

**Highlight Article****External factors affecting polymorphic crystallization of lipids<sup>†</sup>**

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## Abstract

Many physical properties (e.g., hardness, texture, rheology, and spreadability) of lipid-based products largely depend on the extent of crystallization and transformation of lipids, and their network formation. Therefore, many studies have focused on controlling the crystallization of lipids in order to determine the functionality of lipid crystals. Both internal and external factors greatly affect the processes of lipid crystallization. The most important internal factors are polymorphism, which depends on variation in fatty acid moieties, and the composition or blending of different lipid materials. Important external factors are thermal treatment, additives, application of shear, sonication, and pressure. This paper briefly reviews recent advances in research on these external factors. We discuss the results by considering the relationships between external factors and thermodynamics, as well as kinetic properties of the crystallization and transformation of polymorphic forms of lipid crystals.

## 1. Introduction

Lipids are widely employed in the food, pharmaceutical, and cosmetic industries as lipophilic materials. The physical properties (e.g., melting, morphology, rheology, and texture) of many lipid-based solid products (e.g., confections, creams, and spreads) are greatly influenced by the polymorphism of lipid crystals and the crystallization processes. To control the physical properties of lipids, many researchers have focused on (i) the determination of molecular and crystal structures, (ii) the influence of external factors on crystallization and transformation, (iii) the formation of crystal networks from nanoscale primary crystals to mesoscale and macroscale structures, and (iv) their rheological and texture properties, which are basically determined by the crystal networks of lipids [1].

Nutritional concerns have resulted in a growing demand to reduce the content of *trans* and saturated fatty acids in fat-based food products. The crystallization rates of *trans* and saturated fats are higher than those of low-melting fats, so it is necessary to enhance the crystallization rates and thus strengthen the crystal network of *trans*-free and low-saturated products by applying external factors to the crystallization processes of lipids. Therefore, many studies have been conducted to clarify the influences of various external factors on the polymorph-dependent crystallization of lipids.

This paper briefly reviews recent advances in research on such external factors as dynamic temperature variation (e.g., cooling, heating, and thermal cycle), additives, shear, sonication, and pressure, all of which greatly affect the polymorphic crystallization of lipids, especially triacylglycerols (TAGs). Readers who are interested in the polymorphism of various lipids and their crystal structures, and their physical properties are directed to recent book chapters and reviews [2-5].

## 2. Elementary processes of crystallization and transformation of polymorphic lipids

The macroscopic aspects of the polymorphism of lipid crystals involve crystallization and the subsequent transformation, which involve the major elementary processes illustrated in Fig. 1. Considering certain driving forces expressed by supercooling ( $\Delta T$ ) ( $= T_m - T_c$ , where  $T_m$  is melting temperature and  $T_c$  is crystallization temperature), we can initiate nucleation of crystals, followed by crystal growth from supercooled liquid. The rates of nucleation and crystal growth are highly dependent on polymorphic forms, as discussed later. Based on polymorph-dependent crystallization, one may find optimal crystallization conditions in accordance with estimated crystallization rates of the polymorphic forms, when attempting to crystallize a specific polymorphic form. Knowing the details of the transformation mechanisms, we can control the transformation processes either to maintain the first-occurring polymorphic form if it is the desired polymorphic form, or to cause transformation from undesired forms to the most desired forms after crystallization.

To apply external factors to crystallization, it is necessary to predict and clarify how every elementary process is affected by every external factor. For example, applying hydrostatic pressure raises  $T_m$ , thus increasing  $\Delta T$  and subsequently the rates of nucleation and crystal growth. The pressure effect must be polymorph-dependent, because the extent of the increase in  $T_m$  due to pressure differs from one polymorph to another; thus, the crystallization and transformation rates can be affected by pressure differently among the polymorphic forms (see below).

### 2.1 Nucleation and crystal growth

Crystallization is initiated with the nucleation of crystals from supercooled liquid, which occurs through stepwise processes of formation of molecular clusters and transformation into crystal nuclei [6]. Many important structural properties of lipid crystals (e.g., number and size distribution, morphology, and spatial distribution) are

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directly influenced by nucleation behavior.

A major problem in controlling the polymorphic crystallization of lipids is clarification of how a specific polymorphic form starts to nucleate in comparison with other polymorphic forms. The Ostwald step rule is useful in clarifying the behavior of multiple polymorphic forms when they are crystallized from vapor, solution, and melt phases. This rule dictates that less stable polymorphic forms crystallize much faster than more stable forms when the driving force for crystallization is supplied by decreasing the temperature, and that less stable forms stepwise transform to more stable forms during the storage processes after crystallization.

Actually, the kinetic behavior of polymorphic nucleation of various systems (e.g., biominerals [7, 8] and drug materials [9, 10]) often follows this rule. For example, a metastable vaterite form crystallizes faster than the more stable calcite in  $\text{CaCO}_3$ . However, more detailed studies are needed to examine the effects of external factors, and it is necessary to establish some rules regarding the influences of kinetics on polymorphic crystallization and to study situations where the Ostwald step rule may be complemented.

Recent studies on the polymorphic crystallization of lipids under various external factors have indicated that the Ostwald step rule is insufficient, and more detailed studies are needed to examine the effects of external factors on polymorphic crystallization. One example is the effects of cooling rate on the competitive nucleation rates of three polymorphs of a TAG (Fig. 2) [1].

A general tendency in melting temperature ( $T_m$ ) is that the  $T_m$ s of less stable forms are lower than those of more stable forms. For the three typical polymorphs of TAGs,  $T_m$  is lowest for  $\alpha$ , intermediate for  $\beta'$ , and highest for  $\beta$ . When crystallization occurs from the neat liquid, the relative rates and extent of crystallization of these three polymorphs are determined by the rate of nucleation, which is governed by the magnitude of activation free energy for nucleation,  $\Delta G^\ddagger$ .

We may reasonably assume that the values of  $\Delta G^\ddagger$  are smallest for  $\alpha$ , intermediate for  $\beta'$ , and largest for  $\beta$  (Fig. 2a). The nucleation rate thus increases with increasing  $\Delta T$  most rapidly for  $\alpha$  and most slowly for  $\beta$  (Fig. 2b). The rate of nucleation  $J$  is most simply expressed as

$$J = A \exp(-\Delta G^\ddagger/RT), \quad (1)$$

where  $\Delta G^\ddagger$  is the activation free energy for nucleation,  $A$  is a kinetic factor including transfer rates of mass and heat and attachment/detachment rates of lipid molecules among clusters and liquid,  $R$  is the gas constant, and  $T$  is temperature [6].  $\Delta G^\ddagger$  can be expressed by

$$\Delta G^\ddagger = K\gamma^3/(\Delta\mu)^2, \quad (2)$$

where  $K$  is a constant,  $\gamma$  is interfacial energy between the crystal nucleus and liquid, and  $\Delta\mu$  is the chemical potential difference between the supercooled liquid and crystal expressed in

$$\Delta\mu = \Delta H_m \Delta T / T_m, \quad (3)$$

where  $\Delta H_m$  is enthalpy of melting.

Having determined the polymorph-dependent nucleation rates, we may now determine the nucleation rates for the three typical polymorphs of TAG ( $\alpha$ ,  $\beta'$ , and  $\beta$ ) at different crystallization temperatures. Since  $T_m$  is highest for  $\beta$ , nucleation starts in the range of  $T_c$  far above those of  $\alpha$  and  $\beta'$ . Then  $\beta'$  and  $\alpha$  start to nucleate with decreasing  $T_c$ , and competitive nucleation occurs in a range of  $T_c$  below  $T_m$  of  $\beta'$  and  $\alpha$  (Fig. 2b). From graphical consideration, we assume that the most preferred nucleation of  $\beta$  may occur in the range of  $T_c$  between  $T_m$  of  $\beta$  and the crossing temperature of the two nucleation rates of  $\beta$  and  $\beta'$ . On further cooling, the nucleation of  $\alpha$  starts quite rapidly below  $T_m$  of  $\alpha$ . Therefore, it can be assumed that the preferred nucleation for  $\beta'$  may occur in the range of  $T_c$  between the crossing temperatures of the nucleation rates of  $\beta$  and  $\beta'$  and those of  $\alpha$  and  $\beta'$ .

From the polymorph-dependent nucleation rate and the effects of the cooling rate on preferred nucleation, we can assume that the preferred nucleation may change from  $\alpha$  to  $\beta'$  to  $\beta$  with a decreasing cooling rate. This behavior was recently observed for many TAGs, as discussed below.

