1. INTRODUCTION

Triacylglycerols (TAGs) are the major components of fats and oils and biologically important organic molecules along with proteins and carbohydrates. In industrial applications, TAGs are the main components in cream, margarine, and confectionery fats in foods and as matrix materials in pharmaceuticals and cosmetics. The physical behavior of TAGs influences the physical properties of fat-based products, such as appearance, texture, plasticity, morphology, and rheology. Most fat-based products are multicomponent TAG mixtures, containing different kinds of fatty acid moieties. Their complex physical properties are ascribed to polymorphism of individual TAG components and their mixing behavior. Therefore, research into the physical properties of the fat-based products usually starts with an understanding of individual TAG molecules and subsequently moves on to an understanding of the mixed systems, while combining this microscopic information with the macroscopic properties of texture, crystal morphology, and rheology. The macroscopic properties of fats and oils will be discussed in other chapters of this volume.
This chapter describes the polymorphism of the principal TAGs with saturated and unsaturated fatty acid moieties and their binary mixtures.

2. BASIC CONCEPTS OF POLYMORPHISM OF FATS

TAGs are three-fold esters of glycerol and fatty acids, having the general formula shown in Figure 1. There is a number of fatty acid moiety, as indicated in Figure 1. According to Figure 1, TAGs can be divided into two classes depending on the fatty acid composition. TAGs having only one type of fatty acid are called monoacid TAGs, and those having two and three types of fatty acids are, respectively, called diacid and triacid TAGs, and both are categorized as mixed-acid TAGs. Almost all natural fats and oils are mixed-acid TAGs. In addition, the diacid TAGs can be divided into two types: symmetric and asymmetric TAGs. In the asymmetric diacid TAGs, chiral properties are revealed: For example, \( sn\)-R1R1R2 and \( sn\)-R2R1R1 are stereochemically different from each other, in which \( sn\) means a stereospecific number. The same chiral properties occur in the triacid TAGs. It is noteworthy that polymorphism of the symmetric TAGs is largely different from that of the asymmetric TAGs.

The physical properties of TAGs are determined by the types of fatty acids that compose them; for example, the number of saturated and unsaturated chains, \( cis\)-and \( trans\)-double bonds, short and long chains, chains with even and odd numbers of carbon atoms, and esterified positions of fatty acids with glycerol carbon atoms. Fats are modified by hydrogenation, interesterification, and fractionation to produce desirable physical properties for fat-based products.

2.1. Polymorphism of Triacylglycerols

Multiple melting points of fats had already been discovered in the nineteenth century. Clarkson and Malkin showed that this melting behavior resulted from the polymorphism of TAGs (1). In the crystalline state, TAG molecules adopt the ideal

\[
\begin{align*}
\text{sn-1} & \quad \text{CH}_2\text{O} \rightarrow \text{CO} \rightarrow R_1 \\
\text{sn-2} & \quad \text{CH} \rightarrow \text{O} \rightarrow \text{CO} \rightarrow R_2 \\
\text{sn-3} & \quad \text{CH}_2 \rightarrow \text{O} \rightarrow \text{CO} \rightarrow R_3
\end{align*}
\]

Mono-acid TAG \((R_1 = R_2 = R_3)\)
* saturated: length, even-odd
* unsaturated: length, even-odd, number-position-conformation of double bond

Mixed-acid TAG \((R_1 \neq R_2 \neq R_3)\)
* different length
* saturated and unsaturated
* different \(sn\)-positioned

Figure 1. A triacylglycerol molecule \((R\): fatty acid moiety, \(sn\): stereospecific number).
conformation and arrangement in relation to their neighbors to optimize intramolecular and intermolecular interactions and accomplish an efficient close-packing. On the basis of the structural studies by Larsson (2), the three fundamental polymorphs are called $\alpha$, $\beta'$, and $\beta$. The significance of the definition of polymorphism of the TAGs lies in unification of otherwise confused nomenclature of the polymorphic forms of the fats differently named by researchers, such as sub-$\alpha$ form, vitreous phase, and so on. In addition, the polymorphic nomenclature makes it convenient to characterize the crystalline properties of fats employed in many applications. For example, the structure and texture of ice cream is caused by a network of partially coalesced $\alpha$-form crystals and ice crystals that surround air bubbles to form discontinuous foams (3). The small needle-like $\beta'$ crystals impart good plasticity that is desirable in products such as margarine, shortening, and baking fats (4). Cocoa butter replacers (CBR) and cocoa butter substitutes (CBS) can crystallize without tempering into their stable $\beta'$ polymorph upon simple cooling. Tempering is required for $\beta$ form, which is used for chocolate, cocoa butter, and cocoa butter equivalents (CBE) (5).

One may characterize the polymorphic forms of TAGs by thermal stability, subcell packing, and chain-length structure as described below.

