

Effects of adding foreign particles on crystallization and physical properties of fat-based products

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CONTENTS

Chapter 1	1
General introduction	
Chapter 2	25
Promotional effects of new types of additives on fat crystallization	
Chapter 3	47
Fat crystallization with talc particles is influenced by particle size, concentration, and cooling rate	
Chapter 4	67
Adding talc particles improves physical properties of palm oil-based shortening	
Summary	89
Acknowledgements	93
List of Publications	95

List of Abbreviations

CNT	Carbon nanotube
DSC	Differential scanning calorimetry
EAD	Ellagic acid dihydrate
10G6P6O	Decaglycerin hexapalmitoyl hexaoleate
10G8P4O	Decaglycerin octapalmitoyl tetraoleate
10G12P	Decaglycerin dodecapalmitate
LLL	Trilauroylglycerol
MC	Molecular compound
MMM	Trimyristoylglycerol
OOL	1,2-dioleoyl-3-linoleoyl- <i>rac</i> -glycerol
OOO	Trioleoylglycerol
OPO	1,3-dioleoyl-2-palmitoyl glycerol
PET	Polyethylene terephthalate
PO	Palm oil
POM	Polarized optical microscope (microscopy)
POO	1-palmitoyl-2,3-dioleoyl glycerol
POP	1,3-dipalmitoyl-2-oleoyl glycerol
PPO	1,2-dipalmitoyl-3-oleoyl glycerol
PPP	Tripalmitoylglycerol
PS	Palm stearin
RPO	Refined palm oil
SEM	Scanning electron microscope
SO	Soybean oil
SSHE	Scraped surface heat exchanger
TAG	Triacylglycerol
XRD	X-ray diffractometry (diffraction)

Chapter 1

General Introduction

1 Crystal structure and Bragg's condition

In the nature system, organic and inorganic materials change phases according to their atomic or molecular arrangement. The crystal phase high in spatial regularity occurs when the arrangement is highly ordered. Therefore, crystal structure is describable by translational repetitions of the unit cell; the minimum unit is termed “single unit cell”. The parallelepiped shape of such unit cells is defined by the three-dimensional vectors from one lattice point to another in the same surroundings (Fig. 1.1). As shown in Table 1.1, these unit cells are distributed into seven lattice systems with the 14 Bravais lattices, based on the fundamental structures with lattice parameters for the edge length (a , b , and c) and formed angle (α , β , and γ) [1]. In addition, elements in the same nature or condition make a local lattice structure called “sub-cell”.

Due to the spatial regularity, all of the lattice points can lie at intervals in a set of evenly-spaced parallel planes. These “lattice planes”, arbitrarily selectable in numberless patterns, are collectively addressed by the Miller index [2]. Assuming that one of the lattice planes crosses the xyz crystal axes at coordinates $(u, 0, 0)$, $(0, v, 0)$, and $(0, 0, w)$, the Miller index (hkl) of the plane is decided as a minimum integer ratio of a/u , b/v , and c/w . If the plane is parallel to some of these axes, the intercept values are regarded as infinite and thus, returning zeros to the index numbers. Interception with the

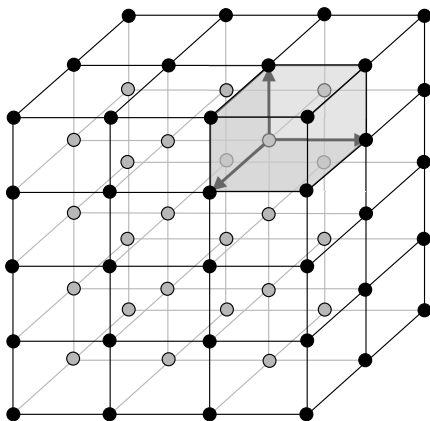
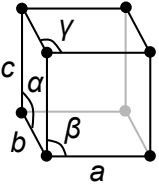
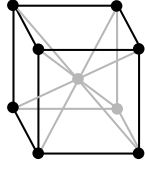
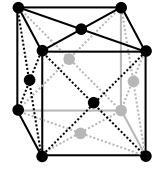
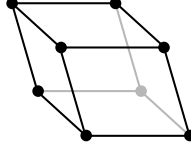
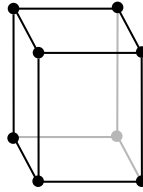
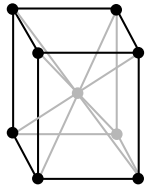
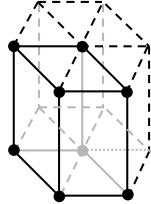
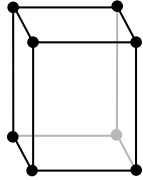
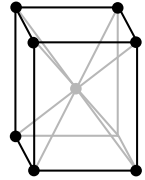
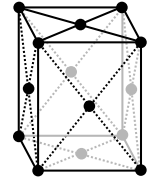
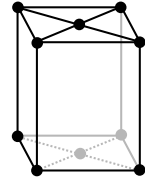
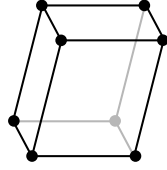
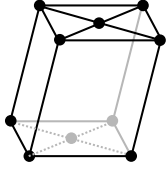
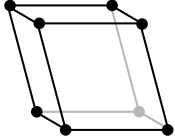


Figure 1.1 Schematic illustration of a unit cell (gray colored) in three-dimensional crystal structure. Arrows indicate translational vectors, and circle symbols denote lattice points.

Table 1.1 Classification of unit cells into seven lattice systems with 14 Bravais lattices.

Lattice system	Lattice parameter	Bravais lattice			
		Simple	Body-centered	Face-centered	Base-centered
Cubic	$a = b = c$ $\alpha = \beta = \gamma = 90^\circ$				
Rhombohedral	$a = b = c$ $\alpha = \beta = \gamma \neq 90^\circ$				
Tetragonal	$a = b \neq c$ $\alpha = \beta = \gamma = 90^\circ$				
Hexagonal	$a = b \neq c$ $\alpha = \beta = 90^\circ$ $\gamma = 120^\circ$				
Orthorhombic	$a \neq b \neq c \neq a$ $\alpha = \beta = \gamma = 90^\circ$				
Monoclinic	$a \neq b \neq c \neq a$ $\alpha = \gamma = 90^\circ$ $\beta \neq 90^\circ$				
Anorthic (Triclinic)	$a \neq b \neq c \neq a$ $\alpha, \beta, \gamma \neq 90^\circ$				

plane at negative sides of the axes gives negative values to the index numbers, which are labeled by placing minus signs over the numbers. As an example, Fig. 1.2 depicts various planes in an orthorhombic lattice together with their Miller indices. Parallel planes and planes identical in the space-group symmetry are crystallographically regarded as equal.

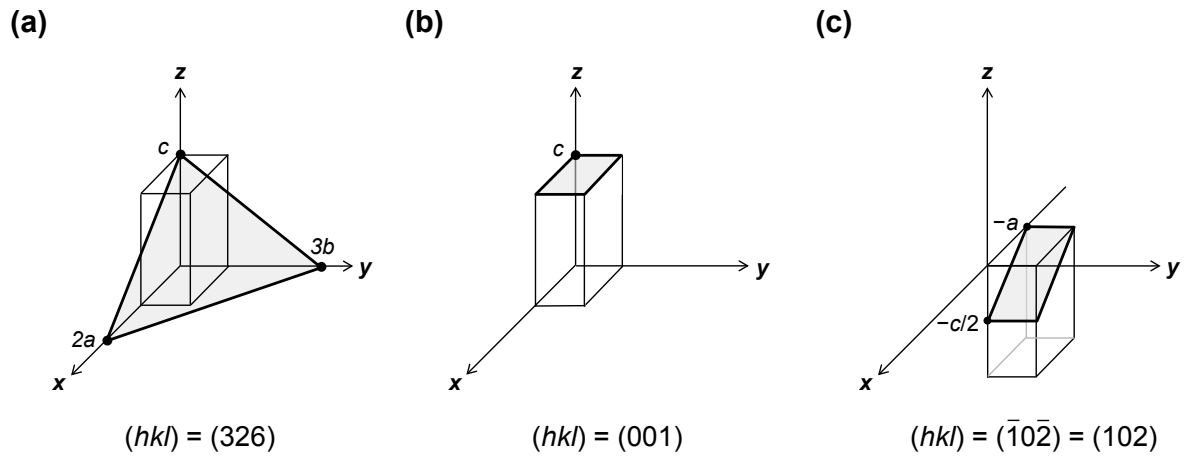


Figure 1.2 Miller indices (hkl) of various planes in an orthorhombic lattice.

To resolve the spatial lattice structure of crystals, diffraction methods of X-ray, electron and atomic beams, and neutron are popularly used as strong tools. Application of X-rays on materials induces the scattering from electrons, such as the Thomson scattering, Compton scattering, and X-ray fluorescence. Among these scatterings, the Thomson scattering with elastic nature exhibits a diffraction phenomenon due to interference, available for the analysis of the lattice structure [3]. When monochromatic X-rays with a single wavelength have reached atoms of a subject material, some of the outer electrons are forced to vibrate in the oscillating electric field. These electrons thus excited act as sources of dipole radiation to generate electromagnetic waves. In this Thomson scattering, the applied X-rays partly convert to the scattering waves without changing the wavelength (elastic), though uniformly shifting their phases by 180° . Provided that differences in the total path length among the associating electrons are integer times of the wavelength, the scattering waves perfectly match their phases so as to make the constructive interference strongest; as a consequence, the X-ray diffraction occurs most intensely in a specific direction.

Here, we consider the conditions for such X-ray diffraction from single and multiple lattice planes (Fig. 1.3), wherein the adjacent lattice-point distance w and lattice-plane distance d are defined. Using arbitrary positive integers j and m , the distances between two randomly selected lattice points and planes are expressed as jw and md , respectively. When parallel beams of the applied X-rays turn at two lattice points in a single plane (Fig. 1.3a), the path-length difference ΔL_1 is expressed as follows:

$$\Delta L_1 = OQ - PR = jw (\cos \delta - \cos \varepsilon) \quad \text{<Eq. 1.1>},$$

where δ is the depression angle of incident X-rays, and ε is the elevation angle of a scattering direction

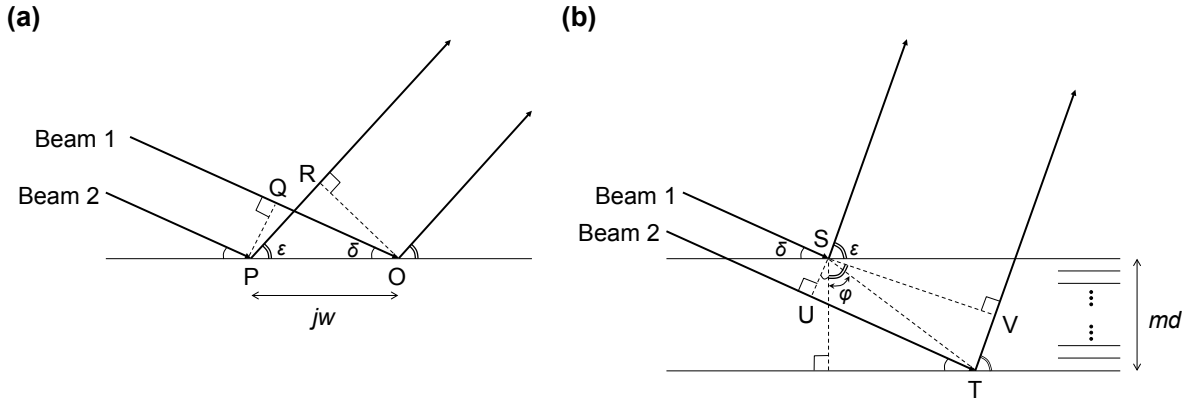


Figure 1.3 Case study for the path-length difference between two parallel X-ray beams, turning at lattice points in (a) a single plane and (b) multiple planes.

from the plane. The sum of these angles corresponds to the so-called scattering angle. As mentioned above, the X-ray diffraction occurs most intensely as long as the path-length difference is always integer times of the wavelength, regardless of the j value. One may notice from Eq. 1.1 that this condition is always met when the scatterings occur in symmetric reflection manners ($\delta = \varepsilon$).

In the other case that the parallel beams turn at two lattice points in discrete lattice planes (Fig. 1.3b), the path-length difference ΔL_2 is expressed as follows:

$$\Delta L_2 = TU + TV = md \frac{\sin(\delta + \varphi) + \sin(\varepsilon - \varphi)}{\cos \varphi} \quad \text{<Eq. 1.2>},$$

where φ is the glancing angle from the point S to T. If the scatterings occur in asymmetric reflection manners ($\delta \neq \varepsilon$), the glancing angle φ is decisive for the phase difference between the scattering waves. This means that the asymmetric scatterings from multiple lattice planes interfere randomly, depending on the relative positions of lattice points therein; consequently, these scatterings offset one another to make the resultant X-ray diffraction negligible.

For the scatterings in symmetric reflection manners ($\delta = \varepsilon = \theta$), Eq. 1.2 transforms to

$$\Delta L_2 = md \frac{\sin(\theta + \varphi) + \sin(\theta - \varphi)}{\cos \varphi} = 2md \sin \theta \quad \text{<Eq. 1.3>},$$

for which the trigonometric addition theorem is used. This indicates that all of the path-length differences in the symmetric scatterings are decided by lattice-plane distances and depression angle of incident X-rays alone. The condition for these scatterings to make the constructive interference strongest is expressed as follows:

$$2md \sin \theta = n\lambda \quad \text{<Eq. 1.4>},$$

where n is an arbitrary integer, and λ is the wavelength of applied X-rays. When Eq. 1.4 holds for any m value, the X-ray diffraction occurs most intensely. The sufficient condition for this is

$$2d \sin \theta = n\lambda \quad \text{<Eq. 1.5>},$$

known as the “Bragg’s condition”. In the nomenclature, θ is the Bragg angle, and n is the reflection order; additionally, 2θ is referred to as the diffraction angle.

2 Polymorphism of TAG crystals

Fats are the major materials for imparting solid characters to foods, cosmetics, and pharmaceuticals. The main components of fats are triacylglycerols (TAGs), whose crystallization and melting properties largely affect the quality, productivity, and storage stability of fat-based products. Figure 1.4 illustrates the structure of TAG molecules, where three fatty acids ester-bond to one glycerol; the acyl chains vary in length with the carbon number and in shape with the number, position, and conformation of C–C double bonds. Such variety of the fatty acid moieties, together with the stereospecific combination thereof, defines the crystallization and melting properties of TAGs [4].

When TAGs crystallize from the melt or solution, the molecules are highly ordered through the aliphatic chain-chain interactions (Fig. 1.5) [5, 6]. Transforming into a chair shape, TAG molecules in

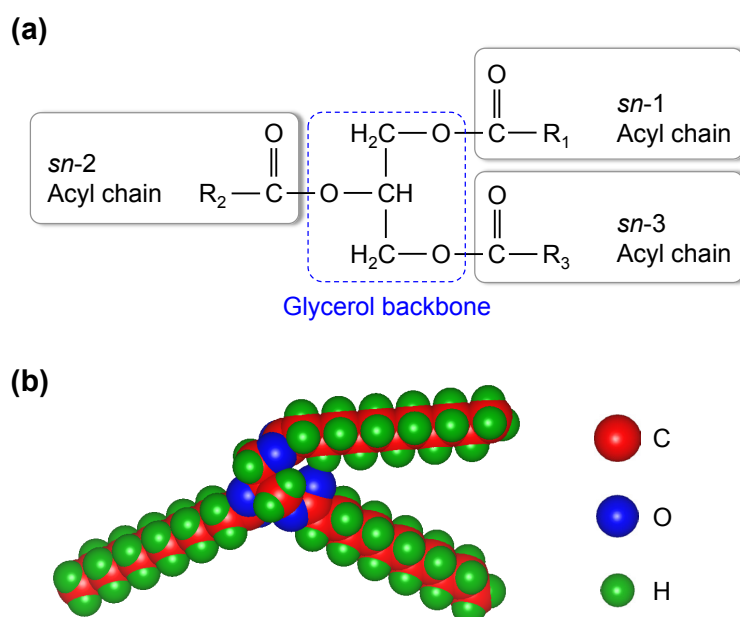


Figure 1.4 Structure of TAG molecules. (a) Representative chemical structure and (b) space-filling model for trioleoylglycerol (LLL).

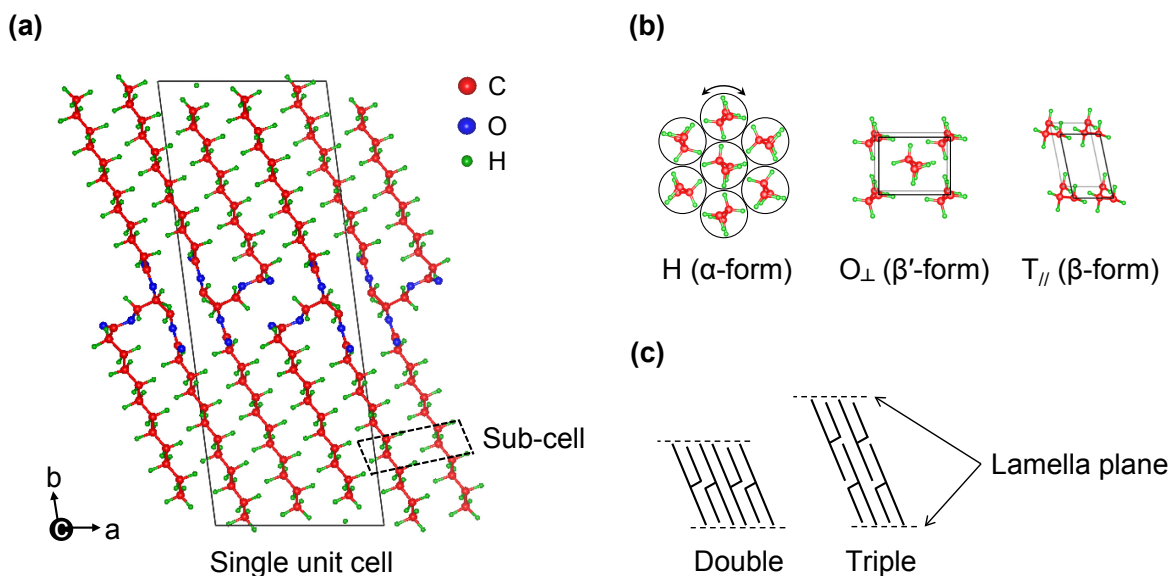


Figure 1.5 Molecular arrangement in TAG crystals. (a) Molecular packing of LLL β [7], drawn by VESTA [8], (b) different types of sub-cells in acyl-chain leaflets, viewed from methyl ends, and (c) chain-length structures.

opposite directions are alternately aligned in parallel rows to make a lamellar layer, which piles up periodically (Fig. 1.5a). Viewed from the methyl ends of TAG molecules, the sub-cell structure in the acyl-chain leaflets is expressed as Fig. 1.5b, where the hydrocarbon chains extend in a front-to-back direction of this paper. According to the spatial layout of the cross-sectional hydrocarbon chains, TAG crystals show polymorphism of the sub-cells; for example, the typical polymorphs α , β' , and β are attributed to the chain packing in hexagonal sub-cell with random arrangement (H), orthorhombic sub-cell with orthogonal arrangement ($O_{\perp}$), and triclinic sub-cell with parallel arrangement ($T_{\parallel}$), respectively. The sub-cell structure influences the chain length between lamella planes formed along the methyl-end groups of TAG molecules (Fig. 1.5c). The lamella planes are often referred to as (001) planes, using the Miller-index notation.

The sub-cell and chain-length structures of TAG crystals are detectable in the X-ray diffractometry (XRD). Figure 1.6 shows the correspondence between these structures and the diffraction pattern of TAG crystals, using the XRD data of LLL β -form crystals. While the structural information on the sub-cell is reflected in a wide-angle region of the diffraction angle 2θ , that on the chain length is reflected in a small-angle 2θ region. Short spacing of the aliphatic-chain packing and long spacing between the lamella planes are calculated from diffraction angles of the XRD peaks and wavelength of the applied X-rays, based on Eq. 1.5 for the Bragg's condition. The typical polymorphs of TAG crystals are characterized by the short-spacing peaks as follows: one strong peak of ~ 0.42 nm

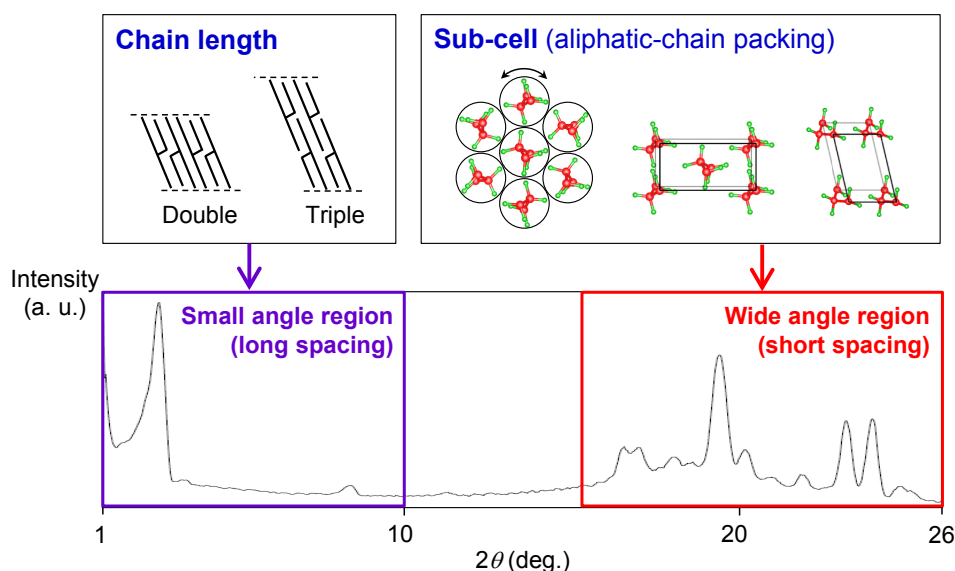


Figure 1.6 Correspondence between structure of TAG crystals and XRD pattern.

for α , two strong peaks of 0.37–0.40 nm and 0.42–0.43 nm for β' , and one strong peak of 0.46 nm and a number of strong peaks of 0.36–0.39 nm for β [9].

The polymorphic transformation of TAG molecules is achieved by the optimization of C–C–C single-bond configurations to minimize the total steric energy. Figure 1.7 illustrates the typical configurations of the adjacent C–C bonds with their torsion angles: *cis* ($\pm 0^\circ$), *gauche* ($+60^\circ$), *gauche'* (-60°), *skew* ($+120^\circ$), *skew'* (-120°), and *trans* ($\pm 180^\circ$). Due to the lower steric energy, the paraffinic chain advantageously takes *trans* configurations in the stable forms, whereas taking *gauche* and *gauche'* ones in the less stable forms. In the *cis*-olefinic chain, the concentration of *gauche* and *gauche'* configurations may rise particular in the paraffinic segment between the *cis*-double bond and methyl end. For two C–C–C bonds sandwiching the *cis*-double bond, not only stable configurations of *skew-cis-skew'* and *skew-cis-skew* but also a unique one of *trans-cis-trans* can be taken with some torsion-angle deviations, which offers diversity in the polymorphism [10]. On the other hand, the C–C–C configuration of the glycerol backbone allows the connecting acyl chains to translocate in a tuning-fork or chair style (Fig. 1.8) [11]. Mismatches of the molecular packing in methyl-end stacking improve with the chain inclination or lamellar twinning, as shown in Fig. 1.9 [6].

In the aspect of thermodynamics, the polymorphic transformation of TAG molecules proceeds to minimize the Gibbs energy of the whole system by reducing their chemical potential [13]. When phase transitions spontaneously occur in the isothermal, isobaric system, the Gibbs-energy variation ΔG at a temperature T is expressed as follows:

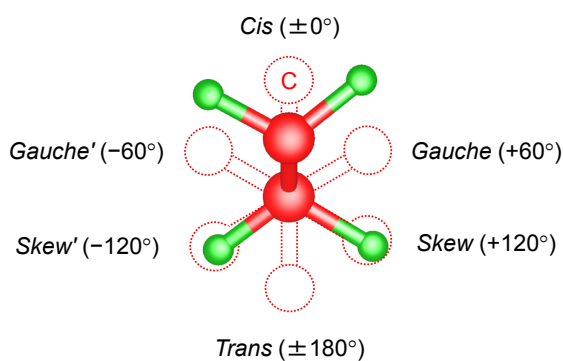


Figure 1.7 Typical configurations of adjacent C–C bonds with torsion angles.

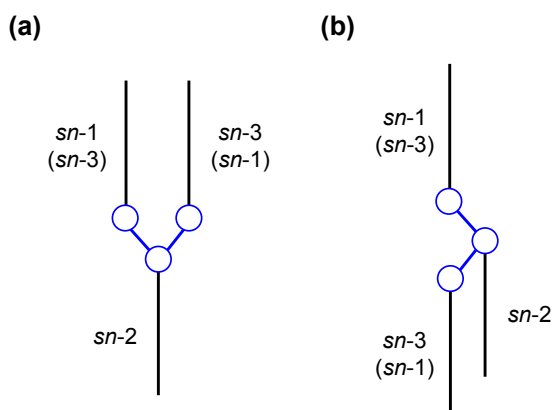


Figure 1.8 Glycerol configurations of TAG molecules in (a) tuning-fork and (b) chair styles. Black lines and blue lines linked with circles represent acyl chains and glycerol backbones, respectively.

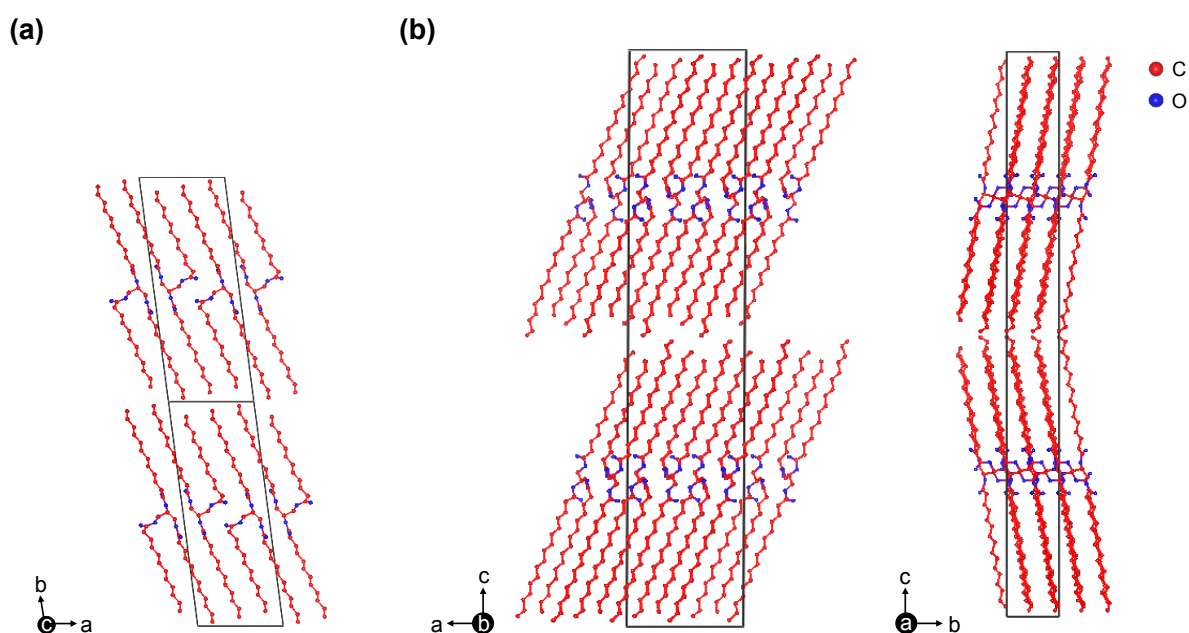


Figure 1.9 Crystal structures of (a) LLL β [7] and (b) 1,2-dipalmitoyl-3-myristoyl glycerol (PPM) β'_2 [12], drawn by VESTA [8]. Hydrogen atoms are not shown.

$$\Delta G = \Delta H - T\Delta S (< 0) \quad \text{<Eq. 1.6>},$$

where ΔH and ΔS are the variations in enthalpy and entropy of the system, respectively. Using the

chemical-potential variation $\Delta\mu_i$ and molar content x_i of the i th system component, the ΔG is also expressed as follows:

$$\Delta G = \sum_i x_i \Delta\mu_i \quad \text{<Eq. 1.7>}$$

Therefore, the chemical-potential difference $\Delta\mu$ between the phases acts as the driving force for phase transitions, provided that the $\Delta\mu$ value is negative. Assuming that the ΔH and ΔS are constant without regard to the temperature, the driving force $\Delta\mu_{L \rightarrow S}$ for the liquid-to-solid phase transition (melt crystallization) at a temperature T is given by

$$\Delta\mu_{L \rightarrow S} = \frac{\Delta H_m (T - T_m)}{T_m} = \frac{\Delta H_m \Delta T}{T_m} \quad \text{<Eq. 1.8>},$$

where ΔH_m and T_m are the molar enthalpy change in fusion and melting point of the solid phase, respectively. In the supercooling region ($\Delta T = T - T_m < 0$), the $\Delta\mu_{L \rightarrow S}$ takes negative values. For a solute in the solution system, Eq 1.8 is translated into

$$\Delta\mu_{L \rightarrow S} = k_B T \ln \frac{C_0}{C} \quad \text{<Eq. 1.9>},$$

where k_B is the Boltzmann constant, C_0 is a saturation concentration of the solute at a temperature T , and C is a concentration of the solute.

