed-acid TAGs exhibited in Table 1.2, the unsaturated and stearic acid moieties form different layers, resulting in the TCL structure.
- Because of the strong van der Waals interactions between the stearic acid layers of the TCL structure, not only the mono-unsaturated fatty acid but also the poly-unsaturated fatty-acid moieties may exhibit extended chain conformation as illustrated in Figure 1.2.
- As a result, the melting points of the two TAGs including the poly-unsaturated fatty-acid moieties are actually decided by van der Waals interactions among the stearic acid moiety, and become much higher than those of the poly-unsaturated fatty acids in the free fatty-acid state.

Van Mechelen *et al.* have recently studied β forms of SOS and POS by using polycrystalline samples grown from the melt phase (van Mechelen *et al.*, 2006a, 2006b), confirming the existence of two β forms of the TCL structure. They claimed that the differences between β_1 and β_2 may mostly be revealed in the layered structures, in which

β_2 is TCL whereas β_1 is a hexa-layer structure composed of two TCL structures which are stacked with the alternation of the inversion-centre relation shown in Figure 1.2.

The peculiar properties of mixed-acid TAGs involving unsaturated fatty-acid moieties may be partly understood in terms of chain-chain interactions between the saturated and the unsaturated fatty-acid moieties (Kaneko *et al.*, 1998; Sato and Ueno, 2001). Much work, however, should be done to clarify the molecular mechanisms that cause such complicated polymorphic occurrence and structures such as those illustrated in Table 1.2 and Figure 1.2.

The variation in polymorphic properties of saturated-unsaturated mixed-acid TAGs (see Table 1.2) may have critical significance for our understanding of the polymorphism of natural oils and fats such as milk fat, palm oil and cocoa butter, which contain large amounts of mixed-acid TAGs (Gunstone, 1997).

1.2.2 Polymorphic crystallisation of fats

The macroscopic aspects of fat polymorphism concern the behaviour at melting and crystallisation and the subsequent transformation. With respect to the melting temperature, T_m , for the three forms of any particular TAG, a general tendency is that T_m is lowest for the α form, intermediate for the β' form, and highest for the β form. In contrast, the crystallisation behaviour is more complicated and is determined primarily by the type of crystallising medium, the crystallisation temperature, and the rate of cooling. In general, the β form is usually crystallised from the solution phase, but all three forms may be crystallised from neat liquid. When crystallisation is from the neat liquid, the relative rates and extents of crystallisation of the three polymorphs are determined by the rate of nucleation, which is highest for the α form, intermediate for the β' form and lowest for the β form. Schematic illustration of polymorphic crystallisation of the three forms of TAG is shown in Figure 1.3. This behaviour was measured with precision for tripalmitoyl glycerol (PPP) (Sato and Kuroda, 1987) and for the other fat crystals (Blaurock, 1999). Stop-and-return DSC method combined with the XRD

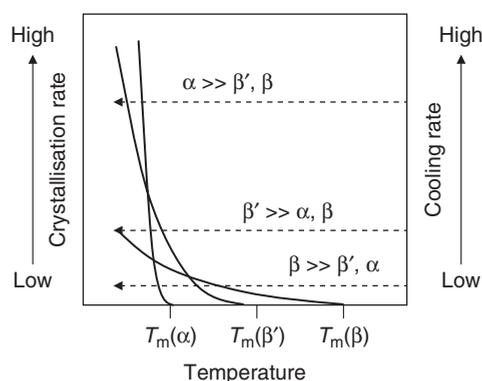


Figure 1.3 Schematic illustration of relative rates of crystallisation of three typical polymorphic forms of TAG in comparison with the rate of cooling.

analysis also showed polymorph-dependent crystallisation kinetics of various natural fats (Foubert *et al.*, 2008). DSC and a synchrotron radiation X-ray diffraction study of POP clearly measured the polymorph-dependent crystallisation rates of OPO, POP, OOO and OOL (L: linoleic) (Bayes-Garcia *et al.*, 2011, 2013a, b).

Based on the polymorph-dependent crystallisation kinetics, it is necessary to find the optimal rate of cooling in accordance with the preferred rate of crystallisation of the three polymorphic forms, when one tries to selectively crystallise a specific polymorphic form from the liquid state. For example, the α form should be crystallised at the highest cooling rate, and the β form can crystallise at the lowest cooling rate. However, it is quite difficult to predict the optimal cooling conditions for specific polymorphic forms, which have been examined by chance or experience rather than by theoretical prediction in the actual production processes of edible fats. Furthermore, concurrent crystallisation of multiple polymorphic forms may occur, because the nucleation of crystals is determined by probabilistic phenomena. From the practical point of view, however, it is often necessary to crystallise the more stable forms much more rapidly with higher selectivity. For this reason, the following methods have been applied to modify the crystallisation behaviour of the polymorphic forms.

1.2.2.1 *Template/additive*

Adding foreign materials on purpose to the crystallising liquid, called templates or additives, is widely applied to modify the crystallisation behaviour of inorganic and organic substances, including fat crystals (Sangwal, 2007). The necessary conditions for the template/additive to successfully modify the polymorphic crystallisation may be summarised as follows:

1. *Similarity in molecular shape and polymorphism.* Similarity in molecular shapes as saturated or unsaturated fatty acids or their chain lengths between the template/additive and fat crystals is required. The same properties may be applied to the polymorphic structures between the template/additive and fat crystals.
2. *Thermal stability.* Template/additive materials should not dissolve when they are added to supercooled liquid of lipids, having higher melting points than the fat materials to crystallise.
3. *Optimal supercooling.* When the supercooling is high enough to induce spontaneous nucleation, the effects of the template/additive may be minimised, because unwanted polymorphic crystals are formed without the effects of templates/additives. Therefore, the rate of cooling and the range of supercooling ΔT ($= T_m - T_C$, T_m and T_C are melting and crystallisation temperatures) may be moderate so that spontaneous nucleation is limited.
4. Very limited studies have been performed on the effects of templates or additives on the polymorphic crystallisation of fats. The effects of crystal seeding of high-melting TAGs on the crystallisation of cocoa butter were studied (Hachiya *et al.*, 1989a, 1989b; Koyano *et al.*, 1990). The effects of the addition of high-melting emulsifiers on the fat crystallisation in oil-in-water (O/W) emulsion were also examined, as will be described in Section 1.2.2.2.

1.2.2.2 Dynamic temperature variation (tempering)

It has been understood that dynamic temperature change after the crystallisation of the metastable forms is effective to obtain the optimal polymorphic forms through melt-mediation or solid-state transformation (see Section 1.2.3.1). Such a process is called *tempering*, as applied in the polymorphic crystallisation of cocoa butter in chocolate production (Timms, 2003). Also, the tempering method was applied to form β -fat gel, in which tiny crystals of β polymorph of high-melting fats were formed to exhibit organogel, which is composed of several wt.% of high-melting fat crystals and >95% liquid oil (Higaki *et al.*, 2003, 2004). During this tempering process, the least stable α form crystals of high-melting fats were formed by very rapid cooling, and subsequent re-heating caused the melt-mediated transformation into the β form, and thus the formed β crystals were so tiny, compared with those formed by simple cooling, that the organogel was formed.

Figure 1.4 illustrates the formation process of the β -fat gel composed of high-melting fat (fully-hydrogenated rapeseed oil, rich in behenic acid, FHR-B) and sal fat olein (SFO) (Higaki *et al.*, 2003). The melting temperatures (T_m) of α and β forms of FHR-B crystals decreased with decreasing concentration of FHR-B, with the differences between $T_m(\alpha)$ and $T_m(\beta)$ of 18°C unchanged at all the FHR-B concentrations in the

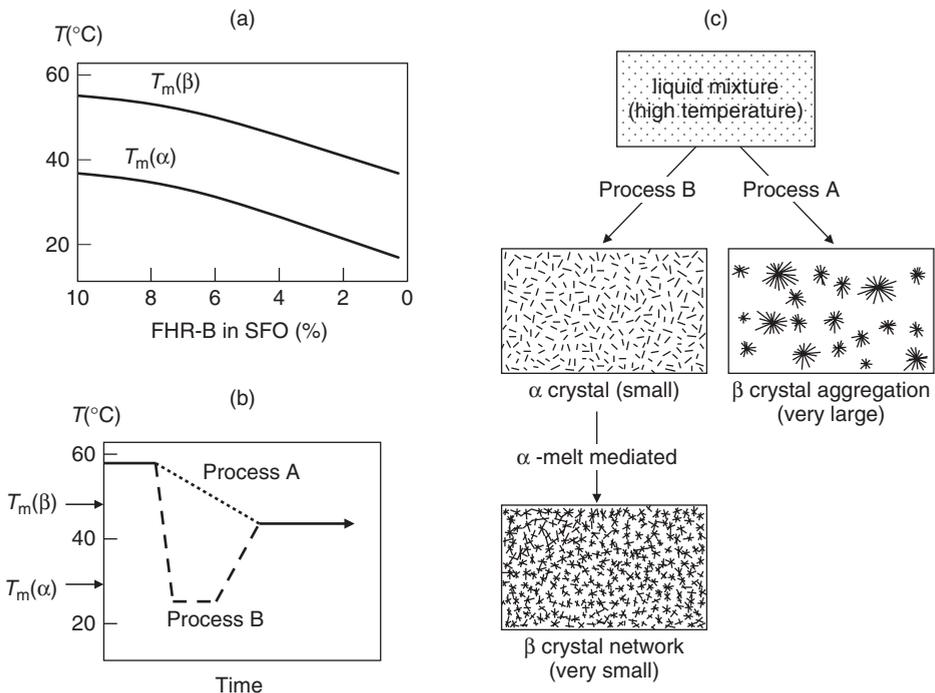


Figure 1.4 Formation mechanisms of β -fat gel made of a high-melting fat (FHR-B) and liquid oil (SFO) mixture (abbreviation, see text). (a) Phase behaviour of α and β forms; (b) temperature variation scheme; and (c) crystallisation behaviour.

mixtures of FHR-B and SFO examined (Figure 1.4 (a)). After the crystallisation process A, during which the molten mixture was slowly cooled to the temperature below $T_m(\beta)$, large β crystals of FHR-B were formed and the gel phase was not formed (Figure 1.4 (b) and (c)). By contrast, the gel phase was formed after the crystallisation process B, in which the molten mixture was rapidly cooled to below $T_m(\alpha)$ and heated to the temperature between $T_m(\alpha)$ and $T_m(\beta)$. After this tempering process, many small β crystals were formed and randomly distributed in the SFO oil which was entrapped by the β crystal network of FHR-B. Such a network formation was caused by the melt-mediated transformation from α to β forms, because the nucleation rate of α crystals was high enough to make the transformed β crystals randomly distributed, rather than aggregated as formed by the slow cooling process A.