Crystal growth proceeds through the incorporation of lipid molecules at the interface of growing crystals after nucleation, which takes place at molecular-level step-kink positions (Fig. 1). We may predict that the additives may retard the incorporation of the lipid molecules at the step-kink positions when they are adsorbed at the kink position and reject the incorporation of lipid molecules.

## 2.2 Solid-state and melt-mediated transformations

Two types of transformations can occur from less stable to more stable polymorphic forms; Fig. 3 depicts the crystal free energies of these two forms. Here, each polymorphic form has its own  $T_m$ ; polymorphism having this property is called monotropism. Many lipid crystals exhibit monotropic polymorphism.

Solid-state transformation occurs when the metastable form A is stored below its  $T_m$ . The rate of solid-state transformation is basically determined by the magnitude of activation free energy barrier  $\Delta G_{ss}^\#$ .  $\Delta G_{ss}^\#$  may include the energies to enable conversions in the subcell and chain length structures, and other molecular structural changes that are necessary to cause transformation in a solid state.

Another type of polymorphic transformation is melt-mediated transformation, which was originally defined by Sato and Garti specifically for lipid crystals [11]. Melt-mediated transformation occurs as the temperature rises just above  $T_m(A)$ , where the melting of A occurs, and crystallization of form B soon follows. In this case, the rate of transformation is determined by the magnitude of activation free energy barriers of melting  $\Delta G_m^\#$ , and crystallization of  $\Delta G_c^\#$ .

One may expect that  $\Delta G_m^\#$  of form A is much smaller than  $\Delta G_c^\#$  of form B, so the rate of melt-mediated transformation from A to B may actually be governed by  $\Delta G_c^\#$ .  $\Delta G_c^\#$  includes activation energies for nucleation and crystal growth of the more stable forms from the liquid that forms soon after melting of the less stable forms. Comparing the rates of solid-state transformation and melt-mediated transformation is not easy, since the factors included in  $\Delta G_{ss}^\#$  and  $\Delta G_c^\#$  are quite different. However, it can be expected that heterogeneous nucleation of the more stable forms reduces the values of  $\Delta G_c^\#$ ; thus, melt-mediated transformation occurs more rapidly than in the solid state, as observed for SOS [12].

When applying polymorphic transformation to the processing of solid lipid products, tempering may correspond with melt-mediated transformation for margarine, fat spreads, and chocolate.

From these fundamental points of view, it is quite important to understand how external factors affect crystallization and transformation of lipids, which are quite complicated because multiple processes of nucleation, crystal growth, and transformation are involved. This paper attempts to shed light on these phenomena from the viewpoints illustrated in Figs. 2 and 3.

### 3. Effects of external factors

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### 3.1 Thermal treatment

The metastable and more stable polymorphic forms of lipids are greatly influenced by dynamic temperature variations, such as cooling rate, heating rate, and thermal thawing (tempering). These effects are highly significant for applications in the pharmaceutical, biomedical, and food areas, since specific polymorphic forms should be crystallized by tailoring the most efficient thermal treatment.

#### (1) Effects of cooling and heating rates

The role of thermal treatment in the crystallization and transformation of lipids has been studied for model fats [13-17], natural lipids [18-24], emulsion [25], and end products [26, 27]. Perez-Martinez et al. studied the quantitative evolution of crystallization of cocoa butter (CB) with liquid oils at different cooling rates. They demonstrated that  $T_c$  and cooling rate had different effects on the three-dimensional organization of the crystal network, and on the proportion and size of  $\beta'$  and  $\beta$  crystals of CB [24]. Tippetts and Martini studied the effects of cooling rate on the stabilization of oil-in-water (o/w) emulsions by combining the effects of the oil content (a mixture of anhydrous milk fat (AMF) and soybean oil), homogenization conditions, and  $T_c$ . They observed that higher oil content increased the stability of emulsions when the cooling rate was high, whereas emulsion stability increased with decreasing cooling rate for emulsions formulated with lower amounts of oil [25]. The key to understanding this opposing effect may be the nucleation and further growth of crystals fats, which affect emulsion stability.

Cooling rates may act through the effects of  $\Delta T$ , which increases with increasing cooling rates, causing increased crystallization rates. Transmission electron microscope (TEM) observation of nanometer-scale fat crystals grown from organic solution indicated that fast cooling rates and application of shear significantly decreased the dimensions of fat crystals [28, 29]. Figure 4 depicts changes in the length, width, and thickness of the crystals of fully hydrogenated canola oil (FHCO) [29]. FHCO was mixed with high oleic sunflower oil (HOSO) at different concentration ratios (solid mass fraction (SMF)), which was defined by SFC/100 (SFC: solid fat content), and crystallization was conducted at fast (10 °C/min) and slow (1 °C/min) cooling rates. Nanocrystal dimensions clearly became smaller as the cooling rate increased, and the extent of decrease was more pronounced at lower SMFs.

Many researchers have focused on the kinetic processes of the crystallization of fats at

varying cooling rates to address crystallization behavior under isothermal and non-isothermal conditions [30-37]. Most fats employed in actual production are complex systems including different TAG components, each of which possesses multiple polymorphic forms. Therefore, crystallization behavior under non-isothermal conditions becomes quite complicated, due to crystallization of different TAG components and their polymorphic forms. During cooling, the  $\Delta T$ s of polymorphic forms of TAG components, and thus the rates of nucleation and crystal growth, vary differently with time, depending on the cooling rate. Marangoni et al. discussed the time-dependent driving force of nucleation by taking into account  $\Delta T$ , the induction time of nucleation, and the cooling rate [34].

The effects of heating rates after crystallization appear either in solid-state or melt-mediated transformations, whose kinetics are determined by activation energies for the transformation and the heating rates.

Recently, particular interest has focused on the effects of cooling and heating rates on the nucleation and transformation of polymorphic forms of OPO (1,3-dioleoyl-2-palmitoyl glycerol) [38], POP (1,3-dipalmitoyl-2-oleoyl glycerol) [39] and OOO (trioleoyl glycerol) and OOL (1,2-dioleoyl-3-linoleoyl-*rac*-glycerol) [40] by directly observing the crystallization processes with DSC and synchrotron radiation X-ray diffraction (SR-XRD).

For example, POP possesses seven polymorphic forms ( $T_m$ ):  $\alpha$  (15.2°C),  $\gamma$  (27.0°C),  $\delta$  (29.2°C),  $\beta'_2$  (30.3°C),  $\beta'_1$  (33.5°C),  $\beta_2$  (35.1°C), and  $\beta_1$  (36.7°C) [41]. The crystallization and transformation pathways of the polymorphs of POP indicate that more stable forms developed in higher quantities when POP was slowly cooled and heated, whereas less stable forms developed at higher cooling and heating rates. Figure 5 presents DSC cooling and heating patterns and wide-angle SR-XRD patterns during cooling at 2°C/min, and heating at 2°C/min and 1°C/min. The SR-XRD patterns indicate that  $\alpha$  was crystallized by cooling and transformed to  $\gamma$  and  $\beta$  (here, the two  $\beta$  forms were not separated), and finally  $\beta$  melted during the heating. However, the transformation mechanisms from  $\alpha$  to  $\gamma$  differ between the heating rates of 2°C/min and 1°C/min, since the melting of  $\alpha$  and successive crystallization of  $\gamma$  occurred at a heating rate of 2°C/min (melt-mediated transformation). This can be shown as an endothermic peak of melting of  $\alpha$ , which was associated with an exothermic of re-crystallization of  $\gamma$  shown in Fig. 5(b). However, solid-state transformation occurred from  $\alpha$  to  $\gamma$  at a



heating rate of 1°C/min, as indicated by an exothermic peak in the DSC pattern in Fig. 5(a). Table 1 indicates the crystallization and transformation behavior of POP polymorphs at different cooling and heating rates observed using DSC and SR-XRD [39].

Specifically, less stable  $\alpha$  and  $\gamma$  forms were directly obtained at cooling rates of 15 to 0.5°C/min. More stable forms of  $\beta'$  ( $\beta'_1$  and  $\beta'_2$ ) or  $\beta$  ( $\beta_1$  and  $\beta_2$ ) did not develop even at a cooling rate of 0.5°C/min. Polymorphic transformations occurred either in solid-state or melt-mediated transformation, which was influenced mainly by heating rate.