Figure 1.10 illustrates a model diagram of the Gibbs energy increasing with decreasing temperature at a constant pressure, drawn for the liquid and solid phases of a single-TAG system [14]. In Fig 1.10a, the typical polymorphs α , β' , and β in a descending order of the Gibbs energy (i.e., ascending order of thermodynamic stability) are presented as the solid phases; the non-crossing energy curves indicate the monotropic nature of TAG crystals that the molecules therein transform in an irreversible direction toward stable polymorphs. However, the polymorphic transformation may be disturbed by the steric hindrance requiring large energy for the molecular rearrangement, which allows TAG crystals to stay in metastable polymorphs till melting. The equilibrium between the liquid and solid phases is reached at the intersections of their energy curves, giving different melting points T_m unique to the polymorphs.

Figure 1.10b–d shows example Gibbs-energy pathways of the TAG system during cooling and heating. In the melt crystallization (Fig. 1.10b), the supercooled liquid first transforms to the closest solid phase with respect to the Gibbs energy due to the lowest energy barrier for nucleation (see

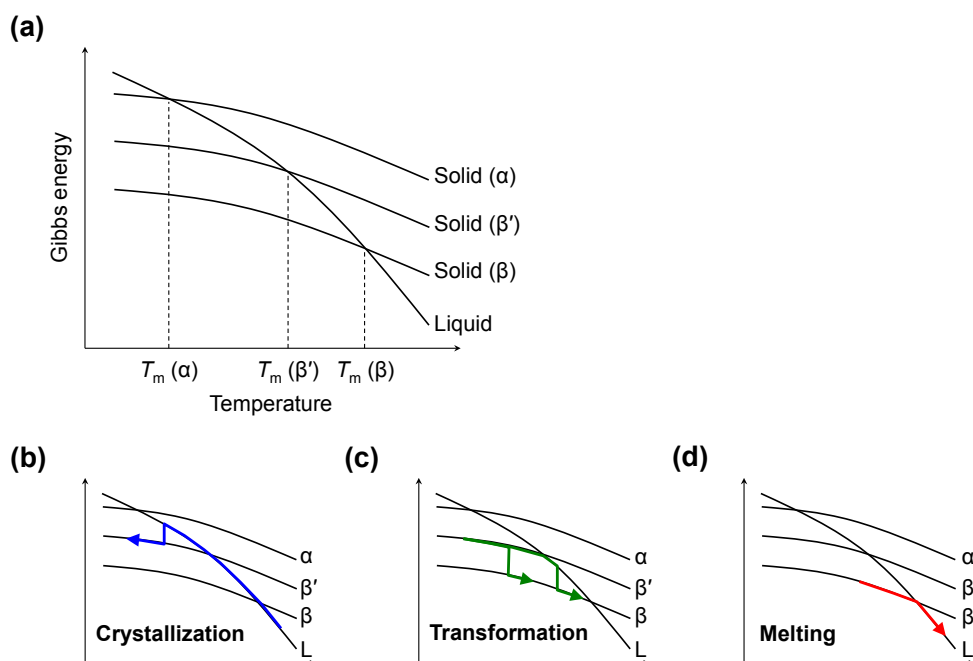


Figure 1.10 (a) Model diagram of the Gibbs energy of a single-TAG system and (b–d) its example pathways accompanying phase transitions.

below). The degree of supercooling decisive for the first-occurring polymorph is variable, depending on the TAG species and external factors (e.g., cooling rate, pressure, and contamination of impurities) [9, 14, 15]. In Fig. 1.10c, the crystals thus formed transform to the more stable phases in a solid state or through melt mediation; the solid-state transformation can occur in the process of cooling as well as heating. In Fig. 1.10d, the crystals start to melt with heating at the intersections of the energy curves between the solid phases and the liquid phase. Figure 1.11 summarizes the possible pathways of phase transition in this TAG system. For multi-component TAG systems including natural fats, the phase behavior becomes much more complicated due to the mutual solubility and compatibility of the constituent TAGs in different concentrations [5, 11, 15–26]. In either case, the enthalpy change ΔH involved in the phase transition is detectable in the differential scanning calorimetry (DSC).

The polymorphism of TAG crystals provides variety in the crystal features. As an example, Table 1.2 shows the representative features of TAG crystals corresponding to the three main polymorphs, α , β' , and β [27, 28]. In terms of the thermodynamic stability, the crystals are least stable in α , intermediately stable in β' , and most stable in β . The more stable the crystals are thermodynamically, the higher melting points of the crystals are. Generally in the case of TAGs, large, thick crystals with high density tend to be formed, as the thermodynamic stability increases. Other than these three polymorphs, several polymorphs (e.g., sub- α , γ , and δ) and subtypes like β_2 and β_1

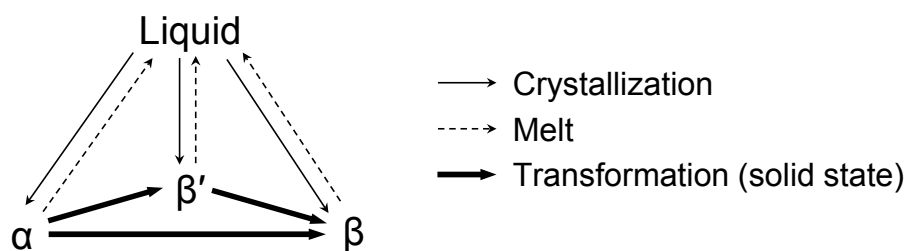


Figure 1.11 Phase transition pathways of TAGs with typical polymorphs.

Table 1.2 Features of TAG crystals in main polymorphs.

	Polymorph		
	α	β'	β
Thermodynamic stability	→ Stable		
Melting point (T_m)	→ High		
Density	→ High		
Size	→ Large		
Shape	→ Thick		

(melting point; $\beta_2 < \beta_1$) have hitherto been defined for TAG crystals [29, 30]. It is said that the β' -form crystals are suitable for the moderate plasticity and consistency of margarine and shortening, while the β_2 -form crystals compatibly impart the desirable sharp-melting, snapping, and demolding properties to chocolate. The $\beta' \rightarrow \beta$ and $\beta_2 \rightarrow \beta_1$ transformations are thought to be one of the causes to degrade these products, giving undesirable properties of low plasticity, sandy mouthfeel, poor meltability, matte or white-spotted surface (so-called fat bloom), and so forth [28].

In this way, the polymorphic difference of TAG crystals extends as far as the macroscopic physicochemical properties of fat-based products, because the growing crystals develop their own geometric structure into high-order networks [14, 31, 32]. Figure 1.12 schematically presents sequential development processes of the crystal structure in TAG systems. At the early stage, TAG molecules in the liquid phase lose their mobility with decreasing temperature, and then assemble into clusters through the van der Waals force. With further cooling, embryos of these clusters evolve into the crystal nuclei, which subsequently undergo a growth phase. At the middle stage, progress in the crystal growth accompanies the assembly and solid-bridge formation (sintering) between the crystals. At the later stage, the crystal networks thus formed may be reconstructed by the change in the crystal

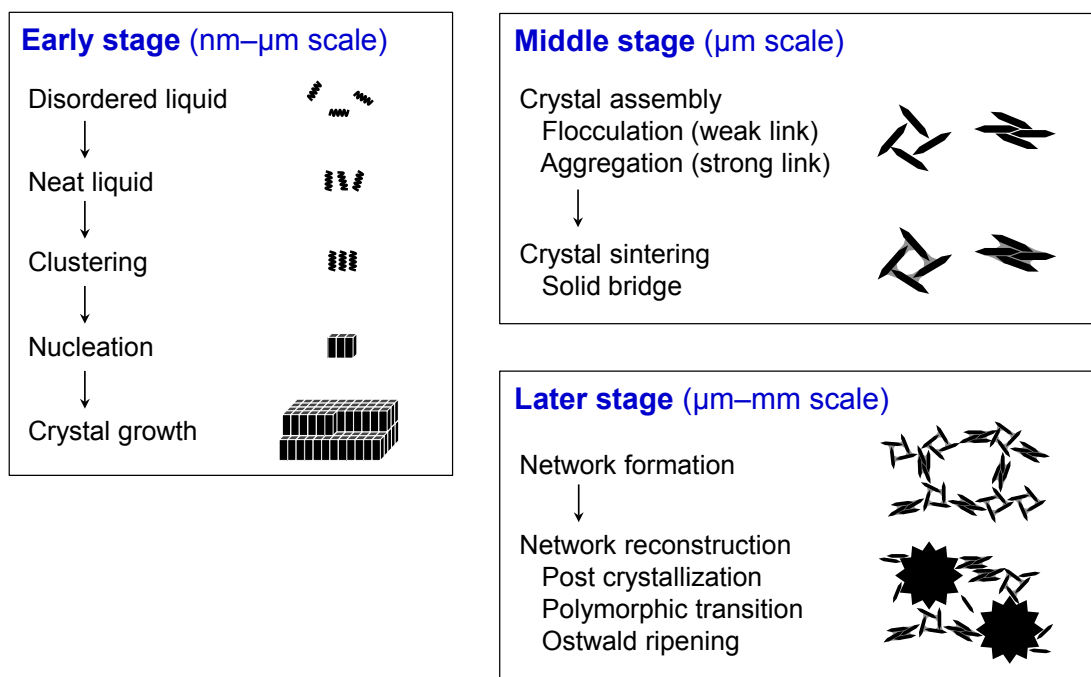


Figure 1.12 Schematic presentation of sequential development processes of the crystal structure in TAG systems.

condition, which arises from the thermal fluctuation and long-term storage. Among all these processes, the nucleation occurring at the initial stage of crystallization is influential over the downstream sequence, affecting the crystal-structure development and the resultant texture of final products; therefore, the way to control this nucleation has been pursued for a long time.

3 Nucleation kinetics

In the formation of a solid phase from a supercooled or supersaturated liquid, the “primary nucleation” occurs independently of the previously formed crystals, while the “secondary nucleation” is induced by these crystals and the chipped fragments. Further, the primary nucleation occurs either spontaneously in the bulk phase (homogeneous nucleation) or inducibly at the foreign solid surfaces (heterogeneous nucleation), as shown in Fig. 1.13 [9, 33–35].

Creation of new interfaces between the liquid and solid phases requires extra energy of molecules to be adsorbed on the solid surfaces. In one case that a spherical cluster with a radius r forms spontaneously from the mother liquid, the classical nucleation theory expresses the accompanying Gibbs-energy change $\Delta G_{\text{hom}(r)}$ as follows:

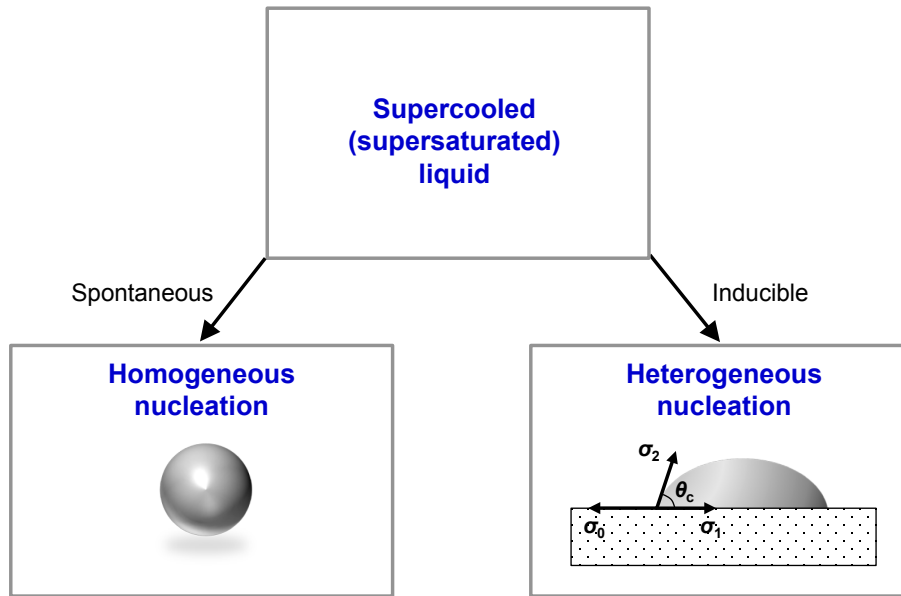


Figure 1.13 Two manners of primary nucleation.

$$\Delta G_{\text{hom}(r)} = \Delta G_{\text{vol}(r)} + \Delta G_{\text{sur}(r)} \quad \text{<Eq. 1.10>},$$

where $\Delta G_{\text{vol}(r)}$ and $\Delta G_{\text{sur}(r)}$ are the corresponding variations in the volume and surface Gibbs energies, respectively. Using ΔG_v as the partial volume-Gibbs-energy difference (per unit volume) between the liquid and solid phases and σ as the interfacial energy per unit area, this equation transforms to

$$\Delta G_{\text{hom}(r)} = \frac{4\pi r^3}{3} \Delta G_v + 4\pi r^2 \sigma \quad \text{<Eq. 1.11>}.$$

One may notice that the predominant factor for $\Delta G_{\text{hom}(r)}$ is the positive value of σ in a small r range, whereas the negative value of ΔG_v in a large r range; thus, the $\Delta G_{\text{hom}(r)}$ exhibits its maximum value of ΔG_{hom}^* at a critical nucleus radius r^* , as shown in Fig. 1.14. In the region of $r < r^*$, the cluster growth to increase r is discouraged by the incidental elevation in $\Delta G_{\text{hom}(r)}$, letting the cluster stay an unstable embryo. In the region of $r > r^*$, the cluster is conversely ready to grow into a stable nucleus for the Gibbs-energy downhill. Therefore, the ΔG_{hom}^* refers to the energy barrier for homogeneous nucleation, which causes the supercooling phenomenon. At the critical point of $r = r^*$, the variation rate of $\Delta G_{\text{hom}(r)}$ with respect to r equals zero ($\partial \Delta G_{\text{hom}(r)} / \partial r = 0$), giving a relational expression as follows:

$$4\pi r^{*2} \Delta G_v + 8\pi r^* \sigma = 0 \quad \text{<Eq. 1.12>}.$$

Accordingly, one obtains

$$r^* = -\frac{2\sigma}{\Delta G_v} \quad \text{<Eq. 1.13>}.$$

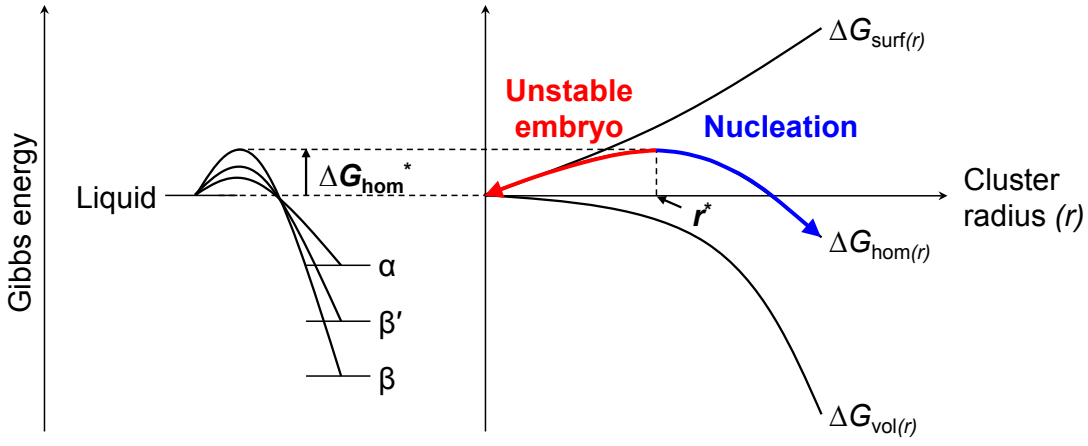


Figure 1.14 Model diagram of the Gibbs-energy change for homogeneous nucleation from a liquid phase.

By substituting this r^* for the r in Eq. 1.11, the ΔG_{hom}^* is expressed as follows:

$$\Delta G_{\text{hom}}^* = \frac{16\pi\sigma^3}{3(\Delta G_v)^2} \quad \text{<Eq. 1.14>}$$

Assuming that the liquid and solid phases show the same molar volume v , the ΔG_v is given by

$$\Delta G_v = \frac{\Delta\mu_{L \rightarrow S}}{v} \quad \text{<Eq. 1.15>}$$

where $\Delta\mu_{L \rightarrow S}$ is the chemical-potential difference between the liquid and solid phases. From Eq. 1.14 and Eq. 1.15, one derives

$$\Delta G_{\text{hom}}^* = \frac{16\pi\sigma^3 v^2}{3(\Delta\mu_{L \rightarrow S})^2} \quad \text{<Eq. 1.16>}$$

For homogeneous nucleation in different shapes, this equation is normalized to

$$\Delta G_{\text{hom}}^* = \frac{f\sigma^3 v^2}{(\Delta\mu_{L \rightarrow S})^2} \quad \text{<Eq. 1.17>}$$

where f is a shape factor (e.g., $f = 32$ for a cube with $2r$ edge length). From Eq. 1.8 and Eq. 1.17, one finds

$$\Delta G_{\text{hom}}^* = \frac{f\sigma^3 v^2 T_m^2}{(\Delta H_m)^2 (\Delta T)^2} \quad \text{<Eq. 1.18>}$$

Due to the all polymorph-dependent factors included in the right side of this equation, the energy barrier for nucleation differs between the crystal polymorphs, as indicated in Fig. 1.14. Additionally, the polymorphic order in the energy-barrier level may possibly change with the extent of supercooling ΔT .

In the other case that a cluster forms in a cap shape inducibly at a surface of the foreign substrate, the Young's equation gives a relational expression as follows:

$$\sigma_0 = \sigma_1 + \sigma_2 \cos \theta_c \quad \text{<Eq. 1.19>},$$

where σ is an interfacial energy (σ_0 for liquid–substrate, σ_1 for cluster–substrate, and σ_2 for liquid–cluster) and θ_c is a contact angle of the cluster with respect to the substrate surface. By regarding this cap-shaped cluster as a spherical segment, the energy barrier for this heterogeneous nucleation (ΔG_{het}^*) is derived as follows:

$$\Delta G_{\text{het}}^* = \Delta G_{\text{hom}}^* \left\{ \frac{1}{2} - \frac{3}{4} \cos \theta_c + \frac{1}{4} (\cos \theta_c)^3 \right\} \quad \text{<Eq. 1.20>},$$

which indicates that ΔG_{het}^* is always smaller than ΔG_{hom}^* and the ratio of the both depends on the contact angle θ_c (e.g., $\Delta G_{\text{het}}^*/\Delta G_{\text{hom}}^* = 1/2$ for $\theta_c = 90^\circ$). High affinity between the cluster and substrate surface results in small θ_c and ΔG_{het}^* ; as a consequent of the low energy barrier, nucleation and subsequent crystallization will be promoted. Therefore, the substrate surface is thought to act as a template for the heterogeneous nucleation. In the field of fat crystallization, it has been believed that such template effects are mainly attributed to the molecular similarity between crystallizing TAGs and the foreign substrates [14, 36].

The nucleation rate, the increasing number of nuclei per unit time and volume, is defined by these energy barriers in combination with the other factors, which make it difficult to fully understand the nucleation kinetics. Although some descriptions have been made for the nucleation rate [37–39], the most essential factors may be summarized as follows. (i) The frequency that one cluster incorporates one single molecule (monomer). (ii) The concentration of clusters over the critical size for nucleation. (iii) The concentration of molecules to be incorporated, which, due to the thermal vibration, acquire temporal energy enough to overcome the energy barrier for nucleation; the existence probability of such molecules can be calculated from the Boltzmann distribution. As the subsidiary factors, diffusion and association of molecules as well as collapse and coalescence of clusters should be also considered.

For fat systems, it is experimentally indicated that the nucleation rate, inversely proportional to the induction time of crystallization, exponentially increases with decreasing temperature [40–44]. Figure 1.15 schematically illustrates the relationship between the nucleation rate and temperature for

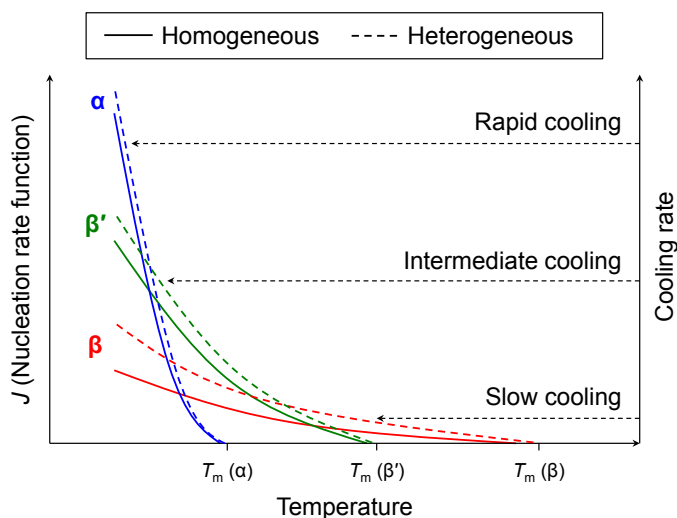


Figure 1.15 Relationship between the nucleation rate and temperature for three main polymorphs α , β' , and β when a single TAG system is non-isothermally cooled at different rates.

the three main polymorphs (α , β' , and β) of TAG crystals when a single TAG system is non-isothermally cooled at different rates. With slow cooling, nucleation occurs preferentially in stable polymorphs at high temperatures due to the high melting points. As the cooling rate increases, the preferential nucleation occurs in less stable polymorphs at lower temperatures. This is probably because (i) TAG molecules in the liquid phase are, by fast cooling, deprived of the chance to acquire extra energy enough to overcome the energy barrier for nucleation in stable polymorphs and (ii) the energy barrier is relatively low in less stable polymorphs in a low temperature range, as indicated in Fig. 1.14. The polymorph-dependent acceleration in the nucleation rate with decreasing temperature may result from different degrees of the energy-barrier reduction with extending supercooling ΔT (see Eq. 1.18). After the first nucleation, nucleation and subsequent crystallization, including polymorphic transformation of the formed crystals, progress in basic accordance with the Ostwald's step rule that the phase transitions from metastable to stable occur with the lowest energy barrier [45].

In comparison with homogeneous nucleation, heterogeneous nucleation is expected to exhibit steep acceleration in the nucleation rate for the low energy barrier (see Eq. 1.20), as indicated by dotted curves in Fig. 1.15. Large difference in the nucleation rate between homogeneous and heterogeneous nucleation has a potential to alter the order of preferential nucleation, which may be introduced by the addition of foreign substrates having high affinity to the forming nuclei. Even then, homogeneous nucleation may still occur away from the foreign substrates, particular with rapid cooling. The possible factors to affect the predominance of heterogeneous nucleation over homogeneous one are the cooling rate, concentration and positional distribution of foreign substrates, degree of their affinity to forming nuclei, and also external forces (e.g., shear and pressure) applied to the system. From the standpoint of

practical use in fat-based products, fats are often expected to crystallize early in stable polymorphs for the desirable quality and storage stability of the end products. Therefore, foreign substrates to promote fat crystallization in stable polymorphs have been explored for decades.

4 Classical fat-crystallization promoters

To date, some fat-related additives were found to promote fat crystallization by acting as templates for the heterogeneous nucleation; the typical examples are high-melting fat crystals, emulsifiers, and waxes, all of which have fatty acid moieties in their molecules [36, 46–53]. Due to the molecular similarity to fats, these additives are capable of dissolving in the molten fats and liquid oils. As illustrated in Fig. 1.16, these additives in a crystalline state serve as templates for fat crystallization, yet those in a solution state unwantedly disturb the clustering of TAG molecules [14, 50]. These conflicting effects compete with each other whether to promote or retard fat crystallization.

In one case that an equally blended fat mixture of palm stearin (PS) and soybean oil (SO) was cooled from the melt at a rate of 2°C/min, the addition of decaglycerin dodecapalmitate (10G12P, melting point: 46.8°C) retarded the crystallization at a concentration of 0.05 wt% but promoted the crystallization at concentrations of 0.5 wt% and 1.0 wt% [50]. In contrast, the addition of decaglycerin octapalmitoyl tetraoleate (10G8P4O, melting point: 33.7°C) and decaglycerin hexapalmitoyl hexaoleate (10G6P6O, melting point: 23.7°C) retarded the mixture crystallization at all concentrations of 0.05 wt%, 0.5 wt%, and 1.0 wt%.

From these results, one may assume that high-melting additives with low solubility in fat systems can act as both retardants at low concentrations and promoters at high concentrations for fat

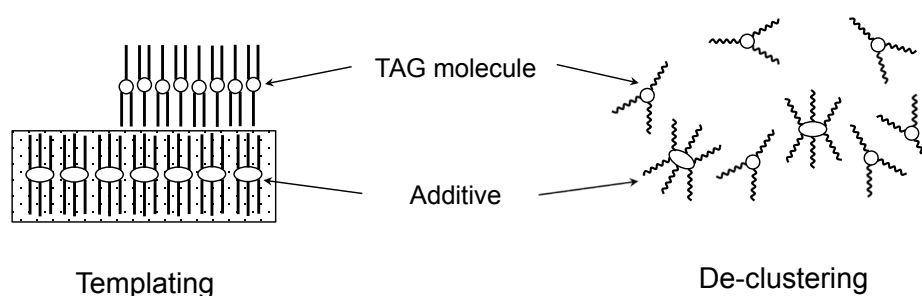


Figure 1.16 Schematic illustration of conflicting effects of additives on TAG nucleation in fat systems.

crystallization. For low-melting additives with high solubility in fat systems, the retardation effects on fat crystallization may be attributed to the molecular interactions between fats and additives in their supercooled liquid, which are likely to be maximized by the highest similarity in their fatty acid moieties. Even with these low-melting additives, promotional effects on fat crystallization might also be expected, provided that the additives possess the melting points higher than those of the crystallizing fats and are used at high concentrations so as to crystallize (or remain in a crystalline state) prior to fat crystallization. In either case, these additives must be used at high concentrations far exceeding their solubility limit in fat systems in order to ensure the promotional effects on fat crystallization. Besides, there exists a problem in employing fat-related additives that their effects on fat crystallization are sensitive to the cooling condition applied, which varies the solubility limit as well as fat crystallization temperature. Indeed, decrease in the cooling rate from 2°C/min to 0.1°C/min let the mixed fat system of PS/SO = 1/1 crystallize in the more stable polymorph at higher temperature in the absence of additives; consequently, the addition of 10G12P, 10G8P4O, and 10G6P6O (at concentrations of 0.05 wt%, 0.5 wt%, and 1.0 wt%) all retarded fat crystallization in the system.