1.2.2.3 Application of shear

The study of fat crystallisation under shear has been carried out over many years, as reviewed by us (Sato and Ueno, 2001). Since this review was published, more detailed work on cocoa butter and milk fat has been reported by many groups (MacMillan *et al.*, 2002; Mazzanti *et al.*, 2003, 2007, 2008, 2009, 2011; Padar *et al.*, 2009). In these studies, it was evident that the rates of polymorphic crystallisation and transformation were largely increased by applying shear. Furthermore, Mazzanti *et al.* (2003) discovered the preferred orientation of fat crystal particles under shear by observing XRD patterns taken during the crystallisation processes with two-dimensional (2D) detectors.

For example, variations in the XRD intensity were monitored during the crystallisation processes of cocoa butter at 18°C under sheared condition (1440 sec^{-1}) as shown in Figure 1.5 (Mazzanti *et al.*, 2003). Of the six polymorphic forms of cocoa butter (Forms I–VI) (Wille and Lutten, 1966), Form III first crystallised, and

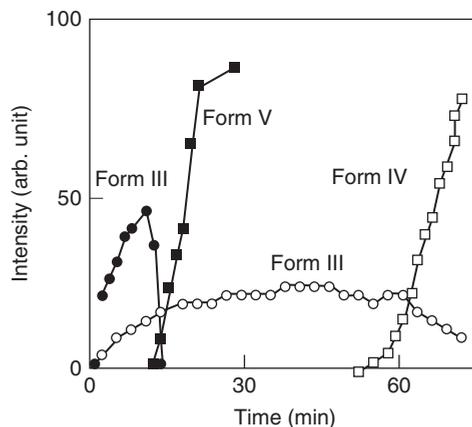


Figure 1.5 Effects of shear application (shear rate 1440 s^{-1}) on crystallisation of cocoa butter at 18°C . Without shear (open) and with shear (filled).

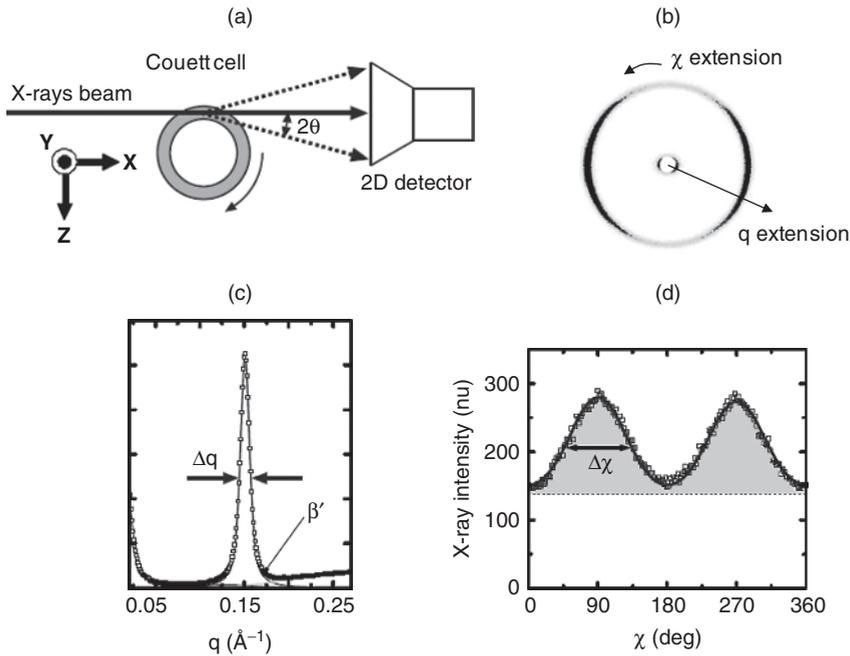


Figure 1.6 Synchrotron radiation XRD patterns from milk fat crystals crystallising under shear. (a) Experimental set-up; (b) two-dimensional XRD pattern; (c) θ -extension pattern; and (d) χ -extension pattern.

conversion into Form IV occurred during further crystallisation when shear was not applied. However, the conversion from Form III to Form V was observed and the rate of crystallisation was increased when shear was applied.

Figure 1.6 shows the effects of shear on the orientation of tiny crystals of anhydrous milk fat (AMF) present in the shear flow, which was determined by synchrotron radiation XRD analysis (Mazzanti *et al.*, 2009). AMF was crystallised in a Couett cell under sheared condition (1440 sec^{-1}) after 1 hour at 17°C . By using two-dimensional X-ray detector, information on the crystals growing in the Couett cell was obtained: (1) polymorphism revealed by observing the q -extension, in which $q = 2\pi/d = \sin\theta \times 4\pi/\lambda$ (where d = lattice parameter; θ = diffraction angle; λ = wavelength of X-ray beam); and (2) orientation of the crystals with respect to the shear direction by observing the χ -extension. Figures 1.6 (c) and (d) show that the AMF crystals grown under shear are of β' polymorph and the crystals are highly oriented, since sharp arc XRD patterns with narrow $\Delta\chi$ values are observed. These results contrast with the crystallisation without shear, since α and β' forms crystallised at the same time and no crystal orientation was observed (Mazzanti *et al.*, 2009).

The effects of shear have a practical significance for the crystallisation in edible fats, most remarkably for the crystallisation of cocoa butter in chocolate production (Dhonsi and Stapley, 2006; Maleky and Marangoni, 2011; Maleky *et al.*, 2011, 2012).

1.2.2.4 Irradiation of ultrasound wave (sono-crystallisation)

For decades, ultrasound has been applied in different applications of the characterisation of microstructures and the process control of materials. In the areas of food fats, there has recently been an increasing interest in the application of ultrasound waves to the crystallisation of fats (sono-crystallisation). Sono-crystallisation has been examined in pure TAGs, confectionery fats, vegetable fats and milk fats, indicating that the rates of polymorph-dependent crystallisation, crystal size and morphology are modified by sono-crystallisation (Higaki *et al.*, 2001; Martini *et al.*, 2005, 2008; Suzuki *et al.*, 2010; Ueno *et al.*, 2003).

The sono-crystallisation of tripalmitin (PPP) and cocoa butter has been studied (Higaki *et al.*, 2001). The main observations of these preliminary experiments can be summarised as follows: (1) the nucleation rate of PPP was enhanced and the induction time was shortened by the ultrasound application; and (2) the polymorphic Form V of cocoa butter was directly crystallised when the ultrasound was applied under optimal conditions of temperature and short period of sonication.

Martini *et al.* recently applied high-intensity ultrasound (HIU) to the crystallisation of palm kernel oil (PKO), anhydrous milk fat (AMF) and shortening (Martini *et al.*, 2005, 2008; Suzuki *et al.*, 2010). They observed the following results. HIU induced primary and secondary nucleation of fat crystals, generating smaller crystals. As a consequence, harder materials were formed when HIU was applied at higher crystallisation temperatures as observed for AMF, and when HIU was applied after the first crystals were formed as observed for PKO and shortening. In addition, the fat crystal network in AMF and shortening obtained after HIU application revealed steeper and sharper melting profiles compared with not-sonicated samples. For example, Figure 1.7 shows the effects of the application of HIU at different temperatures on the induction

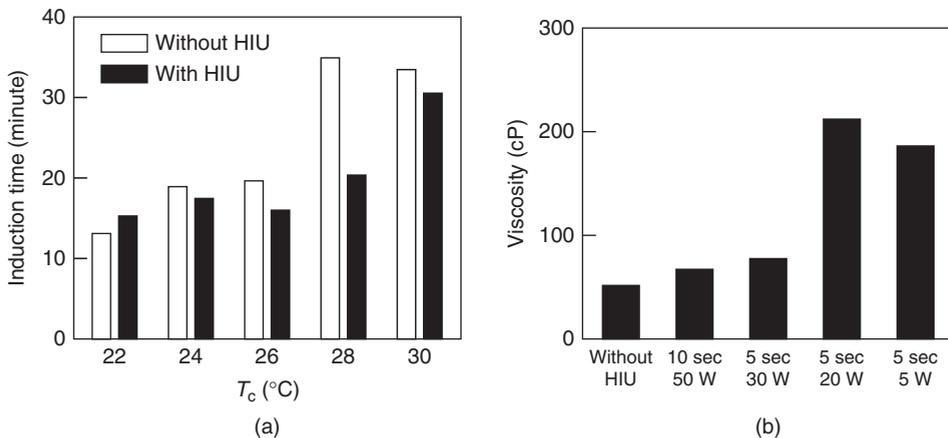


Figure 1.7 Effects of application of high intensity ultrasound (HIU) on crystallisation behaviour of anhydrous milk fat (AMF). (a) Induction time for crystallisation at a different crystallisation temperature (T_c); (b) viscosity of crystallised AMF with different sonication conditions at $T_c = 30^\circ\text{C}$.

time and viscosity of a crystallised sample of AMF. It was evident that the induction time was shortened when HIU was applied at 26°C and 28°C, and viscosity increased as the sonication time and HIU power were decreased.