### 2.1.1. Thermal Stability

Among the three main polymorphic forms of TAGs and their mixtures, generally, $\beta$ is the most stable, $\beta'$ is less stable, and $\alpha$ is the least stable form (6, 7). A diagram of the Gibbs free energy ($G = H - TS$, in which $H$, $S$, and $T$ are enthalpy, entropy, and temperature) versus $T$ for TAG polymorphs is shown in Figure 2. The $G$-$T$ relationship determines the transformation pathways among the polymorphs and liquid (8). The polymorphism of TAGs is monotropic, and the $G$ values are largest for $\alpha$, intermediate for $\beta'$, and smallest for $\beta$ in the solid

![Figure 2. A schematic diagram of Gibbs energy ($G$) and temperature of three polymorphs of a triacylglycerol.](image)
phase domain at low temperature. Each polymorph has its own melting temperature \((T_m)\) that is defined as the temperature where the G value of crystal becomes lower than that of liquid. These thermodynamic conditions influence the kinetic aspects of crystallization and transformation of TAGs.

The three basic polymorphic forms shown in Figure 2, which may apply to the saturated monoacid TAGs, are largely modified when the shape of a TAG molecule becomes more heterogeneous. For example, TAGs containing unsaturated fatty acid moieties or saturated diacid moieties exhibit two \(\beta'\) or \(\beta\) forms. In other cases, \(\beta\) does not occur and \(\beta'\) becomes most stable with the highest \(T_m\) instead. These properties will be discussed in Section 3.

A primary concern is polymorphic crystallization in which the Ostwald step rule is very useful (9). This rule predicts that phase changes occur step by step by way of successively more stable phases. For the relative rate of nucleation of polymorphic crystals shown in Figure 2, it follows that nucleation of the metastable forms such as \(\alpha\) and \(\beta'\) occurs first before the most stable \(\beta\) form, when nucleation occurs under a large supercooling or high supersaturation. When the amount of supercooling or supersaturation is decreased, the law is broken and the most stable form tends to nucleate at a relatively slow rate.

Because of its monotropic nature, the polymorphic transformation occurs irreversibly from the least stable \(\alpha\) form to the most stable \(\beta\) form. The rate of transformation is both time- and temperature-dependent. There are two modes of polymorphic transformation processes: solid-solid and melt-mediated transformations. Solid-solid transformations occur below the melting points of all the polymorphs involved. In contrast, melt-mediated crystallization occurs when the temperature is above the melting points of the less stable forms. Melt-mediated crystallization involves the following processes:

1. Melting of the less stable form
2. Nucleation and growth of the more stable forms
3. Mass transfer in the liquid formed by melting of the less stable form

It has been observed in some TAGs that the rate of melt-mediated crystallization is often much higher than that of solid-solid transformation (10–14).

### 2.1.2. Subcell Structure

Subcell structure defines a lateral packing mode of the hydrocarbon chains (2, 15, 16). Three typical subcell structures are shown in Figure 3. The \(\alpha\), \(\beta'\), and \(\beta\) forms have hexagonal (H), orthorhombic perpendicular (\(O_\perp\)), and triclinic parallel (\(T_\parallel\)) subcell structures, respectively (2).

In the hexagonal subcell structure, the two-dimensional lattice is hexagonal and gives rise to a 0.41-nm wide-angle X-ray diffraction (XRD) pattern. The chain packing is loose, and the specific chain-chain interactions are lost because of the ability of the carbon atoms to rotate several degrees and form disordered conformations of hydrocarbon chains. The two-dimensional lattice of an orthorhombic perpendicular (\(O_\perp\)) subcell structure is rectangular, and this represents a tightly
packed lattice with specific chain-chain interactions. The subcell parameters of O_{⊥} are typically shown in two wide-angle XRD patterns at 0.37 nm and 0.41 nm. Triclinic parallel subcell structure (T_{∥}) has an oblique two-dimensional lattice and represents tightly packed chains, in which there are specific chain-chain interactions. This subcell structure of T_{∥} is characterized by a strong wide-angle XRD pattern at 0.46 nm and week patterns at 0.39 nm and 0.38 nm. The values given for these wide-angle XRD patterns of the three polymorphs are typical for the saturated monoacid TAGs; they vary when the fatty acid moieties change from saturated to unsaturated acids.

2.1.3. Chain Length Structure

The TAG crystals form chain-length structures, in which a repetitive sequence of the hydrocarbon chains is involved in a unit lamellar along the c-axis (Figure 4) (17). One unit layer made up of one hydrocarbon chain is called a leaflet. Several types of chain-length structures can form as shown in Figure 4. The TAGs with the same or very similar fatty acids might form a double chain-length structure. A triple chain-length structure is formed when the chemical natures of one or two of the fatty acids are much different from the others. A quarto-chain-length structure consists of two double chain-length structures, which are combined end-to-end. A hexa-chain-length structure consists of two
triple chain-length structures. The quarto- and hexa-chain-length structures were observed in asymmetric saturated diacid TAGs, as discussed below.