5 Scope of this research

Based on these contexts, I launched a hypothesis that fat crystallization might be promoted by the addition of foreign solid materials having high affinity to fats but poor meltability and solubility in fats, even at low concentrations of these materials. At the beginning of this research, a number of solid materials, regardless of the molecular similarity to fats, were examined for the promotional effects on fat crystallization. Specifically, particles of these materials were screened for the ability to raise the crystallization temperatures of TAGs in the DSC experiments. As to one of the materials effectively promoting the TAG crystallization, influential factors on the promotional effects and the addition effects on the physical properties of fat-based products were then investigated for the purpose of practical application.

Chapter 2 reports the new findings that six materials promoted the crystallization of monoacid saturated TAGs in stable polymorphs, despite the molecular dissimilarity to the TAGs. Chapter 3 describes the influences on the promotional effects of adding talc particles when the size,

concentration, and anisotropic surfaces of the particles, cooling rate applied, and type of the target TAGs were modified. Chapter 4 demonstrates the effects of adding talc particles on the physical properties of fat-based products, adopting palm oil and its shortening as the model systems. In conclusion, brief summaries on these results will follow.

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Chapter 2

Promotional effects of new types of additives on fat crystallization

1 Introduction

Fats are used in foods, cosmetics, and pharmaceuticals, in which the fats are the main ingredients of the solid materials [1]. The crystallization properties of fats largely affect the microstructural and physical properties and help to establish the quality, productivity, and preservability of the products [2, 3]. For example, in the commercial manufacturing cooling processes for fat products, such as margarine, shortening, and chocolate, the crystal nucleation of the fats occurs first, followed by crystal growth. Then, the dispersed crystals form networks [4]. Thus, the textures of the products are determined by the structural properties of the networks and the size distribution and shapes of the fat crystals. Above all, the initial stage of crystallization significantly contributes to the entire processes of crystallization; therefore, numerous studies have been conducted to better understand and control the crystal nucleation [2, 5–9].

Nowadays, there is a growing demand to reduce the content of *trans* and saturated fatty acids in fat-based food products [10–13]. The crystallization rates of *trans* and saturated fats are higher than other low-melting fats; therefore, it is necessary to enhance the crystallization rates of *trans*-free and low-saturated fats to strengthen their crystal networks by improving the fat crystallization processes. The use of additives has been one of the promising methods to promote fat crystallization.

The recent studies on the effects of additives on fat crystallization were reviewed by Smith *et al.* [14]. After the publication of this review, further researches on the effects of additives have been conducted [15–19]. However, most of the additives were hydrophobic materials, such as high-melting fats, emulsifiers, and waxes, mostly containing fatty acid or other hydrophobic residues like the fats. Consequently, it has been recognized that the hydrophobic interactions owing to the similarities between the fats and additives may have promoted the fat crystallization. On the other hand, some impurities affect crystallization in natural systems [20]; however, no additive, with very different chemical structures than fats, has been shown to significantly improve fat crystallization.

A major objective of this study was to find such new types of additives that promote the crystallization of typical triacylglycerols (TAGs). After studying several materials, I found that the inorganic materials of talc, carbon nanotube (CNT), and graphite, and the organic materials of theobromine, ellagic acid dihydrate (EAD), and terephthalic acid remarkably promoted the crystallization of TAGs. The most unique property of these additives is that they do not contain hydrocarbon chains, which are the main components of TAGs along with the glycerol backbone. Talc is a crystalline hydrated magnesium silicate belonging to the 2:1 layer clays of the phyllosilicate family, with the chemical formula $\text{Si}_4\text{Mg}_3\text{O}_{10}(\text{OH})_2$ (Fig. 2.1a) [21]. CNT and graphite are well-known inorganic materials, comprising layered carbon atoms with conjugated honeycomb structure. Theobromine, with the chemical formula $\text{C}_7\text{H}_8\text{N}_4\text{O}_2$, is one of the main alkaloids present in cacao plant [22]. EAD, with the chemical formula $\text{C}_{14}\text{H}_6\text{O}_8 \cdot 2\text{H}_2\text{O}$, is the dihydrate of ellagic acid, a type of polyphenol present in berries, pomegranate, etc. [23]. Terephthalic acid, with the chemical formula $\text{C}_6\text{H}_4(\text{COOH})_2$, is a well-known precursor for polyethylene terephthalate (PET) [24]. The crystals of theobromine, EAD, and terephthalic acid have honeycomb-like patterns (Fig. 2.1b–d).

In this study, I found that the above six additives greatly affected the crystallization and polymorphic transformation behavior of the saturated TAGs of trioleoylglycerol (LLL), trimyristoylglycerol (MMM), and tripalmitoylglycerol (PPP). In particular, the rates of crystallization increased similarly to or more remarkably than those by the previously reported additives.

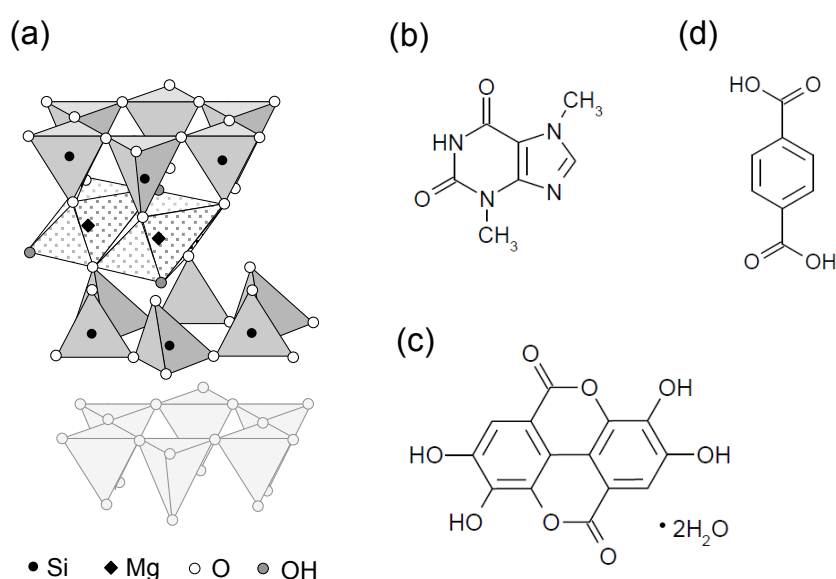


Figure 2.1 (a) Atomic arrangements in the crystals of talc, and molecular structures of (b) theobromine, (c) ellagic acid dihydrate (EAD), and (d) terephthalic acid.

2 Experimental

2.1 Materials and sample preparation

The TAG samples of LLL, MMM, and PPP with $\geq 99\%$ purity were purchased from Sigma-Aldrich (St. Louis, USA), and used as received without further purification. The melting temperatures (T_m) of α , β' , and β forms of the three TAGs are the following: α (15.0°C), β' (35.0°C), and β (46.5°C) for LLL [25]; α (33.0°C), β' (46.5°C), and β (57.0°C) for MMM [25]; and α (44.7°C), β' (56.6°C), and β (66.4°C) for PPP [25, 26]. The fine powder sample of talc (NANO ACE® D-600) was supplied by Nippon Talc (Osaka, Japan). The CNT powder sample (single-walled CNT ($> 55\%$), < 2 nm diameter, and 5–15 μm length) was purchased from Tokyo Chemical Industry (Tokyo, Japan). The fine powder sample of graphite, with 99.9995% purity and passed through a 200 mesh sieve, was purchased from Alfa Aesar (Ward Hill, UK). The fine powder samples of theobromine with $> 98\%$ purity ($T_m = 357^\circ\text{C}$ [27]), EAD with $> 98\%$ purity ($T_m > 360^\circ\text{C}$ as ellagic acid [27]), and terephthalic acid with $> 99\%$ purity (sublimation temperature, 402°C [27]) were purchased from Tokyo Chemical Industry (Tokyo, Japan).

The scanning electron microscope (SEM) observation showed the following particle sizes of the additives: Talc: several hundred nanometers of primary particles and several micrometers of aggregated secondary particles; CNT: ~ 10 μm primary particles and 10–100 μm secondary stuck particles; graphite: < 100 μm platelet-shaped crystals; theobromine: < 10 μm primary particles and several ten micrometers of aggregated secondary particles; EAD: < 50 μm columnar-shaped crystals and several ten micrometers of aggregated secondary particles; and terephthalic acid: < 200 μm layer-shaped crystals.

Each additive was separately added to each TAG sample at room temperature (1 wt% with respect to the TAG sample). The temperatures of the mixtures were increased to 80°C for LLL and MMM and 90°C for PPP to melt the TAG samples. Because the six additives are sparingly soluble in the molten TAGs and their T_m values are much higher than 90°C , the six additives were left as crystals in the molten TAGs.

The crystallization and melting experiments were conducted as follows: (i) holding at 80°C or 90°C for 10 min, (ii) cooling from 80°C or 90°C to 0°C at a rate of $1^\circ\text{C}/\text{min}$, and (iii) heating from

0°C to 80°C or 90°C at a rate of 5°C/min soon after step (ii).

2.2 Thermal analysis

The thermal behaviors of the mixtures were analyzed by differential scanning calorimetry (DSC) using a DSC III calorimeter (Rigaku, Tokyo, Japan) attached to an X-ray diffractometer. Temperature and heat flow were calibrated with reference to the melting points and enthalpies of lead, tin, indium, and biphenyl. Each mixture (10 mg) was weighed on an aluminum pan and placed in the measuring chamber filled with dry nitrogen gas (flow rate 50 ml/min).

Particular attention was paid to observe the increase in the crystallization rate with the additives by determining the crystallization temperature, which is defined as the initial temperature (T_i) of the first exothermic DSC peak during the cooling process. To compare the T_i values in the presence and absence of the additives, ΔT_i was defined as the increase in the T_i caused by the additives. The enthalpy change involved in the crystallization, polymorphic transformation, and melting was calculated using the software attached to the DSC apparatus.

2.3 Polymorphism and morphology of fat crystals

The polymorphic behavior of the TAGs in the presence and absence of the additives was examined by the X-ray diffraction (XRD)-DSC analysis. An Ultima IV X-ray diffractometer (Rigaku, Tokyo, Japan) equipped with a DSC unit was employed. The measurements were performed by the reflection method in the 2θ range of 1–30° using Cu-K α radiation (0.154 nm wavelength, 40 kV, 40 mA). The polymorphic structures were determined by observing the XRD short-spacing patterns, which are clearly different among the α , β' , and β forms. With regard to the long-spacing patterns, the values of the long spacing of the β' and β forms are so close to each other that the experimental errors were not negligible for defining the peaks of the (001) reflections. Therefore, the long-spacing patterns were not employed for determining the polymorphic forms.

The morphology of the fat crystals was observed using a polarized optical microscope (POM), VHX-600 digital microscope (Keyence, Osaka, Japan). The total magnification was $\times 500$. The mixtures (4–8 μ L) were placed on glass plates and gently covered with cover slips. The temperature of

the mixtures under the POM observation was controlled using a T95 system controller (Linkam Scientific Instruments, Tadworth, UK).

3 Results and discussion

3.1 DSC thermograms

Figure 2.2 shows the DSC cooling and heating thermograms of LLL in the presence and absence of the three inorganic additives. In the cooling thermograms (Fig. 2.2a), pure LLL showed a single exothermic peak. LLL + talc and LLL + CNT also showed single exothermic peaks; however, LLL + graphite showed a broad-shouldered exothermic peak. According to the XRD and POM experiments, the exothermic peak of pure LLL is attributed to only the crystallization in the β' form from the melt; however, the corresponding peaks of LLL in the presence of the three additives result from the successive crystallization in the β' and β forms including the solid-state $\beta' \rightarrow \beta$ transformation, which occurred during the cooling processes.

Notably, the peak position of pure LLL shifted to higher temperatures when the three additives were employed, as indicated by the arrows in Fig. 2.2. The T_i value of pure LLL was 27.3°C, whereas it was increased to 29.9°C by talc, 31.2°C by CNT, and 35.4°C by graphite. The ΔT_i values are listed in Table 2.1. Thus, the three additives promoted the crystallization of LLL in the order: graphite > CNT > talc.

In the heating thermograms (Fig. 2.2b), pure LLL showed a small exothermic peak at 29.9°C and a large endothermic peak at 46.7°C with an end point at 54.8°C as the full-melting temperature. The exothermic and endothermic peaks are attributed to the solid-state $\beta' \rightarrow \beta$ transformation and the melting behavior of the β -form crystals, respectively, as shown in the XRD experiments.

However, all the mixtures with the three inorganic additives showed very weak and broad peaks at ~29.9°C and large endothermic peaks at ~46.7°C. This is because the $\beta' \rightarrow \beta$ transformation of LLL in the presence of the three additives almost completed during the cooling processes and early stages (far below 29.9°C) of the heating processes as observed in the XRD and POM experiments. The endothermic peak position and the full-melting temperature did not change by the use of the three

additives.

Figure 2.3 shows the DSC cooling and heating thermograms of LLL in the presence and absence of the three organic additives. In the cooling thermograms (Fig. 2.3a), LLL + theobromine and LLL + EAD showed single exothermic peaks similar to pure LLL; however, LLL + terephthalic acid showed a broad double exothermic peak. The exothermic peaks of LLL in the presence of the three additives result from the successive crystallization in the β' and β forms including the

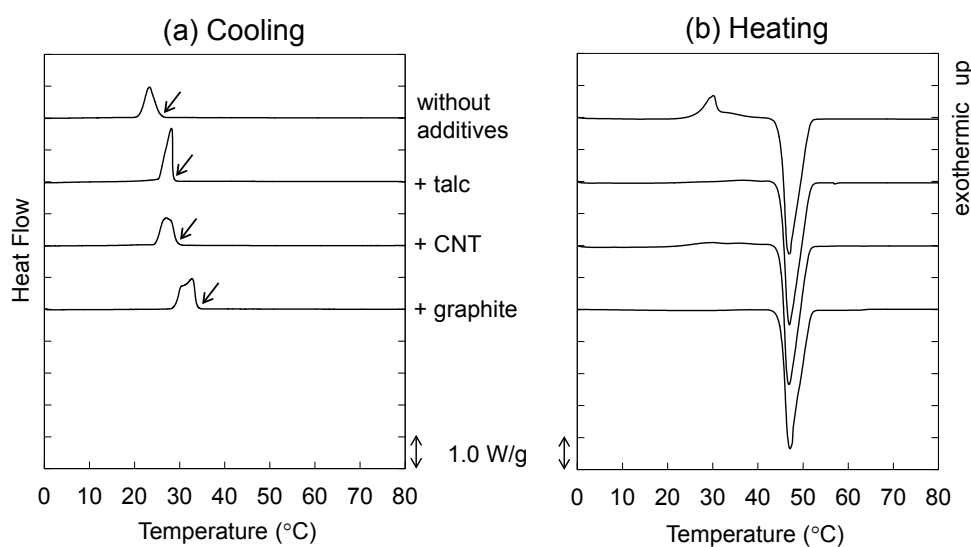


Figure 2.2 DSC thermograms of LLL in the presence and absence of the inorganic additives of talc, CNT, and graphite. (a) Cooling at 1°C/min and (b) subsequent heating at 5°C/min. Arrows in (a) indicate initial temperatures of the crystallization (T_i).

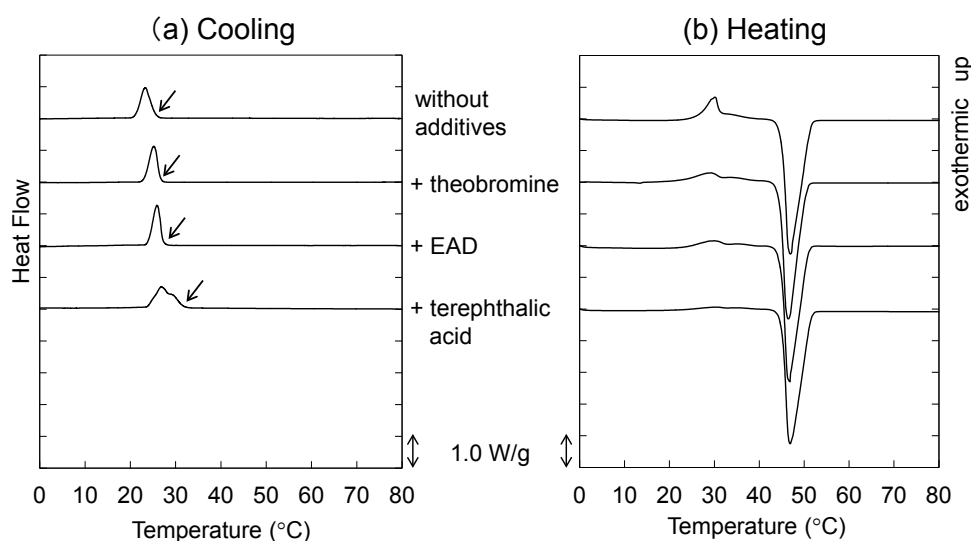


Figure 2.3 DSC thermograms of LLL in the presence and absence of the organic additives of theobromine, EAD, and terephthalic acid. (a) Cooling at 1°C/min and (b) subsequent heating at 5°C/min. Arrows in (a) indicate T_i .

solid-state $\beta' \rightarrow \beta$ transformation, as observed in the XRD and POM experiments. Notably, the peak position of pure LLL shifted to higher temperatures when the three additives were employed: the T_i value of 27.3°C for pure LLL was increased to 28.4°C by theobromine, 29.3°C by EAD, and 34.1°C by terephthalic acid. The ΔT_i values are listed in Table 2.1, indicating that the three additives promoted the crystallization of LLL in the order: terephthalic acid > EAD > theobromine.

In the heating thermograms (Fig. 2.3b), the exothermic peaks at ~29.9°C, as observed in pure LLL, appeared in a small scale when theobromine and EAD were added. However, the corresponding peak became very weak and broad when terephthalic acid was added. This is because the $\beta' \rightarrow \beta$ transformation almost completed during the cooling processes or early stages (far below 29.9°C) of the heating processes. The large endothermic peaks at ~46.7°C were observed for all the three mixtures without changing the peak position and full-melting temperature.

The same experiments were conducted for MMM and PPP in the presence and absence of the six additives (data not shown here). The results are almost the same as those of LLL: the six additives promoted the crystallization of MMM and PPP, as shown by the T_i and ΔT_i values listed in Table 2.1. Thus, all the six additives increased the ΔT_i values because of their promotional effects; in addition, they also promoted the crystallization in more stable forms and the transformation from metastable to more stable forms. The details of these effects were most clearly shown by the XRD and POM observations, as explained below.

I compared my data with those reported in the recent publications. The crystallization

Table 2.1 T_i and increases in T_i (ΔT_i) of LLL, MMM, and PPP in the presence and absence of the six additives obtained by the DSC cooling (1°C/min) thermograms.

	LLL		MMM		PPP	
	T_i (°C)	ΔT_i (°C)	T_i (°C)	ΔT_i (°C)	T_i (°C)	ΔT_i (°C)
without additives	27.3	–	39.7	–	47.0	–
Inorganic additives						
+ talc	29.9	+ 2.6	43.2	+ 3.5	49.1	+ 2.1
+ CNT	31.2	+ 3.9	41.4	+ 1.7	48.3	+ 1.3
+ graphite	35.4	+ 8.1	45.3	+ 5.6	50.8	+ 3.8
Organic additives						
+ theobromine	28.4	+ 1.1	41.2	+ 1.5	48.6	+ 1.6
+ EAD	29.3	+ 2.0	42.4	+ 2.7	50.4	+ 3.4
+ terephthalic acid	34.1	+ 6.8	44.9	+ 5.2	52.1	+ 5.1

temperature increased by 1.1–8.1°C with the additives, as shown in Table 2.1, whereas the addition of monopalmitin (1 wt%) to palm oil and polyglycerine fatty acid ester (1 wt%) to palm stearin increased the crystallization temperatures by 1.7°C [18] and 1.0–3.9°C [19], respectively.

3.2 XRD experiments

The XRD profiles of LLL in the presence and absence of the three inorganic additives are shown in Fig. 2.4. When pure LLL was cooled, the initial crystallization occurred at 26.3–25.3°C in the β' form as shown by the occurrence of short-spacing peaks (0.42 nm and 0.39 nm). The T_i value in Table 2.1 corresponds to this crystallization; however, the temperature at which these XRD peaks appeared first (defined as T_i in XRD) was 1–2°C lower than the T_i in DSC. This is because XRD is less sensitive in detecting the occurrence of crystallization than DSC. Both the crystallization in any other polymorphs and the $\beta' \rightarrow \beta$ transformation were not detected during the cooling process.

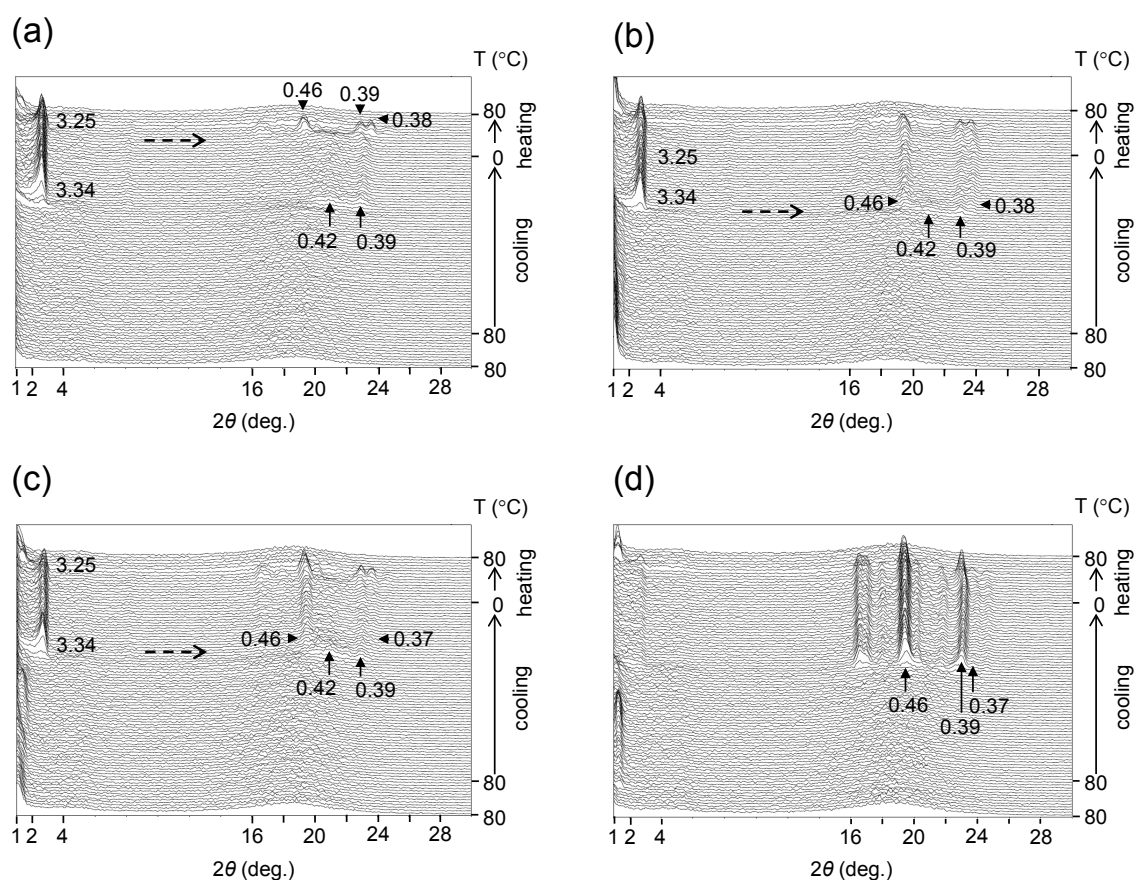


Figure 2.4 XRD patterns of LLL in the presence and absence of the inorganic additives during the cooling (1°C/min) and subsequent heating (5°C/min) processes. (a) Without additives (pure LLL), (b) LLL + talc, (c) LLL + CNT, and (d) LLL + graphite. Units are nanometers.

On subsequent heating, the XRD patterns showed the occurrence of the $\beta' \rightarrow \beta$ transformation as shown by the change in the short-spacing patterns at ~ 22.5 – 28.6°C (shown by the dotted arrow in Fig. 2.4a), which corresponds to the small exothermic DSC peak at 29.9°C (Fig. 2.2b). The transformation into the β form was shown by the appearance of the short-spacing peaks (0.46 nm, 0.39 nm, and 0.38 nm), as shown by the arrows in Fig. 2.4a. The β -form crystals obtained melted by further heating as shown by the endothermic DSC peak at 46.7°C (Fig. 2.2b) and the disappearance of the XRD peaks for the β form (Fig. 2.4a).

The additive effects of the three inorganic materials on LLL are shown as the remarkable differences in the XRD patterns obtained during the cooling and heating processes, summarized as follows. (i) The T_i in XRD was increased by the three additives, i.e., 28.4 – 27.7°C by talc, 28.2 – 27.2°C by CNT, and 33.1 – 32.1°C by graphite. These values were almost identical to those in DSC (Table 2.1). (ii) The occurrence of the β form was promoted by the three additives during the cooling processes. In the cases of LLL + talc and LLL + CNT, LLL first crystallized in the β' form. However, the short-spacing peaks for the β form appeared at ~ 27.6 – 25.8°C (LLL + talc) and ~ 24.2 – 23.1°C (LLL + CNT) during the cooling processes, as shown by the dotted arrows in Fig. 2.4b and 2.4c. The intensities of these peaks for the β form increased as the mixtures were cooled, and finally the peaks for the β' form disappeared. This change in the short-spacing peaks was attributed to the solid-state $\beta' \rightarrow \beta$ transformation. In the case of LLL + graphite, the short-spacing peaks for the β form appeared soon after the crystallization started, and the peaks for the β' form were not detected, as shown in Fig. 2.4d. (iii) The solid-state $\beta' \rightarrow \beta$ transformation during the heating processes was not detected when the three additives were employed. This was because the $\beta' \rightarrow \beta$ transformation was mostly completed during the cooling processes.