To observe the kinetic influences of ultrasound irradiation, *in-situ* observation of the crystallisation processes of PPP and LLL was performed by using synchrotron radiation time resolved small-angle X-ray diffraction (SAXD) and wide-angle X-ray diffraction (WAXD) simultaneous measurement (Ueno *et al.*, 2003). Without ultrasound application, both forms of β' and β crystallised in the melt of each substance. With ultrasound treatment of the melt, the following effects were observed: (1) a marked decrease of induction times for crystallisation of both PPP and LLL; (2) an increased nucleation rate; and (3) crystallisation of only β forms for both PPP and LLL under conditions of initial crystallisation temperature of 50°C and 30°C, respectively, and applied ultrasound of 2 sec. Based on the dynamic nucleation of PPP and LLL crystals induced by collapsing cavitation bubbles, we argued that a pronounced decline in induction times, and an increase in the nucleation rate, result from the melting points shift due to high pressure pulses associated with collapsing bubbles.

The studies reviewed above clearly indicate that ultrasound does affect the crystallisation behaviour of fats in many ways. However, it is still unclear which particular mechanisms are responsible for these effects. To improve our knowledge and predictability in terms of the desired polymorphism, induction times, and nucleation rates that are all influenced by sono-crystallisation, a better understanding of the following issues is crucial: (1) to establish a P-T phase diagram for polymorphic forms, since the primary effect of sono-crystallisation may be due to high pressure when the sonication-induced cavity is collapsed; (2) the stability (lifetime) of different polymorphic forms as a function of the supercooling temperature; (3) the mechanism and lifetime of collapsing cavities; and (4) the basic mechanism of the dynamic nucleation, in the vicinity of a collapsing bubble (Ueno *et al.*, 2003).

1.2.3 Polymorphic transformation of fats

Once the less-stable forms are crystallised, they transform to more stable forms in a post-crystallisation process in solid phase or through liquid mediation (Sato *et al.*, 1999). As a consequence, the morphology of fat crystals is determined by the polymorphic modification, by the thermal processes of crystallisation and by subsequent transformation. It is worthy of note that various morphologies of β' -form PPP crystals have been found for different temperature treatments. In particular, the β' -form showed needle-like crystals after slow crystallisation, similar to β -form crystals, which usually exhibit a long needle shape (Kellens *et al.*, 1992).

Recent studies have shed light on the microscopic structures of polymorphic transformation of fats, which are reviewed as follows.

1.2.3.1 Solid-state and melt-mediated transformations

Two types of transformation processes occur from less stable to more stable polymorphic forms, when crystal-free energy values of α , β' and β forms are depicted,

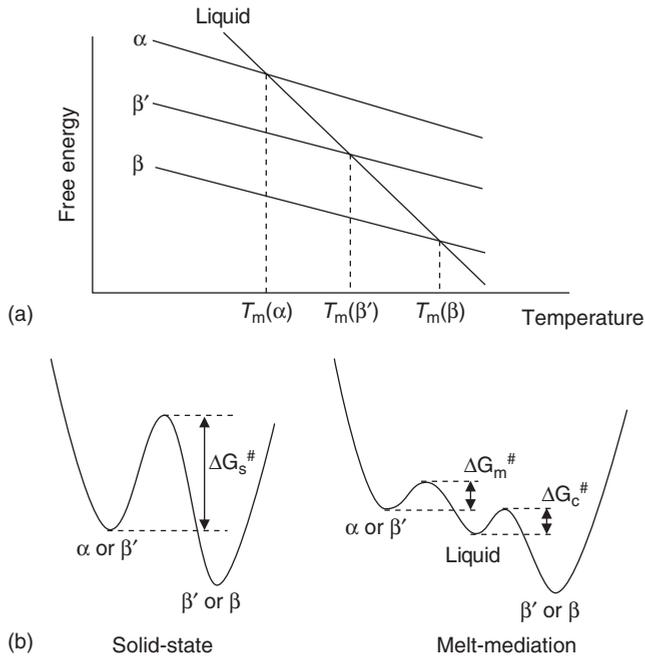


Figure 1.8 Schematic illustration of polymorphic transformations in TAG. (a) Thermal properties of three forms; (b) activation free energy barriers for solid state transformation and melt-mediated transformation.

as in Figure 1.8 (a). As each polymorphic form has its own melting temperature T_m , polymorphism having this property is called monotropism.

Solid-state transformation occurs when the metastable form of α or β' is stored below their T_m . The rate of solid-state transformation is basically determined by the magnitude of the activation free energy barrier $\Delta G_s^\#$, which may include the energies to enable the conversions in the subcell and chain length structures and other molecular structural changes, as illustrated in Figure 1.1.

By contrast, melt-mediated transformation occurs when α or β' forms are heated to the temperatures just above their T_m , where melting of α or β' is associated with the crystallisation of the more stable forms of β' or β . In this case, the rate of transformation is basically determined by the magnitude of activation free energy barriers of melting $\Delta G_m^\#$, and crystallisation $\Delta G_c^\#$, as depicted in Figure 1.8 (b). As one may expect that $\Delta G_m^\#$ is much smaller than $\Delta G_c^\#$, the rate of melt-mediated transformation may actually be governed by $\Delta G_c^\#$. $\Delta G_c^\#$ may include activation energies for nucleation and crystal growth of the more stable forms from the liquid which is formed soon after the melting of the less stable forms. We cannot simply compare the rates of solid-state transformation and melt-mediated transformation, as the factors included in $\Delta G_s^\#$ and $\Delta G_c^\#$ are quite different. However, it can be expected that heterogeneous nucleation of the more stable forms will reduce the values of $\Delta G_c^\#$, and thereby the melt-mediated transformation occurs more rapidly than that in the solid

state (Kashchiev and Sato, 1998). This property was observed for the polymorphic transformation in SOS (Ueno *et al.*, 1997). The effects of heating rates on the polymorphic transformation on the transformation pathways, either through solid-state or melt-mediation, were observed for POP polymorphs (Bayes-Garcia *et al.*, 2013a).

As for the application of the polymorphic transformations in the processing of food fats, so-called *tempering* corresponds to the melt-mediated transformation from α to β' in the case of margarine, fat spread and shortening, and from Form IV to Form V in the case of cocoa butter crystals in chocolate.

1.2.3.2 Molecular aspects of solid state transformation examined with SR- μ -XRD

Particular interest has been focused on the solid-state transformation mechanisms, including the variations in molecular orientation of the long-chain axes with respect to the lamellar plane and the subcell axes between α or β' and β . However, little information on molecular-level understanding of the transformation mechanisms has been available due to the difficulty of growing single crystals of TAGs, both for the metastable and stable polymorphs, unlike the cases of saturated fatty-acid crystals (Larsson *et al.*, 2006). Another interest concerning the solid-state transformation may involve the formation of spherulites of fats, which are the main causes of the deterioration of texture in fat-based products, for example, granular crystal formation in margarine, fat spread and chocolate (fat bloom). It was believed that the polymorphic transformation from β' to β is related to the formation of spherulites, but no microscopic information has yet been obtained to verify this.

We recently reported on the microstructure of spherulites of trilaurin, which are formed by crystallisation and solid-phase transformation, and which were measured by the synchrotron radiation microbeam X-ray diffraction method (SR- μ -XRD) (Ueno *et al.*, 2008). This was the first study involving the structural analysis of the texture of fat crystals using an X-ray microbeam method. Before this work, there had been no study using SR- μ -XRD in the research area of food science except for the study of the microstructure of starch (Buleon *et al.*, 1997; Chanzy *et al.*, 2006; Lemke *et al.*, 2004).

The basic principle of SR- μ -XRD technique relies on X-ray focusing optics and the synchrotron radiation X-ray source, enabling us to use highly brilliant X-rays and to generate an intense X-ray microbeam with a divergence small enough to perform X-ray diffraction studies. By scanning the X-ray microbeam on a thin section of the sample in two dimensions with steps on the order of the beam size, and by collecting each two-dimensional (2D) X-ray diffraction pattern with a 2D X-ray sensitive area detector, we can construct 2D images of a micrometer-dimension in real space.