Figure 6 illustrates the relationships between the nucleation rates of  $\alpha$ ,  $\gamma$ , and  $\beta'$  of POP and the cooling rates and suggests that  $\alpha$  can preferentially be nucleated in a low  $T_c$  region ( $\alpha$ -dominating region),  $\gamma$  in an intermediate  $T_c$  region ( $\gamma$ -dominating region), and  $\beta'$  in the highest  $T_c$  region ( $\beta'$ -dominating region). Therefore, we may expect that the cooling rates of 15°C/min and 2°C/min may reach the  $\alpha$ -dominating region, and that those of 1°C/min and 0.5°C/min may reach the  $\gamma$ -dominating region. More stable polymorphs of the  $\beta'$  and  $\beta$  forms could be obtained only by cooling POP at rates much lower than 0.5°C/min.

The complex transformation pathways observed during heating (summarized in Table 1) can be understood by comparing the induction time for the transformation pathways with the time allocated to the samples for transformation (transformation time) at a defined heating rate [39].

Induction time ( $\tau$ ) can be defined for solid-state transformation ( $\tau_{ss}$ ), melt-mediated transformation ( $\tau_{mm}$ ), and melting ( $t_m$ ) as follows.

$$\tau_{ss} = A_1 \exp(\Delta G_{ss}^{\#}/RT)$$

$$\tau_{mm} = A_2 \exp(\Delta G_{mm}^{\#}/RT) = A_2 \exp[(\Delta G_m^{\#} + \Delta G_c^{\#})/RT]$$

$$\tau_m = A_3 \exp(\Delta G_m^{\#}/RT).$$

Here,  $A_1$  to  $A_3$  are coefficients,  $R$  is the gas constant,  $T$  is temperature,  $\Delta G_{ss}^{\#}$  is the activation Gibbs free energy for solid-state transformation,  $\Delta G_{mm}^{\#} = \Delta G_m^{\#} + \Delta G_c^{\#}$  is that for melt-mediated transformation including melting of less stable forms and crystallization of more stable forms, and  $\Delta G_m^{\#}$  is that for melting.

Obviously, the induction time for the transformation increases as  $\Delta G^{\#}$  increases. It is reasonable that  $\Delta G_m^{\#}$  is much smaller than the others, and that  $\Delta G_{ss}^{\#}$  and  $\Delta G_c^{\#}$  increase when the polymorphic form becomes more stable, as the crystal packing of the more

stable form is more stabilized than that of less stable forms. This may apply to  $\alpha$ ,  $\gamma$ ,  $\beta'$ , and  $\delta$  of POP in such a manner that  $\Delta G_{ss}^\#$  for  $\gamma \rightarrow \beta'$ ,  $\gamma \rightarrow \delta$ , and  $\delta \rightarrow \beta$  may be greater than that of  $\alpha \rightarrow \gamma$ . Similarly,  $\Delta G_c^\#$  is clearly greater for  $\beta'$  and  $\gamma$  than for  $\alpha$ . Therefore, the following relationships can be obtained.

$$\tau_{ss}(\alpha \rightarrow \gamma) < \tau_{ss}(\gamma \rightarrow \delta) < \tau_{ss}(\gamma \rightarrow \beta') < \tau_{ss}(\delta \rightarrow \beta)$$

$$\tau_{mm}(\alpha \rightarrow \gamma) < \tau_{mm}(\gamma \rightarrow \beta') < \tau_{mm}(\gamma \rightarrow \delta) < \tau_{mm}(\delta \rightarrow \beta) < \tau_{mm}(\beta' \rightarrow \beta).$$

As the heating rate is increased, the time for the polymorphic form to transform into more stable forms is decreased, as expressed in  $r^{-1}$ , where  $r$  is the heating rate. There are three typical relationships between heating rate and induction time for the transformations.

Case 1.  $r^{-1} < \tau_{mm} < \tau_{ss}$

This is applied to simple melting of  $\gamma$  at heating rates of 15 and 2°C/min.

Case 2.  $\tau_{mm} < r^{-1} < \tau_{ss}$

This is applied to the melt-mediated transformation pathways of  $\alpha \rightarrow \beta'$  (15°C/min) and  $\alpha \rightarrow \gamma$  (2°C/min),  $\gamma \rightarrow \beta$  (2°C/min and 1°C/min),  $\gamma \rightarrow \beta'$  (0.5°C/min),  $\gamma \rightarrow \beta' + \delta$  (0.1°C/min), and  $\beta' + \delta \rightarrow \beta$  (0.1°C/min).

Case 3.  $\tau_{ss} < r^{-1}$

This is applied to very low heating rates of  $\alpha \rightarrow \gamma$  (1°C/min and 0.1°C/min) and  $\gamma \rightarrow \delta$  (0.1°C/min).

Finally, the transformation pathways clearly tend to change from solid-state to melt-mediated and simple melting when the starting polymorph changes from  $\alpha$  to  $\gamma$  and the heating rate is increased (Table 1).

## (2) Thermal thawing (tempering)

Thermal thawing (cooling-heating-cooling cycle) can produce optimal polymorphic forms through melt-mediated or solid-state transformation. Such a process is often called *tempering*, as applied to confectionary fats [42] and creams [43]. The polymorphic crystallization of CB in chocolate production can be summarized as follows. CB crystals have six polymorphic forms From I through Form VI [44]. During tempering, the samples were rapidly cooled from elevated temperatures in order to cause crystallization of metastable Form IV of  $\beta'$  type CB. The samples were then heated just above the  $T_m$  of Form IV in order to cause melt-mediated transformation from Form IV to Form V, and cooled again so that the CB crystals in chocolate were

eventually crystallized in Form V during final cooling.

The dispersion stability of oil droplets containing semi-solid fats in oil-in-water (O/W) emulsion is affected by tempering [45]. Numbers and dimensions of lipid crystals change during heating and subsequent cooling processes, when melting and crystallization of the fractions of lipid crystals present in the oil droplets occur. As a result, susceptibility for partial coalescence among the semi-solid droplets in the O/W emulsion is largely affected by the tempering.

Tempering was also applied to form organogels using high-melting fats [46, 47]. During this tempering process, the least stable  $\alpha$  crystals of high-melting fats were formed by very rapid cooling, and subsequent reheating caused melt-mediated transformation into  $\beta$  form. The  $\beta$  crystals were so tiny compared with those formed by simple cooling, that organogel ( $\beta$ -fat gel) was formed.

Figure 7 illustrates the formation process of  $\beta$ -fat gel composed of high-melting fat (fully-hydrogenated rapeseed oil rich in behenic acid (FHR-B)) and liquid oil (sal fat olein) [46]. The  $T_m$  of  $\alpha$  form FHR-B crystals is 29°C and that of  $\beta$  form is 47°C at a concentration of 5wt.% of FHR-B in liquid oil. When crystallization was performed through process A, the molten mixture was slowly cooled to a temperature below  $T_m(\beta)$ , large  $\beta$  crystals of FHR-B were formed, and no gel phase was formed. In contrast, the gel phase was formed after crystallization process B, in which the molten mixture was rapidly cooled to below  $T_m(\alpha)$  and heated to a temperature between  $T_m(\beta)$  and  $T_m(\alpha)$ . After this tempering process, many small  $\beta$  crystals were formed and randomly distributed in liquid oil, which was entrapped by the  $\beta$  crystal network of FHR-B. Such a network was formed due to melt-mediated transformation from  $\alpha$  to  $\beta$  forms, because the nucleation rate of  $\alpha$  crystals was high enough to cause the transformed  $\beta$  crystals to be randomly distributed, rather than aggregated as with slow cooling process A.

The above fundamental studies on the polymorphic crystallization of fats using pure TAG samples suggest that **slow** cooling rates do not crystallize more stable  $\beta'$  and  $\beta$  forms, and that successive heating may cause transformation into more stable forms as the heating rate is decreased. Other external effects such as additives or shear in addition to different thermal treatments may modify these properties, which may be open to further research.

### 3.2 Additives

Foreign materials have been added to liquid before or during crystallization in order to modify the crystallization behavior of inorganic and organic substances [48]. In the area of lipid crystallization, Smith et al. recently published a comprehensive review of research on the effects of minor components and additives [49]. The minor components and additives they considered were free fatty acids; mono-, di- and triacylglycerols; phospholipids; and such emulsifiers as sorbitan fatty acid esters and sucrose fatty acid esters. Their effects on crystallization included microscopic aspects (e.g., nucleation, crystal growth, morphology, heat capacity, and polymorphic stability), as well as macroscopic aspects (e.g., visual aspects, melting profiles, post-hardening, and rheology of lipid crystals). Since this review, further studies have been reported [50-53].