A remarkable difference in the XRD patterns was observed in the relative intensity of the long- and short-spacing peaks of LLL between graphite and the other two inorganic additives. In the cases of pure LLL, LLL + talc, and LLL + CNT, the intensity of the long-spacing peaks was always stronger than those of the short-spacing peaks. However, the intensity of the long-spacing peak was extremely lower than those of the short-spacing peaks in the case of LLL + graphite. I assume that the adsorption patterns of the LLL molecules on the surfaces of graphite crystals may be different from those on the other additives (e.g., talc) as shown in Fig. 2.5. In the case of adding talc, the long-chain axes of the LLL molecules are arranged normal to the crystal surfaces, making the lamellar planes

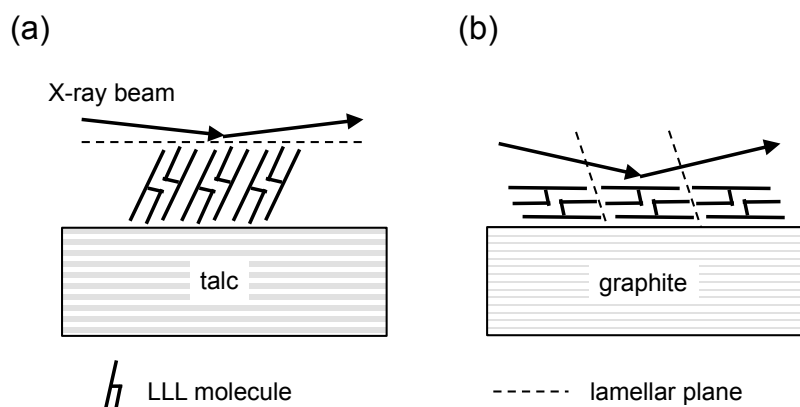


Figure 2.5 Postulated molecular arrangements of LLL on the surfaces of the additive crystals of (a) talc and (b) graphite.

parallel to the surfaces. In contrast, the long-chain axes of the LLL molecules are parallel to the surfaces of the graphite crystals, making the lamellar planes normal to the surfaces. In these cases, the X-ray beams can be strongly diffracted by the lamellar planes of the LLL molecules on talc, whereas strongly diffracted by the sub-cell structures of the LLL molecules on graphite. Therefore, the intensities of the short-spacing peaks for the LLL crystals on graphite were stronger than that of the long-spacing peaks. Such a result has often been observed in the long- and short-spacing peaks for the long-chain compound crystals with relatively different intensity, named as “morphology and orientation effects”.

Although Fig. 2.5 simply shows as if the surfaces of the additive particles are atomically/molecularly flat, the surfaces where certain catalytic interactions occur to promote the heterogeneous nucleation may not be as simple as shown in Fig. 2.5. In fact, the additive surfaces should be constructed by steps/kinks/vacancies/holes, some of which may be effective for the heterogeneous nucleation. Figure 2.5 shows that the molecular orientations of the fat crystals are very different among the additives, as shown by the XRD patterns. I believe that this result is one of my most important findings in this study because of two reasons: (i) no such finding on the additive effects has been reported so far and (ii) the orientations of the fat crystals on the additive surfaces should be the key to better understand the molecular-level interactions between the fat molecules and additive surfaces, which will be studied in the future.

In the cases of adding the three organic materials, the XRD patterns showed that all of them promoted the crystallization of LLL during the cooling processes: the T_i values in XRD were increased to 26.4–25.7°C by theobromine, 26.6–25.7°C by EAD, and 30.9–29.9°C by terephthalic

acid. These values were almost identical to those in DSC (Table 2.1). However, the effects on the polymorphic crystallization of LLL were different between terephthalic acid and the other two additives. Figure 2.6a and 2.6b shows that the first-occurring polymorph was the β' form, soon followed by the occurrence of the β form during the subsequent cooling processes at 16.0–14.9°C (LLL + theobromine) and at 14.6–13.4°C (LLL + EAD), as represented by the dotted arrows. The two forms coexisted during the cooling processes, as shown by the presence of their short-spacing patterns. In contrast, LLL started to crystallize in the β form from the beginning when terephthalic acid was added, as shown by the occurrence of the short-spacing patterns for the β form (Fig. 2.6c).

The XRD patterns obtained during the heating processes also showed the effects of the three organic additives. The $\beta' \rightarrow \beta$ transformation did not complete during the cooling processes when theobromine and EAD were added. Therefore, the solid-state $\beta' \rightarrow \beta$ transformation occurred again during the subsequent heating processes, as shown by the disappearance of the short-spacing patterns for the β' form at 27.3°C (LLL + theobromine) and at 29.9°C (LLL + EAD). These changes

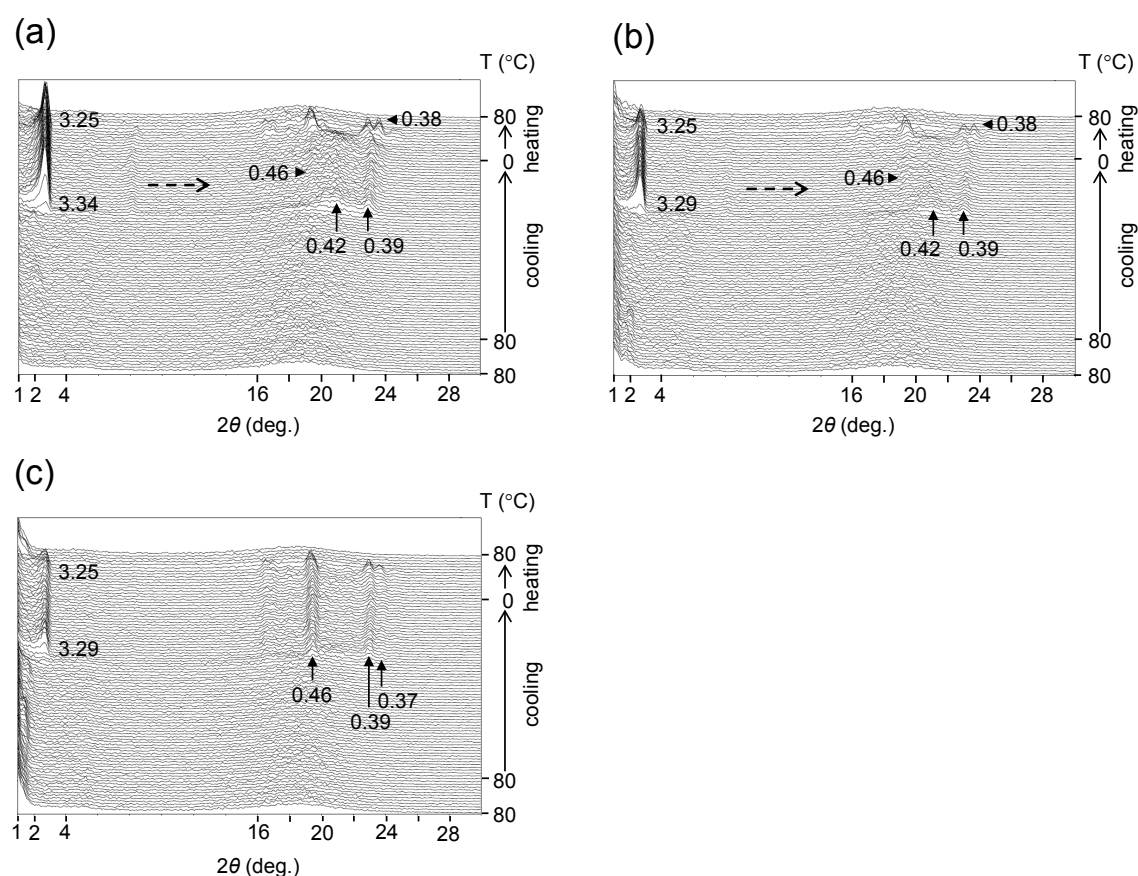


Figure 2.6 XRD patterns of LLL in the presence of the organic additives during the cooling (1°C/min) and subsequent heating (5°C/min) processes. (a) LLL + theobromine, (b) LLL + EAD, and (c) LLL + terephthalic acid. Units are nanometers.

correspond to the exothermic DSC peaks at $\sim 29.9^{\circ}\text{C}$ (Fig. 2.3b). However, the crystallization in the β' form was not detected in LLL + terephthalic acid; thus, no $\beta' \rightarrow \beta$ transformation was observed.

The same experiments were conducted for MMM and PPP in the presence and absence of the six additives (data not shown here). The results obtained are discussed along with those of the POM observation.

3.3 POM observation

Figures 2.7 and 2.8 show the results of the in-situ observation on the crystallization processes of LLL in the presence and absence of the additives, which was conducted by the temperature-controlled POM methods. All the figures clearly demonstrate that the additives promoted the crystallization of LLL and modified their polymorphic crystallization behavior, thus supporting the results obtained by the DSC and XRD studies. The same experiments were conducted for all the three TAGs in the presence and absence of the six additives (data not shown here).

Figure 2.7 shows the typical POM images obtained at the early stages of the crystallization of LLL in the presence and absence of the six additives. In pure LLL, the bright image of LLL crystals in a spherulite pattern became visible at 25.5°C , which was $\sim 2^{\circ}\text{C}$ below the corresponding T_i in DSC (Table 2.1). This was because the image shown in Fig. 2.7a was recorded slightly after the crystallization started. The spherulite pattern was the same as that obtained for the β' -form crystals of LLL reported previously [28].

The temperature at which the LLL crystals appeared was increased by the six additives: 30.4°C by talc (Fig. 2.7b), 30.4°C by CNT (Fig. 2.7c), 35.4°C by graphite (Fig. 2.7d), 27.5°C by theobromine (Fig. 2.7e), 27.5°C by EAD (Fig. 2.7f), and 32.9°C by terephthalic acid (Fig. 2.7g), which were almost equal to the corresponding T_i in DSC (Table 2.1). In Fig. 2.7b–g, the bright images shown by the solid arrows represent the LLL crystals, whereas the images shown by the dotted arrows represent the additive particles. The bright images of the LLL crystals started to grow at the edge of the additive particles.

It was very interesting to observe the changes in the color of the bright crystal images under the crossed Nicols condition for POM and in the crystal aggregation behavior in accordance with the progress in the crystal growth. By combining these observations with the results of the DSC and XRD

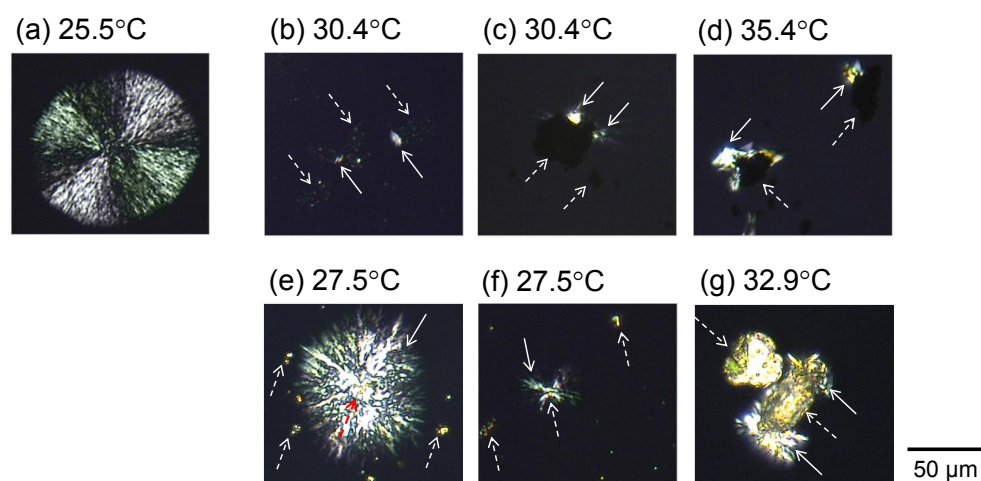


Figure 2.7 POM images of LLL in the presence and absence of the inorganic (b–d) and organic (e–g) additives at the initial stages of the crystallization in the cooling (1°C/min) processes. (a) Without additives (pure LLL), (b) LLL + talc, (c) LLL + CNT, (d) LLL + graphite, (e) LLL + theobromine, (f) LLL + EAD, and (g) LLL + terephthalic acid.

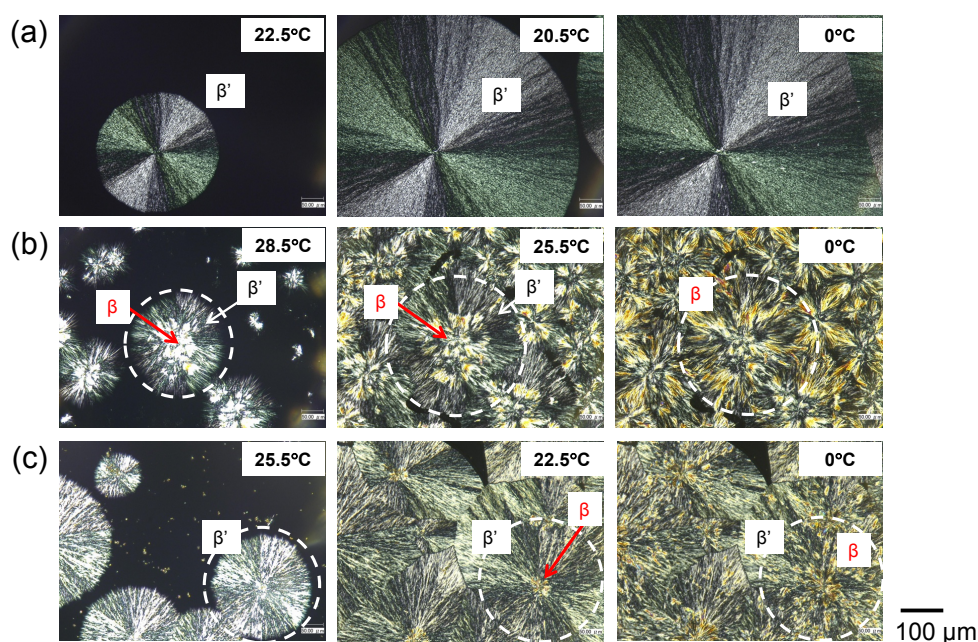


Figure 2.8 POM images of LLL in the presence and absence of the additives at the later stages of the crystallization in the cooling (1°C/min) processes. (a) Without additives (pure LLL), (b) LLL + talc, and (c) LLL + theobromine.

studies, I concluded that these changes directly corresponded to the changes in the polymorphic structures of the three TAGs. Thus, I could also distinguish the crystal polymorphs by the POM images.

Figure 2.8 shows three examples of the POM images taken for LLL in the presence and absence of the additives at the later stages of crystallization in the cooling processes. In pure LLL (Fig. 2.8a), the bright images of crystals radially grew with increasing crystallization time and decreasing

temperature to 0°C, finally forming large spherulite crystals. The spherulite patterns for the β' -form crystals were maintained during the cooling process.

When LLL + talc was cooled from 80°C, the bright images of the β' -form crystals started to appear at 30.4°C, as shown in Fig. 2.7. At 28.5°C, the growth of the β -form crystals followed that of the β' -form crystals from the central position of the spherulite, as shown by the different colors in the POM image (represented by the dotted circle in Fig. 2.8b). On further cooling, both β' - and β -form crystals continued to grow, and simultaneously, the $\beta' \rightarrow \beta$ transformation occurred during the crystal growth. The growth of the other spherulites proceeded in the same manner, and no free spaces for the growth of the β' -form crystals were left (see the image taken at 25.5°C, Fig. 2.8b). However, the β -form crystals still continued to grow and finally occupied all the crystal areas below 9.5°C. The results of the DSC (Fig. 2.2) and XRD (Fig. 2.4b) studies show that the growth of the β -form crystals can be attributed to the solid-state $\beta' \rightarrow \beta$ transformation.

In the case of LLL + theobromine (Fig. 2.8c), the growth of the β' -form crystals at the outer area of the spherulites followed by the growth of the β -form crystals at the center of the spherulites were also observed during the cooling process. However, the change in the images from the β' to β form was so slow compared to that of LLL + talc that the β' -form crystals remained at 0°C without any changes in the growth patterns, as shown in the spherulite represented by the dotted circle in Fig. 2.8c. This observation agreed well with the results obtained by the XRD analysis (Fig. 2.6a), in which the occurrence of the β form was detected at a temperature far below that of the first crystallization in the β' form and the short-spacing patterns for the β' and β forms coexisted at 0°C.

The POM observation was also conducted on LLL + CNT, LLL + graphite, LLL + EAD, and LLL + terephthalic acid. The main results obtained are as follows: the β -form crystals started to grow from the beginning of the crystallization in LLL + graphite and LLL + terephthalic acid, whereas the results of LLL + CNT and LLL + EAD are similar to that of LLL + theobromine.

Regarding the crystallization of MMM and PPP, the POM observation was also conducted in the same manner as that on LLL. The general behavior of MMM was almost identical to that of LLL. However, the behavior of PPP was different from those of LLL and MMM, because the least stable α form occurred along with the β' and β forms. In either case, a clear trend was observed: the six additives promoted the crystallization of the TAGs in more stable forms. The main results are summarized in Table 2.2.

Table 2.2 Crystal polymorphs of LLL, MMM, and PPP in the presence and absence of the six additives at the initial and last stages (0°C) of the crystallization in the cooling processes (1°C/min).

	LLL		MMM		PPP	
	initial	last	initial	last	initial	last
without additives	β'	β'	β'	β'	β'	$\alpha \gg \beta'$
Inorganic additives						
+ talc	$\beta' \sim \beta$	β	β	$\beta' \ll \beta$	β'	$\beta' \gg \beta$
+ CNT	$\beta' \sim \beta$	$\beta' < \beta$	β'	$\beta' \gg \beta$	β'	$\alpha \ll \beta'$
+ graphite	β	β	β	$\beta' \sim \beta$	β	$\beta' \gg \beta$
Organic additives						
+ theobromine	β'	$\beta' > \beta$	β'	$\beta' \gg \beta$	β'	$\alpha \ll \beta'$
+ EAD	$\beta' \sim \beta$	$\beta' > \beta$	β	$\beta' \sim \beta$	$\beta' \sim \beta$	$\beta' > \beta$
+ terephthalic acid	β	β	$\beta' \sim \beta$	$\beta' \gg \beta$	β	$\beta' \sim \beta$

3.4 Enthalpy change

It is very interesting to compare the enthalpy change obtained by the DSC analysis for crystallization, polymorphic transformation, and melting behavior among the liquid and β' and β forms of LLL (Fig. 2.9 and Table 2.3). The solid-state $\beta' \rightarrow \beta$ transformation and the crystallization in the β' and β forms contribute to the enthalpy change in the exothermic DSC peaks (ΔH_{exo}), whereas the heat of melting of the β -form crystals comprises the enthalpy change in the endothermic DSC peaks (ΔH_{endo}). Then, if I obtain the exothermic peaks during the cooling processes, ΔH_{exo} may include the heat of the solid-state $\beta' \rightarrow \beta$ transformation and that of the crystallization in the β' and β forms. In contrast, the ΔH_{exo} during the heating processes only includes the heat of the solid-state transformation occurring at $\sim 29.9^\circ\text{C}$. Therefore, two values of ΔH_{exo} measured during the cooling and heating processes are defined as $\Delta H_{\text{exo}}(\text{cool})$ and $\Delta H_{\text{exo}}(\text{heat})$, respectively.

The simplest case is as follows: the crystallization in the β' form occurs in the cooling process, the solid-state $\beta' \rightarrow \beta$ transformation occurs in the heating process, and melting of the β -form crystals occurs on further heating. In this case, $\Delta H_{\text{exo}}(\text{cool})$ and $\Delta H_{\text{exo}}(\text{heat})$ correspond to the heat of the crystallization in the β' form and solid-state $\beta' \rightarrow \beta$ transformation, respectively. Then, the following relation should operate:

$$\Delta H_{\text{exo}}(\text{cool}) + \Delta H_{\text{exo}}(\text{heat}) = \Delta H_{\text{endo}} \quad \langle \text{Eq. 2.1} \rangle,$$

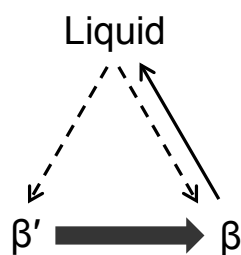


Figure 2.9 Phase-transition cycles among the liquid and the β' and β forms of LLL. Solid arrow means melting, bold arrow means polymorphic transformation, and dotted arrows mean crystallization.

Table 2.3 Enthalpy change (kJ/mol) in the exothermic and endothermic DSC peaks for LLL in the presence and absence of the six additives (cooling at 1°C/min and subsequent heating at 5°C/min).

	Cooling	Heating	
	$\Delta H_{\text{exo}}(\text{cool})$	$\Delta H_{\text{exo}}(\text{heat})$	ΔH_{endo}
without additives	– 98.8	– 29.8	+ 123.5
Inorganic additives			
+ talc	– 125.0	– 8.6	+ 130.0
+ CNT	– 111.4	– 15.3	+ 124.1
+ graphite	– 127.6	– 1.3	+ 125.4
Organic additives			
+ theobromine	– 98.7	– 26.4	+ 119.3
+ EAD	– 100.1	– 15.0	+ 118.0
+ terephthalic acid	– 118.5	– 13.7	+ 125.6

as observed for pure LLL (Table 2.3). In fact, Eq. 2.1 could be applied to all the cases, as shown in Table 2.3. However, interesting changes were observed in the relative values of $\Delta H_{\text{exo}}(\text{cool})$ and $\Delta H_{\text{exo}}(\text{heat})$. The first case is the remarkable increases in the absolute values of $\Delta H_{\text{exo}}(\text{cool})$ compared to pure LLL at the expense of $\Delta H_{\text{exo}}(\text{heat})$. This case was applied to LLL + talc, LLL + graphite, and LLL + terephthalic acid, which promoted the crystallization mostly in the β form, as shown by the XRD results (Figs. 2.4 and 2.6). The second case is the moderate increases in the absolute values of $\Delta H_{\text{exo}}(\text{cool})$ at the expense of $\Delta H_{\text{exo}}(\text{heat})$, which was applied to LLL + CNT. The third case is that the values of $\Delta H_{\text{exo}}(\text{cool})$ and $\Delta H_{\text{exo}}(\text{heat})$ did not change from those of pure LLL, as observed for LLL + theobromine and LLL + EAD. In the case of LLL + EAD, I assume that the solid-state $\beta' \rightarrow \beta$ transformation occurred so slowly that the DSC analysis could not detect the heat of transformation. Therefore, the absolute value of the $\Delta H_{\text{exo}}(\text{heat})$ decreased. Moreover, each value of ΔH_{endo} agreed well with that in the literatures (114.3–116.4 kJ/mol in [26] and 120.8–130.2 kJ/mol calculated from the previous studies [29, 30]).

3.5 Polymorphic nucleation from the melt

The additive effects of the six materials on LLL, MMM, and PPP can be well understood by considering the polymorph-dependent nucleation rate (J) as a function of temperature (T). The crystallization in the β' and β forms is discussed here because the two forms were commonly observed in the three TAGs. Figure 2.10 shows the schematic illustrations of the additive effects on J of the β' and β forms with decreasing temperature.

In the absence of the additives, J increases with decreasing temperature in such a manner that the slope of the T - J curve for the β' form is larger than that for the β form, as shown by the solid lines in Fig. 2.10. This is because the rate of nucleation of the β' form is higher than that of the β form [31]. Such a relationship was experimentally confirmed for 1,3-dioleoyl-2-palmitoyl glycerol (OPO) [32], 1,3-dipalmitoyl-2-oleoyl glycerol (POP) [33], and trioleoylglycerol (OOO), and 1,2-dioleoyl-3-linoleoyl-*rac*-glycerol (OOL) [34]. Because of the T - J relationship as shown in Fig. 2.10, the initially occurring polymorphic form observed at a cooling rate of $1^\circ\text{C}/\text{min}$ was the β' form for all the TAGs examined, as shown in Table 2.2.

However, the T - J relationship largely varied depending on the additive-TAG combinations as shown in Tables 2.1 and 2.2. Because the six additives promoted the crystallization in the β' and β forms of the three TAGs as shown by the increases in the T_i values, the slopes of the T - J curves for

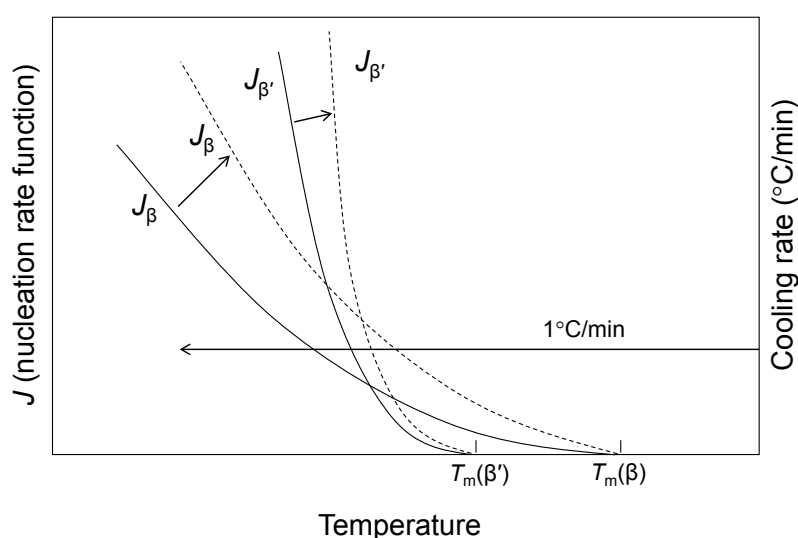


Figure 2.10 Schematic illustrations of the additive effects on the polymorph-dependent nucleation rate function (J) for the β' and β forms with decreasing temperature. Solid lines mean the cases in the absence of the additives, dotted lines mean the cases in the presence of the additives, and T_m means the melting temperatures.

both the β' and β forms became steep in the presence of these additives (represented by the dotted lines in Fig. 2.10), indicating that the rates of the nucleation of the β' and β forms increased in the presence of the additives. Furthermore, the degree of the increase largely depended on the different additive–TAG combinations, as seen from Table 2.2.

I may divide the relative nucleation behavior of the β' and β forms at the initial stages of the crystallization into three groups in accordance with the combinations of six additives and three TAGs (see columns “initial” in Table 2.2). In the first group, the initially occurring polymorph was the β' form alone. The combinations of talc–PPP, CNT–MMM (–PPP), and theobromine–LLL (–MMM and –PPP) belong to this group.

In the second group, the initial crystallization occurred in the β' and β forms, which is expressed as “ $\beta'\sim\beta$ ” in Table 2.2. The combinations of talc–LLL, CNT–LLL, EAD–LLL (–PPP), and terephthalic acid–MMM belong to this group.

In the third group, the nucleation of the β form dominated over that of the β' form, containing talc–MMM, graphite–LLL (–MMM and –PPP), EAD–MMM, and terephthalic acid–LLL (–PPP). In this group, the slope of the T – J curve for the β form may increase more than that for the β' form; therefore, the initially occurring polymorph converted from the β' to β form at a cooling rate of $1^\circ\text{C}/\text{min}$, as shown in Fig. 2.10.

The crystallization behavior in the later stages of the cooling ($1^\circ\text{C}/\text{min}$) processes was more complicated than that expected from the T – J relationships because certain types of transformation as well as the crystallization in the β' and β forms (and the α form for PPP) occurred. The crystal polymorphs at 0°C for all the additive–TAG combinations are shown in the columns “last” in Table 2.2.

4 Conclusions

This study has shown for the first time that the heterogeneous nucleation of fat crystals can be greatly promoted by new types of additives. Such additives have great potential to promote the fat crystallization by hydrophilic as well as hydrophobic molecular interactions between the fats and additives.