In the study of spherulite analysis, we applied a microbeam small-angle X-ray diffraction (μ -SAXD) technique using an X-ray microbeam having a width of $5 \times 5 \mu\text{m}^2$ so that the lamellar planes of the fat crystals within a droplet could be clearly observed. Figure 1.9 illustrates the information obtained from a 2D μ -SAXD pattern from the fat crystals present in a 2D space of the microbeam area. The polymorphic structure can be determined by a lattice parameter by calculating the diffraction angles (2θ s) shown in Figure 1.9 (a), which is basically identical to the

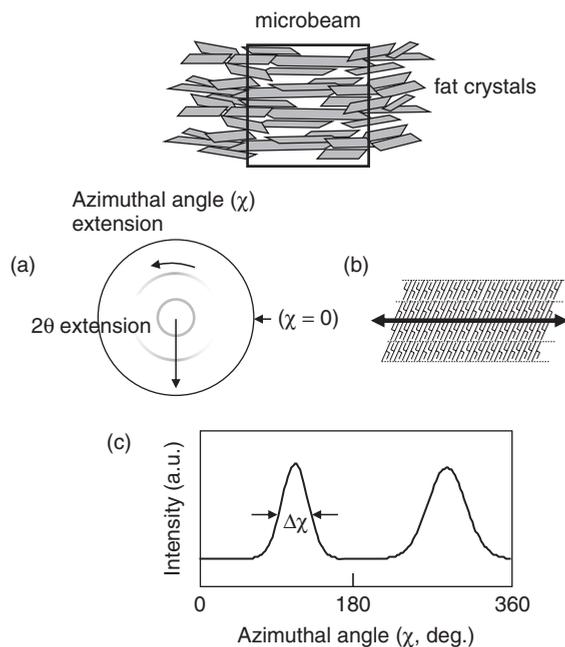


Figure 1.9 Relationships between lamellar plane direction noted by an arrow in (b) and the polymorphism of fat crystals and two-dimensional small-angle XRD patterns examined by SR- μ -XRD.

q-extension shown in Figure 1.6. When all the fat crystals are arranged in a highly ordered manner, two sharp 2D diffraction peaks (arc peaks) should appear. In this case, the average direction of the lamellar planes of the fat crystals is directed normal to the direction connecting the two arc peaks (Figure 1.9 (b)). When the fat crystals are randomly oriented, however, the 2D diffraction patterns must appear in almost all directions with equal intensity. In our previous study, we successfully used a scanning μ -SAXD method to analyze the spatial distribution of the lamellar planes on the fat crystals in spherulites by observing the occurrence and direction of lamellar planes expressed in sharp arc peaks. These properties are basically the same as those presented in Figure 1.6 to observe the effects of shear on fat crystallisation.

Figure 1.10 shows the results of optical and SR- μ -XRD analysis of the solid-state β' to β transformation occurring in the same spherulite of trilaurin grown within thin spaces between PET films (Ueno *et al.*, 2008). The following results were obtained. The two-dimensional spherulites of β' were composed of nanometer-sized crystals in which the lamellar planes were oriented parallel to the radial direction of the spherulites, except for the center position. At the center position, the lamellar planes of the first-occurring crystals are oriented randomly, indicating that numerous crystal nuclei of β' form are formed with random orientation at the first stage of spherulite formation. It was also verified that, following the solid-state transformation from β' to β , the orientations of the long-chain axes of the β form remained unchanged with respect

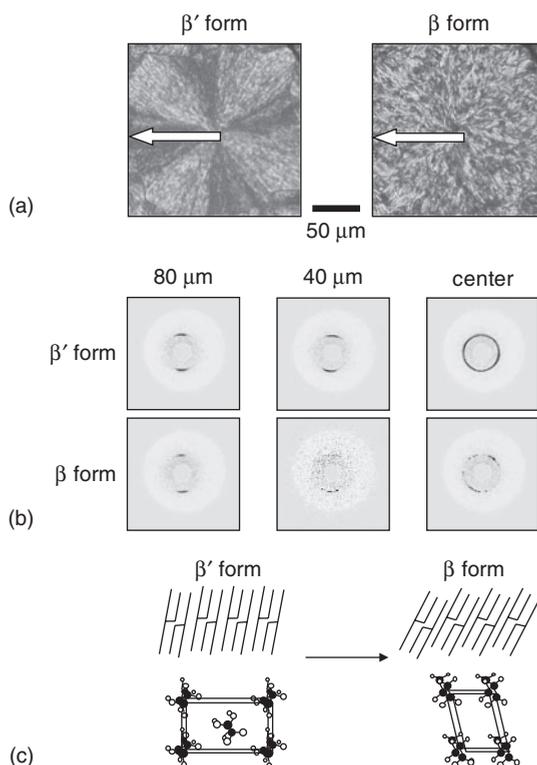


Figure 1.10 (a) Polarised optical micrographs of spherulite crystals of β' and β forms of trilaurin; (b) small-angle SR- μ -XRD patterns taken at the positions from the centre to left directions noted by arrows in (a) of the same spherulite before and after the β' to β transformation; (c) variation in lamellar structures and subcell structures during solid-state $\beta' \rightarrow \beta$ transformation.

to those of the β' form. This suggests that the molecular arrangements of trilaurin during the $\beta' \rightarrow \beta$ polymorphic transformation occurred through template effects of the lamellar structures of the mother phase of β' as shown in Figure 1.10 (c).

1.2.4 Phase behaviour of fat mixtures

Naturally occurring fats and lipids are mixtures of different types of TAG. The complicated behaviour they exhibit with regard to melting, crystallisation and transformation, crystal morphology and aggregation are partly a result of the physical properties of the component TAGs (discussed above) and, more importantly, partly a result of the phase behaviour of the mixture. To resolve this complexity in mixed-fat systems, a fundamental study of the binary and ternary mixtures of specific TAG components is necessary (Rossel, 1967).

Three typical mixture phases occur in binary solid mixtures of fats in the case where the two components are miscible, for all concentration ratios, in the liquid state.

These are: solid solution phase, eutectic phase and compound formation. For TAG mixtures, two factors affect the mixing phase behaviour simultaneously: chain–chain interactions and polymorphism. Chain–chain interactions are influenced by the chemical nature of the component TAGs varying with chain length (N_c), the saturation or unsaturation and the isomeric conformation (*cis* or *trans*) of the unsaturated chains. The effect of polymorphism is revealed in the formation of miscible and eutectic phases, miscible mixtures being formed with less stable polymorphs (the α and β' forms) and eutectic phases tending to occur with the stable polymorph (the β form). In addition, differences in chain-length structure (DCL or TCL) affect the mixing systems, the formation of the miscible phase for fats with different chain-length structure being prohibited. Three examples illustrate the effects of polymorphism and structure on the binary mixture behaviour of TAGs (Table 1.3).

In the mixture of saturated mono-acid TAGs, a eutectic phase with a limited region of miscible phase was formed for the stable polymorph when the difference in N_c is no greater than 2, as shown for the mixture of PPP and SSS. However, a miscible mixture was formed for the α and β' polymorphs in the mixture of PPP and SSS. This means that, when the mixed PPP and SSS liquid is chilled to form the α or β' form, and when further polymorphic transformation into the β form is induced, the mixture changes from being miscible to separated. This was clearly demonstrated by an *in-situ* X-ray diffraction measurement by using a synchrotron radiation X-ray beam (Kellens *et al.*, 1991). The same results were observed in the mixtures of PPP, MMM and LLL (Takeuchi *et al.*, 2003). These results indicate the importance of the effect of polymorphism on the mixture system and must be kept in mind when one is seeking an optimal blend of TAGs for use in food where the phase separation of fats is not preferred.

The formation of molecular compound crystals has been observed in mixtures of saturated/unsaturated mixed-acid TAGs. The formation of a molecular compound may be viewed as a special case of eutectic mixing systems that occurs as a result of specific molecular interactions between the component TAGs. Two examples are shown in Table 1.3: for an SOS/OSO mixture (Koyano *et al.*, 1992) and for a POP/PPO mixture

Table 1.3 Typical binary mixing behaviour of triacylglycerols (TAGs).

TAG polymorphic form ^a	Mixing phase and polymorphic form
PPP α -2, β' -2, β -2	Miscible phase: α -2 and β' -2
SSS α -2, β' -2, β -2	Eutectic phase: β -2
PPP α -2, β' -2, β -2	Eutectic phase
MMM α -2, β' -2, β -2	
SOS α -2, γ -3, β' -3, β_2 -3, β_1 -3	Compound formation: α -2, β' -2, β -2
OSO α -2, β' -2, β -3	
POP α -2, γ -3, β' -3, β_2 -3, β_1 -3	Eutectic phase
OOP α -3, β' -3	

Note: PPP, tripalmitoylglycerol; SSS, tristearoylglycerol; MMM, trimyristoylglycerol; SOS, 1,3-distearoyl-2-oleoyl-*sn*-glycerol; OSO, 1,3-dioleoyl-2-stearoyl-*sn*-glycerol; POP, 1,3-dipalmitoyl-2-oleoyl-*sn*-glycerol; OOP, 1,2-dioleoyl-3-palmitoyl-*rac*-glycerol.

^aSuffixes 2 and 3 refer to double and triple chain-length structures, respectively.

(Minato *et al.*, 1997). In both mixture systems, a common result was obtained in that the polymorphic behaviour was largely different for the component TAGs, yet the molecular compound was formed for all three polymorphic forms. It is worthy of note that all of the molecular compounds were packed in the DCL structure, although DCL and TCL structures are revealed in the component TAGs. A mechanistic treatment has been carried out on the formation of molecular compound systems of SOS/OSO and POP/PPO, taking into account chain–chain interactions (Kaneko *et al.*, 1998; Sato *et al.*, 1999). The significance of the formation of a molecular compound in food applications is that the rate and extent of the β' – β transformation in molecular compound systems are remarkably higher than those occurring in the component TAGs.

In this regard, quite interesting results were observed in the binary mixtures of SOS-OOS and POP-OOP, which are the mixtures of symmetric saturated-oleic-saturated mixed acid TAGs (SOS and POP) and asymmetric oleic-oleic-saturated mixed acid TAGs (OOS and OOP) (Zhang *et al.*, 2007, 2009). The two mixtures showed the same results as summarised in the following.