2.2. Phase Equilibria

Figure 5 shows three cases that are generally applicable to many materials, and observed in TAG binary mixtures: solid-solution mixture, eutectic mixture, and molecular compound forming mixture (18). Here we summarize basic properties of the three mixture phases. Various binary mixture systems of TAGs will be discussed in Section 4.

2.2.1. Solid-Solution Mixture  In this system, a binary mixture is cooled but neither component solidifies without containing some of the other component: Both components are deposited simultaneously, and the deposited solid phase is a solid-solution. Only two phases can exist in such a system: a homogeneous liquid-solution and a solid-solution. The equilibrium phase diagram is shown in Figure 4. Typical variations in the chain-length structures of triacylglycerol crystals. An arrow means a leaflet.
Figure 5a. All mixtures of the two components have melting points intermediate between the melting points of the pure components.

TAGs that have similar physical and chemical properties, for example, similar melting points, chain-length, polymorphic form, and molecular volume, form solid-solution mixtures.
2.2.2. Eutectic Mixture  A general example for a eutectic mixture system is shown in Figure 5b, where curves PQ and RQ represent the temperatures at which homogeneous liquid-solutions begin to crystallize. Above the curves P-Q-R, the two components are liquid. Line TQU represents the temperature at which solid mixtures of A-molecule and B-molecule begin to melt, and the two components are completely solid below the line T-Q-U. The small and large areas of PQ and RQU represent mixtures of A-molecule crystals in liquid A/B-molecule and solid B-molecule crystals in liquid A/B-molecule, respectively. It is important to note that the eutectic is a physical mixture, not a molecular compound. Below the eutectic temperature, all mixtures are solid.

Among binary TAG mixtures, the eutectic system is most common. Eutectic systems tend to occur when the TAGs differ in chain-length, molecular volume, shape, or polymorphic form, but they have similar melting points.

2.2.3. Molecular-Compound-Forming Mixture  A-molecule and B-molecule of a binary system sometimes combine to form a molecular compound. If a molecular compound can coexist in equilibrium with a liquid of the same composition, the compound has a congruent melting point shown by point R (Figure 5c). The phase diagram for this system can be split into two subdiagrams: A-molecule/molecular compound and molecular compound/B-molecule, and each of these subdiagrams may be considered as a eutectic mixture as shown in Figure 5c. Points P and Q are the other two eutectic points of the subdiagrams that are placed in a juxtaposition manner.

A molecular compound-forming mixture can occur in particular combinations of TAGs, being based on the possibility of achieving specific interactions between both molecules in the crystalline state. Even now, there is no general explanation why and how specific combinations of TAGs can result in molecular compound-forming mixtures, but several examples will be provided in Section 4.

2.3. Basic Methods for Studying the Polymorphism of Fats

Methodology for studying the polymorphism of fats, among which thermal analysis, most typically, differential scanning calorimetry (DSC), X-ray diffraction (XRD), neutron diffraction, infrared absorption spectroscopy, and nuclear magnetic resonance (NMR), are briefly mentioned here.

DSC analysis provides the data of temperatures, enthalpy and entropy values of melting, crystallization, and polymorphic transitions, which are prerequisites for isolation of individual polymorphic forms and their thermal stability.

Molecular structural information, lamellar distance (long spacing), and subcell structure (the short spacing) are calculated by small-angle and wide-angle diffraction patterns from a powder XRD study using polycrystalline powder sample. Atomic-level crystal structure is revealed by XRD using a high-quality single crystal.

One of the most exciting methodologies that has recently been applied to fat polymorphism is synchrotron radiation XRD (SR-XRD). It has made it possible to perform real-time (in situ) observations of polymorphic transformations at rapid
rates of temperature variation as high as 5°C/min under external stimuli of shear (19) and ultrasonication (20). Furthermore, a combined study of SR-XRD small-angle X-ray scattering (SAXS) and wide-angle X-ray scattering (WAXS) with DSC is now one of the most powerful methods for clarifying the kinetics of the polymorphic transitions of TAGs in single-component and mixture systems. Observing the correspondence of the DSC thermopeaks and variations in the SAXS-WAXS patterns during rapid temperature change has clarified the mechanisms of complicated polymorphic transformations of binary mixtures of TAGs or liquid-crystal to polymorphic crystal conversion, which had been overlooked with conventional laboratory-scale XRD apparatus. Sections 3 and 4 will address these issues.

The use of neutron diffraction provides structural information about fats in liquid and crystalline states through interactions of neutrons with atomic nucleus that is different from the information provided by X-ray diffraction. Neutron diffraction studies with selective deuteration of glycerine and fatty acid chains of a TAG indicated nematic-type liquid crystal organization of the TAG molecules in the liquid phase (21, 22).