In basic agreement with the main findings presented in the review by Smith et al., here we elaborate on the effects of additives on the polymorphic crystallization of lipids. It has often been observed that some additives promote the process, whereas others retard it, even when the adding conditions are similar [54].

Conditions related to the effects of additives on the nucleation of lipid crystals (Fig. 8) may be understood by considering the following four factors.

(1) Similarity in molecular shape and polymorphism

It is reasonable to assume that similarity in molecular shape between the additive and the lipid, especially the fatty acid moiety, is required, as Smith et al. indicated [49]. For example, when the lipid contains long-chain saturated fatty acid moiety, an additive having the same or similar acyl chain structure may affect crystallization more than those containing short-chain or unsaturated fatty acid moieties. Such effects were observed for polyglycerine fatty acid esters having different fatty acid moieties [55].

(2) Concentration of additive

There may be a critical concentration of the additive, as determined by the solubility of the additive in supercooled liquid of the lipids at  $T_c$ . When the solubility of the additive is high or the concentration of the additive is lower than the solubility limit, it may not crystallize prior to the lipid during cooling, but prevent the formation of crystal nuclei of the lipid through attractive molecular interactions between the additive and the lipid molecules, due to the similarity in molecular shape (de-clustering) (Fig. 8a). Many food emulsifiers exhibit such retardation effects when added to the lipid at low concentrations [56, 57].

In contrast, an additive having low solubility may crystallize prior to the lipid when the concentration of the additive exceeds its solubility limit. Additive may then act as a

“template” promoting crystallization (templating) (Fig. 8b), as observed in bulk [53] and emulsion [50]. The same effect may alter the preferred nucleation of polymorphic crystallization from that without the additive, if the crystal structure of the additive can act as a template for the nucleation of a specific polymorphic form of lipid (Fig. 9), as already reported for bulk [58] and emulsion [59, 60].

### (3) Cooling rate

The cooling rate also influences the additive effects, as noted by Smith et al. [49], and such effects may occur through the following two mechanisms.

First, additive effects are minimized when  $\Delta T$  is high enough to induce spontaneous nucleation because undesired polymorphic crystals are formed without the effects of additives. Therefore, the cooling rate and the range of  $\Delta T$  may not exceed optimal values, over which spontaneous nucleation can occur and diminish the effects of the additives.

Second, the cooling rate is related to the preferred nucleation of the polymorphic forms. For example, the nucleation rate of  $\alpha$  is higher than that of  $\beta'$ ; the relative occurrence of  $\alpha$  exceeds that of  $\beta'$  as  $T_c$  is lowered and the cooling rate is increased, as explained earlier (Fig. 2). However, this effect may be altered when the additive acts as a template and thus increases the nucleation rate of the more stable form more effectively than that of the less stable form. In this case, the nucleation rate of specific polymorphic form may increase with an increasing concentration of the additive and decreasing rate of cooling (Fig. 9).

### (4) Polymorphic matching

When the additive acts as a template to promote the nucleation of lipid crystals, it may selectively promote the nucleation of specific polymorphic forms. A prerequisite for this to occur may be polymorphic matching between the template crystals and the lipid crystals. The results of previous studies [60, 61] may be interpreted by this mechanism.

## 3.3 Application of Shear

The study of fat crystallization under shear was initiated half a century ago, as we described earlier [62]. Since this review, detailed work on natural fats has been reported by many researchers [52, 63-83]. These studies clearly demonstrate that applying shear increases the rates of polymorphic crystallization and transformation of lipids, and modifies the aggregation of nanocrystals of the crystal network.

Figure 10 depicts variations in XRD intensity, which were monitored during the crystallization of CB at 18°C with shear applied (1440sec<sup>-1</sup>) [64]. Form III was the first of the six polymorphic forms of CB (I through VI) to crystallize. The rate of increase of the XRD peak of Form III without shear was much lower than that with shear, confirming that shear promotes the crystallization of Form III. In addition, Form III converted to Form IV during further crystallization when no shear was applied. However, conversion from Form III to Form V was observed to be much more rapid than transformation without shear.

The mechanisms of the effects of shear on the increase in rates of crystallization and polymorphic transformation of lipids are still unclear. The promotion may be due to increased rates of heat and mass transfer, which reduce  $\Delta G^\ddagger$  for nucleation (Fig. 2a), thus promoting nucleation. Tiny nanocrystals then formed, causing transformation from less stable to more stable forms through recrystallization of more stable forms onto the surfaces of the less stable forms. This transformation may be facilitated by the enormous increase in surface area compared to the volume of nanocrystals. Promotion of polymorphic transformation may also be due to melt-mediated transformation, as shear may increase the local temperature within the crystallizing medium above  $T_m$  of Form III.

Exciting findings related to shear include preferred orientation of fat crystal particles, which was discovered by *in-situ* observation of SR-XRD patterns acquired during the crystallization of various fats with two-dimensional (2D) detectors [64, 68]. Such effects resulted in oriented crystal network formation; therefore, porosity and oil migration were affected when fat crystallized in a laminar flow.

Figure 11 indicates the effects of shear on the orientation of tiny fat crystals present in the shear flow, as determined by SR-XRD analysis. The fat was crystallized in a Couett cell with shear applied at different temperatures (Fig. 11a). The polymorphic structure and the orientation of the crystals growing in a Couett cell were obtained simultaneously using a 2-dimensional X-ray beam detector. The former was determined by observing the diffraction angle of  $2\theta$  ( $2\theta$ -extension), and the orientation of crystals was determined with respect to shear direction by observing diffraction intensity at varying azimuthal angle positions ( $\chi$ -extension) at the fixed  $\theta$  angle. The degree of orientation can be assessed by defining  $\Delta\chi$  (Fig. 11b).

For example, AMF crystals grown with shear applied (1440sec<sup>-1</sup>) at 17°C are  $\beta'$

polymorph, and the crystals are highly oriented, since sharp arc XRD patterns with narrow  $\Delta\chi$  values are observed [73]. These results are in contrast to crystallization without shear, since  $\alpha$  and  $\beta'$  forms crystallized at the same time and no crystal orientation was observed.

The effects of shear have practical significance for crystallization in edible fats because various macroscopic physical properties of fat crystals are changed [75-78, 81].

The effects of laminar shear on crystalline orientation and the nanostructure of fat crystal networks of CB were quantified using cryogenic scanning electron microscopy (Cryo-SEM), which indicated oriented sheets of crystalline CB in the sample obtained in the laminar shear crystallizer, while spherulitic structures were observed in the statically crystallized sample. These effects increased breaking force, Young's modulus, and elastic storage modulus ( $G'$ ) of the solid CB materials [76, 77].

Furthermore, the effect of laminar shear on the migration kinetics of liquid oil through a polycrystalline colloidal fat crystal network was characterized and quantified. A shear rate of  $340\text{sec}^{-1}$  was applied, and the CB was crystallized from melt ( $60\text{ }^\circ\text{C}$ ) to  $20\text{ }^\circ\text{C}$ . The resulting solid sheet of CB was stored for seven days at  $20\text{ }^\circ\text{C}$ , and the crystal dimensions of CB were observed using TEM [76, 77]. Statistical analysis demonstrated that shear processing significantly reduced the dimensions of the nanoparticles of CB so that a length of  $2085 \pm 128\text{nm}$  and a width of  $164 \pm 6.4\text{nm}$  without shear were reduced to a length of  $310 \pm 7.3\text{nm}$  and a width of  $138 \pm 5.3\text{nm}$  under laminar shear. The aspect ratio (length/width) was thus greatly reduced, meaning that the crystal morphology changed from needle shape to plate shape. We can reasonably assume that the combined effect of the orientation and the morphological changes of CB crystals caused reduced rates of oil migration due to the formation of a more tightly packed crystal network.

This study suggested that the migration rate of liquid oil through a polycrystalline fat crystal network can be modified using laminar shear flow. A lower migration rate was observed in shear-crystallized samples having smaller and better-oriented nanocrystals, which retard oil migration (Fig. 12) [4, 78].

### 3.4 Sonication

Ultrasound has been applied for characterizing microstructures and process control of food materials [84, 85]. For lipids, ultrasound waves have been used to characterize the physical properties of lipid crystals such as SFC [86-93] and to control the crystallization of lipids (sonocrystallization) [94-99]. Sonocrystallization modifies the

rates of polymorph-dependent crystallization, crystal size, and morphology in pure TAGs, confectionery fats, vegetable fats, and milk fats.