To elucidate the mechanism of the promotional effects of the additives on the crystallization of the three TAGs described in this study, some questions need to be solved with regard to the following points: (i) the effects of the particle sizes and shapes of the additives, (ii) the interactions between the surface structures of the additive particles and the TAG molecules, (iii) the adsorption patterns and molecular arrangement of the TAGs on the surfaces of the additives (e.g., the postulated images are shown in Fig. 2.5), (iv) the atomistic/molecular mechanisms of the heterogeneous nucleation of the TAGs on the additive crystal surfaces, (v) the effects of the additives on the rates of the crystal growth and solid-state $\beta' \rightarrow \beta$ transformation, (vi) the effects of the water–oil interfaces in emulsified systems, (vii) the effects of external factors such as the cooling rates and shear, etc.

Moreover, it is expected that the present findings may be used to develop novel manufacturing technologies for fat products in food, cosmetic, and other applications. For this purpose, further studies using different types of fats are required.

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Chapter 3

Fat crystallization with talc particles is influenced by particle size, concentration, and cooling rate

1 Introduction

Fat-based products (e.g., foods, cosmetics, and pharmaceuticals including solid fats) depend on fat crystallization for their physical properties and textures [1, 2]. Nucleation is a key process in crystallization, because of its remarkable effects on the polymorphism, the crystal size and distribution, and the formation of crystal networks [3]. Therefore, many researchers have tried to control the nucleation in fat systems effectively with additives. Typical additive examples are high-melting fats, emulsifiers, and waxes, as reviewed by Smith *et al.* [4]. This review generated further studies on additive effects [5–11]. However, most of these additives have fatty-acid or other hydrophobic residues in their molecules, like fats; thus, I assume that van der Waals interactions between additives and fats play important roles in promoting or retarding fat crystallization.

Recently, I demonstrated that six new additives (talc, carbon nanotubes, graphite, theobromine, ellagic acid dihydrate, and terephthalic acid) effectively promoted the crystallization of three monoacid saturated triacylglycerols (TAGs): trioleoylglycerol (LLL), trimyristoylglycerol, and tripalmitoylglycerol (PPP) [12]. Especially notable is the fact that these additives facilitated the occurrence of stable polymorphs. Because of the low solubility of these six additives in the liquid phases of TAGs, these additives may act as templates for heterogeneous nucleation, despite their dissimilarity to TAGs in chemical structure. This is supported by the direct observation of crystallization using a polarized optical microscope, which confirmed initial crystallization at the surfaces of additive particles [12]. However, the mechanisms of the additive effects (e.g., the effects of size and concentration of the additive particles and cooling rate) have not been clarified. Moreover, further studies using different types of TAGs are required in order to apply these findings to various fat-based products.

A major objective of this study was to precisely examine the effects of size and concentration of

talc particles and cooling rate on the crystallization of mono-acid saturated TAGs. In addition, the effects of cooling rate were also examined for the combination of talc particles and a mixed-acid saturated/unsaturated TAG. For this purpose, I employed LLL and PPP as the monoacid saturated TAGs, and 1,3-dipalmitoyl-2-oleoyl glycerol (POP) as the mixed-acid saturated/unsaturated TAG. POP is the main component of palm oil [13]; however, it sometimes causes an unfavorable texture with fat blends containing palm oil by forming granular crystals during storage [14].

Talc, a crystalline hydrated magnesium silicate with the chemical formula $\text{Si}_4\text{Mg}_3\text{O}_{10}(\text{OH})_2$, was employed as the additive because of its wide approval as a food-grade additive. Talc has a 2:1 layered structure in crystals and perfect cleavage occurs along the interlayers, as shown by an arrow in Fig. 3.1 [15]. With grinding, talc particles exhibit anisotropic surfaces: their cleavage surfaces parallel to (001) planes are hydrophobic, and their edge surfaces are hydrophilic [16, 17].

2 Materials and methods

2.1 Materials and sample preparation

Powder samples of talc particles (TALC MS ($D50 = 14 \mu\text{m}$, $SA = 4.5 \text{ m}^2/\text{g}$), MICRO ACE[®] SG-95 ($D50 = 2.5 \mu\text{m}$, $SA = 15.0 \text{ m}^2/\text{g}$), NANO ACE[®] D-1000 ($D50 = 1.0 \mu\text{m}$, $SA = 20 \text{ m}^2/\text{g}$), and NANO ACE[®] D-600 ($D50 = 0.6 \mu\text{m}$, $SA = 24 \text{ m}^2/\text{g}$)) were supplied by Nippon Talc (Osaka, Japan). The values of $D50$ are the representative ones of the median particle size, and the values of SA are the

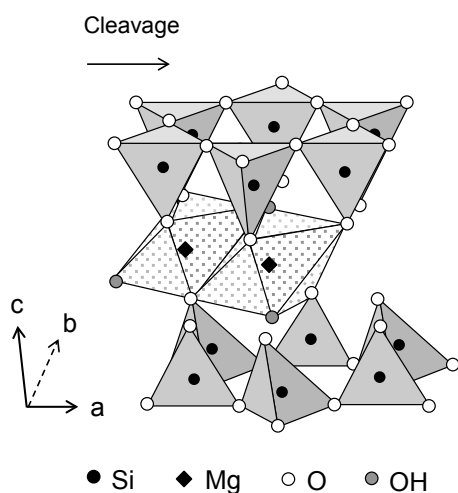


Figure 3.1 2:1 layered structure of talc crystals.

representative ones of the specific surface area (BET method). These talc samples are referred to as “talc (*D50*)” below. In order to examine the molecular interactions between talc and TAGs, hydrophobic surface modification was done to talc (0.6 μm) with four kinds of silane coupling agents: epoxysilane, methacryloxysilane, aminosilane, and alkoxysilane. In this modification, the hydrophilic edge surfaces of talc particles are coated with the layered agents via dehydrating condensation. The modified talc particles showed median sizes ranging from 0.8 μm to 1.1 μm , and the additive effects were compared with those of unmodified talc (0.6 μm).

The TAG samples of LLL and PPP were purchased from Sigma-Aldrich (St. Louis, MO, USA), and that of POP was purchased from Tsukishima Food Industry (Tokyo, Japan). These TAG samples with $\geq 99\%$ purity (pure TAGs) were used as received without further purification. Both LLL and PPP exhibit crystal polymorphs with melting temperatures (T_m) as follows: α (15.0°C), β' (35.0°C), and β (46.5°C) for LLL [18]; and α (44.7°C), β' (56.6°C), and β (66.4°C) for PPP [18, 19]. The polymorphism of POP is more complicated because an oleic acid chain exists in the *sn*-2 position [20]. The crystal polymorphs with T_m and long spacings are α (15.2°C, 4.65 nm), γ (27.0°C, 6.54 nm), δ (29.2°C, 6.25 nm), β'_2 (30.3°C, 4.24 nm), β'_1 (33.5°C, 4.24 nm), β_2 (35.1°C, 6.10 nm), and β_1 (36.7°C, 6.10 nm) [21].

Talc samples were added to pure TAGs at room temperature. The mixtures were heated to 80–90°C in order to melt LLL, PPP, and POP. Talc is a clay mineral, so talc crystals did not dissolve into the molten TAGs. The thermal procedures in all the experiments were (i) holding at 80–90°C for 10 min, (ii) cooling to 0°C at the designated rates (0.2–6.3°C/min), and (iii) heating to 80–90°C at a rate of 5°C/min soon after step (ii).

2.2 Differential scanning calorimetry (DSC)

The thermal behavior of the mixtures was analyzed using a differential scanning calorimeter (DSC III) (Rigaku, Tokyo, Japan) attached to an X-ray diffractometer. Temperature and heat flow were calibrated with reference to the melting points and enthalpies of lead, tin, indium, and biphenyl. Each mixture (10 mg) was weighed on an aluminum pan and placed in the measuring chamber where dry nitrogen gas flowed constantly at a rate of 50 ml/min.

Nucleation of the first-occurring crystals was evaluated using the onset temperatures of the first

exothermic peaks in the DSC cooling thermograms, which were defined as initial crystallization temperatures (T_i). The peak-top temperatures (T_p) of the same exothermic peaks were also determined. The mean values of T_i and T_p were calculated from new multiple measurements conducted for this study using talc and TAGs samples in the same lots. Promotional effects on crystallization were compared using these mean values. For simplicity, ΔT_i was calculated as the increase in T_i , and ΔT_p was calculated as the increase T_p caused by adding talc particles. Enthalpy changes were calculated as the peak areas using the software in the DSC apparatus.

2.3 X-ray diffractometry (XRD)

The polymorphic behavior of TAGs with and without talc particles was examined simultaneously by XRD and DSC (XRD-DSC). An Ultima IV X-ray diffractometer (Rigaku, Tokyo, Japan) equipped with a DSC unit was employed. Measurements were performed using the reflection method in the 2θ range of 1–30° with Cu-K α radiation (0.154 nm wavelength, 40 kV, 40 mA). Crystal polymorphs were determined by observing the small-angle pattern, which reflects the long spacing of the lamellar structure, and the wide-angle pattern, which reflects the short spacing of the sub-cell structure.

2.4 Polarized optical microscopy (POM)

Crystal morphology of the TAGs was observed by POM using a VHX-600 digital microscope (Keyence, Osaka, Japan). The mixtures (4–8 μ L) were placed on glass plates and gently covered with cover slips. Preparations undergoing POM observation were thermally controlled using a T95 system controller (Linkam Scientific Instruments, Tadworth, UK).

3 Results and discussion

3.1 Effects of particle size and concentration

The additive effects of talc particles having different median sizes and concentrations were compared

in XRD-DSC experiments conducted on LLL with and without talc particles under 1°C/min cooling and subsequent 5°C/min heating conditions.

Figure 3.2 depicts the DSC thermograms of LLL with and without 1 wt% talc particles having different median sizes (0.6–14 μm) taken during cooling and subsequent heating. In the cooling thermograms (Fig. 3.2a), pure LLL exhibited a single exothermic peak. In contrast, LLL + talc (14 μm) exhibited a shouldered exothermic peak at higher temperatures, and LLL + talc (2.5 μm , 1.0 μm , and 0.6 μm) exhibited sharp and single exothermic peaks at more elevated temperatures. According to the XRD study (see below), the exothermic peak of pure LLL is attributed to crystallization in β' from the melt. Corresponding peaks of LLL with talc particles, regardless of the median sizes, were due to successive crystallization in β' and β including solid-state $\beta' \rightarrow \beta$ transformation.

Mean values of T_i (indicated by arrows in Fig. 3.2a) and T_p are listed in Table 3.1, as well as ΔT_i and ΔT_p values. Considering experimental errors within $\pm 0.3^\circ\text{C}$, I conclude that talc (14 μm) remarkably promoted the crystallization of LLL, and that the promotional effect was enhanced when the median size decreased to 2.5 μm . However, no additional effects were obtained by decreasing the median size to less than 2.5 μm . Because of the similar particle shapes of all the talc samples having aspect ratios of 20–30, the additive effects were assumed to increase as the total surface area increased with decreasing particle size. Aggregation of small particles may have canceled the additive effects by hiding the active sites on the surfaces. Actually, aggregation was observed by POM and scanning

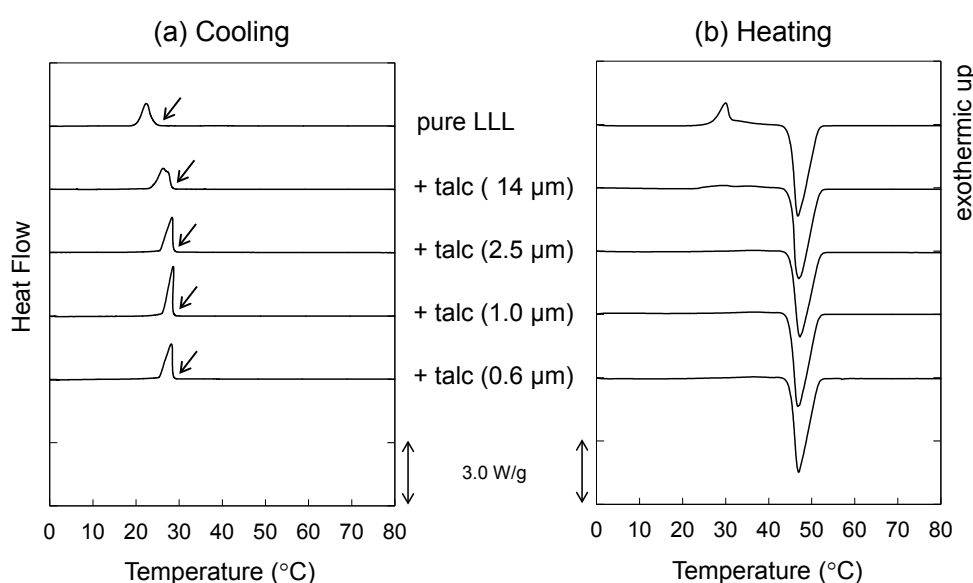


Figure 3.2 DSC thermograms of LLL with and without 1 wt% talc particles having different median sizes (0.6–14 μm) during (a) 1°C/min cooling and (b) subsequent 5°C/min heating. Arrows in (a) indicate the initial crystallization temperatures (T_i).

Table 3.1 Mean values of T_i and peak-top temperatures (T_p) of LLL with and without talc particles obtained from DSC cooling (1°C/min) thermograms.

		T_i (°C)	ΔT_i (°C)	T_p (°C)	ΔT_p (°C)
Pure LLL		26.7	–	22.6	–
+ talc (14 μm)	1 wt%	29.7	+ 3.0	26.3	+ 3.7
+ talc (2.5 μm)	1 wt%	30.3	+ 3.6	28.4	+ 5.8
+ talc (1.0 μm)	1 wt%	29.9	+ 3.2	28.6	+ 6.0
+ talc (0.6 μm)	1 wt%	29.9	+ 3.2	28.3	+ 5.7
+ talc (0.6 μm)	0.1 wt%	28.5	+ 1.8	26.0	+ 3.4
+ talc (0.6 μm)	0.01 wt%	28.3	+ 1.6	26.3	+ 3.7

electron microscopy (data not shown).

In the heating thermograms (Fig. 3.2b), pure LLL exhibited a small exothermic peak at 30.0°C, followed by a large endothermic peak at 46.8°C. These peaks are attributed to solid-state $\beta' \rightarrow \beta$ transformation and melting of β from the following results. (i) Although POM images of the growing crystals are not shown here, grown β' spherulites occupied all area within the cooling process. With subsequent heating, β nucleated from the inside and grew at the expense of β' . (ii) In the XRD patterns (see below), the 0.42 nm short-spacing peak of β' disappeared when the peaks of β occurred at 27.3–33.1°C in the heating process. (iii) In the DSC heating thermogram in Fig. 3.2b, only a single peak was observed as an endothermic peak despite the large difference in the melting points of β' (35.0°C) and β (46.5°C). The exothermic peak at 30°C was remarkably weakened and broadened by adding talc particles, which promoted the occurrence of β and thereby decreased the concentration of β' to transform in this peak, as observed in the XRD study. Change in exothermic peaks is expressed as enthalpy variations as follows: –35.6 kJ/mol for pure LLL, –15.6 kJ/mol for LLL + talc (14 μm), –8.3 kJ/mol for LLL + talc (2.5 μm), –7.2 kJ/mol for LLL + talc (1.0 μm), and –8.6 kJ/mol for LLL + talc (0.6 μm).

Figure 3.3 presents the DSC thermograms of LLL with and without talc (0.6 μm) having different concentrations (0.01–1 wt%) acquired during cooling and subsequent heating. In the cooling thermograms (Fig. 3.3a), LLL with talc particles, regardless of the concentrations, exhibited single exothermic peaks, like pure LLL. However, these peaks occurred at higher temperatures than that of pure LLL because talc particles promoted crystallization in the same manner as indicated in Fig. 3.2a. The mean values of T_i (indicated by arrows in Fig. 3.3a) and T_p are presented in Table 3.1. These

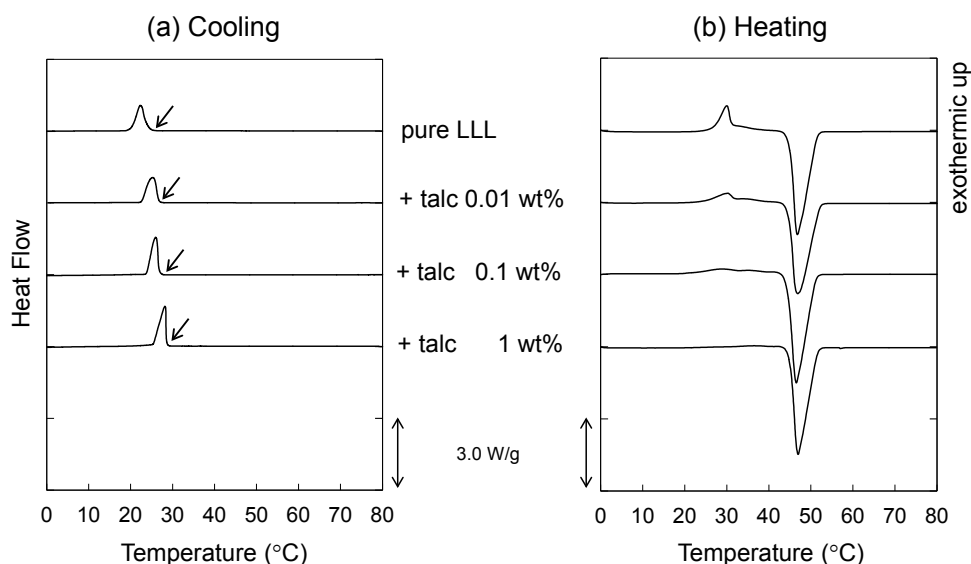


Figure 3.3 DSC thermograms of LLL with and without talc (0.6 μm) having different concentrations (0.01–1 wt%) during (a) 1°C/min cooling and (b) subsequent 5°C/min heating. Arrows in (a) indicate T_i .

results confirm that talc (0.6 μm) promoted the crystallization of LLL even at the concentration of 0.01 wt%, and that the 1 wt% addition promoted crystallization the most.

In the heating thermograms (Fig. 3.3b), the exothermic peak at 30°C involved in the solid-state $\beta' \rightarrow \beta$ transformation of LLL crystals weakened and broadened with increasing concentration of talc (0.6 μm). This change is expressed as the following enthalpy variations: -35.6 kJ/mol for pure LLL, -25.8 kJ/mol for LLL + talc 0.01 wt%, -22.2 kJ/mol for LLL + talc 0.1 wt%, and -8.6 kJ/mol for LLL + talc 1 wt%. These results can be understood by the promotion of β (Fig. 3.2b).

Figure 3.4 depicts the wide-angle XRD patterns of pure LLL, LLL+ 1 wt% talc (14 μm), and LLL + 1 wt% talc (0.6 μm) acquired during cooling and subsequent heating. Small-angle XRD patterns were also observed for the three samples; however, only a slight difference between β' and β was obtained. In contrast, wide-angle XRD patterns exhibited clear evidence of the occurrence of two polymorphs. Therefore, I present here the temperature variations in the wide-angle XRD patterns of the three samples.

For pure LLL (Fig. 3.4a), short-spacing peaks (0.42 nm and 0.39 nm) of β' first occurred at 23.5–22.7°C during cooling. On subsequent heating, these peaks changed to peaks (0.46 nm, 0.39 nm, and 0.38 nm) of β at 27.3–33.1°C and then disappeared at 50.4–57.1°C after melting. The change at 27.3–33.1°C was caused by solid-state $\beta' \rightarrow \beta$ transformation because only the exothermic peak was observed as the corresponding DSC peak (Fig. 3.2b). For LLL + talc (14 μm) (Fig. 3.4b), peaks of β'

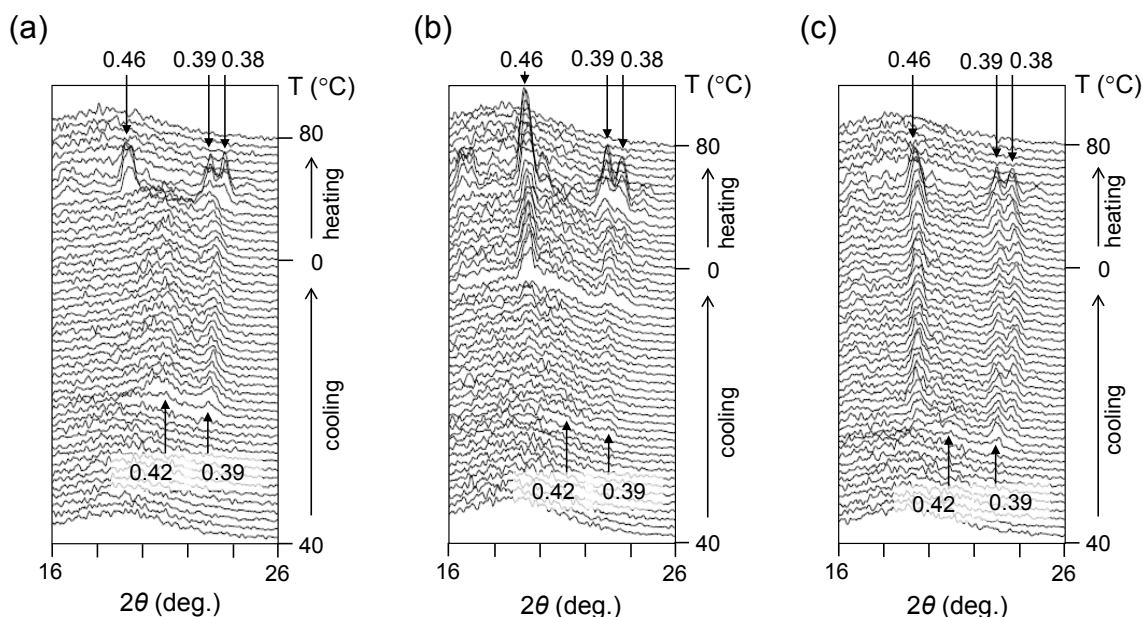


Figure 3.4 Wide-angle XRD patterns of LLL with and without 1 wt% talc particles having different median sizes (0.6–14 μm) during 1°C/min cooling and subsequent 5°C/min heating. (a) Pure LLL, (b) LLL + talc (14 μm), and (c) LLL + talc (0.6 μm). Units are nanometers.

first occurred at 26.1–24.7°C and were replaced by peaks of β at 7.2–6.1°C during cooling due to the $\beta' \rightarrow \beta$ transformation. For LLL + talc (0.6 μm) (Fig. 3.4c), soon after the occurrence of peaks of β' at 28.4–27.7°C during cooling, peaks of β occurred due to $\beta' \rightarrow \beta$ transformation. All these data supported the DSC results in that 1 wt% talc particles promoted the crystallization of LLL and the following $\beta' \rightarrow \beta$ transformation with decreasing particle size (Fig. 3.4b and 3.4c).

3.2 Effects of cooling rate

The nucleation rates (J) of first-occurring polymorphs can be determined by plotting T_i against different cooling rates [22–24] because J is expressed as a function of supercooling [25]. To obtain T_i values, DSC cooling experiments were conducted on LLL with and without 1 wt% talc (0.6 μm) while changing the cooling rate. β' and β were differentiated by comparing the crystal morphologies of first-occurring crystals in POM images (see below) in which bright images of LLL crystals appeared at almost the same temperatures as T_i in DSC.

Figure 3.5 plots variations in T_i of pure LLL and LLL + talc, obtained from DSC and POM experiments under different cooling conditions at rates of 0.2–5.8°C/min. For pure LLL, T_i values increased nonlinearly with decreasing cooling rates. First-occurring polymorphs were β' in the

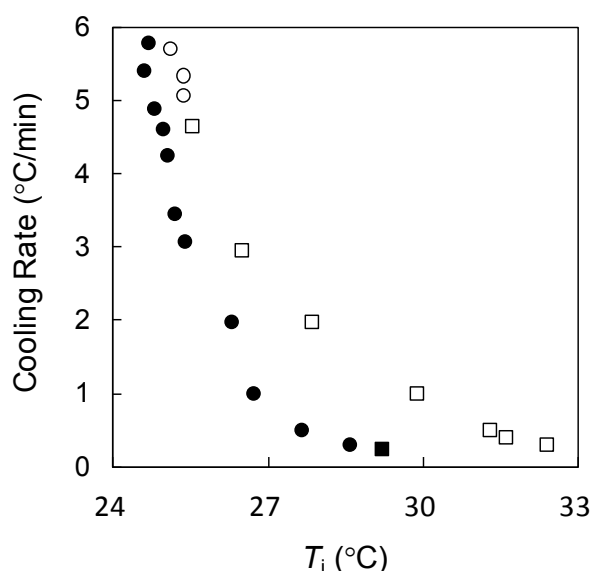


Figure 3.5 T_i versus cooling rate of LLL with and without 1 wt% talc (0.6 μm) obtained from DSC and POM experiments.

0.3–5.8°C/min cooling (plotted as black circles (●) in Fig. 3.5) and β with 0.2°C/min cooling (plotted as a black square (■) in Fig. 3.5). Adding talc particles caused the following changes: (i) all T_i values of pure LLL increased, (ii) the extent of the increase in T_i by decreasing the cooling rate was enhanced, and (iii) the occurrence of β was enhanced, as evidenced by the change in first-occurring polymorphs to β' during 5.1–5.7°C/min cooling (plotted as white circles (○) in Fig. 3.5) and β during 0.3–4.6°C/min cooling (plotted as white squares (□) in Fig. 3.5). Based on these results, I can conclude that talc particles promoted the first nucleation of LLL in stable polymorphs at all the cooling rates applied and that degree of the promotional effects depended on the cooling rate.

Figure 3.6 presents POM images of pure LLL and LLL + talc acquired in the initial stages of crystallization when cooled at different rates. LLL crystals appeared as bright images under the crossed Nicols condition, whereas talc particles were observed as faint spots. The POM observation was repeated to ensure crystal morphologies just after nucleation. When cooled at 1°C/min (Fig. 3.6a), pure LLL exhibited first crystals at 25.5°C and grown crystals in a spherulite pattern at 25.3°C, as previously reported for β' -form crystals of LLL [26]. However, LLL + talc exhibited first crystals at 30.4°C and grown crystals in a grainy-pellet pattern at 29.5°C. The grainy-pellet pattern was identified as that of β , as reported in my previous study [12]. The same morphologies were observed with 3°C/min cooling (Fig. 3.6b). Quite similarly to T_i in DSC (Fig. 3.5), the temperatures at which LLL crystals were first observed decreased with increasing cooling rates for both pure LLL and LLL + talc.

The same experiments were conducted on PPP with and without 1 wt% talc (0.6 μm) while

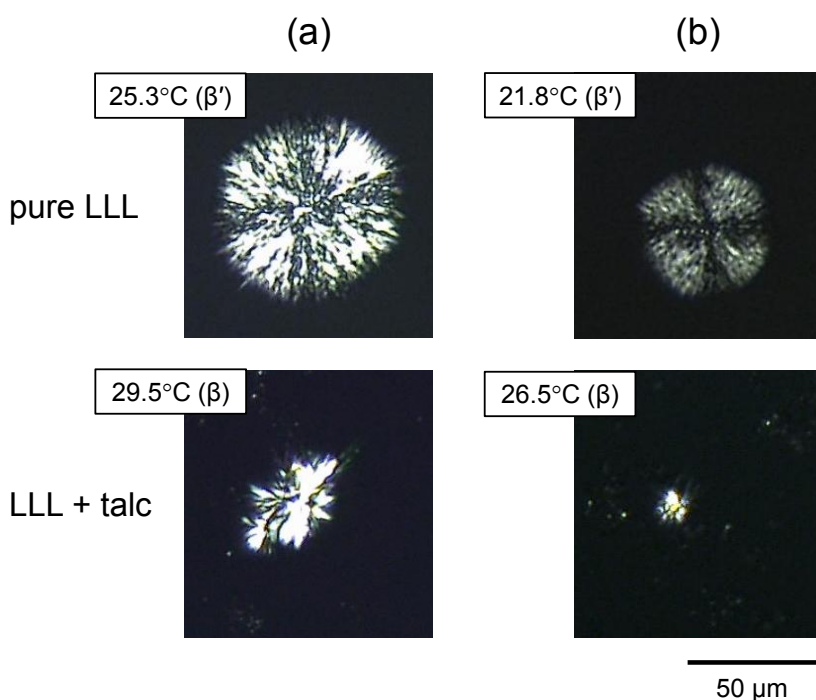


Figure 3.6 POM images of LLL with and without 1 wt% talc (0.6 μm) acquired in the initial stages of crystallization when cooled at (a) 1°C/min and (b) 3°C/min.

changing the cooling rates (0.4–6.3°C/min) (data not shown). Talc particles also promoted the nucleation of PPP in stable polymorphs, and the change in the first-occurring polymorphs and the increase in T_i depended on the cooling rates. First-occurring polymorphs were α (3–5.9°C/min cooling) and β' (0.4–2°C/min cooling) for pure PPP, and β' (3–6.3°C/min cooling) and β (0.4–2°C/min cooling) for PPP + talc.