For molecular properties of the TAG polymorphs, local molecular structural information such as methyl-end group, olefinic conformation, and chain-chain interaction are unveiled by infrared (IR) spectroscopy, especially Fourier-transformed infrared spectroscopy (FT-IR) (23, 24). Compared with a pioneering work by Chapman (25), great progress has been achieved by using various FT-IR techniques, such as polarized transmission FT-IR, reflection absorption spectroscopy (RAS), and attenuated total reflection (ATR) (26–28).

NMR, especially cross-polarization and magic-angle spinning NMR (CP/MAS NMR), is also a powerful tool for studying the molecular conformations of the TAGs in a crystalline state, because CP/MAS NMR spectra give detailed information about the local environment and mobility of specific carbon sites (29–33).

3. POLYMORPHISM OF MONOACID TRIACYLGLYCEROLS

3.1. Saturated Monoacid Triacylglycerol

Saturated monoacid TAGs are of the simplest chemical shape and, therefore, have been examined as model substances for the study of the complex fats.

The atomic-level crystal structures of the β form of saturated monoacid TAGs were first clarified almost four decades ago (34–36). Based on these structural data, Lutton postulated the β form structures of saturated monoacid and diacid TAGs (37).

Quite recently, van Langevelde et al. examined the crystal structures of the β form of tripalmitin (PPP) in comparison with the results of tricaprin (CCC) (38, 39), trilaurin (LLL) (36), and predicted the β form structure of trimyristin (MMM) (38). As shown in Table 1, the unit cell parameters, double-chain length structure, and T // subcell structure of the three TAGs are almost the same, except
TABLE 1. Unit Cell Parameters of $\beta$ Form of Tricaprin (CCC), Trilaurin (LLL), and Tripalmitin (PPP) ($V$; Unit Cell Volume, $D$; Density).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CCC</th>
<th>LLL</th>
<th>PPP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Space group</td>
<td>P$\bar{1}$</td>
<td>P$\bar{1}$</td>
<td>P$\bar{1}$</td>
</tr>
<tr>
<td>a axis (nm)</td>
<td>1.218</td>
<td>1.208</td>
<td>1.195</td>
</tr>
<tr>
<td>b axis (nm)</td>
<td>3.156</td>
<td>3.661</td>
<td>4.684</td>
</tr>
<tr>
<td>c axis (nm)</td>
<td>0.549</td>
<td>0.547</td>
<td>0.545</td>
</tr>
<tr>
<td>$\alpha$ (deg.)</td>
<td>73.4</td>
<td>73.4</td>
<td>73.8</td>
</tr>
<tr>
<td>$\beta$ (deg.)</td>
<td>100.7</td>
<td>100.5</td>
<td>100.2</td>
</tr>
<tr>
<td>$\gamma$ (deg.)</td>
<td>119.2</td>
<td>118.7</td>
<td>118.1</td>
</tr>
<tr>
<td>$V$ (nm$^3$)</td>
<td>1.7613</td>
<td>2.0292</td>
<td>2.5811</td>
</tr>
<tr>
<td>$D$ (g cm$^{-3}$)</td>
<td>1.04</td>
<td>1.04</td>
<td>1.04</td>
</tr>
</tbody>
</table>

Figure 6. Two types of triacylglycerol conformations in crystal: (a) tuning folk conformation and (b) chair conformation. Numbers correspond to carbon atoms of a glycerine group.
for the chain length parameter (b-axis). Therefore, van Langevelde et al. concluded that, as long as the series of C\textsubscript{n}C\textsubscript{n}C\textsubscript{n}, in which n means even-number of carbon atoms, all structure models can be predicted by extrapolation of the cell parameters and copying of the atomic coordinates.

There are two types of molecular conformation of TAG molecules in the crystal (39): tuning fork and chair, as shown in Figure 6. In a tuning fork conformation, the two outer acyl chains (sn-1 and sn-3) point in one direction and the middle acyl chain (sn-2) in the opposite direction. In contrast, a chair conformation has the two neighboring acyl chains (sn-1 and sn-2) pointing in one direction and the third acyl chain (sn-3) in the opposite direction. In the β form of CCC, LLL, and PPP, asymmetric tuning fork conformation was revealed.

For β' forms of saturated monoacid TAGs, no crystal structure has so far been determined, because of difficulty in growing single crystals suitable for atomic-level structure determination. However, information about the unit cell can be obtained or calculated. For example, Figure 7 shows the density of liquid, β' and β forms of saturated monoacid and diacid TAGs as a function of the number of carbon atoms (20). The densities of the β' forms of CCC, LLL, MMM (trimyristin), and PPP could be determined by applying a least-square fitting procedure based on the density of the β' form of tristearin (SSS) (40) and crystal structure data of saturated diacid TAGs (41, 42).