The kinetic processes of sonocrystallization of tripalmitin (PPP) and CB were examined with SR-XRD and optical microscopy [94]. The main observations of these preliminary experiments indicate that the nucleation rate of PPP was enhanced and induction time was shortened by ultrasound application when it was irradiated properly, and that the polymorphic Form V of CB was directly crystallized when ultrasound was applied under optimal conditions of temperature and a short period of sonication.

Martini et al. recently applied high-intensity ultrasound (HIU) to the crystallization of palm kernel oil (PKO), AMF, and shortening [96, 97]. The following results were observed.

- (a) HIU induced primary and secondary nucleation of fat crystals, generating smaller crystals.
- (b) Consequently, harder materials were formed when HIU was applied at higher  $T_c$ , as observed for AMF, and when HIU was applied after the first crystals were formed, as observed for PKO and shortening.
- (c) In addition, fat crystal networks in AMF and shortening obtained after HIU application exhibited steeper and sharper melting profiles than did nonsonicated samples.

Figure 13 presents the effects of applying HIU at different temperatures on induction time and viscosity of the crystallized sample of AMF [96]. Induction time was clearly shortened when HIU was applied at 26°C and 28°C, and viscosity increased as sonication time and HIU were decreased.

*In-situ* observation of the sonocrystallization of PPP and LLL using SR-XRD indicated that polymorphic crystallization was remarkably modified by the irradiation of ultrasound [95]. Without ultrasound application,  $\beta'$  and  $\beta$  forms were crystallized in the melt of each TAG. With ultrasound treatment of the melt, the following effects were observed: (i) marked decrease of induction times for crystallization of both PPP and LLL, (ii) increased nucleation rate, and (iii) crystallization of only  $\beta$  forms for both PPP with an initial crystallization temperature of 50°C and LLL with that of 30°C, and with ultrasound applied for 2sec.

Based on the dynamic nucleation of PPP and LLL crystals induced by collapsing cavitation bubbles, we proposed that a pronounced decline in induction times and an



increase in nucleation rate result from the melting point shift due to high pressure pulses associated with collapsing bubbles [95].

The studies reviewed above clearly indicate that ultrasound does affect the crystallization of fats in many ways. However, we still do not know what mechanisms are responsible for these effects. To improve our knowledge and predictability in terms of desired polymorphism, induction times, and nucleation rates that are influenced by sonocrystallization, a better understanding of the following issues is crucial: (i) the establishment of a P-T phase diagram for polymorphic forms, since a primary effect of sonocrystallization may be due to high pressure when a sonication-induced cavity is collapsed, (ii) stability (lifetime) of different polymorphic forms as a function of  $\Delta T$  and temperature, (iii) mechanism and lifetime of collapsing cavities, and (iv) the basic mechanism of dynamic nucleation near a collapsing bubble.

### 3.5 Pressure

The application of pressure has been useful in food engineering, particularly because of the viability of sterilization and protein denaturation [100]. However, quite limited trials have been conducted to analyze lipid crystallization, due to difficulty in applying high pressure on a factory scale. Most machines that apply high pressure work only in batch processing, which is unsuitable for large-scale production of food lipids.

However, in 2002 innovative technology was presented to produce food emulsion under ambient high pressure of 10 to 150MPa [101]. This high pressure was applied to promote fat crystallization in the emulsion, while the entire process was performed in continuous production lines, not by batch processing. This technology increased the rate of fat crystallization and decreased undesirable problems of grain growth of fat crystals that occur after crystallization. The result was improved efficiency of the production process in terms of time and energy.

Quite recently, Wierschem et al. conducted fundamental studies of pressure effects on fat crystallization [102, 103]. They observed induction times for crystallization (induction time for nucleation) and rates of increase in crystal size (crystal growth rate) of various fats, while applying pressure and observing the crystals using an optical microscope. The results suggested that the induction time was shortened and the growth rate was increased with increasing pressures.

The above two studies clearly indicate that the application of hydrostatic pressure promotes nucleation and crystal growth of lipids. It is reasonable to assume that the

underlying principle of these pressure effects is the increase in  $T_m$  by pressure, as defined by the Clausius-Clapeyron equation:

$$dp/dT = \Delta H_m/T\Delta V, \quad (4)$$

where  $dp/dT$  gives the slope in a p-T phase transition diagram,  $\Delta H_m$  is the molar enthalpy of melting, T is the temperature, and  $\Delta V$  is the difference in molar volume between crystal and liquid states. When pressure is applied rapidly without any change in  $T_c$ , it reaches the entire volume of supercooled liquid at the sonic speed and increases  $T_m$ .  $\Delta T$  then increases and enhances the driving force for nucleation and crystal growth (see Eq. 3).

It is necessary to determine how pressure increases  $\Delta T$  for lipids. The lack of comprehensive data on pressure-temperature thermodynamic properties based on Eq. 4 until recently is surprising. Greiner et al. are the first to perform molecular dynamic (MD) simulation on the density of liquid of principal TAGs to draw the solid-liquid phase equilibria of principal TAGs of LLL (trilauroyl-glycerol), PPP (tripalmitoyl-glycerol), AAA (triarachidoyl-glycerol), SOS (1,3-dipalmitoyl-2-oleoyl-glycerol), POS (1-palmitoyl-2-oleoyl-3-stearoyl-rac-glycerol), and SOS (1,3-distearoyl-2-oleoyl-glycerol) (Fig. 14) [104, 105].  $T_m$  increased almost linearly with pressure, and the increase in  $\Delta T$  with a pressure difference of 1000bar ranged from 16K (POP) to 27K (PPP) and 37K (LLL), which is large enough to increase the rates of nucleation and crystal growth

One specific advantage of applying pressure is that, as mentioned above, the pressure is applied to the whole body of liquid of lipids quite quickly (1 $\mu$ sec per centimeter). This may be in contrast with thermal treatment, whose kinetics is governed by thermal conductivity of the liquid of lipids (4min per centimeter). However, the machines used to apply high pressure in the continuous production line must tolerate an inner pressure as high as several hundred bars, which may be the effective pressure range to promote fat crystallization.

Additional fundamental and application studies on the effects of pressure, including the technical problems of the production processes, are needed to improve the crystallization of lipids. In particular, to experimentally determine the values of  $\Delta H_m$  and  $\Delta V$  appearing in eq. (4) for different lipid materials is quite important, since the effects of pressure on  $T_m$  can be predicted by the two values which may vary with the pressure and polymorphic forms of lipids.

#### 4. CONCLUSION

The crystallization and transformation of polymorphic lipid materials require further study regarding fundamental and application aspects. Several additional issues were not mentioned in this review.

Novel additives that exhibit hydrophobic as well as hydrophilic interactions with lipid molecules may open new windows for further research. As for the polymorphism of lipid crystals, some basic questions remain unanswered (e.g., why symmetric and asymmetric TAGs have different polymorphic stability). Labor-consuming work on single crystal-based crystal structure determination using simple- and complex-shaped lipid materials and detailed observation of the polymorphic transformation pathways will provide decisive answers. Molecular-level observation and understanding of crystal growth processes with various external factors will provide new and deeper insight into polymorphic crystallization.

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Figure captions

Figure 1. Elementary processes of crystallization and transformation of polymorphic lipids.

Figure 2. Schematic illustrations of (a) activation free energy for nucleation ( $\Delta G^\ddagger$ ) of polymorphic forms of  $\alpha$ ,  $\beta'$  and  $\beta$  of triacylglycerols and (b) nucleation rate and cooling rate. In (b), solid and dotted lines refer to nucleation rates and cooling rates, respectively.

Figure 3. Relative stability of two polymorphic forms showing typical transformation pathways and corresponding activation energy for transformation ( $\Delta G^\ddagger$ ).

Figure 4. Effects of cooling rates on length (L), width (W) and thickness (T) of fat crystals in the blends of Fully hydrogenated canola oil (FHCO) and high oleic sunflower oil (HOSO).

Figure 5. Polymorphic behavior of POP, (a) cooling at 2 °C/min and heating at 2 °C/min and (b) cooling at 2 °C/min and heating at 1 °C/min. For both, A; DSC patterns and B; wide-angle SR-XRD patterns.

Figure 6. Relationship between nucleation rates of POP polymorphs and different cooling rates .