Thermal and X-ray diffraction experiments on binary mixtures of SOS-OOS and POP-OOP exhibited immiscible monotectic or peritectic mixing behaviour. The differences between the SOS-OOS and POP-OOP mixtures were in the polymorphic behaviour of the fractions of POP and SOS. No difference was found in the mixing behaviour between optically active (*sn*-OOS) and racemic mixture (*rac*-OOP) as an asymmetric oleic-oleic-saturated acid TAG. From the two results, it was concluded that an immiscible phase was formed in the binary mixtures of symmetric saturated-oleic-saturated TAGs and asymmetric oleic-oleic-saturated TAGs, both for racemic and optically active molecules of asymmetric oleic-oleic-saturated TAGs. This result stands in contrast to mixtures of SOS-OSO, SOS-SSO, POP-OPO and POP-PPO, all of which exhibited molecular-compound-forming behaviour with molecular compound crystals in an equal ratio to the binary mixtures. Molecular-level mechanisms to explain this difference are discussed, based on the possible roles of glycerol groups acting during the mixing processes of saturated-unsaturated mixed-acid TAGs.

Figure 1.11 presents a hypothetical structural model of a molecular compound of SOS and *sn*-OOS, though such mixing behaviour was not observed. Figure 1.11 illustrates that there would be difficulty either in arranging the glycerol groups or with lateral chain packing of stearic and oleic acid moieties, if we draw the molecular compound crystal as a double-chain-length structure. In model (1), the arrangements of the glycerol groups may have a discrepancy in the directions of the glycerol groups, as denoted by arrows, although the oleic acid and stearic acid moieties are packed in separated leaflets. In model (2), however, the aliphatic chain packing may have steric hindrance because of the coexistence of stearic acid and oleic acid chains in each leaflet, though the glycerol groups are well arranged. To conclude, it is difficult to construct a molecular compound crystal with SOS and *sn*-OOS, and therefore eutectic mixing behaviour was observed for SOS-OOS and POP-OOP mixtures.

The effects of chain length, saturation and unsaturation of fatty-acid moiety, and glycerol structure on the mixing behaviour of TAGs are summarised in Figure 1.12. The mixing properties thus summarised may provide important indications of the fat

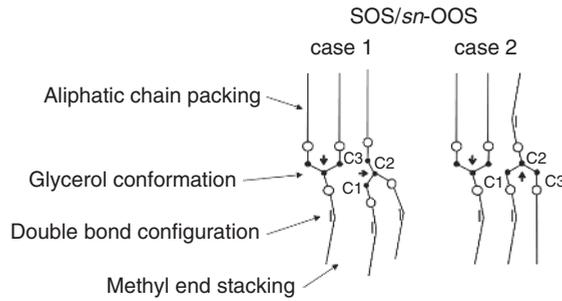


Figure 1.11 Structural models illustrating glycerol groups of a hypothetical molecular compound structure of SOS-*sn*-OOS.

	SSS-SOS	SSS-PPP (β)	SSS-MMM	POP-OOP
Eutectic				
Miscible	SOS-SLS	SSS-PPP (α, β')		
Molecular compound	SOS-OSO			

Figure 1.12 Note: For abbreviations, see Table 1.3.

blending of edible and confectionery fats, and the separation of high-melting and low-melting fractions of natural semi-solid oils such as palm oil and milk fat.

It should be noted that the formation of a molecular compound in the binary mixture of POP-OPO was observed, not only in the neat liquid but also in the diluted n-dodecane solution (Ikeda *et al.*, 2010).

1.2.5 Microstructure, texture and rheological properties

One of the most important macroscopic physical properties of food fats is the rheology, affecting the spreadability of margarine and other spreads, the ‘snap’ of chocolate and the smoothness, mouth feel and stability of bulk fats and emulsion products

(de Man, 1999; van den Tempel, 1961). In addition, control of rheological properties is necessary in the production processes in a factory.

The rheological properties of food fats are determined by many factors that can be grouped into two categories (Marangoni and Hartel, 1998):

- internal factors, involving the molecular compositions of fats (TAGs, ingredients and additives), the polymorphism of crystals of the constituent TAGs and the microstructure of fat crystals (morphology, crystal size distribution and crystal network formation);
- external processing conditions, involving temperature variation, shear, flow velocity, and so on.

Of the internal factors, the microstructure of the fat crystals greatly influences the rheological properties (Marangoni, 2005; Marangoni *et al.*, 2012).

Much progress has been made by Marangoni and his colleagues on the analysis of fat crystal microstructure. These workers discussed the macroscopic physical properties of food fats in terms of formation and internal assembly of fat microstructures, the rheological properties of the final products being assessed by conventional analytical methods such as thermal measurements, and the determination of solid fat content (SFC) and turbidity. One aspect of their research was focused on the hardness of chocolate made with Salatrim[®] (a trademark of Pfizer), which is much softer than chocolate made with cocoa butter (Narine and Marangoni, 1999). This phenomenon of hardness is caused by differences in the microstructure of chocolate fats. The TAGs involved in Salatrim[®] consist of asymmetric molecules (C₃-C₃-C₁₈) that result in strong repulsive interactions between TAG molecules in the crystal and induce platelet structures. In contrast, cocoa butter consists of symmetric TAGs such as POP and SOS that pack together densely to make tightly packed crystals. Hence, macroscopic structure is a consequence of the interaction of microstructures and is thus affected by microstructure characteristics. In the case of Salatrim[®], a random macrostructure is formed because there are few attractive interactions between the constituent microstructures, thus the macroscopic structure is weak. In the case of cocoa butter, a strong three-dimensional crystal network is formed.

The effects of chemical interesterification on crystallisation, SFC, fat microstructure and rheological properties of various fat blends have also been examined quantitatively with a framework of fractal concepts by Marangoni and co-workers (Marangoni and Rousseau, 1998a, 1998b; Rousseau *et al.*, 1998; Tang and Marangoni, 2006).

Most recently, cryo-transmission electron microscopic (cryo-TEM) observation was carried out on edible fat crystals (Acevedo and Marangoni, 2010a, 2010b; Acevedo *et al.*, 2011). Blends of tristearoylglycerol (SSS) and trioleoylglycerol (OOO) were prepared in proportions between 20 and 100% w/w to achieve a wide range of supersaturations. Spherulites of SSS were formed by cooling of the molten fat blends, and subjected to mechanical disruption using isobutanol at 10°C for visualisation with the cryo-TEM process. Spherulite structures were broken down into their primary crystals of nanoplatelets of approximate sizes of 150 × 60 × 30 nm³ to 370 × 160 × 40 nm³

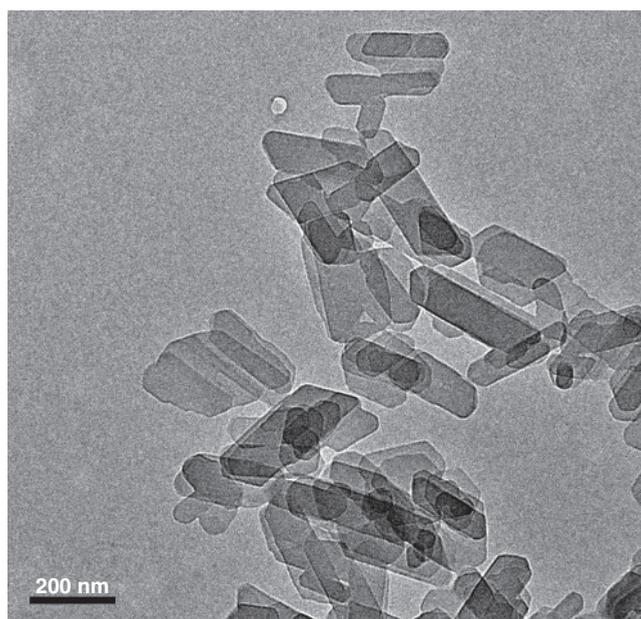


Figure 1.13 Primary crystal particles of SSS in OOO taken with cryo-TEM observation. *Source:* N. C. Acevedo. Reproduced with permission of Dr. N. C. Acevedo, Iowa State University, Ames, IA, USA.

depending on supersaturation conditions (Figure 1.13). This method also allowed the visualisation of bimolecular triacylglycerol lamellae within a cross-section of a nanoplatelet. Acevedo and Marangoni claim that their work opens up the possibility of nanomanipulation of the microstructure of fats to target specific physical properties which govern the texture and rheological properties of edible fats.

1.3 Structure–function relations in food fats

In this section we discuss how the fat structures discussed above influence the macroscopic functions of foods in bulk, in oil-in-water (O/W) emulsion states and in water-in-oil (W/O) emulsion states, looking at specific example materials for each case.

1.3.1 *Fats in bulk phase*

First, we discuss the physical properties of fats in a bulk phase, taking cocoa butter in chocolate as a model. Cocoa butter forms a continuous fat phase in chocolate, with small particles of sugar, cocoa mass, milk powder (in milk chocolates) and other ingredients, including food emulsifiers, dispersed within it. Compositional effects on rheological and textual qualities in chocolate are largely affected by fats together with sugar, milk and other dairy components and emulsifiers. In particular, sensory

perception of chocolate is determined by the melting behaviour of cocoa butter, which is affected by the extent of crystallisation of the cocoa butter at ambient temperature, and by the formation of O/W emulsion through phase inversion from W/O emulsion with saliva within the mouth (Afoakwa *et al.*, 2007).

1.3.1.1 Crystallisation processes

In the preparation of most commercial-grade chocolates, the temperature treatment of the sample, called tempering, is varied in the manner shown in Figure 1.14, after the mixing, blending, grinding and conching processes. In the following we will describe the events occurring at each stage of tempering in which the extent and polymorphism of cocoa butter crystals are most critical. There are six polymorphs of cocoa butter: Forms I to VI (Wille and Lutton, 1966). The polymorphism of the cocoa butter crystals described above is of Form IV (β' type) and Form V (β type); fat bloom is caused by the transformation from Form V to Form VI. Note that both are of a β -type polymorph. The snapping, appearance and demoulding properties of chocolate are best when the cocoa butter crystals are of Form V (β type), but the formation of fat bloom is a serious problem and is a result of the growth of needle-like crystals after the transformation to and crystal growth of Form VI.