**Figure 7.** Relationship between the density of a TAG and the number of carbon atoms present in the acyl chain of its molecule. Closed circle: liquid state; open triangle: β' form; and open square: β form. Solid and dotted lines are the least-squares fitting curves for each state.
3.2. Unsaturated Monoacid Triacylglycerol

Natural fats and oils having $T_m$ below ambient temperature contain the TAGs with unsaturated fatty acid moieties. Crystallization properties of unsaturated monoacid TAGs were examined six decades ago (43, 44). Compared with the saturated monoacid TAGs, polymorphism of unsaturated monoacid TAGs is more complicated, because of diverse variations in the number and the position of double bonds of their acyl-chain moieties, as clarified by Hagemann et al. (45). The polymorphism of the series of positional isomers of the TAGs having cis- and trans-octadecenoic acids ($C_{18}$, with one double bond) is summarized as follows:

1. $\alpha$ form occurred except for cis $\Delta_{12}$, $\Delta_{13}$, $\Delta_{15}$, and trans $\Delta_{10}$, where $\Delta$ means the position expressed as the number of the carbon atom counted from the glycerol backbone at which a double bond is placed,

2. For cis-type TAGs, three $\beta'$ forms were observed for cis $\Delta_{7}$, $\Delta_{9}$, $\Delta_{11}$, and $\Delta_{13}$, but not for $\Delta_{5}$ and $\Delta_{15}$,

3. For trans-type TAGs, two $\beta'$ forms were observed for trans $\Delta_{11}$ and $\Delta_{14}$, one $\beta'$ form for $\Delta_{13}$, whereas $\beta'$ did not appear for $\Delta_{4}$, $\Delta_{5}$, $\Delta_{6}$, $\Delta_{7}$, $\Delta_{8}$, $\Delta_{9}$, $\Delta_{10}$, $\Delta_{12}$, and $\Delta_{15}$,

4. $\beta$ form was observed in all of the TAGs.

The complexity of polymorphism, especially with regard to the occurrence of multiple $\beta'$ form, may be ascribed to the variety of positional isomers and cis-trans-conformation.

Triolein (OOO) has been studied over many years. Some inconsistency, however, still remains among several reports; for example, Wheeler et al. (43) and Ferguson and Lutton (44) observed an intermediate form, whereas three $\beta'$ forms, $\beta'_1$, $\beta'_2$, $\beta'_3$, were isolated by Hagemann et al. (45). This kind of inconsistency for the presence of $\beta'$ form may be caused by inconsistency in thermal treatment and purity of the samples employed in the experiments. We have worked on the polymorphism of high-purity OOO (> 99 %, supplied from Nippon Oil and Fats Co.) using DSC, X-ray diffraction, and FT-IR (Ueno and Sato, unpublished work). Six polymorphs: $\alpha$, $\beta'_2$, $\beta'_1$, $\beta_2$, and $\beta_1$ were isolated, and thermal and structural properties of the six forms are shown in Table 2. Figure 8 shows the polymorphic transition pathways among the six polymorphs and melt of OOO. Two types of transitions were observed: liquid $\rightarrow \alpha \rightarrow \beta'_1 \rightarrow \beta'_2 \rightarrow \beta_2$ and liquid $\rightarrow \beta'_1 \rightarrow \beta_1$. The former transition occurred after rapid cooling (20°C/min) to about −80°C and subsequent

<table>
<thead>
<tr>
<th>Polymorph</th>
<th>$\alpha$</th>
<th>$\beta'_3$</th>
<th>$\beta'_2$</th>
<th>$\beta'_1$</th>
<th>$\beta_2$</th>
<th>$\beta_1$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_m$ (°C)</td>
<td>−37.5</td>
<td>−24.9</td>
<td>−15.5</td>
<td>−5.8</td>
<td>4.7</td>
<td>5.9</td>
</tr>
<tr>
<td>Subcell</td>
<td>$H$</td>
<td>$O_\perp$</td>
<td>$O_\perp$</td>
<td>$O_\perp$</td>
<td>$T_{//}$</td>
<td>$T_{//}$</td>
</tr>
<tr>
<td>$\Delta H$ (kJ/mol)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>110</td>
<td>120</td>
</tr>
</tbody>
</table>

Melting point: $T_m$, enthalpy of fusion: $\Delta H$. 
heating, in which crystallization of $\alpha$ and successive transformations to $\beta_2$ through intermediate two $\beta'$ occurred. By contrast, with slow cooling, the liquid crystallized in $\beta'_1$ form, which transformed to $\beta_1$ by subsequent heating. It was interesting to observe that no direct transformation occurred from $\beta'_2$ to $\beta'_1$ and from $\beta_2$ to $\beta_1$.

**Figure 8.** Polymorphic transition pathways of triolein.

**Figure 9.** Synchrotron-radiation X-ray diffraction patterns of polymorphic transformation of trielaidin taken during temperature variation shown in an inserted figure (unit, nm). $Q$: wave vector.
It is not easy to understand how such a kind of individual transformation pathway occurs.