3.3 Effects on POP

XRD-DSC and POM experiments were conducted on POP with and without 1 wt% talc (0.6 μm) under cooling and subsequent 5°C/min heating conditions. Cooling rates of 5°C/min and 1°C/min were employed.

Figure 3.7 presents the DSC thermograms of pure POP and POP + talc acquired during cooling and subsequent heating. In the cooling thermograms (Fig. 3.7a), pure POP and POP + talc exhibited single exothermic peaks, regardless of the cooling rates. For 5°C/min cooling, T_i values (indicated by arrows in Fig. 3.7a) increased from 13.5°C to 16.1°C after adding talc particles. However, the change in T_i was within experimental error ($\pm 0.3^\circ\text{C}$) in the 1°C/min cooling. These differences due to the

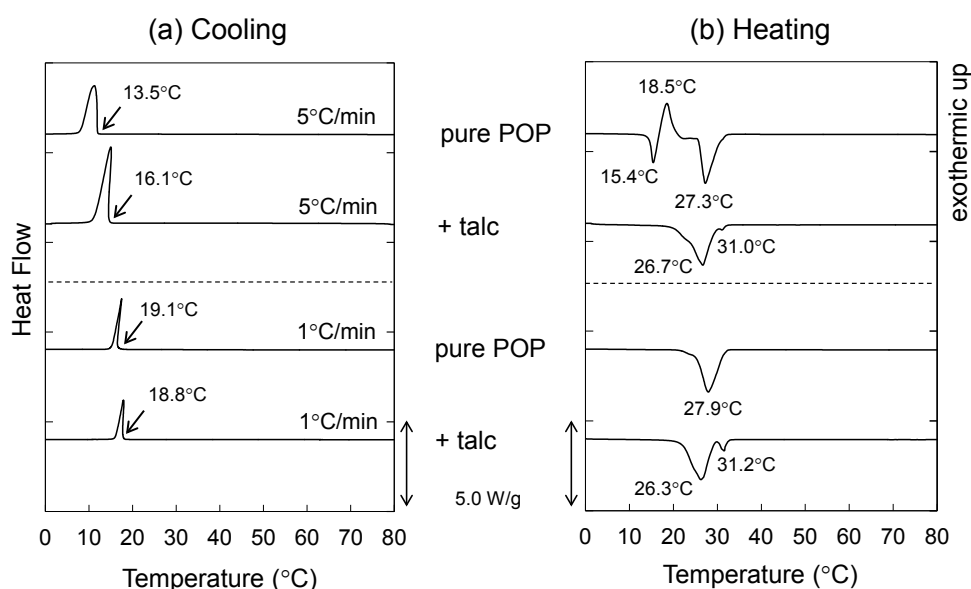


Figure 3.7 DSC thermograms of POP with and without 1 wt% talc (0.6 μm) during (a) 5°C/min and 1°C/min cooling and (b) subsequent 5°C/min heating. Arrows in (a) indicate T_i .

cooling rates may have resulted from differences in first-occurring polymorphs. According to XRD and POM studies (see below), polymorphic occurrence during cooling is α (5°C/min cooling of pure POP); β' , small amounts of β , and α (5°C/min cooling of POP + talc); γ (1°C/min cooling of pure POP); and β' and small amounts of β (1°C/min cooling of POP + talc). It was difficult to determine the subtypes of β' and β of POP from all the results, so I collectively refer to them as β' and β in this study.

The heating thermograms exhibited quite complicated patterns (Fig. 3.7b), because the polymorphic behavior involved in transformation and melting was modified by adding talc particles and the cooling rates before heating. The XRD and POM results explain these thermograms as follows. (i) Pure POP cooled at 5°C/min exhibited melting of α (an endothermic peak at 15.4°C) and subsequent crystallization in γ (an exothermic peak at 18.5°C). The obtained γ melted with further heating (an endothermic peak at 27.3°C with an end at 33.6°C). (ii) POP + talc cooled at 5°C/min exhibited melting of α and β' (a broad endothermic peak at 26.7°C) followed by melting of β (an endothermic peak at 31.0°C with an end at 33.5°C). (iii) Pure POP cooled at 1°C/min exhibited only melting of γ (an endothermic peak at 27.9°C with an end at 33.6°C). (iv) POP + talc cooled at 1°C/min exhibited melting of β' (an endothermic peak at 26.3°C) followed by melting of β (an endothermic peak at 31.2°C with an end at 34.2°C).

Figure 3.8 presents the XRD patterns of pure POP and POP + talc during 5°C/min cooling and subsequent 5°C/min heating. When pure POP was cooled at 5°C/min (Fig. 3.8a), a 0.42 nm

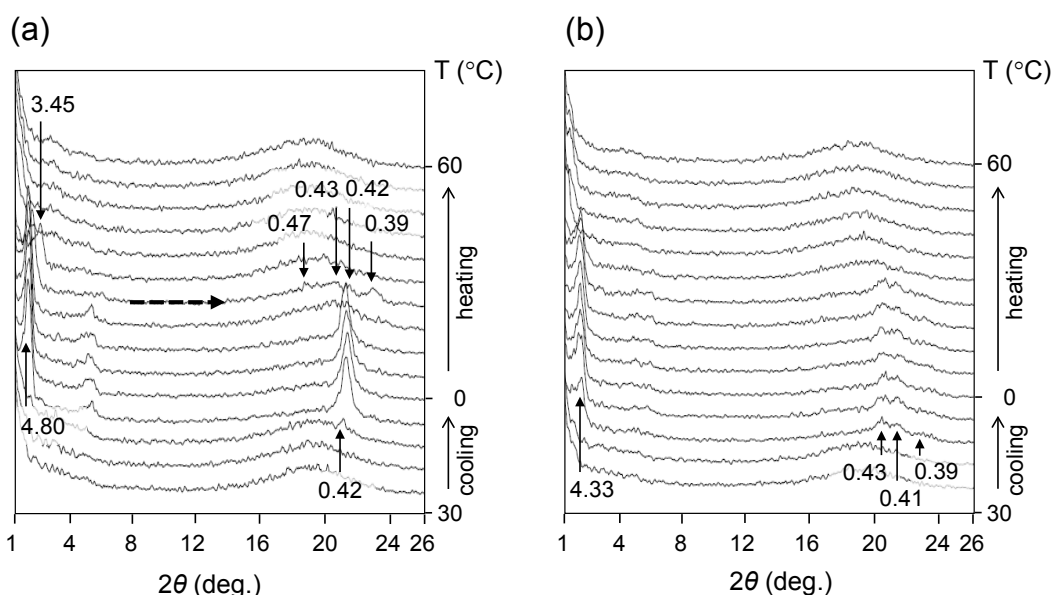


Figure 3.8 XRD patterns of POP with and without 1 wt% talc (0.6 μm) during 5°C/min cooling and subsequent 5°C/min heating. (a) Pure POP and (b) POP + talc. Units are nanometers.

short-spacing peak of α was first detected at 14.7–11.4°C. A 4.80 nm long-spacing peak occurred at the same time as a (001) reflection, exhibiting the double chain-length of α . The short- and long-spacing peaks did not change with further cooling. During subsequent heating, the short-spacing peak changed to peaks of γ (0.47 nm, 0.43 nm, 0.42 nm, and 0.39 nm) at 19.2–24.5°C, denoted by a dotted arrow in Fig. 3.8a. The corresponding DSC peaks are the endothermic peak at 15.4°C and the following exothermic peak at 18.5°C (Fig. 3.7b), so the change in the short-spacing peaks is attributed to melt-mediated $\alpha \rightarrow \gamma$ transformation. The change in the crystal structure is also indicated in the shift of the long-spacing peak. Namely, the 4.80 nm long-spacing peak shifted to a 3.45 nm long-spacing peak as a (002) reflection, indicating change in the chain-length from double to triple. The short- and long-spacing peaks of γ disappeared with further heating without detecting β which was observed in the POM experiments.

When POP + talc was cooled at 5°C/min (Fig. 3.8b), 0.43 nm, 0.41 nm, and 0.39 nm short-spacing peaks were first detected at 14.5–9.9°C. These peaks are attributed to crystallization in β' because of the peak pattern and the 4.33 nm long-spacing peak as a (001) reflection of the double chain-length. The short- and long-spacing peaks did not change until they disappeared during subsequent heating. However, DSC and POM results indicated other polymorphs as well as β' and the complex behavior of polymorphic transformation.

Figure 3.9 presents the XRD patterns of pure POP and POP + talc during 1°C/min cooling and

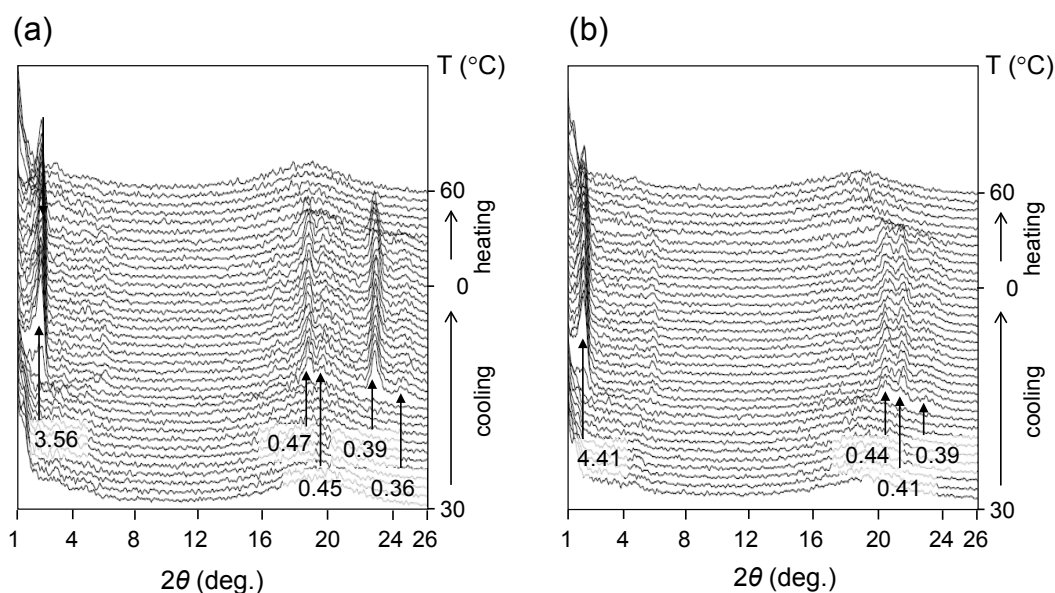


Figure 3.9 XRD patterns of POP with and without 1 wt% talc (0.6 μm) during 1°C/min cooling and subsequent 5°C/min heating. (a) Pure POP and (b) POP + talc. Units are nanometers.

subsequent 5°C/min heating. When pure POP was cooled at 1°C/min (Fig. 3.9a), short-spacing peaks of γ (0.47 nm, 0.45 nm, 0.39 nm, and 0.36 nm) were first detected at 16.5–15.3°C. The 3.56 nm long-spacing peak as a (002) reflection appeared at the same time, exhibiting the triple chain-length of γ . The short- and long-spacing peaks did not change until they disappeared during subsequent heating.

When POP + talc was cooled at 1°C/min (Fig. 3.9b), 0.44 nm, 0.41 nm, and 0.39 nm short-spacing peaks were first detected at 17.7–16.7°C. These peaks are attributed to crystallization in β' because of the peak pattern and the 4.41 nm long-spacing peak as a (001) reflection of the double chain-length. The short- and long-spacing peaks did not change until they disappeared during subsequent heating. However, DSC and POM results indicated β as well as β' .

Figure 3.10 presents POM images of the POP crystals grown with and without talc particles. At 0°C after cooling, pure POP exhibited typical morphologies of α -form crystals (5°C/min cooling, Fig. 3.10a) and γ -form crystals (1°C/min cooling, Fig. 3.10b). When talc particles were added, the most dominant crystals varied to β' -form crystals in both cases, as observed in the XRD results. However, other crystals having different morphologies were also observed, with polymorphs identified as α and β (5°C/min cooling, Fig. 3.10a) and β (1°C/min cooling, Fig. 3.10b) from the disappearing temperatures during subsequent heating (16.7°C for α and 34°C for β). These crystals became visible just after the β' -form crystals, as indicated by arrows in Fig. 3.10 (β -form crystals are in dotted circles). Therefore, POP + talc exhibited a complex morphological transition during heating. At the end of a

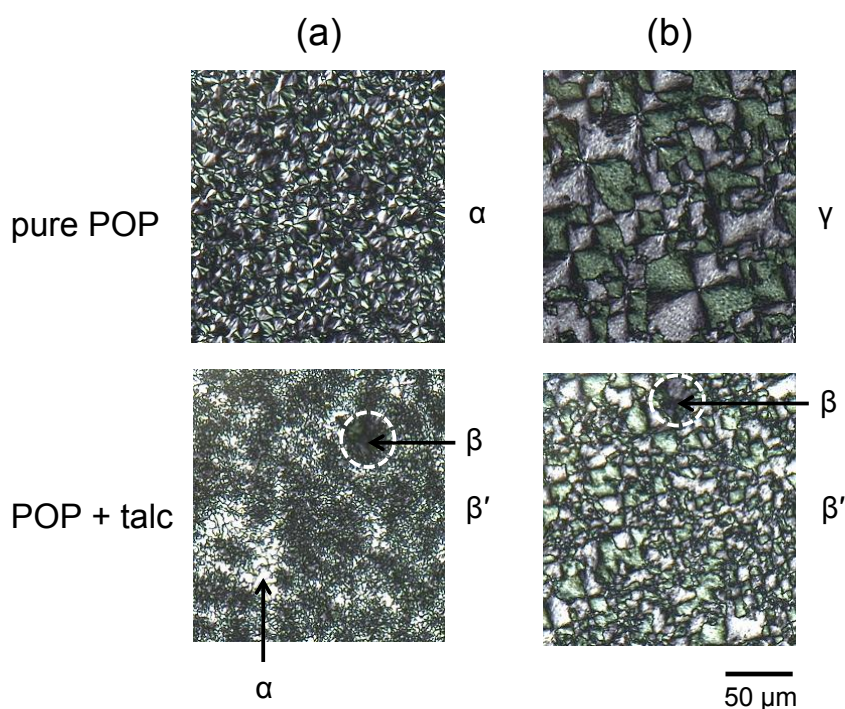


Figure 3.10 POM images of POP with and without 1 wt% talc (0.6 μm) taken at 0°C after (a) 5°C/min cooling and (b) 1°C/min cooling.

series of transitions, needle- and urchin-like crystals, probably β_2 -crystals, formed from the melt at 29°C, and then melted at 34°C together with the original β -form crystals. The melting of these β -form crystals corresponded to endothermic peaks at 31.0–31.2°C in the DSC heating thermograms of POP + talc (Fig. 3.7b). The main results of the POM observation are that adding talc particles caused crystallization of POP in stable polymorphs, which agrees with the DSC and XRD results.

3.4 Model of heterogeneous nucleation

To elaborate on the mechanisms of talc-induced heterogeneous nucleation of TAG crystals, the XRD-DSC experiments were conducted on LLL with 1 wt% talc (0.6 μm) whose hydrophilic edge surfaces were hydrophobically modified with the silane coupling agents.

Figure 3.11 shows the DSC thermograms of LLL + talc (0.6 μm) with and without epoxysilane coupling treatment. It is obvious from the peak shapes and positions that treated talc exhibited almost the same effects as untreated one. LLL + treated talc showed a single exothermic peak at 27.8°C (T_p) with the T_i value of 29.3°C in the 1°C/min cooling (Fig. 3.11a) and a large endothermic peak at 46.8°C in the subsequent 5°C/min heating (Fig. 3.11b). Prior to this large endothermic peak, a remarkably

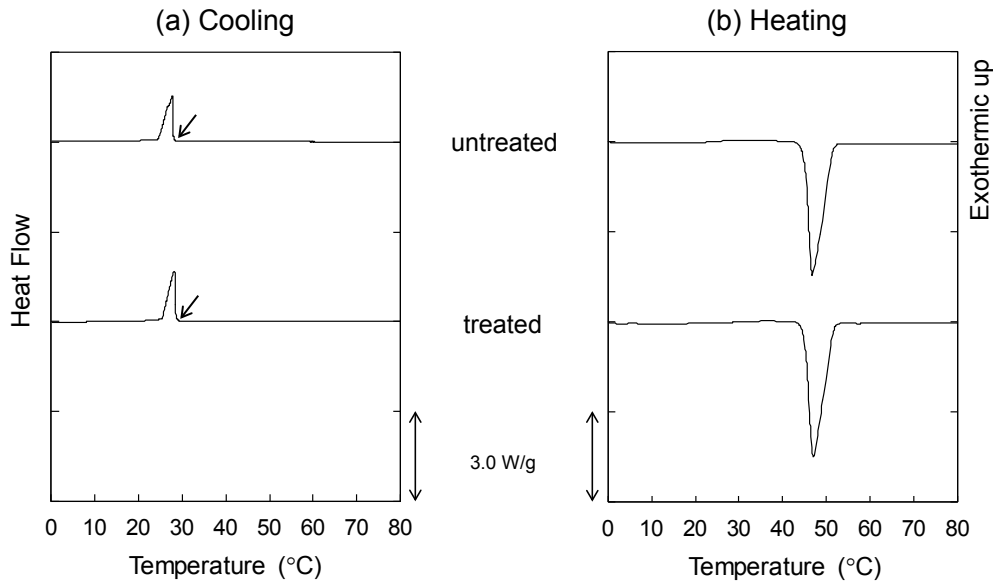


Figure 3.11 Influence of epoxysilane coupling treatment of talc on DSC thermograms of LLL + talc (0.6 μm) 1 wt% during (a) 1°C/min cooling and (b) subsequent 5°C/min heating. Arrows in (a) indicate T_g .

weakened and broadened exothermic peak due to $\beta' \rightarrow \beta$ transformation was also observed (Fig. 3.11b); the enthalpy change was -13.2 kJ/mol while the corresponding values were -35.6 kJ/mol for pure LLL and -8.6 kJ/mol for LLL + untreated talc. Although not shown here, the XRD patterns for LLL + treated talc were the same as those for LLL + untreated talc, as observed in Fig. 3.4c. When the other silane coupling agents having different functional groups were applied, the same XRD-DSC results were obtained (data not shown). All of these results mean that both hydrophobically unmodified and modified talc particles were effective and that the active sites for the heterogeneous nucleation may be present not on the hydrophilic edge surfaces but on the hydrophobic cleavage surfaces of talc particles.

Foreign particles have potential to act as templates for heterogeneous nucleation of fats, and the occurrence of such template effects depends on the affinity to the fats and driving force of nucleation [27]. This is well understood by considering energy barriers for homogeneous and heterogeneous nucleation, which are expressed in the following Eqs. 3.1 and 3.2:

$$\Delta G_{\text{hom}}^* = \frac{f\sigma^3 V^2}{(\Delta\mu_{\text{L} \rightarrow \text{S}})^2} \quad \text{<Eq. 3.1>}$$

$$\Delta G_{\text{het}}^* = \Delta G_{\text{hom}}^* \left\{ \frac{1}{2} - \frac{3}{4} \cos \theta_c + \frac{1}{4} (\cos \theta_c)^3 \right\} \quad \text{<Eq. 3.2>},$$

where ΔG^* (ΔG_{hom}^* and ΔG_{het}^*) is an energy barrier to overcome for forming a nucleus of critical size,

f is a factor specific to the shape, v is a molecular volume in the nucleus, σ is an interfacial energy between the nucleus and the mother phase, and θ_c is a contact angle of a nucleus with respect to the surface of a foreign particle. This contact angle varies from 0 to 180° according to the wettability which relates to molecular affinity between the mother phase and the foreign materials; namely, high affinity gives low values of θ_c and enables decrease in ΔG^* according to Eq. 3.2. Probably, this is why some seed crystals of high-melting fats and emulsifiers, having fatty acid moieties in their molecules, acted as templates and promoted the nucleation of TAGs. $\Delta\mu_{L \rightarrow S}$ is the difference in the Gibbs free energy between liquid phase (L) and solid phase (S), called as driving force of nucleation. From Eqs. 3.1 and 3.2, the energy barrier for nucleation lowers as the driving force is enhanced, whether the nucleation occurs homogeneously or heterogeneously. Here, $\Delta\mu_{L \rightarrow S}$ is expressed as functions of supercooling degree and supersaturation ratio in the following Eqs. 3.3 and 3.4:

$$\Delta\mu_{L \rightarrow S} = \Delta H_m \frac{(T - T_m)}{T_m} \quad \text{<Eq. 3.3>}$$

$$\Delta\mu_{L \rightarrow S} = k_B T \ln \frac{C_0}{C} \quad \text{<Eq. 3.4>},$$

where ΔH_m is the enthalpy change per mole in melting, k_B is the Boltzmann constant, C is concentration of a solute, and C_0 is saturation concentration of the solute. In these equations, $\Delta\mu_{L \rightarrow S}$ is proportional to the supercooling degree of $(T - T_m)/T_m$ and the reciprocal of the supersaturation ratio of C/C_0 .

Therefore, it is reasonable to assume that the cooling rate influences heterogeneous nucleation as well as homogeneous one. Although above four equations hold for all polymorphs, some terms including T_m differ among them, which gives different ΔG^* and thereby determines prior polymorphs for the first nucleation. However, the polymorphic priority may be reversed by some factors such as cooling conditions and different types of foreign materials. In fact, it is known that less stable polymorphs preferentially occur with rapid cooling in the case of homogeneous nucleation [28]. These factors may also change the priority between homogeneous and heterogeneous nucleation.

New types of additives including talc remarkably promoted the crystallization of TAGs in stable polymorphs as compared to the conventional seed crystals [12]. Thus, I believe that these additives have high affinity to the TAGs to reverse the polymorphic priority, despite the molecular dissimilarity between them. From the results using modified talc particles, I presume strong

interactions between TAG molecules and cleavage surfaces of talc, which make it possible for talc to attract and adsorb TAG molecules in stable polymorphs. Adsorbed TAG molecules may have allowed the liquid TAGs to nucleate in stable polymorphs at low supercooling by acting as templates. Although further research is needed to determine the precise arrangements of long-chain TAG molecules with respect to the cleavage surfaces (e.g., normal or parallel to the surfaces), the parallel arrangement may cause steric mismatch between 0.15 nm C–C bonds of hydrocarbon chains in the TAG molecules and 0.305 nm O–O bonds on the cleavage surfaces [29]. One possible interaction between TAG molecules and talc surfaces is nucleophilic attack on carbonyl carbon of the TAG molecules from hydroxyl groups of talc, which are located at the bottom of pseudo-hexagonal cavities in the cleavage surfaces and known to act as electron donors [30].

4 Conclusions

I found that talc promoted the crystallization of LLL in stable polymorphs with decreasing particle size and increasing concentration. This result indicates that the additive effects positively correlate with total surface area of the particles. When different cooling rates were applied, the promotional effects were also observed but the degree depended on the cooling rates, as revealed in first-occurring polymorphs and initial crystallization temperatures (T_i). The same behavior was observed for PPP and POP, and the additive effects of talc particles were understood by making T_i –cooling rate diagrams like LLL. These effects on nucleation also affected the crystal size and distribution of TAG crystals. The results for LLL with surface-modified talc particles gave us a new insight into mechanisms of the heterogeneous nucleation, in which TAG molecules may be adsorbed on the cleavage surfaces of talc. To develop these findings, further studies on the mechanisms and applications to fat-based products are required.

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Chapter 4

Adding talc particles improves physical properties of palm oil-based shortening

1 Introduction

Shortening is a fat-based product that provides food applications with desirable functions such as a tender texture, lubrication, and creaming properties [1]. Liquid oils, emulsifiers, and other additives like antioxidants can be formulated in shortening to reinforce these functions and enhance its applicability. Among various types of shortenings, plastic shortening is typical for bakery use and exhibits semi-solid character at room temperature [1, 2].

Fat crystals play important roles in defining the functions and physical properties of plastic shortening. Triacylglycerols (TAGs) are the main components of fats. TAG crystals exhibit monotropic polymorphism: the typical polymorphs are α , β' , and β forms in ascending order of thermodynamic stability [1, 3–5]. The least stable α easily transforms to β' and β , though the tiny, thin crystals of less than several micrometers make an amorphous mass with smooth surfaces [4–6]. The metastable β' is most desirable for plastic shortening because the small needle-like crystals less than 5 μm long contribute to the plasticity, smooth texture, oil-holding capacity, and creaming properties [1, 2, 6]. The most stable β usually forms needle-like crystals, but these crystals tend to grow to make large platelets (20–100 μm) and further large grainy clumps, losing plasticity and creaming properties [4–6].

The microstructure (e.g., size, shape, and spatial distribution) of fat crystals depends on the polymorph but sometimes differs even in the same polymorph [1, 3–5]. This mesoscale structure made of nanocrystals interlinks to form high-order networks whose mechanical strength basically defines the macroscopic physical properties of fat-based products, such as their viscoelasticity and hardness [3, 7–10]. It is widely believed that fractal aggregation via the London-van der Waals force and crystal sintering (i.e., the formation of irreversible solid bridges between crystals) contribute to the mechanical strength, together with the geometry of the networks [8–10].

The physical properties of fat-based products are often evaluated by deformation tests, where the rheological response to an applied stress or strain is analyzed. Among the various measuring techniques, an oscillatory measurement with small deformation gives the dynamic viscoelasticity, whereas a penetration test with large deformation yields the hardness [11]. These two parameters are important measures of texture and are thus employed for the quality assessment of fat-based foods [10, 12].

Palm oil (PO) is an ideal fat material for plastic shortening because of its easy availability, competitive price, naturally occurring semi-solidity, and β' -crystallization tendency [2, 13]. However, the use of PO causes a serious problem of slow crystallization, which is attributed to the unique TAG composition and appreciable amounts of diacylglycerols [2]. As a consequence of this slow crystallization, post-hardening due to crystal sintering often makes PO-based products hard and brittle beyond the control of manufacturers [2, 14]. Another problem with PO-based products is the occurrence of granular β -form crystals, accounting for the unacceptable sandy mouth-feel and poor plasticity [5, 13–15]. It is considered that the granular crystals form mainly by aggregation of β' -form crystals of the high-melting fraction, such as tripalmitoylglycerol (PPP) and 1,3-dipalmitoyl-2-oleoyl glycerol (POP), and the following $\beta' \rightarrow \beta$ transformation of these crystals [16–19].