- Stage A (cooling period): This consists of the nucleation and crystal growth of metastable forms of cocoa butter as a result of heterogeneous interactions between cocoa butter molecules and preexisting high melting fats. The metastable forms such as Form IV cause fat bloom if no further tempering is applied.
- Stage B (reheating): This comprises the transformation from the metastable forms to the stable form (Form V) of cocoa butter crystals by raising the temperature. The Form V crystals dominantly formed at this stage serve as seed crystals, leading the rest of cocoa butter liquid to crystallise in Form V. Transformation and further crystal growth of cocoa butter in Form V are accelerated by shear force operated in the tempering machine (Ziegleder, 1985; MacMillan *et al.*, 2002).
- Stage C (cooling): crystal growth of Form V develops, with the consequence that shrinkage of the cocoa butter occurs so that demoulding is enabled. Rheological properties at this stage are extremely important in producing chocolate bars or indeed any other enrobed chocolates,² the critical step being delicately determined by the solid fat content of the fat.
- Stage D (storage): storage of chilled chocolate enables stabilisation of the fat crystal network and size distribution.

Various techniques have been applied to control the crystallisation and transformation processes of cocoa butter. Blending of different fats with cocoa butter, in

²Enrobing is a process involving covering nuts or baked snacks, for instance, with chocolate. In this process, stabilisation of the chocolate viscosity is critical in ensuring the chocolate coating is of uniform thickness.

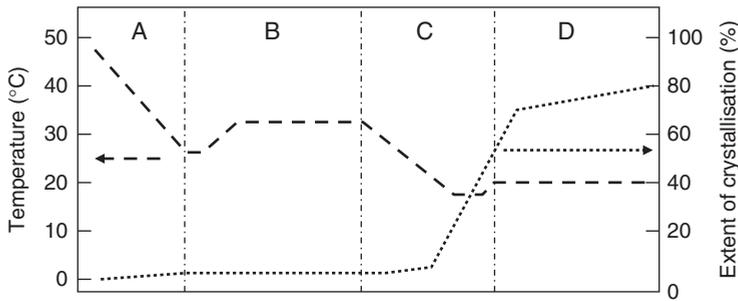


Figure 1.14 Temperature variation with time during chocolate production.

accordance with regulations for blending (which differ from one country to another) is one of the main ways to modify the melting and crystallisation properties of chocolate fats (Timms, 1980, 2003; Faulkner, 1981; Hogenbirk, 1984; Uragami *et al.*, 1986, Koyano *et al.*, 1993; Ali and Dimick, 1994; Sabariah *et al.*, 1998; Narine and Marangoni, 1999). Emulsifiers are added to chocolate fats to modify the rheological properties so that interactions between fat crystals, hydrophilic particles such as sugar and milk powders and water phases are mediated through the emulsifiers (Katsuragi, 1999). The emulsifiers also affect fat bloom stability, acting at growing crystal surfaces of cocoa butter (Aronhime *et al.*, 1988).

The addition of high-melting fats having the same crystal structure as Form V, namely form β_2 of 1,3-dibehenoyl-*sn*-2-oleoyl-glycerol (BOB), is quite effective in crystallising cocoa butter in Form V without the need for tempering (crystal seeding; Hachiya *et al.*, 1989a, 1989b; Koyano *et al.*, 1990). This property was employed to produce enrobing chocolate at temperatures as high as 38 °C at which the viscosity of the chocolate was so decreased that enrobing conditions became very stable. The addition of high-melting fractions of cocoa butter, particularly SOS β crystals, is also employed as a crystal seeding technique. In this case, the temperature at which the seed crystals are added to the molten chocolate liquor should not be as high as those for the case of BOB seed crystals, because of the lower melting temperature of SOS crystals.

External forces, such as shear stress and ultrasound irradiation, are also effective in modifying the crystallisation rate and the polymorphic transformation of cocoa butter, as already described above.

1.3.1.2 Fat bloom problems

Even after the whole production process has been completed, the fat bloom phenomena may arise, causing further problems (Padley, 1997). This can become a serious problem if, during the winter, the chocolate is stored in warehouses, sales outlets or in the home of the consumer, where temperatures can drop to extremely low levels. Similarly, during the summer, temperatures may rise considerably.

Without a doubt, fat bloom is an unwanted phenomenon, which causes serious deterioration of the surface as well as the inner texture of chocolate. Fat bloom frequently occurs when chocolate is stored over a long period at an elevated temperature (Sonwai

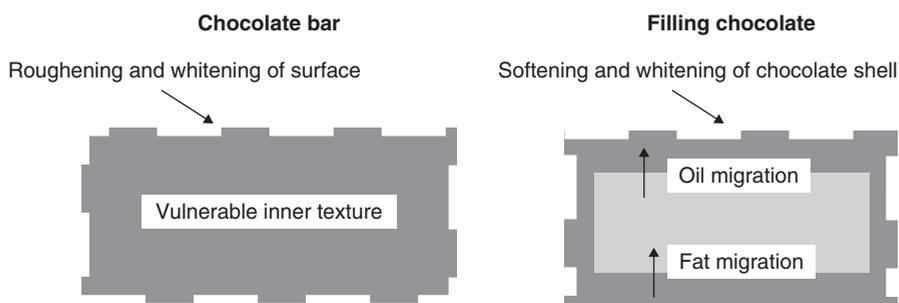


Figure 1.15 Fat bloom phenomena in chocolate.

and Rousseau, 2006) or when optimal tempering was not applied during the production of the chocolate (Lonchampt and Hartel, 2006). Two types of fat bloom are well known, depending on whether chocolate includes an oily filling in it (filling chocolate) or not (chocolate bar), as depicted in Figure 1.15. In both cases, fat bloom makes the chocolate surface white due to light scattering (Briones and Aguilera, 2005), and the inner texture vulnerable due to coarsening of the cocoa butter crystals. Therefore, not only a deteriorated physical appearance but also a sandy taste and worse organoleptic feeling are caused by fat bloom. Many factors are involved in the formation processes of fat bloom (Timms, 2003; Lonchampt and Hartel, 2004) and there is not sufficient space to fully discuss them in this section. Instead, some remarks will briefly be mentioned about fat bloom in terms of crystallisation and polymorphic transformation of cocoa butter crystals.

Common properties of fat crystals in bloomed chocolate may be summarised as follows:

1. Polymorphic transformation from Form V to Form VI of cocoa butter occurs in tempered chocolate. In the case of incompletely tempered chocolate, the transformation from Form IV to Form V also causes fat bloom.
2. Recrystallisation including the polymorphic transformation increases the average size of the cocoa butter crystals from sub- μm (normal chocolate) to tens of μm (bloomed chocolate). As a result, the physical appearance becomes worse and the sharp melting feature disappears.

An interesting observation was made using an atomic force microscope (Rousseau, 2006), that, when the chocolate was stored at temperatures at $25\sim 27^{\circ}\text{C}$, porous surface structures were revealed and crystal growth occurred in the vicinity of the pores. Rousseau indicated a relation of the presence of pores in cocoa butter crystal network in chocolate to the fat bloom formation.

As to the fat bloom in filling chocolate, the migration of liquid oil from inside to outside the chocolate shell and also the migration of the cocoa butter into the filling core occur at the same time (Smith *et al.*, 2007). As the oil content increases by

migration into the chocolate, recrystallisation of cocoa butter is promoted, causing serious fat bloom. The driving forces for such migration processes come from the difference between the melting points of liquid oils included in the filling and the cocoa butter (Khan and Rousseau, 2006). Then the rate of oil migration-driven fat bloom is influenced by the rate of diffusion of liquid oil in chocolate, the interfacial area of the contact of filling with chocolate, the crystallinity of the cocoa butter crystals in chocolate, and the overall rate of recrystallisation of the cocoa butter.

Methods to retard fat bloom formation are, in general, summarised as follows:

1. Decrease the storage temperature (Depypere *et al.*, 2009).
2. Select oil components in the filling so that the driving force for oil migration is minimised.
3. Increase the perfection of the cocoa butter crystal network to avoid the formation of defects through which liquid oil may migrate.
4. Use cocoa butter equivalent (CBE) to retard the recrystallisation rate of the chocolate fats.
5. Use food emulsifiers which may promote cocoa butter crystallisation so that tiny and well-dispersed crystals are formed and retard the polymorphic transformation of cocoa butter and the associated re-crystallisation (Lonchamp and Hartel, 2004).

In addition, stability against fat bloom can be improved by fat blending (Lohman and Hartel, 1994; Tietz and Hartel, 2000; Timms, 2003). The effects of the addition of milk fat on the retardation of fat bloom formation have been well known for a long time, but the exact mechanism is still open to question. The true mechanism that underlies the effect of bloom inhibition may involve interactions between the various TAGs involved in milk fats and the polymorphic nature of cocoa butter, and some other components such as minor lipids. It has been reported that the high-melting fraction in milk fat exhibits an anti-bloom effect (Kaylegian, 1997), and that the lipid content, in particular polar lipids such as diacylglycerols and phospholipids, involved in milk fat can have a significant effect on the retardation of fat bloom (Tietz and Hartel, 2000). Bricknell and Hartel (1998) also studied the effects of chocolate microstructures with respect to sugar particles on fat bloom formation. Whatever components are found to improve anti-bloom properties, a microscopic understanding of the fat bloom processes is needed (Sato and Koyano, 2001).