Figure 7. Formation mechanisms of  $\beta$  fat gel made of high-melting fat and liquid oil mixture. (a) Temperature variation scheme and (b) crystallization behavior.

Figure 8. Schematic illustrations of the effects of additive on nucleation of lipid crystals.

Figure 9. Schematic illustration of promotion mechanisms of nucleation rates of metastable A and more stable B polymorphs (solid lines: without additive, dotted lines: with additive).

Figure 10 Effects of shear application (shear rate  $1440 \text{ sec}^{-1}$ ) on crystallization of cocoa butter at  $18 \text{ }^\circ\text{C}$ . Without shear (open) and with shear (filled).

Figure 11 Observation of orientation of fat crystals grown under shear with SR-XRD techniques. (a) Diffraction experiments with a Couett cell and (b) analysis of 2-dimensional diffraction patterns ( $\theta$ : diffraction angle,  $\chi$ : azimuthal angle).

Figure 12. A schematic illustration of the tortuosity of the diffusive path in cocoa butter crystallized under various shearing conditions. (a) Shear-mixed cocoa butter crystals and (b) oriented cocoa butter crystals formed under laminar shear.

Figure 13 Effects of application of high intensity ultrasound (HIU) on crystallization behavior of anhydrous milk fat (AMF). (a) Induction time for crystallization at different  $T_c$  and (b) viscosity of crystallized AMF with different sonication conditions at  $T_c=30 \text{ }^\circ\text{C}$ .

Figure 14 Plots of the slope in the predicted phase co-existence diagrams for (a) saturated monoacid triacylglycerols and (b) saturated-unsaturated mixed-acid triacylglycerols. Abbreviations; see text.

Figure 1.

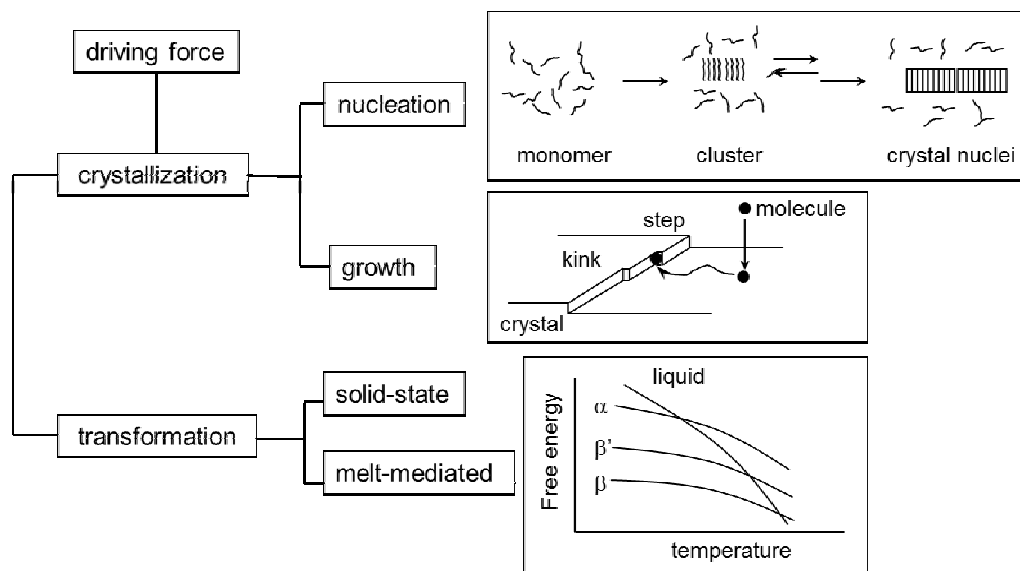


Figure 2.

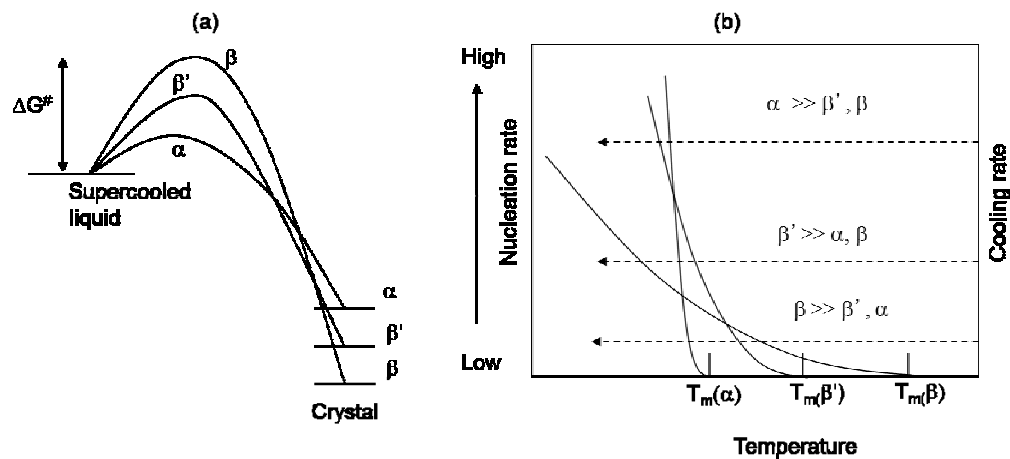


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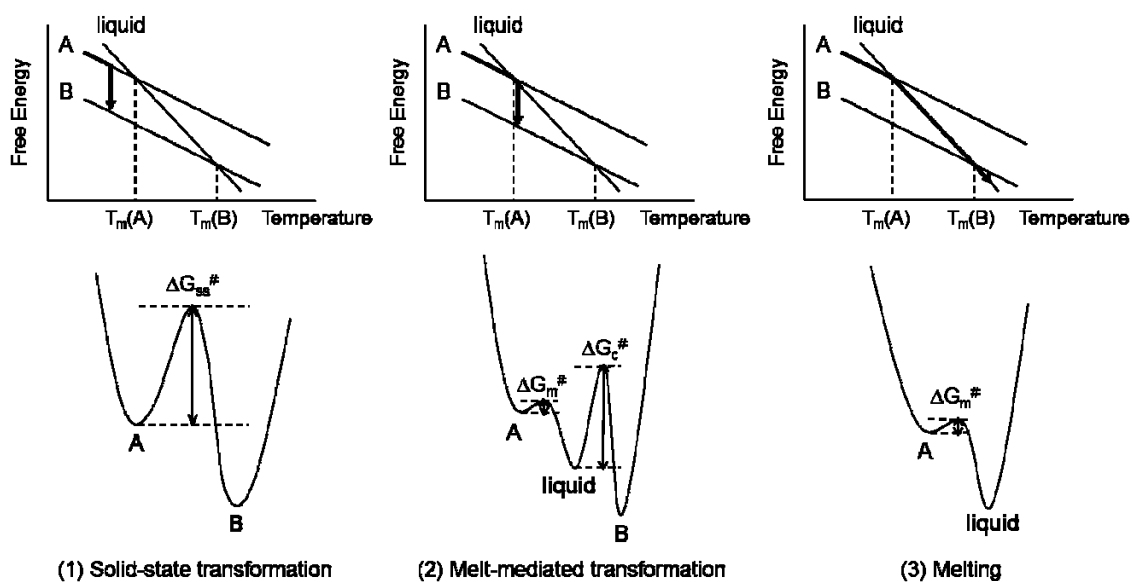


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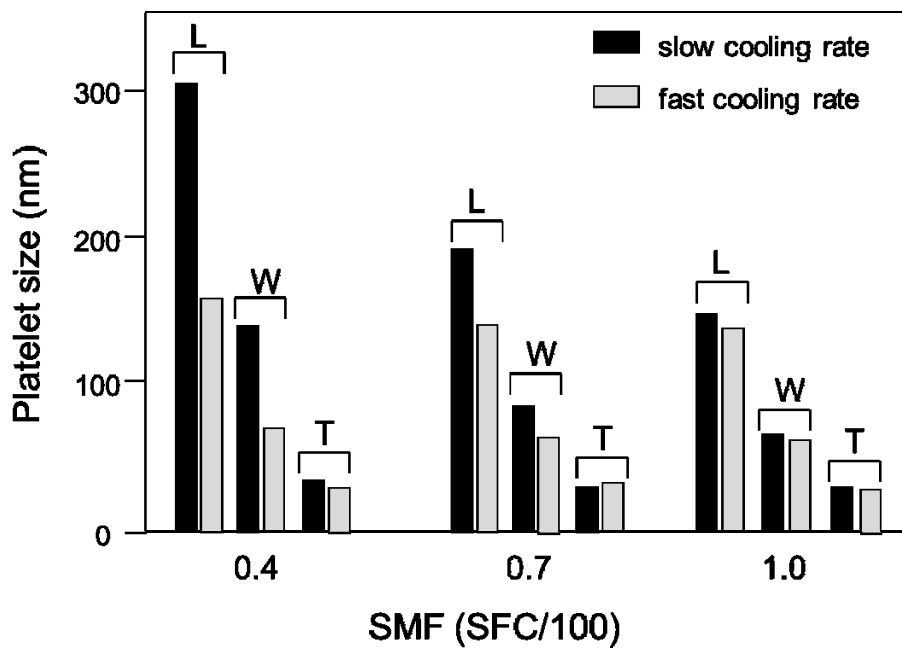


Figure 5.