As a typical trans-monounsaturated TAG, trielaidin (EEE) with trans-\(\omega\)-9- octadecenoic acid has been examined. Carter and Malkin (46) reported three polymorphs, \(\alpha\), \(\beta'\), \(\beta\). Since then, an argument was made to whether the intermediate \(\beta'\) is present or not. In 1990, Desmedt et al. (47) re-examined the existence of the \(\beta'\) form by using DSC and the powder XRD pattern, showing that the \(\beta'\) form was indeed crystallized after the melting of \(\alpha\) form, but soon transformed to \(\beta\). Therefore, it is difficult to obtain the XRD patterns or FT-IR data of the \(\beta'\) form of EEE at some fixed temperature.

To further investigate the existence of \(\beta'\) form of EEE, we have carried out time-resolved synchrotron radiation X-ray diffraction (SR-XRD) (Ueno and Sato, unpublished). Figure 9 shows the time-resolved small-angle SR-XRD patterns, which reveals the occurrence of a polymorph having the long spacing value of 5.1 nm on cooling, its conversion to the polymorph with the long spacing value of 4.6 nm on heating, and disappearance of the second polymorph during further heating. According to previous reports (46, 47), it was evident that the former polymorph corresponds to the \(\alpha\) form that crystallized when temperature was quenched from 50°C to 13.6°C, and the second form is \(\beta\). When temperature was jumped to 22°C, \(\alpha\) melted and the \(\beta\) form crystallized, which then melted when temperature increased to 48°C. During this temperature variation, \(\beta'\) form was not observed by the in situ SR-XRD study. Therefore, our conclusion is that trielaidin has the \(\beta'\) form, which is very unstable.

### 4. POLYMORPHISM OF MIXED-ACID TRIACYLGLYCEROLS

Understanding the polymorphism of mixed-acid TAGs is of significance because the fatty acid compositions of natural fats are generally heterogeneous; namely, combinations of fatty acids in TAGs are diverse with respect to carbon number, mixing of saturated-unsaturated chains, the position and the number of double bond of the unsaturated fatty acids, and so on. This group of mixed-acid TAGs is classified to two types: (1) saturated mixed acid type and (2) saturated and unsaturated mixed acid type.

#### 4.1. Saturated Mixed Acid Triacylglycerols

##### 4.1.1. Polymorphic Behavior

In this section, the polymorphism of the \(C_{16}-C_{16}-C_n\), \(C_nC_{n+2}C_n\), and \(C_nC_2C_n\) TAG series is discussed in terms of their diversity in occurrence of polymorphic structures and their thermodynamic stability and molecular structures.

\(C_{16}-C_{16}-C_n\) represents a series of homologous TAGs, in which \(n\), the carbon number of even-numbered carbon atoms of the \(sn\)-3 fatty acid chain, varies from 0 to 16. Systematic research on \(C_{16}-C_{16}-C_n\) by using XRD, DSC, and FT-IR
techniques (42, 48–54) showed remarkable diversity in their polymorphism. The α form was present in C₁₆-C₁₆-Cₙ. For the β' form, however, there were many variations. One β' form was present in C₁₆-C₁₆-C₂ through C₁₆-C₁₆-C₈, but three β' forms (β₁', β₂', β₃') were observed in C₁₆-C₁₆-C₁₀, whereas two β' forms (β₂', β₃') were isolated in C₁₆-C₁₆-C₁₂ and C₁₆-C₁₆-C₁₄. As for β form, one β form was present in C₁₆-C₁₆-C₂ through C₁₆-C₁₆-C₁₂. There is, however, no β form in C₁₆-C₁₆-C₁₄, which revealed the most stable form as β₁'. Moreover, chain-length structures of the polymorphic forms in C₁₆-C₁₆-Cₙ were complicated. For example, single-chain-length structure appeared in the α form of C₁₆-C₁₆-C₄, C₁₆-C₁₆-C₆, and C₁₆-C₁₆-C₈, and hexa- and quarto-chain-length structures were observed in β₁' of C₁₆-C₁₆-C₁₀ and β₂' of C₁₆-C₁₆-C₁₄. Correspondingly, the melting point decreased with increasing carbon number of n in C₁₆-C₁₆-Cₙ varying from 0 to 6, whereas it increased with n from 8 to 16. The unique properties of the series of C₁₆-C₁₆-Cₙ, in particular for C₁₆-C₁₆-C₁₄ have been discussed elsewhere (51, 54). Here we discuss the polymorphic structures and thermal transformation pathways of C₁₆-C₁₆-C₁₀ (50), as a typical example of C₁₆-C₁₆-Cₙ.