1.3.2 *Fats in oil-in-water emulsions*

O/W (i.e. water-continuous) emulsions in food are observed in the main body of whipped cream, ice cream, coffee cream, and so on, and their production processes involve pre-emulsification, homogenisation, pasteurisation, rehomogenisation and cooling. Although some details differ from one product to another, the most preferable physical properties of O/W emulsions containing a fat phase are optimal melting and solidification properties, emulsion stability when chilled during storage and during

the final usage stage, and a good crystal morphology and network exhibiting optimal rheological and whipping properties. Partial coalescence of oil droplets is one of the prerequisites for making whipped cream and ice cream. Many factors are involved in the partial coalescence of O/W emulsion droplets, and fat crystallisation is the key to them (Frederick *et al.*, 2010). The interactions of emulsifiers and proteins in the oil and water phases are of immense significance but are beyond the scope of this chapter.

The crystallisation of solid fats in O/W emulsion droplets influences the stability, rheology and appearance of emulsions (Boekel and Walstra, 1981; Boode *et al.*, 1991; Dickinson and McClements, 1996, Thanasukarn *et al.*, 2004, 2006). Furthermore, fat crystallisation largely influences the flavour release (Ghosh *et al.*, 2006, 2007) and the digestibility of lipids (Mun *et al.*, 2007). Therefore, it is important to analyse the fat crystallisation processes in O/W emulsions. The rate and extent of crystallisation, the effect of polymorphism and emulsifiers, the influence of emulsion droplet size and droplet–droplet interactions and the effect of rate of cooling and subsequent temperature history on fat crystallisation behaviour must be clarified (Povey, 2001; Coupland, 2002).

The elucidation of these complicated crystallisation processes in O/W emulsions can be achieved through two-step studies: (1) by monitoring *in situ* the crystallisation process under a well-defined simple model; (2) by extending this model to more complicated systems containing polymorphic fats under varying temperature treatments, and so on.

For this purpose, ultrasonic velocity measurement has been employed to monitor *in situ* crystallisation processes (Dickinson *et al.*, 1991; Gulseren and Coupland, 2007a, 2007b) based on the principle that the event of crystallisation of a liquid oil phase dispersed in a water phase can be monitored by means of the ultrasonic sound velocity, which increases as the transformation from the liquid to the solid phase progresses. In addition, DSC is also effective for monitoring fat crystallisation in O/W emulsions (Katsuragi *et al.*, 2001).

Our recent studies on the kinetic properties of the nucleation processes of palm oil, palm mid-fractions (PMFs) and palm kernel oil in O/W emulsions have shown a remarkable acceleration when highly hydrophobic food emulsifiers such as sucrose fatty-acid oligoesters (SOEs) and polyglyverine fatty-acid esters (PGFEs) are added in the oil phase (Awad and Sato, 2003; Sakamoto *et al.*, 2004; Arima *et al.*, 2007; Arima *et al.*, 2009). Furthermore, the simultaneous addition of two types of SOE additives having hydrophobic and hydrophilic properties to the PMF emulsion remarkably improved the emulsion stability, when the PMF was crystallised in the droplets and fat-crystallised emulsion was stored at a chilled temperature over a long period (Arima *et al.*, 2009). In addition, SR- μ -XRD analysis has unveiled that the texture of the PMF crystals in an emulsion droplet changed with the SOE additive compared with the droplet without the additive, as summarised in the following (Arima *et al.*, 2009).

Scanning small-angle SR- μ -XRD experiments were performed by irradiating a synchrotron radiation X-ray microbeam having a width of $5 \times 5 \mu\text{m}^2$ at different positions in a $50 \mu\text{m}$ -diameter emulsion droplet after the crystallisation of PMF by chilling the emulsion at 5°C . Every SR- μ -XRD pattern was recorded with a two-dimensional

(2D) detector, which enabled spatial analysis of polymorphic structures and the orientation of lamella planes of PMF crystals at different positions inside the emulsion droplet. Particular attention was paid to compare the crystallisation behaviour of the PMF in two types of emulsion droplets: hydrophilic polyoxyethylene sorbitan monooleate (Tween 80) alone (Tween 80 emulsion), and Tween 80 and hydrophobic sucrose palmitic acid oligoester (P-170) (Tween 80+P-170 emulsion). The DSC study revealed that the crystallisation temperature of PMF in the Tween 80+P-170 emulsion droplets increased by 3°C compared to the Tween 80 emulsion because of the effects of the P-170 additive in promoting the crystallisation of PMF in the emulsion droplets.

The SR- μ -XRD studies revealed the following results:

1. The lamella planes of the PMF crystals near the outer edges of the droplet in the Tween 80+P-170 emulsion were mostly parallel to an oil-water interface, whereas the lamella planes of the PMF crystals were not always aligned with the oil-water interface in the Tween 80 emulsion droplet.
2. The degree of orientation of the lamellar planes of the PMF crystals, which were evaluated from the values of full width at half maximum of SR- μ -XRD patterns with respect to the χ -extension ($\Delta\chi$, see Figure 1.6 (c)), was remarkably higher in the Tween 80+P-170 emulsion than in the Tween 80 emulsion.
3. Polymorphic transformation from α to β' of PMF in the Tween 80+P-170 emulsion was retarded, compared to that in the Tween 80 emulsion.

The conclusion (1) was drawn from the χ -extension patterns acquired at the pixel positions in the two droplets of Tween 80 emulsion and Tween 80+P-170 emulsion noted by A through H, both of which are placed more or less equidistantly along the circles near the oil-water interface (Figure 1.16). In the case of the Tween 80+P-170 emulsion droplet, every pixel had single or double peaks, and the double peaks included sub-peaks separated by 50° at most. Furthermore, the differences in the χ values of the χ -extension peaks corresponded well with the differences in the positions of every pixel in the anticlockwise χ direction, except for pixel G. This tendency is clearly indicated by the arrows in Figure 1.16 (b), as the χ values of the peaks increase straightforwardly as the position moves from A to H except for pixel G. By contrast, the χ -extension patterns from the Tween 80 emulsion droplets showed broader peaks and no correlation between the χ values of the χ -extension peaks with the differences in the positions in the droplet, as shown in Figure 1.16 (a).

From these results, it was confirmed that the P-170 additive caused interfacial heterogeneous crystallisation (Krog and Larsson, 1992) through hydrophobic interactions at the oil-water interfaces in the emulsion, which influenced the arrangements of fat crystals so that the lamellar planes of fat crystals were parallel to the oil-water interface (Figure 1.17). Thus, crystallised PMF emulsion droplets were stabilised against partial coalescence due to the irregular morphology of the fat crystals in the droplet, which may occur in the droplets without the additives.

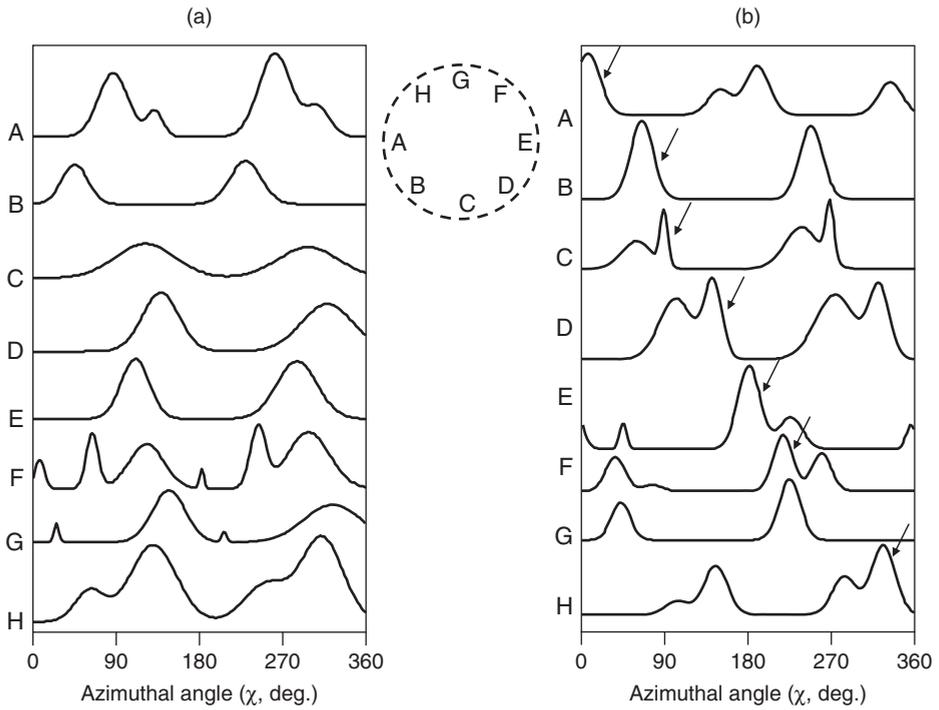


Figure 1.16 χ -extension of the SR- μ -XRD patterns of PMF crystals in emulsion droplets. (a) eight positions of the Tween 80 emulsion droplet; (b) eight positions of the Tween 80+P-170 emulsion droplet.

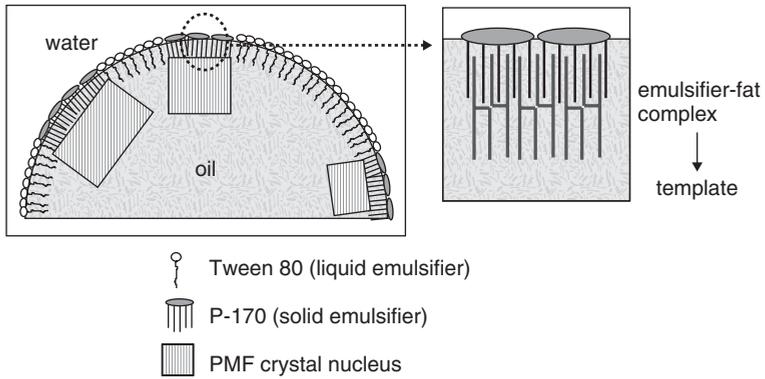


Figure 1.17 A model of interfacial heterogeneous crystallisation of fats in O/W emulsion with the additives.