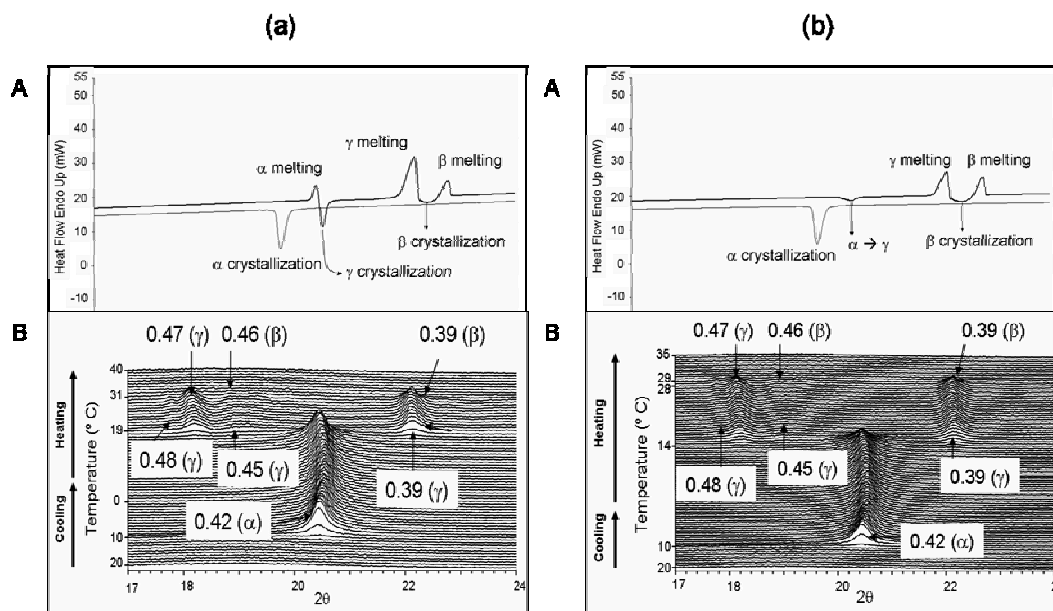


Figure 6.

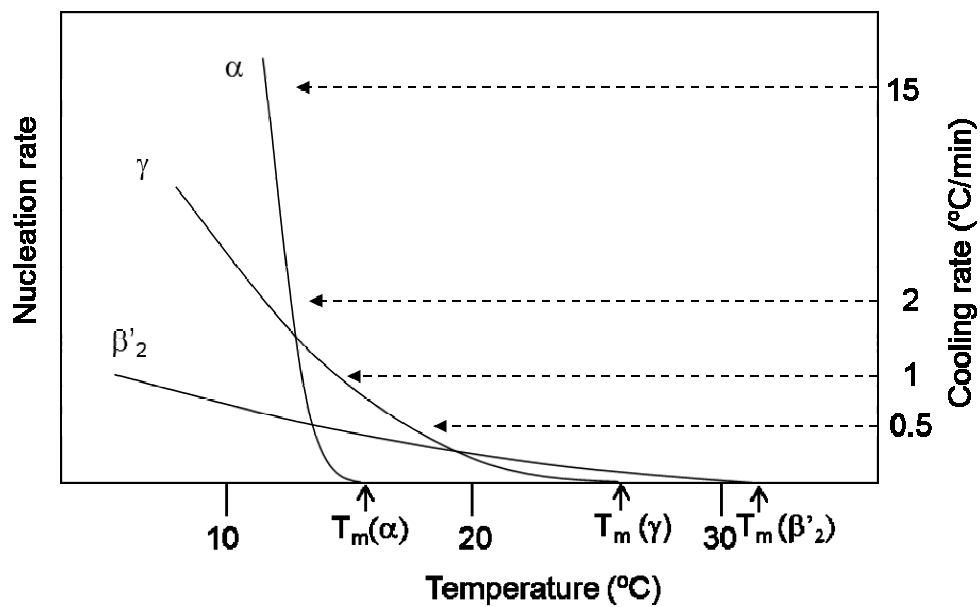


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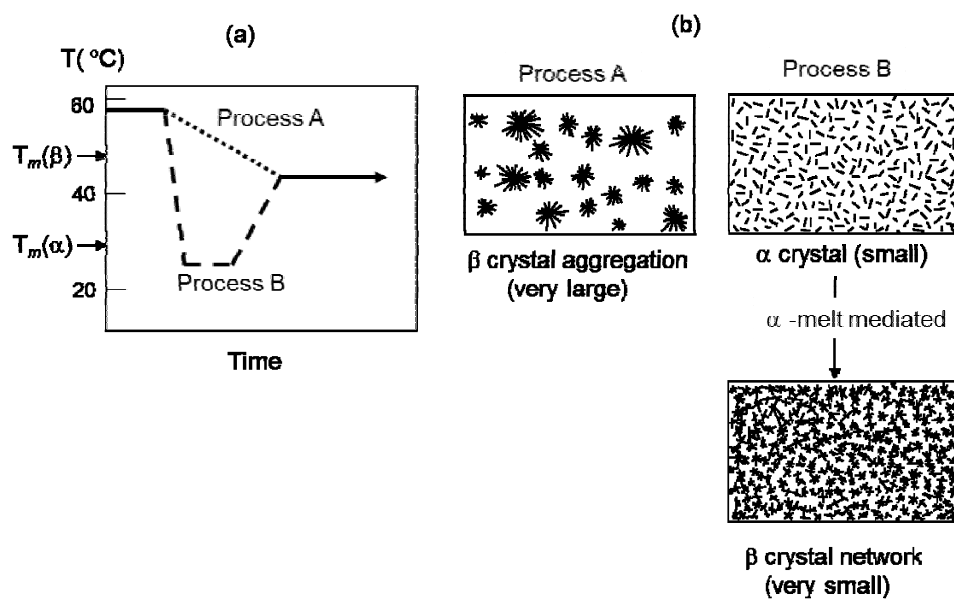


Figure 8.

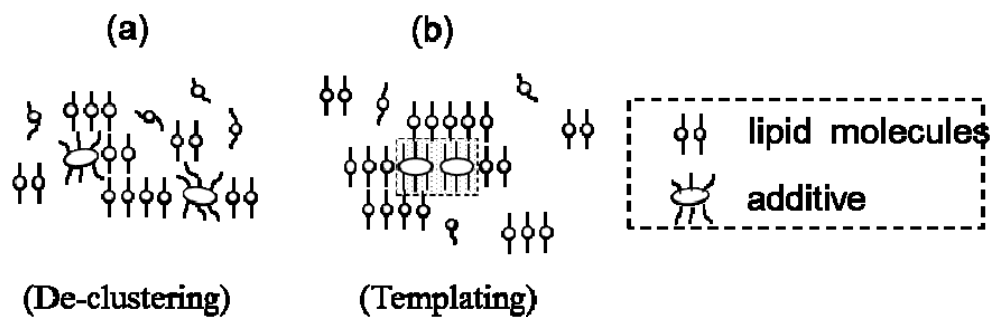


Figure 9.