Figure 10 shows the polymorphic transformation pathways of five polymorphs of C₁₆-C₁₆-C₁₀ together with melt. On quenching of melt, the isotropic liquid produced α form with a double chain-length structure, which melts at 22°C. There are two transformation pathways starting from the α form. The first transformation pathway is α → β₁' → β₂' → β, in which the subcell structure changed from H to two O₂, keeping the double chain length structure in α → β₁' → β₂'. Then, the chain length structure changed from double to triple, and the subcell structure changed from O₂ to T₁ when β₂' transformed to β. In the second transformation pathway of α → melt → β₁', the subcell structure and chain-length structure are changed.
drastically. In both cases, many details of the polymorphic transformation mechanisms of C_{16}-C_{16}-C_{10} remain uncertain. Similarly, interesting phenomena revealed in the polymorphic behavior of C_{16}-C_{16}-C_{n} are not understood, e.g., the occurrence of the \( \alpha \) form (single-chain-length structure) and the \( \beta' \) form (tripe-chain-length) in C_{16}-C_{16}-C_{4}, C_{16}-C_{16}-C_{6} and C_{16}-C_{16}-C_{8}, and disappearance of \( \beta \) in C_{16}-C_{16}-C_{6} and C_{16}-C_{16}-C_{8}. All of these properties indicate that intramolecular and intermolecular interactions are largely affected by changing the molecular shape of the fatty acid moieties placed at the \( sn-3 \) position. Understanding the polymorphism of asymmetrical mixed-acid TAGs such as C_{16}-C_{16}-C_{n} must be valuable for the formation of structured fats.

In the C_{n}C_{n}+\_2C_{n}-type TAGs in which \( n \) is even-numbered and varying from 10 to 16, the most stable form is the \( \beta' \) form, not the \( \beta \) form. The long spacing values and melting points of the \( \beta' \) forms of these TAGs linearly increase with increasing number of \( n \) (41, 55).

The C_{n}C_{2}C_{n}-type TAGs, in which \( n \) is even-numbered varying from 10 to 18, were investigated by Zacharis et al (56). Three polymorphs, the lowest melting, the intermediate, and the highest melting forms, were observed by thermal analysis. As for the highest melting form, the following physical properties were found: (1) The subcell is T\(_{\parallel}\), (2) enthalpy of fusion, enthalpy of resolidification, and lamellar spacing increased linearly with increasing length of the acyl chains; and (3) the hexa-chain-length structure was formed in C_{14}C_{2}C_{14} and C_{16}C_{2}C_{16}. Little information has been obtained for the lowest and intermediately melting forms.

### 4.1.2. Atomic-Level Crystal Structures of \( \beta' \) Form

We discuss crystal structures of the \( \beta' \) forms of two saturated diacid TAGs, C_{10}C_{12}C_{10} (41) and C_{16}C_{16}C_{14} (42). Both are the first \( \beta' \) polymorphs of TAG that have been analyzed at the atomic level by using single crystals. Two different types of the \( \beta' \) structures were found as revealed in the unit cell parameters shown in Table 3.

The unit cell structure of C_{16}C_{16}C_{14} \( \beta' \) is shown in Figure 11 (42). The unit cell is stacked in the quarto-chain-length structure, which is constructed by two double layers (I and II in Figure 11) in such a way that the methyl end groups of one double layer are faced with those of another double layer at the center of the unit cell in the a-b plane. The chain axes of the two double layers in the unit cell are alternately

| TABLE 3. Unit Cell Parameters of \( \beta' \) Forms of C_{10}C_{12}C_{10} and C_{16}C_{16}C_{14} |
|---------------------------------|-----------------|-----------------|
| Space group                     | Iba2            | C2              |
| a axis (nm)                     | 5.57368         | 1.6534          |
| b axis (nm)                     | 2.22783         | 0.7537          |
| c axis (nm)                     | 0.56945         | 8.1626          |
| \( \beta \) (deg.)              | 90              | 90.28           |
| density (g cm\(^{-3}\))        | 1.04            | 1.018           |
inclined against the lamellar interface in the b-c plane. The methyl end groups make zigzag arrangements, but the zigzag angles of the outer interface ($\theta_1 = 9.6^\circ$) and inner interface ($\theta_2 = 38^\circ$) are different. A hybrid-type orthorhombic perpendicular subcell is formed, because of the presence of two asymmetric units in a unit cell. The molecules of two asymmetric units of C$_{16}$C$_{16}$C$_{14}$ have the chair conformation defined in Figure 6.

C$_{10}$C$_{12}$C$_{10}$ $\beta'$ crystallizes in a chair conformation with the O$_\perp$ subcell, having a bend at the glycerol moiety as shown in Figure 12 (41). There is no zigzag methyl end stacking with a flat lamellar interface. Based on the crystal structure of C$_{10}$C$_{12}$C$_{10}$ $\beta'$, van Langevelde et al. (39) determined the structure of C$_{14}$C$_{16}$C$_{14}$ $\beta'$, using the powder XRD patterns of polycrystalline samples. They concluded that the two $\beta'$ forms of C$_{10}$C$_{12}$C$_{10}$ and C$_{14}$C$_{16}$C$_{14}$ are identical, except for the chain-length distance.