One approach to overcoming these difficulties is to modify the crystallization kinetics by adding high-melting fats and emulsifiers, whose crystals are expected to act as templates for the heterogeneous nucleation of PO crystals [15, 20–26]. However, a high concentration of these additives is needed for the template effects due to their solubility in fats [3, 23]. My recent study revealed that fat crystallization was promoted especially by adding foreign crystals of talc, carbon nanotube, graphite, theobromine, ellagic acid dihydrate, or terephthalic acid, despite their molecular dissimilarity to fats [27, 28]. Such promotional effects are expected even at low concentrations of these materials because their crystals are thermally stable and sparingly soluble in fats.

The major objective of this study is to examine the effects of adding talc particles on the physical properties of PO-based shortening. Refined PO (RPO) was employed as the fat material and processed to plastic shortening in the presence and absence of talc particles. The hardness of the shortening was measured after different storage periods and the variation was analyzed in relation to the polymorphic transition of RPO crystals. In addition, the microstructure of the fat crystals was observed to be associated with the macroscopic physical properties.

2 Materials and methods

2.1 Materials

Fat material, pure RPO (iodine value: 52), was supplied by Fuji Oil (Osaka, Japan). Its main TAGs are POP and 1-palmitoyl-2,3-dioleoyl glycerol (POO), accounting for about half of the total TAGs overall. POP is known to exhibit seven crystal polymorphs with the following melting temperatures (T_m) and long-spacing values: α (15.2°C, 4.65 nm), γ (27.0°C, 6.54 nm), δ (29.2°C, 6.25 nm), β'_2 (30.3°C, 4.24 nm), β'_1 (33.5°C, 4.24 nm), β_2 (35.1°C, 6.10 nm), and β_1 (36.7°C, 6.10 nm) [29]. Five polymorphs were reported for POO crystals with T_m and long-spacing values as follows: sub- α (not determined, 5.8 nm), α (-4.0°C, 5.7 nm), liquid crystal (not determined, 6.0 nm), β'_2 (not determined, 6.7 nm), and β'_1 (20.3°C, 6.7 nm) [30]. POP and POO form eutectic mixtures where the T_m for each polymorph changes in proportion to the mix ratio [31]. In addition, about 5% PPP is present in the RPO as a high-melting TAG. PPP crystals have three polymorphs with T_m and long-spacing values as follows: α (44.7°C, 4.60 nm), β' (56.6°C, 4.23 nm), and β (66.4°C, 4.09 nm) [32].

As talc particles, a powder sample of NANO ACE[®] D-600 having 0.6 μm median size was supplied by Nippon Talc (Osaka, Japan), which showed the primary particles of several hundred nanometers and their aggregates of several micrometers in the scanning electron microscopy [27]. This talc sample was mixed with the molten RPO in concentrations of 0.1%, 0.2%, and 1% by weight.

2.2 Thermal analysis of bulk fat systems

The thermal behavior of RPOs with and without talc was analyzed by differential scanning calorimetry (DSC) using a DSC III calorimeter (Rigaku, Tokyo, Japan) attached to an Ultima IV X-ray diffractometer (Rigaku, Tokyo, Japan). Calibration of the temperature and heat flow was performed with reference to the T_m and enthalpy changes of lead, tin, indium, and biphenyl. Each sample (10 mg) on an aluminum pan was prepared after being agitated by a touch mixer to disperse talc particles in the molten RPO (80°C), and then placed in the measuring chamber filled with dry nitrogen gas constantly flowing at a rate of 50 ml/min. After the sample was heated at 80°C for 10 min to erase all crystal memory of fats, its heat flow was recorded during cooling from 80°C to 0°C at a

rate of 5°C/min. To examine the melting behavior of fat crystals formed in the cooling process, samples of RPOs with and without 1% talc were subsequently heated to 80°C at a rate of 5°C/min.

In the DSC thermograms thus obtained (cf. Fig. 4.1), the heat of crystallization was detected as exothermic peaks, whereas the heat of fusion was detected as endothermic peaks. Starting temperatures, peak-top temperatures, and areas (i.e., enthalpy changes) of these peaks were calculated using the associated software. The starting temperatures of the first-occurring peaks in the cooling thermograms were defined as the initial crystallization temperatures (T_i).

2.3 Viscoelasticity measurement of bulk fat systems

The viscoelastic behavior of RPOs with and without talc was examined by a temperature-sweep measurement of dynamic viscoelasticity, using an MCR 302 rheometer (Anton Paar, Graz, Austria) equipped with a temperature-controlled P-PTD200 stage (Anton Paar, Graz, Austria) and an H-PTD200 hood (Anton Paar, Graz, Austria). A PP50 parallel plate (Anton Paar, Graz, Austria) with a 50 mm diameter was set above the preheated stage (80°C) on which samples in a dispersion state were placed. The gap was then narrowed to 1 mm and the overflowed samples were removed. After coverage with the hood, this measuring part was heated at 80°C for 10 min and subsequently cooled to 0°C at a rate of 5°C/min. During cooling, the measurement was performed by sinusoidally rotating the

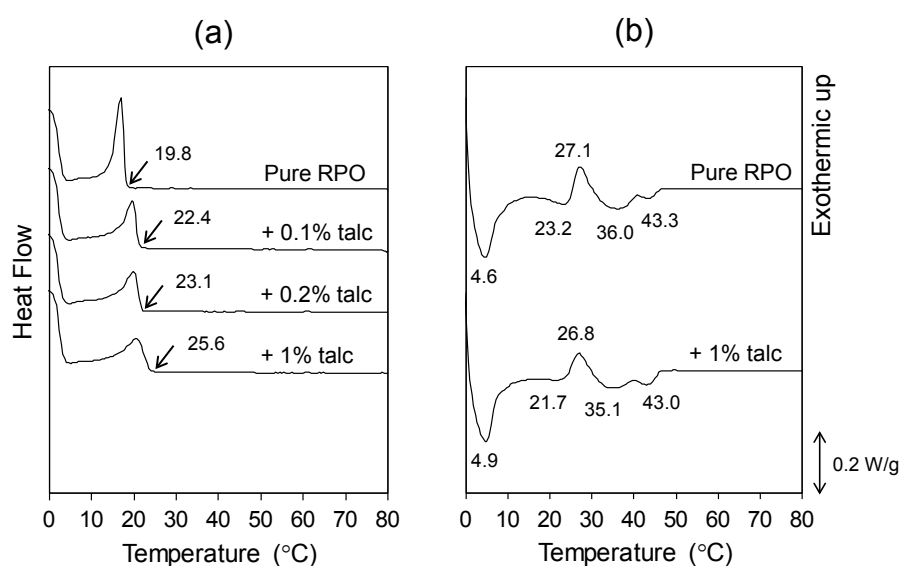


Figure 4.1 DSC thermograms of refined palm oils (RPOs) with and without talc particles. (a) Cooling and (b) subsequent heating at a rate of 5°C/min. Arrows indicate initial crystallization temperatures (T_i). Unit: °C.

plate at a frequency of 1 Hz, where the strain amplitude linearly changed from 30% to 0.01% with decreasing temperature.

The viscoelasticity was evaluated in terms of the storage modulus (G'), loss modulus (G''), and loss tangent ($\tan \delta$), all of which were calculated using the associated software. Elastic factor G' reflects the solid nature of samples, and viscous factor G'' describes the fluid nature of samples [9, 10, 14]. The viscoelastic ratio G''/G' equals $\tan \delta$, which is the tangent of the phase lag (δ) between strain and stress. As $\tan \delta$ decreases, the solid nature becomes more prominent, reducing the rate of energy loss due to conversion from applied force into heat [9]. Duplicate data were collected for each sample in the discrete measurements.

2.4 Shortening preparation

Shortening samples were prepared without gas injection through a Margarine and Spreads Processor (Powerpoint International, Saitama, Japan). The processor consisted of a 10 L hopper, a feed pump, two barrels of scraped surface heat exchangers (SSHEs), and a pin worker, in this order. Processing conditions were as follows: (i) after being heated at 80°C for 10 min, RPOs with and without 0.2% talc were poured into the preheated hopper (60°C); (ii) maintaining the dispersion state of the talc particles by stirring, the mixtures flowed at a rate of 30 kg/hr; (iii) in the SSHEs, the mixtures were rapidly cooled to 3–6°C (average rates: 28–30°C/min) and scraped by the blades on shafts rotating at 800 rpm; and (iv) in the pin worker, the products were well worked by a pin rotor revolving at 1,000 rpm, which produced outlet temperatures of 8–9°C.

The shortenings thus formed were termed ‘pure RPO shortening’ (without talc) and ‘RPO + talc shortening’ (with talc). The fresh-made samples were stored at 5°C for 84 days to examine the variation in the physical properties of the shortenings during storage.

2.5 Polymorphic analysis of shortening

The polymorphism of the shortenings was examined by X-ray diffractometry (XRD) using an Ultima IV X-ray diffractometer (Rigaku, Tokyo, Japan) equipped with a DSC III calorimeter (Rigaku, Tokyo, Japan). 10 mg samples on aluminum pans were set in the measuring chamber without thermal thawing

from 5°C. The measurement was performed by applying X-rays to these samples using the reflection method in a diffraction-angle (2θ) range of 1–30°, with Cu-K α radiation (0.154 nm wavelength, 40 kV, 40 mA). The diffracted X-rays were detected by a scintillation counter (NaI) and electrically converted to one-dimensional patterns.

The XRD patterns thus obtained were analyzed using the associated software in order to define the polymorphs of the RPO crystals formed in the shortenings. Long spacing of the lamellar structure and short spacing of the sub-cell structure were calculated from the 2θ values of XRD peaks in the small- and wide-angle regions, respectively. Polymorphic transitions during the 84-day storage period were compared between the shortenings with and without talc. Complementary data were acquired for the 84-day stored samples by the simultaneous measurement of XRD and DSC (XRD-DSC) during heating from 5°C to 80°C at a rate of 5°C/min.

2.6 Hardness measurement of shortening

The hardness of the shortenings was measured by a penetration test using an RE2-33005S creep meter (Yamaden, Tokyo, Japan) equipped with a 200 N load cell (Yamaden, Tokyo, Japan). A uniaxial disc probe (Rheotech, Tokyo, Japan) with 10 mm diameter and 1 mm thickness was attached to the upper part. Each cylindrical sample, with 100 mm diameter and 35 mm height in a soft cup, was put on a stage in the lower part. By lifting this stage at a speed of 1 mm/sec, the sample was penetrated up to 75% of the thickness with the probe. During penetration, the normal force on the sample was recorded and analyzed using the associated software. In the present study, I defined the maximum force as the hardness.

The measurement was performed at 20°C using shortening samples which were incubated at 20°C for 16 hours after storage at 5°C. The hardness values of the shortenings with and without talc were compared over the 84-day storage period. Triple data were collected for each shortening after different storage periods.

2.7 Observation of fat crystal microstructure

The microstructure of the RPO crystals, formed in preparations of the bulk fat systems (4–8 μ L) and

shortenings (20 mg), was observed by polarized optical microscopy (POM) using a VHX-600 digital microscope (Keyence, Osaka, Japan). The observation was conducted under the crossed Nicols condition while the temperature of the preparations was controlled by a T95 system controller (Linkam Scientific Instruments, Tadworth, UK). The thermal conditions were cooling from 80°C to 0°C at a rate of 5°C/min after heating at 80°C for 10 min (for bulk fat systems), and cooling at 5°C (for shortenings).

3 Results and discussion

3.1 Thermal behavior of bulk fat systems

Figure 4.1 presents DSC thermograms of RPOs with and without talc, recorded during cooling and subsequent heating at a rate of 5°C/min.

In the cooling thermograms (Fig. 4.1a), pure RPO exhibited two exothermic peaks due to crystallization: one peak showed T_i of 19.8°C (indicated by an arrow in Fig. 4.1a) and a peak top at 16.9°C, and the other peak started from 4.7°C. Although not presented here, I confirmed by XRD experiment that α -form crystals having a double-chain-length structure occurred in the first exothermic peak. Such α -crystallization of the high-melting fraction was previously observed for PO under isothermal (cooling at 15°C [24] and 25°C [21]) and non-isothermal (cooling at a rate of 10°C/min [25]) conditions.

In the subsequent heating thermograms (Fig. 4.1b), pure RPO exhibited endothermic peaks at 4.6°C and 23.2°C, followed by an exothermic peak at 27.1°C and endothermic peaks at 36.0°C and 43.3°C. Taking account of the T_m of the major component TAGs, we can interpret these thermopeaks as follows: melting of POO β' -form crystals at 4.6°C, melting of POP and PPP α -form crystals at 23.2°C, β -crystallization of POP and PPP at 27.1°C, melting of POP β -form crystals at 36.0°C, and melting of PPP β -form crystals at 43.3°C. Actually, the melting behavior of the RPO crystals may be far more complicated due to the complex fat composition, as revealed by the broad thermopeaks in Fig. 4.1b.

When the talc particles were added to the RPO, the first-occurring peak in the cooling

thermogram shifted to higher temperatures, while the second peak did not change (Fig. 4.1a). The enthalpy changes in these peaks (-13.8 J/g and -9.7 J/g) and the subsequent heating thermogram of pure RPO were almost unchanged even when 1% talc particles were added (Fig. 4.1a and 4.1b). These results indicate that the talc promoted crystallization of the high-melting fraction (including PPP and POP) without changing the crystal mass and polymorphs, but did not affect crystallization of the low-melting fraction (including POO). This promotional effect was enhanced with increasing concentrations of the talc particles, as revealed in T_i (indicated by arrows in Fig. 4.1a) of 22.4°C for RPO + 0.1% talc, 23.1°C for RPO + 0.2% talc, and 25.6°C for RPO + 1% talc.

3.2 Microstructure of fat crystals in bulk fat systems

Figure 4.2 presents POM images of RPOs with and without 1% talc, taken during cooling at a rate of $5^\circ\text{C}/\text{min}$. Fat crystals were observed as bright images under the crossed Nicols condition.

When pure RPO was cooled (Fig. 4.2a), needle-like crystals first occurred at 20.8°C . As the temperature decreased, these crystals increased in size and number, aggregating to form local networks. With further cooling, other crystals formed from the liquid phase below 2°C .

In contrast, RPO + talc (Fig. 4.2b) exhibited small microcrystals which first occurred at 25.4°C . These crystals rapidly increased in number with little growth because most of the high-melting

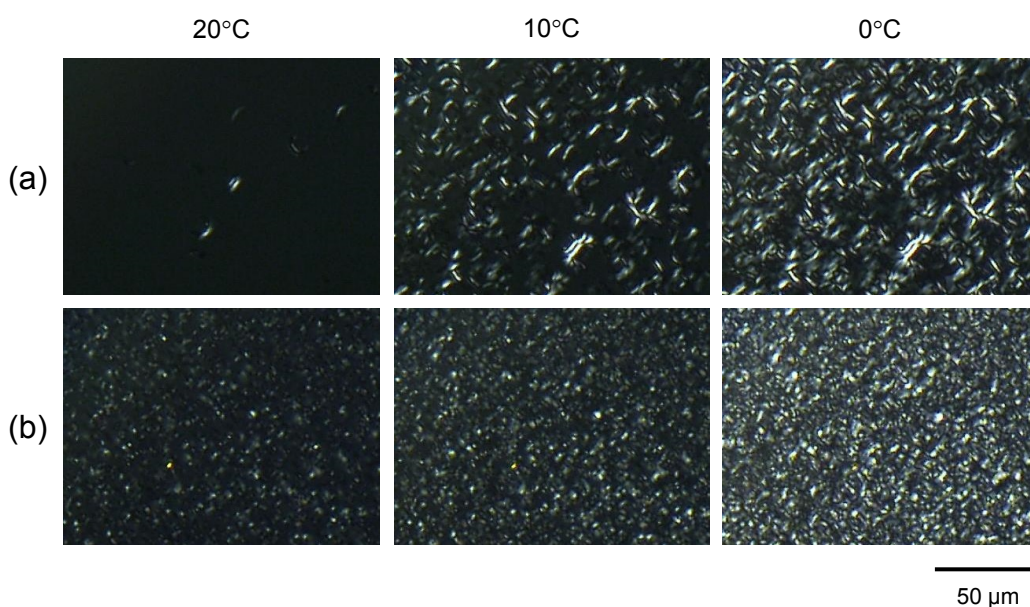


Figure 4.2 POM images of RPOs with and without 1% talc particles, taken at different temperatures during cooling at a rate of $5^\circ\text{C}/\text{min}$. (a) Pure RPO and (b) RPO + talc.

fraction crystallized at the surfaces of the talc particles by heterogeneous nucleation. Within the matrix of a great number of the microcrystals, new crystals formed below 2°C with further cooling.

The results of the POM observation are consistent with those of the DSC experiments (Fig. 4.1a) in that two-step crystallization was observed for pure RPO and only the first step was promoted by talc addition. Therefore, I can conclude that the talc changed the microstructure of the RPO crystals by preferentially promoting crystallization of the high-melting fraction.

3.3 Viscoelastic behavior of bulk fat systems

Figure 4.3 presents temperature-sweep profiles of the dynamic viscoelasticity measured for RPOs with and without talc during cooling at a rate of 5°C/min. Because the duplicate measurements gave quite similar results, the G' , G'' , and $\tan \delta$ values of one data set are logarithmically plotted against the decreasing temperature.

In Fig. 4.3a, the storage modulus G' of pure RPO exhibited three-stage increases (16–12°C (1st stage), 12–5°C (2nd stage), and 5–0°C (3rd stage)). These increases in the G' indicate the development of fat crystal networks with strong links because the elastic factor G' reflects the solid nature of samples, as mentioned before (see Section 2.3). The increasing G' was drastically changed by talc addition in the following manner. First, the onset temperature of the first-stage increase rose from 16°C for pure RPO to 22°C for RPO + 0.1% talc and 24.5°C for RPO + 1% talc, as indicated by the arrows in Fig. 4.3a. Second, the rates of the second- and third-stage increases decreased remarkably, which made the G' values of RPO + talc smaller than those of pure RPO below 11°C. Finally, the G' value at 0°C decreased from 1.3×10^5 Pa for pure RPO to 1.8×10^3 Pa for RPO + 0.1% talc and 4.4×10^2 Pa for RPO + 1% talc. To summarize the effects of talc addition, talc promoted the first-stage increase in G' but suppressed subsequent increases at low temperatures during cooling.

In Fig. 4.3b, the loss modulus G'' of RPOs with and without talc increased in the same manner as their G' after the gradual increase starting from 80°C. This means that networks of the RPO crystals developed with weak links as well as with strong links. The gradual increase in the G'' is attributed to the increasing viscosity of the liquid phase, due to the reduced mobility of the fat molecules. The G'' values at 0°C were 4.1×10^4 Pa for pure RPO, 7.8×10^2 Pa for RPO + 0.1% talc, and 1.7×10^2 Pa for RPO + 1% talc.

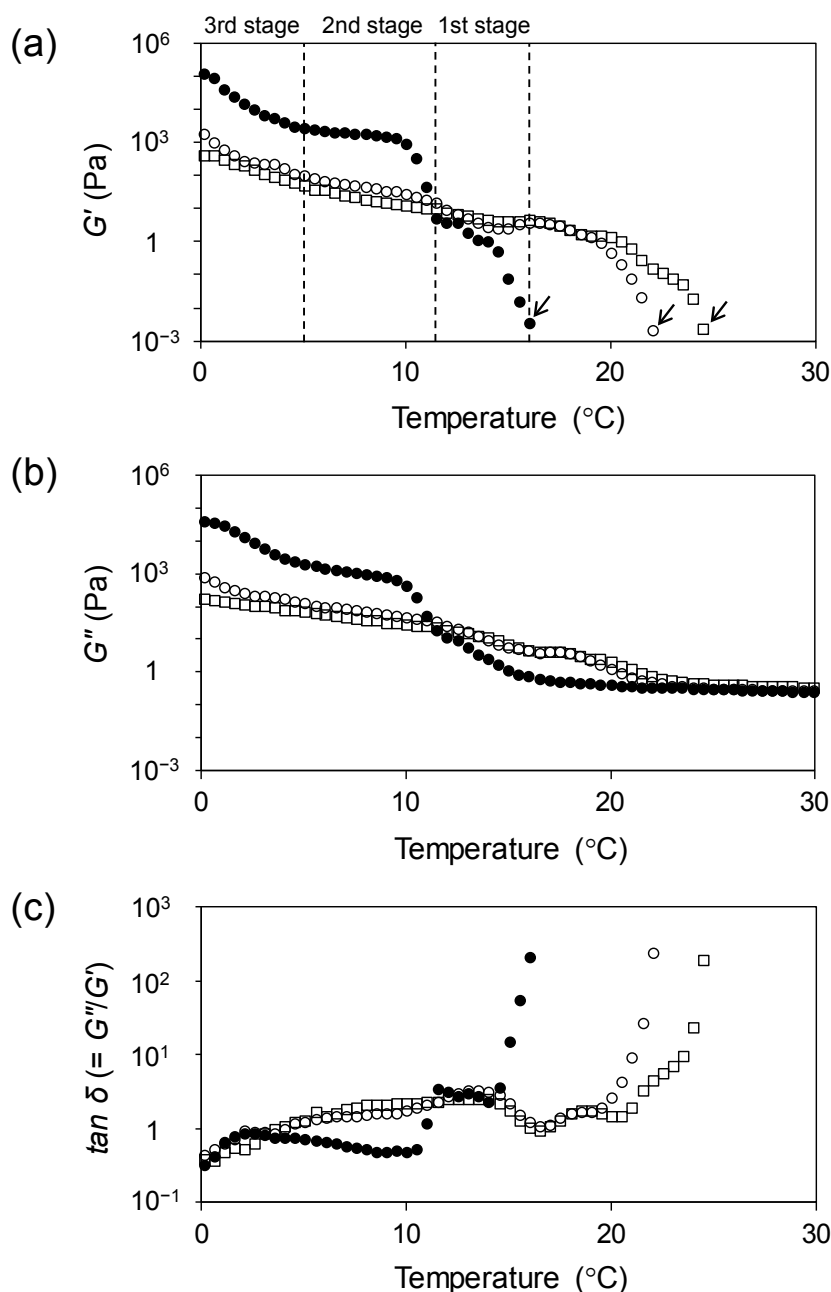


Figure 4.3 Temperature-sweep ($-5^{\circ}\text{C}/\text{min}$) profiles of dynamic viscoelasticity measured for RPOs with and without talc particles. (a) Storage modulus (G'), (b) loss modulus (G''), and (c) loss tangent ($\tan \delta$). Symbols mean pure RPO ($\bullet$), RPO + 0.1% talc ($\circ$), and RPO + 1% talc ($\square$).

In Fig. 4.3c, the loss tangent $\tan \delta (= G''/G')$ of pure RPO decreased stepwise in a zigzag pattern during cooling; specifically, the $\tan \delta$ decreased ($16\text{--}14^{\circ}\text{C}$), increased ($14\text{--}12^{\circ}\text{C}$), decreased ($12\text{--}10^{\circ}\text{C}$), increased ($10\text{--}2^{\circ}\text{C}$), and decreased ($2\text{--}0^{\circ}\text{C}$). By comparing the rates of increases between the G' and G'' of pure RPO, we can interpret this complex $\tan \delta$ behavior as follows. Relatively high rates of the increasing G' caused the decreases in the $\tan \delta$, indicating the predominant development of fat crystal networks with strong links. Conversely, the increases in the $\tan \delta$ indicate the predominant development of the networks with weak links because of relatively high rates of the increasing G'' .

Therefore, based on the $\tan \delta$ curve of pure RPO, I consider that the formation of strong links between the RPO crystals was enhanced in the temperature ranges of 16–14°C, 12–10°C, and 2–0°C while weak links continued to be formed by flocculation of the crystals.

When the talc particles were added to the RPO, the first decrease in $\tan \delta$ occurred in the temperature ranges of 22–16.5°C for RPO + 0.1% talc and 24.5–16.5°C for RPO + 1% talc, showing large drops of the $\tan \delta$ values compared to that of pure RPO. The $\tan \delta$ of RPO + talc subsequently increased (16.5–13.5°C) and then continued to decrease at rates lower than that of the second decrease in the $\tan \delta$ of pure RPO. The $\tan \delta$ values at 0°C were 0.33 for pure RPO, 0.44 for RPO + 0.1% talc, and 0.39 for RPO + 1% talc.

Taking into account the results of DSC (Fig. 4.1a) and POM (Fig. 4.2), I can explain the viscoelastic behavior of RPOs with and without talc by the sequence of development of fat crystal networks, as illustrated in Fig. 4.4.

For pure RPO, an increased number of needle-like crystals of the high-melting fraction aggregated to form local networks after the initial crystallization (Fig. 4.4a). The aggregates thus formed continued to grow and gathered into flocs, which were sintered by subsequently occurring crystals of the high-melting fraction (Fig. 4.4b). Crystals of the low-melting fraction then started to grow and aggregated to form continuous networks together with the previously formed crystal aggregates of the high-melting fraction (Fig. 4.4c). Sintering phenomena between fat crystals were

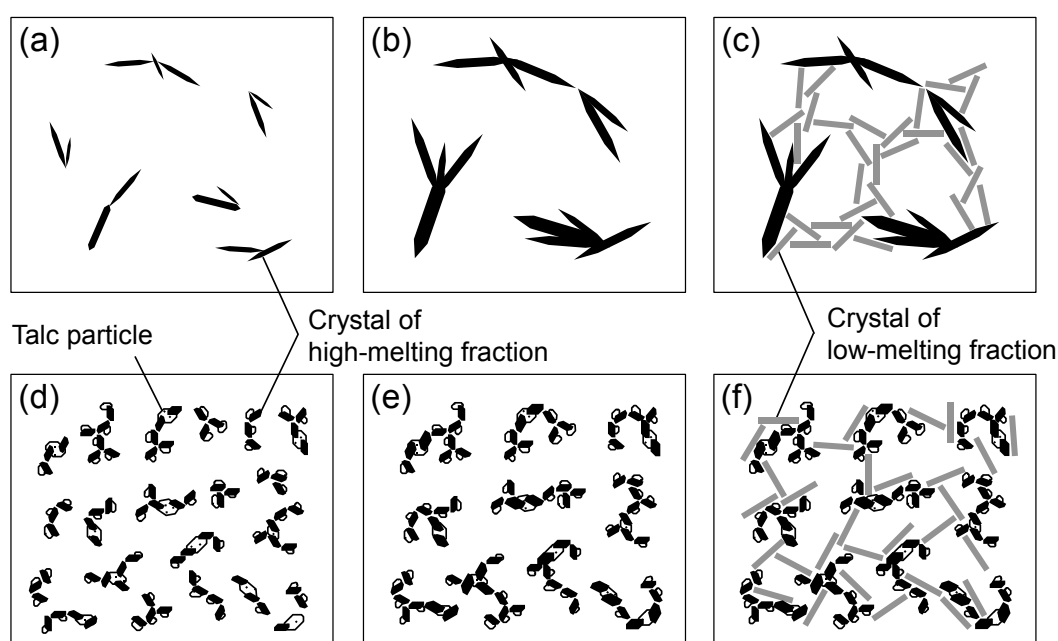


Figure 4.4 Models of fat crystal networks developing in (a)–(c) pure RPO and (d)–(f) RPO + talc.

observed in previous studies [33, 34].