1.3.3 Fats in water-in-oil emulsions

Margarine and spreads are typical food fats found in the form of W/O emulsions and consist of vegetable fats and oils. The optimal functional, and by implication, physical properties required of margarine and spreadable fats are spreadability, plasticity and consistency (de Man, 1983; de Man *et al.*, 1992; Bot *et al.*, 2003; Chrysan, 2005). Stabilisation of water droplets against coalescence and water-oil separation is also very important (Rousseau and Hodge, 2005; Ghosh and Rousseau, 2009; Rousseau *et al.*, 2009). For this reason, semisolid fats are to be preferred, with optimal SFC values of 50%–60% at around 5°C, which gradually decrease with increasing temperature, until complete melt at about 38°C. Furthermore, special texture and spreadability are needed for margarine for industrial uses, such as the roll-in type used in bakeries. Fat blending of high-melting-point (40–55°C), medium-melting-point (20–40°C) and low-melting-point (< 20°C) fats has been tried, and it has been found that the medium-melting-point fats play an important role, exhibiting optimal spreadability for margarine.

The preferable polymorphic form of margarine or spreadable fats is β' , since β' crystals exhibit a very fine crystal network which comprises two types of particle–particle interactions: primary and secondary (de Man, 1982; Naguib-Mostafa *et al.*, 1985). Primary interactions with strong binding forces form a three-dimensional crystal network throughout the continuous fat phase. Secondary interactions result in small crystals with weaker binding forces. The transformation from the β' to the β form causes serious deterioration, giving a sandy texture, hardening, reduced spreadability and oil–fat separation and coalescence of water droplets (emulsion instability) in extreme cases. These properties result from, in part, granular crystals of the β form, which tend to grow with rectangular needle morphology (Bennema *et al.*, 1992).

In connection to the fats to be employed for margarine and fat spread, *trans* fat alternative issues should be discussed. Due to health claims, reduction of the use of *trans* fats, which are produced by partial hydrogenation of vegetable oils, is required. From the point of view of physical and chemical functionality, however, *trans* fats possess such advantageous properties as a fine crystal network, easy control of melting range, and high stability against oxidation. The fats which are employed as *trans* fat alternatives (Kodali and List, 2005), must possess these properties to be employed for frying oil, margarine, fat spread, shortening, confectionery, etc. The technologies for the production of *trans*-free fats or low-*trans* fats are the combination of esterification and full-hydrogenation of vegetable oils, the separation of semi-solid oils such as palm oil and palm kernel oil, the esterification of semi-solid oil, the use of high-melting emulsifiers, the use of wax crystals, etc. In particular, palm oil may be a promising resource for the *trans* fat alternative materials (Smith, 2001; Aini and Miskandar, 2007). Despite numerous efforts, however, there still remain many disadvantageous problems in *trans* fat alternative technologies, one of which is the formation of granular crystals during long storage of the end products.

Figure 1.18 shows the formation of granular crystals in a model margarine containing the oil and fat phase (70% in total), including fully-hydrogenated rapeseed oil (2 wt.%), soybean oil (48 wt.%) and palm oil (20 wt.%). It is evident that granular

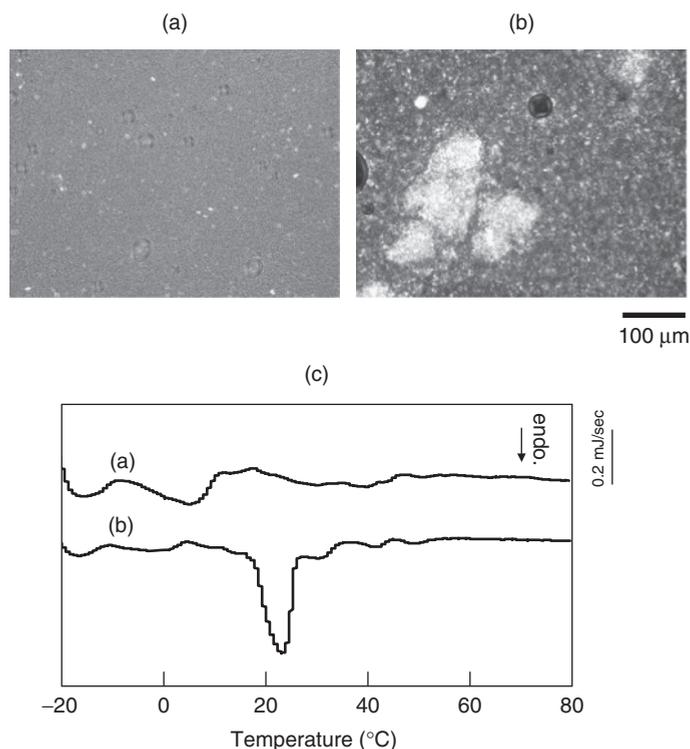


Figure 1.18 Polarised optical microscope images of palm oil-blended margarine. (a) 1 day after keeping at 5°C; (b) 5 days after thermal thawing; and (c) corresponding DSC heating thermopeaks.

crystals grew over 5 days under thermal thawing between 7°C and 15°C for every 12 hours, and the DSC heating thermogram of the granular crystal portions showed a large melting peak around 23°C (Tanaka *et al.*, 2009). Conventional XRD study of the granular crystals showed that β -fat crystals of a TCL structure melted below the melting of β' -fat of a DCL structure. Such granular crystals cause a sandy taste and bad physical appearance of the end products, and formation of such crystals should be avoided.

The formation mechanism of granular crystals in fat blends similar to those of margarine fats containing palm oil and other vegetable oils was analysed at the polymorphic level (Miura and Konishi, 2001; Tanaka *et al.*, 2007; Watanabe *et al.*, 1992). Although palm oil is categorised as a β' -tending fat, fats containing palm-oil fractions show formation of granular crystals in long-term storage. Chemical and physical analyses of TAG compositions, polymorphism and melting points of the granular crystals led to the conclusion that the granular crystals are of β polymorph of POP (1,3-dipalmitoyl-*sn*-2-oleoyl-glycerol). As shown in Figure 1.19, an SR- μ -XRD study showed that the fat crystals of the DCL structure were observed in the granular crystals (region A). However, the fat crystals of the DCL and TCL structures were simultaneously observed

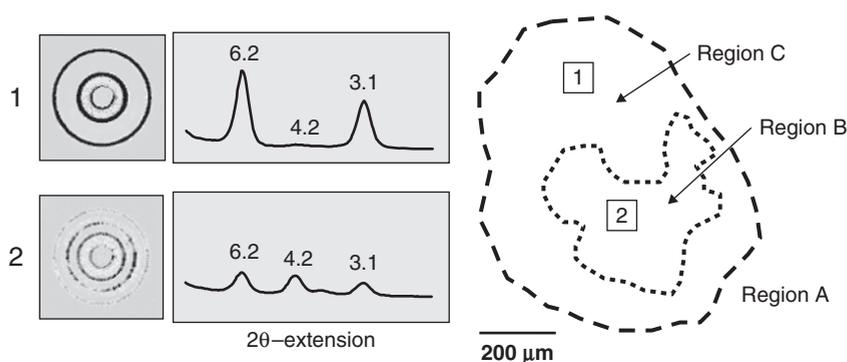


Figure 1.19 SR- μ -XRD patterns taken at two positions in a granular crystal formed in palm-based margarine. Note: Unit nm.

in the central region of a granular crystal (region B, position 2 long spacing values of 6.2 nm and 4.2 nm and 3.1 nm), whereas the fat crystals of the TCL were predominantly present in an outer region of a granular crystal (region C, position 6.2 nm and 3.1 nm). From these results, the microstructures and the formation processes of granular crystals are closely related to the fractional crystallisation of the β form of POP promoted by crystallisation and transformation of PPP and SSS fractions (Tanaka *et al.*, 2009).

Various ways of preventing crystallisation and transformation into the β form in margarine and fat spread have been developed, through many techniques, as reviewed by Chrysan (Chrysan, 2005). For example, one can choose β' -tending fat resources (such as cottonseed oil) and blend them with β' -tending fats (such as soybean, safflower, etc.) (Wiedermann, 1978). One can add food emulsifiers that retard transformations from the β' to the β form (Aronhime *et al.*, 1988; Garbolino *et al.*, 2005; Sato and Kuroda, 1987). One can add diacylglycerols (Mohamed and Larsson, 1992), or one can use interesterification techniques with different fats and oils (Chrysan, 2005).

The formation of fat crystals in a continuous oil phase is largely influenced by food emulsifiers, which are employed not only for emulsification itself, but to control the fat crystallisation occurring at the water-oil interfaces. Such effects were recently observed in W/O emulsions by adding a high-melting monoacylglycerol (Wassell *et al.*, 2012) and by applying shear (Ghosh and Rousseau, 2012).

1.4 Conclusion

The crystallization and transformation properties of fat materials deserve further study regarding fundamental aspects, as discussed in this chapter. It is worthy of note again that the interrelations between polymorphism, solidification kinetics and crystal particle networks underlie the apparently complicated physical behaviour of various food fats. Although not reviewed in this chapter, the physical properties of fats in

organogels and aerated systems (whipped cream, ice cream, aerated confenctions, etc.) are very important.

As for experimental techniques, many advanced methods such as synchrotron radiation X-ray diffraction, ultrasonic velocity techniques, AFM, cryo-TEM technique, etc., have great potential and applicability in such studies. Further research should be carried out in this area.

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