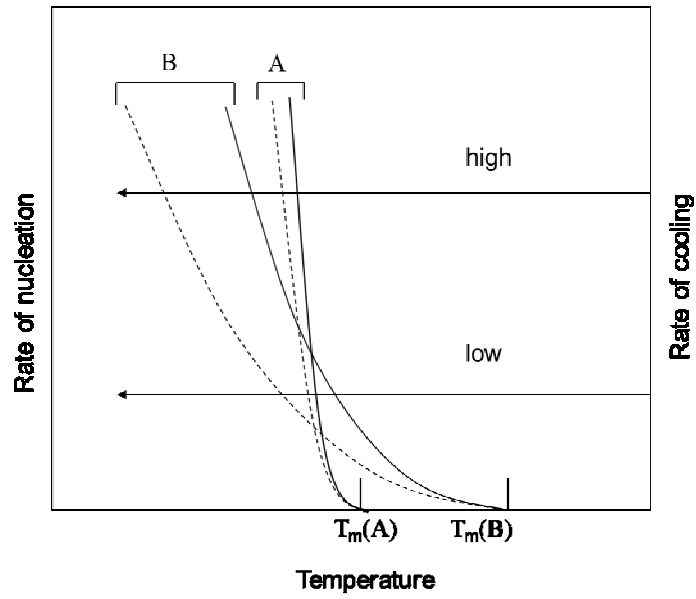


Figure 10

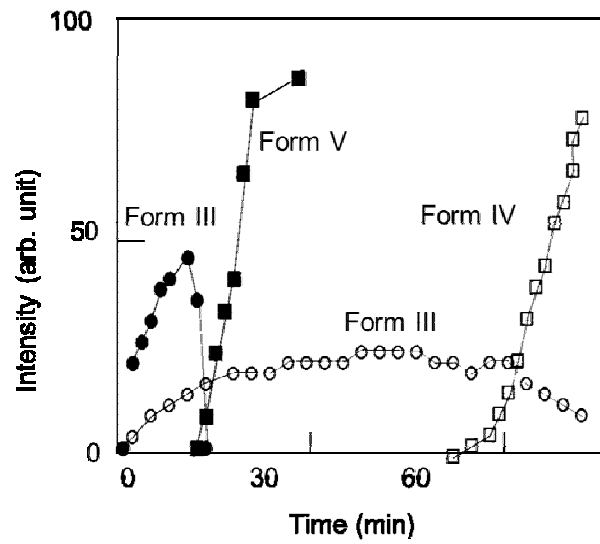


Figure 11

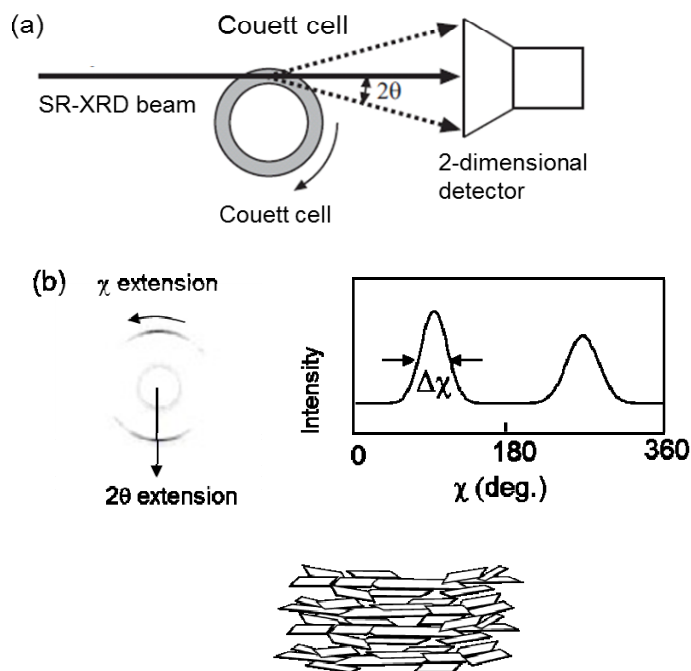


Figure 12

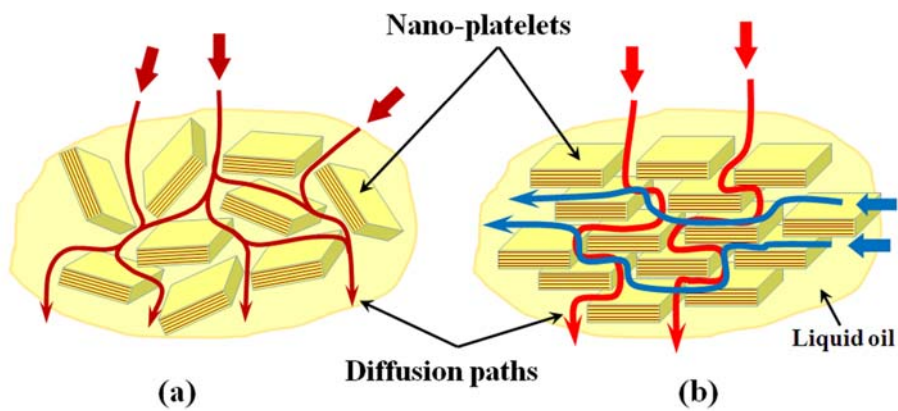




Figure 13

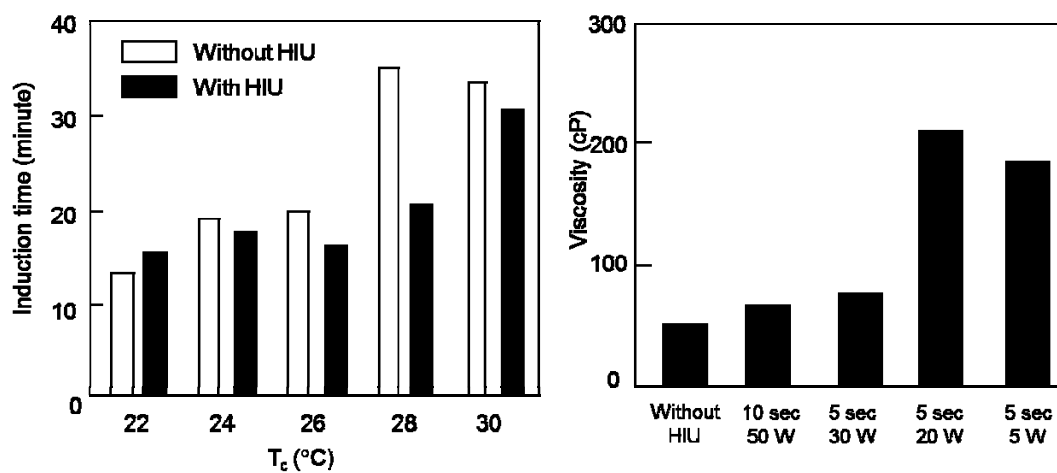


Figure 14

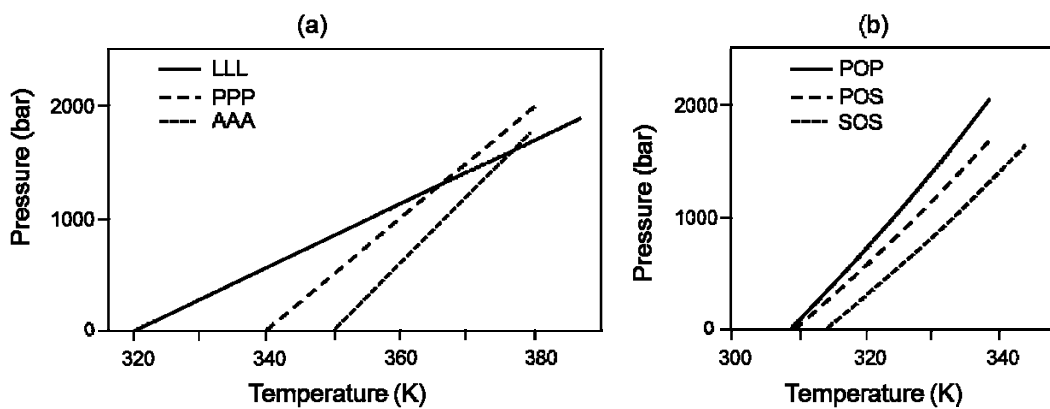


Table 1 Diagram of polymorphic pathways of POP under different cooling and heating rates

Cooling	Heating
liquid 15 °C/min 2 °C/min → α  1 °C/min 0.5 °C/min → γ	15 °C/min → liquid → β' → liquid
	2 °C/min → liquid → γ → liquid → β → liquid
	1 °C/min → γ → liquid → β → liquid
	0.1 °C/min → γ → δ → liquid → β → liquid
	15 °C/min → liquid
	2 °C/min → liquid
	0.5 °C/min → liquid → β' → liquid
	0.1 °C/min → liquid → β' + δ → liquid → β → liquid