For RPO + talc, a great number of small microcrystals of the high-melting fraction readily aggregated to form dense networks soon after the initial crystallization (Fig. 4.4d), being aided by the template effects of the talc particles. The aggregates thus formed flocculated, but sintered insufficiently due to the limited growth of the microcrystals (Fig. 4.4e). The dense networks of these crystals hindered the network formation of subsequently occurring crystals of the low-melting fraction (Fig. 4.4f). Adding talc particles shortened the time lag from the initial crystallization to the network formation of the RPO crystals, as indicated by the reduced difference between the T_i in DSC (indicated by arrows in Fig. 4.1a) and the onset temperatures of the first-stage increase in G' (indicated by arrows in Fig. 4.3a): the differences were 3.8°C for pure RPO, 2.4°C for RPO + 0.1% talc, and 1.1°C for RPO + 1% talc.

Although not shown here, it must be mentioned that RPO + 0.2% talc exhibited viscoelastic behavior close to that of RPO + 1% talc. Adding talc particles at concentrations of 0.2% and 1% most effectively suppressed the increases in the G' and G'' of pure RPO below 11°C. Therefore, I determined the concentration of talc particles to be added to the shortenings as 0.2%, which was enough to improve the physical properties efficiently, as discussed below.

3.4 Polymorphism of shortening

Figure 4.5 presents XRD patterns of the shortenings with and without talc, taken at 5°C after different storage periods.

Pure RPO shortening (Fig. 4.5a) exhibited two long-spacing peaks (6.64 nm and 4.44 nm) and four short-spacing peaks (0.46 nm, 0.43 nm, 0.42 nm, and 0.39 nm) for all of the storage periods. For the 84-day stored sample, the XRD-DSC study attributes these peaks to mixed crystals, mainly of PPP β' , POP β' , POO β' , and molecular-compound (MC) β of POP and 1,2-dipalmitoyl-3-oleoyl glycerol (PPO) (see below). After 21 days of storage, the relative intensity of the 0.46 nm short-spacing peak increased obviously, keeping the proportion between those of the two long-spacing peaks almost unchanged. This is probably because the underlying β' -form crystals of MC_{POP/PPO} transformed to β without changing the double-chain-length structure [35].

The same XRD pattern was observed for 84-day stored RPO + talc shortening (Fig. 4.5b).

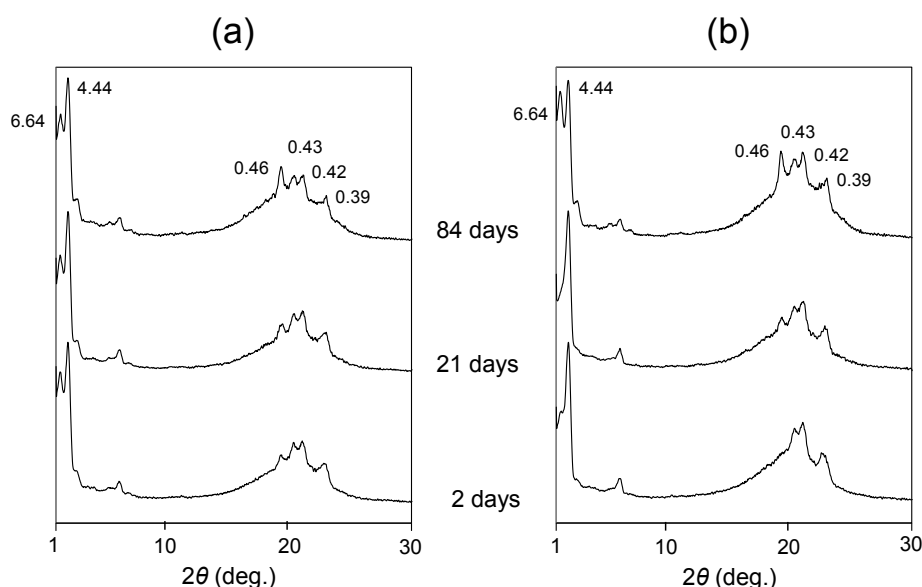


Figure 4.5 XRD patterns of shortenings with and without 0.2% talc particles, taken at 5°C after different storage periods. (a) Pure RPO shortening and (b) RPO + talc shortening. Unit: nm.

However, the 0.46 nm short-spacing peak occurred on the 21st day and the 6.64 nm long-spacing peak clearly appeared on the 84th day. This indicates that the talc delayed crystallization of MC_{POP/POO} β and POO β' compared to that of PPP β' and POP β' . The relative intensity of the 0.46 nm short-spacing peak increased after 21 days of storage, similarly to the case of pure RPO shortening. These results demonstrate that the talc changed the crystallization kinetics of pure RPO shortening by retarding crystallization of the low-melting fraction.

Figures 4.6 and 4.7 present XRD-DSC results for the 84-day stored shortenings with and without talc, taken during heating from 5°C to 80°C at a rate of 5°C/min. To my best knowledge, no literature has been available to report XRD patterns of RPO-based shortening. Changes in the XRD patterns during heating inform us of the polymorphic behavior of the RPO crystals melting in the shortenings.

For pure RPO shortening, the DSC thermogram (Fig. 4.6a) exhibits three major endothermic peaks (at 11.8°C, 20.2°C, and 30.7°C), which overlap with each other and have a shoulder around 38°C (indicated by an arrow in Fig. 4.6a). The simultaneously acquired XRD patterns (Fig. 4.7a) suggest that these thermopeaks are due mainly to the melting behavior of the major TAG crystals in the following sequence. First, POO β' -form crystals having a triple-chain-length structure melted at 11.8°C. Second, β -form crystals having a double-chain-length structure melted at 20.2°C. Among the major TAGs in the RPO and their MCs such as MC_{POP/POO} [35] and MC_{PPO/POO} [30], MC_{POP/POO} β is

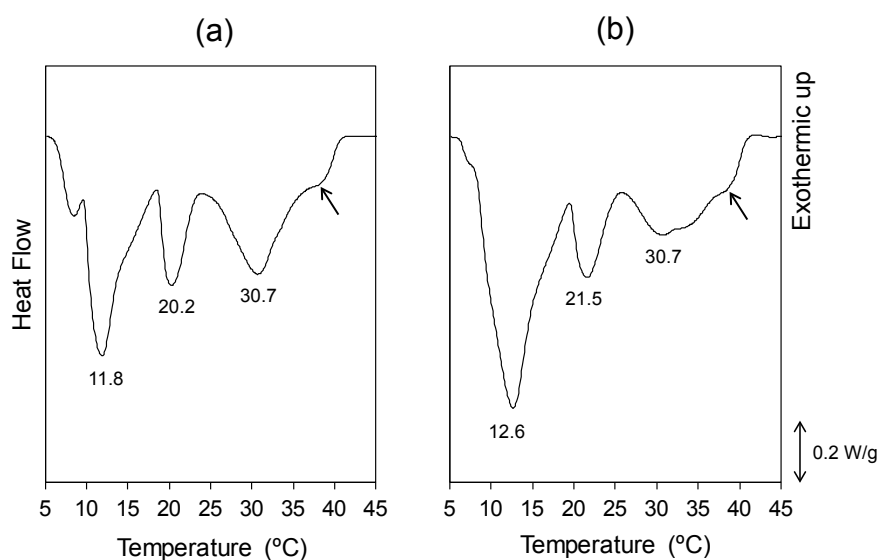


Figure 4.6 DSC heating (5°C/min) thermograms of 84-day stored shortenings with and without 0.2% talc particles. (a) Pure RPO shortening and (b) RPO + talc shortening. Unit: °C.

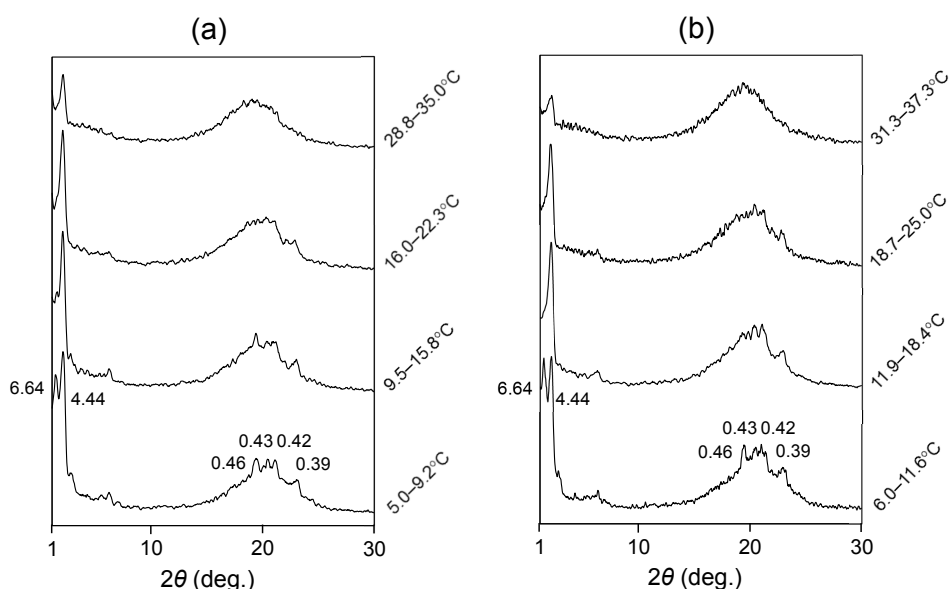


Figure 4.7 XRD patterns of 84-day stored shortenings with and without 0.2% talc particles, taken during heating at a rate of 5°C/min. (a) Pure RPO shortening and (b) RPO + talc shortening. Unit: nm.

most applicable to the β -form crystals. $MC_{POP/PPPO}$ β -form crystals are known to exhibit a 4.1 nm long-spacing peak and 0.46 nm, 0.40 nm, and 0.38 nm short-spacing peaks in the XRD pattern, which indicated melting at 31.2°C in the binary mixture [35]. If all PPO in the RPO formed 1:1 MC crystals with POP, the total amount would be about 10% of the total TAGs. Finally, POP and PPP β' -form crystals having a double-chain-length structure melted at 30.7°C and 38°C, respectively. From the XRD-DSC results in Figs. 4.6a and 4.7a, I can conclude that pure RPO shortening contained crystals of POO β' , $MC_{POP/PPPO}$ β , POP β' , and PPP β' after storage for 84 days.

The XRD-DSC results for 84-day stored RPO + talc shortening (Figs. 4.6b and 4.7b) are almost the same as those for 84-day stored pure RPO shortening (Figs. 4.6a and 4.7a). Namely, POO β' -form crystals having a triple-chain-length structure melted at 12.6°C, MC_{POP/PPO} β -form crystals having a double-chain-length structure melted at 21.5°C, and POP and PPP β' -form crystals having a double-chain-length structure melted at 30.7°C and 38°C (indicated by an arrow in Fig. 4.6b). However, their DSC heating thermopeaks in Fig. 4.6 differed in the enthalpy changes as follows: 48.7 J/g (5.0–18.3°C), 20.8 J/g (18.3–23.8°C), and 49.6 J/g (23.8–42.0°C) for pure RPO shortening; 68.6 J/g (5.0–19.4°C), 22.9 J/g (19.4–25.7°C), and 40.4 J/g (25.7–41.8°C) for RPO + talc shortening. These results indicate that the talc did not change the variety and polymorphs of the main TAGs crystallizing in the 84-day stored shortening, but affected the crystal mass, increasing crystals of the low-melting fraction and decreasing those of the high-melting fraction. This change in crystal mass appears to contradict the XRD result that the talc retarded crystallization of the low-melting fraction (Fig. 4.5b). I assume that this retardation was exerted on the nucleation but acted on the crystal growth to be enhanced.

The melting behavior of the RPO crystals in the 84-day stored shortenings (Fig. 4.6) clearly differs from that in the bulk fat systems (Fig. 4.1b). This means that the crystal polymorphs and mass were influenced by the combination of intense shear and rapid cooling followed by long storage. For pure RPO and 84-day stored pure RPO shortening, the polymorphic behavior of the major TAG crystals during heating (5°C/min) is summarized in Table 4.1.

Table 4.1 Polymorphic behavior of major TAG crystals in pure RPO and 84-day stored pure RPO shortening, analyzed for DSC heating (5°C/min) thermopeaks in Figs. 4.1b and 4.6a.

	Peak-top temperatures				
	4.6°C	23.2°C	27.1°C	36.0°C	43.3°C
Pure RPO (Fig. 4.1b)	POO β' -3 melt	POP α -2 PPP α -2 melt	POP β -3 PPP β -2 crystallize	POP β -3 melt	PPP β -2 melt
Pure RPO shortening (Fig. 4.6a)	11.8°C POO β' -3 melt	20.2°C MC _{POP/PPO} β -2 melt	— —	30.7°C POP β' -2 melt	38°C PPP β' -2 melt

MC, molecular compound.

3.5 Microstructure of fat crystals in shortening

Figure 4.8 presents POM images of the shortenings with and without talc, taken at 5°C after different storage periods.

For pure RPO shortening (Fig. 4.8a), the two-day stored sample exhibited a marble-like pattern of the RPO crystals, with the aggregated crystals and the surrounding crystals dispersed heterogeneously. In the 84-day stored sample, granular crystals with much brighter images were observed in place of the aggregates. Although not presented here, this change had already started in the seven-day stored sample. Nevertheless, polymorphic change did not occur between the 2- and 21-day stored samples, as revealed in their XRD patterns (Fig. 4.5a). These results suggest that the granular crystals were formed by local condensation of the high-melting fraction through rearrangement of the aggregated crystals without changing the polymorphs. Post-crystallization and recrystallization due to Ostwald ripening may have contributed to the growth of the granular crystals.

In contrast, RPO + talc shortening (Fig. 4.8b) exhibited a large number of small granular crystals and interstitial crystals in the two-day stored sample. I assume that many seed crystals of the high-melting fraction, which formed on the surfaces of the talc particles by heterogeneous nucleation, quickly incorporated their peer crystals from the surroundings. The granular crystals thus formed increased in size in the seven-day stored sample but did not grow further, as observed in the 84-day stored sample. In the comparison with the fat crystal microstructure in pure RPO shortening, the homogeneity of the fat crystal dispersion was improved over the 84-day storage period.

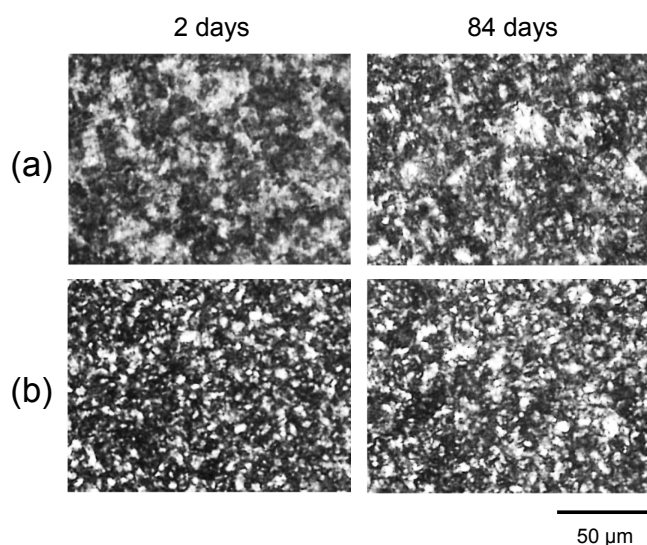


Figure 4.8 POM images of shortenings with and without 0.2% talc particles, taken at 5°C after different storage periods. (a) Pure RPO shortening and (b) RPO + talc shortening.

3.6 Hardness of shortening

Figure 4.9 presents the hardness of the shortenings with and without talc, with the average values plotted against different storage periods.

There is a common trend between the two shortenings in which the average hardness values increased in the early stages of storage; specifically, pure RPO shortening exhibited a long-term increase from 19.5 N (2 days) to 26.3 N (62 days), whereas RPO + talc shortening exhibited a short-term increase from 15.9 N (2 days) to 19.1 N (7 days). According to the XRD-DSC results (Figs. 4.6 and 4.7), crystals of the low-melting fraction seem to have almost melted during incubation at 20°C before the hardness measurements. Therefore, I consider that the increases in the hardness (post-hardening) are due mainly to the growth of granular crystals of the high-melting fraction, which was observed in the POM study (Fig. 4.8). This consideration is supported by the results that adding talc particles shortened the periods of post-hardening and growth of the granular crystals in the same manner. From the result that the average hardness values were always higher in pure RPO shortening than in RPO + talc shortening in proportion to the crystal size, it seems that the hardness depends on the size distribution rather than the number of the RPO crystals.

In addition, the difference between the triple data of the hardness was greatest in the short-term stored samples of pure RPO shortening, as expressed by the long error bars of the standard deviation (S.D.) in Fig. 4.9. This indicates that unstable networks of granular crystals developing in pure RPO

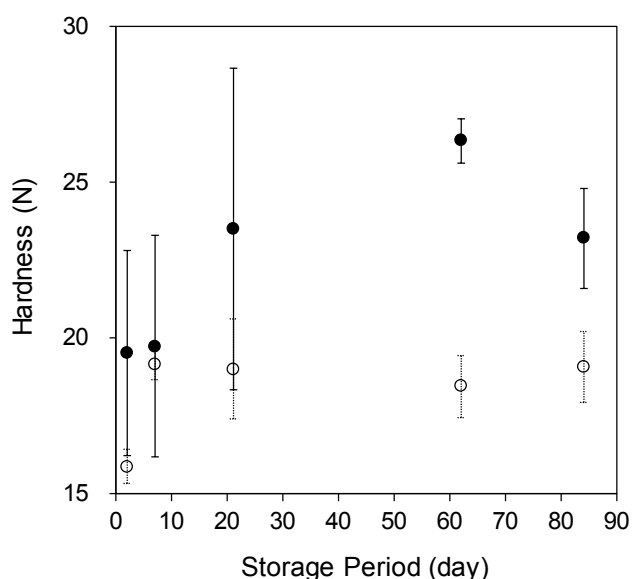


Figure 4.9 Hardness (average $\pm$ S.D.) of shortenings with and without 0.2% talc particles, measured after incubation at 20°C for 16 hours. Symbols mean pure RPO shortening (●) and RPO + talc shortening (○).

shortening were stabilized after 21 days of storage. During the 84-day storage period, RPO + talc shortening exhibited a small difference between the measurements, probably because the network formation of granular crystals was almost completed in the two-day stored samples.

Figure 4.10 displays typical data from the penetration tests, taken for the 62-day stored shortenings with and without talc. Curves of the normal force as a function of the penetration rate are overlaid in Fig. 4.10a, and the primarily differentiated curves with shifted x-axes are presented in Fig. 4.10b.

In Fig. 4.10a, the normal force on pure RPO shortening quickly increased during penetration up to 3% of the thickness, which gave a yield value of 27.1 N (indicated by an arrow in Fig. 4.10a). With further penetration, the normal force dropped significantly and then fluctuated randomly. RPO + talc shortening also exhibited a rapid increase in the normal force during penetration up to 5.3% of the thickness; however, the rate of the increase was low and the yield value of 14.9 N (indicated by an arrow in Fig. 4.10a) was small in the comparison with the normal force on pure RPO shortening. The normal force on RPO + talc shortening basically increased at low penetration rates up to 15.5% and slightly decreased with further penetration, exhibiting relatively uniform fluctuation and a maximum value lower than that for pure RPO shortening.

Although shortening is not an ideal elastic body, the variation in the normal force during penetration is easy to understand if the underlying fat crystal networks are regarded as springs linked

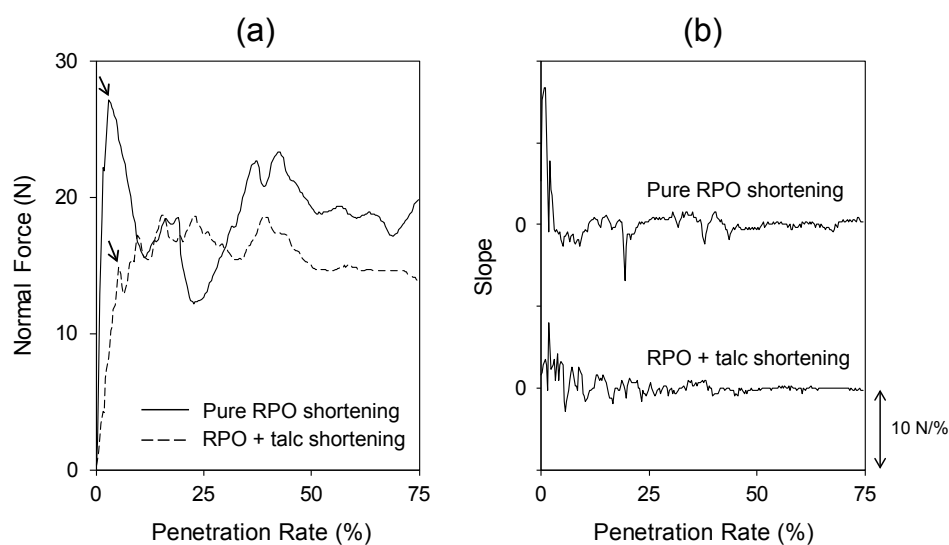


Figure 4.10 Typical data from penetration tests, taken for 62-day stored shortenings with and without 0.2% talc particles. (a) Curves of normal force versus penetration rate and (b) primarily differentiated curves with shifted x-axes.

with each other. According to this, an increase in the normal force represents the storage of applied energy within the networks. The rate of the increase, which corresponds to the spring constant, indicates the stiffness of the network structure. The peak values of the normal force, like the yield values, describe the mechanical strength of local networks, whose breakage causes a decrease in the normal force in proportion to the fracture magnitude. The intervals and size distribution of the peaks may be used to determine the density and geometry of the networks.

From these viewpoints, the effects of talc addition on the physical properties of the 62-day stored shortening are summarized as follows. (i) Crystal networks of the high-melting fraction softened, decreasing the stiffness and mechanical strength. (ii) The brittleness of the networks improved with reduced large fractures during penetration. (iii) The network structure became denser and more homogeneous. We can see the effects of (ii) and (iii) more easily as the decreased intensity and increased frequency of peaks in the primarily differentiated curves in Fig. 4.10b.

4 Conclusions

This is the first experiment to reveal the effects of adding talc particles on the physical properties of fat-based products as well as on the crystallization kinetics of multi-component natural fats. For RPO, talc preferentially promoted the crystallization of the high-melting fraction. Changes in the crystallization kinetics extended to the microstructure and networking processes of the fat crystals, which increased the plasticity of RPO in bulk fat systems and shortening. Further, the results for the shortening indicate the possibility that post-hardening and the formation of granular crystals during storage of PO-based products may be controlled by talc addition.

I consider that the effects of talc addition to improve the physical properties of PO-based shortening must be extensively applied to semi-solid fat-based products, and that further studies on the combined effects with other factors (cooling rates, shear, emulsifiers, etc.) are required. In addition, basic research on the mechanisms whereby the surfaces of talc particles selectively adsorb fat molecules and trigger the template effects for heterogeneous nucleation is expected.

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Summary

Chapter 2

Promotional effects of new types of additives on fat crystallization

I examined the promotional effects of additives on fat crystallization, such as inorganic (talc, carbon nanotube (CNT), and graphite) and organic (theobromine, ellagic acid dihydrate (EAD), and terephthalic acid) materials. The triacylglycerols (TAGs) of trioleoylglycerol (LLL), trimyristoylglycerol (MMM), and tripalmitoylglycerol (PPP) were employed as the fats. The additives (1 wt%) were added to the molten TAGs, and then the mixtures were cooled at a rate of 1°C/min followed by heating at a rate of 5°C/min. The crystallization and melting properties were observed using differential scanning calorimetry, X-ray diffraction, and polarized optical microscope (POM). Consequently, I found that the above six additives remarkably increased the initial temperatures of crystallization (T_i) on cooling without changing the melting temperatures. For example, in the case of LLL, the increases in T_i were 2.6°C (talc), 3.9°C (CNT), 8.1°C (graphite), 1.1°C (theobromine), 2.0°C (EAD), and 6.8°C (terephthalic acid). Very similar effects were observed for the crystallization of MMM and PPP with the six additives. Furthermore, the polymorphs of the first occurring crystals were changed from metastable to more stable forms by many of these additives. The POM observation revealed that the crystallization was initiated at the surfaces of additive particles. This study has shown for the first time that the heterogeneous nucleation of fat crystals can be greatly promoted by new types of additives. Such additives have great potential to promote fat crystallization by not only hydrophobic but also hydrophilic molecular interactions between the fats and additives.

Chapter 3

Fat crystallization with talc particles is influenced by particle size, concentration, and cooling rate

I recently found that new types of additives including talc promoted the crystallization of monoacid saturated triacylglycerols (TAGs) in stable polymorphs. This study reports the effects of size and

concentration of talc particles and cooling rate on the crystallization of trioleoylglycerol (LLL), tripalmitoylglycerol (PPP), and 1,3-dipalmitoyl-2-oleoyl glycerol (POP). Talc particles were mixed with molten TAGs, and the mixtures were then cooled at designated cooling rates. Polymorphic crystallization was observed by simultaneous use of X-ray diffractometry and differential scanning calorimetry and by polarized optical microscopy. The following results were obtained. (i) Talc promoted the crystallization of LLL in stable polymorphs with decreasing particle size and increasing concentration. (ii) The promotional effects were influenced by the cooling rate. (iii) The same behavior was observed for PPP and POP. (iv) Hydrophobic modification of talc surfaces did not affect the promotional effects. These results indicate that talc particles having median sizes of less than 2.5 μ m and concentrations exceeding 0.1wt.% effectively promote fat crystallization, and that the promotional effects can be maximized by choosing proper cooling rates according to the target fats. Furthermore, I propose a model of the heterogeneous nucleation of TAG crystals taking account of the molecular adsorption on cleavage surfaces of talc.

Chapter 4

Adding talc particles improves physical properties of palm oil-based shortening

This study reports the effects of adding talc particles on the physical properties of palm oil (PO)-based shortening formed by working refined PO (RPO) after rapid cooling. I examined the thermal and viscoelastic behavior of RPO in bulk fat systems, and the polymorphism and hardness of the shortening in the presence and absence of talc particles. In addition, the microstructure of fat crystals formed was observed. The effects of talc addition are summarized as follows. (i) When RPO was cooled at a rate of 5°C/min, crystallization of the high-melting fraction, such as tripalmitoylglycerol (PPP) and 1,3-dipalmitoyl-2-oleoyl glycerol (POP), was preferentially promoted to form small-sized microcrystals. During cooling, the increase in the viscoelasticity of RPO was suppressed at low temperatures. (ii) When the shortening was stored at 5°C, β' -crystallization of 1-palmitoyl-2,3-dioleoyl glycerol (POO) and β -crystallization, probably of a molecular compound (MC) of POP and 1,2-dipalmitoyl-3-oleoyl glycerol (PPO), were delayed compared to β' -crystallization of PPP and POP. During storage, the homogeneity of the fat crystal dispersion improved and the hardness of the

shortening decreased. These results indicate that talc improved the physical properties of the PO-based shortening by changing the crystallization kinetics and the networking processes of the fat crystals.

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Kyoto, 2016

吉川 真一

Shinichi Yoshikawa

List of Publications

Original Papers

1. Yoshikawa, S., Kida, H. & Sato, K. Promotional effects of new types of additives on fat crystallization. *J. Oleo Sci.* **63** (4), 333–345, © 2014 Japan Oil Chemists' Society, Tokyo.
2. Yoshikawa, S., Kida, H. & Sato, K. Fat crystallization with talc particles is influenced by particle size, concentration, and cooling rate. *Eur. J. Lipid Sci. Technol.* **117** (6), 858–868, © 2015 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.
3. Yoshikawa, S., Kida, H., Matsumura, Y. & Sato, K. Adding talc particles improves physical properties of palm oil-based shortening. *Eur. J. Lipid Sci. Technol.*, DOI: 10.1002/ejlt.201500283 (in press), © 2015 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

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