The above results of the two $\beta'$ forms indicate that there is diversity in the crystal structures of $\beta'$ that are affected by chain-chain interactions of the diacid TAGs.
4.2. Saturated-Unsaturated Mixed Acid Triacylglycerols

Saturated-unsaturated mixed acid TAGs are the main components of vegetable fats and fish oils. The basic polymorphism of saturated-unsaturated mixed acid TAGs is more complicated than that of the saturated monoacid TAGs (28, 33, 57–65). The chain-chain interactions between the saturated and unsaturated fatty acid moieties are the essential determining factors of this complexity (23, 63).

4.2.1. 1,3-Disaturated-2-Unsaturated Mixed Acid Triacylglycerols

In this section, we consider the polymorphic behavior of a series of Sat.Unsat.Sat.TAGs, in which the $sn$-2 acid moieties are oleic, ricinoleic, and linoleic acids and the even-numbered saturated acids (palmitic, stearic, arachidic and behenic acids) are placed at the $sn$-1 and $sn$-3 positions.

Figure 13 shows a model of the polymorphic forms of SOS (1,3-distearoyl-2-oleoyl-$sn$-glycerol) (58). Polymorphic transformations occur from $\alpha$ to $\beta_1$ through
γ, β', and β₂. Compared with the saturated monoacid-type and diacid-type TAGs, the occurrence of the γ form is the unique feature of this group of TAGs. In the case of SOS, the chain-length structure converts from double to triple, and the subcell structures change in different manners between oleic and stearic acid chains. These changes are the result of steric hindrance of the stearic and oleic acid moieties, making the polymorphic transformation of SOS complicated. The main structural properties of SOS are briefly described below:

1. α form. The double-chain-length structure determined by the SAXS spectra assumes the coexistence of the stearoyl and oleoyl moieties in the same leaflets. The hexagonal subcell shown in the XRD WAXS patterns and FT-IR spectra of δ(CH₂) and r(CH₂) modes lead to a disordered aliphatic conformation. No specific structure was shown for the olefinic conformation, because no detectable IR band of γ(=CH) was seen (61) and the two carbons adjacent to the cis-double bond were equivalent because of the NMR spectra.

2. γ form. The long spacing value of 7.05 nm assumes a triple-chain-length structure, in which the oleoyl and stearoyl leaflets are separated through the chain sorting during the α-γ transformation. The stearoyl leaflet assumes a specific parallel packing, and the hexagonal subcell structure still remains in the oleoyl leaflets, as verified by FT-IR spectral bands of SOS containing fully deuterated stearoyl and hydrogenated oleoyl chains (61).

3. β' form. The long spacing value 7.00 nm determined by the SAXS peak assumes the triple-chain-length structure, which is constructed by the stearoyl leaflet with the O₀ subcell and the oleoyl leaflet with hexagonal subcell, as shown by FT-IR spectral bands of SOS containing fully deuterated stearoyl and hydrogenated oleoyl chains (61). The ¹³C NMR spectra showed clear differences between the two carbons adjacent to the cis-double bond and the three glycerol carbons.

4. two β forms. The long spacing values of the triple-chain-length structure were 6.75 nm for β₂ and 6.60 nm for β₁. The subcell structures of the stearoyl and oleoyl leaflets are T₁ in β₁. The subcell structure in β₂ was very close to T₂ for the two leaflets, but very subtle differences were detectable between the two β forms.

Figure 13. A schematic model of polymorphic transformation of SOS.
POP (1,3-palmitoyl-2-oleoyl-sn-glycerol) is a homologous substitution of the stearoyl moiety in SOS with the palmitoyl moiety. It was anticipated that POP might show the same polymorphic behavior as SOS. However, a few differences were observed regarding intermediate forms as explained in the following (Table 4) (58):

1. Two β' forms appeared, having a double chain-length structure.
2. During the polymorphic transformation from α to β₁ forms, the chain-length structure changes as double (α) → triple (γ) → double (two β' forms) → triple (two β forms). The alternative variations of the chain-length structure among double and triple are only detected in POP.
3. Another intermediate form with triple chain-length structure, δ, was observed. The cause of complexity in the polymorphism of POP is still open to question.

However, the polymorphism of SOS is common to the other Sat.Unsat.Sat.TAGs, as evidenced in the polymorphism of SRS(R, ricinoleoyl) and SLS (L, linoleoyl).

Although SOS, SRS, and SLS share the same polymorphic nature illustrated in Figure 13, remarkable differences are seen in the presence or absence of the stable forms of β' or β among the three TAGs. Namely, SRS has no β form and two β' forms (64), whereas β' and β forms are absent in SLS (65). Thermal data of the

<table>
<thead>
<tr>
<th>Table 4. Polymorphism of POP.</th>
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<tbody>
<tr>
<td>Form</td>
</tr>
<tr>
<td>α</td>
</tr>
<tr>
<td>γ</td>
</tr>
<tr>
<td>γ</td>
</tr>
<tr>
<td>β₂'</td>
</tr>
<tr>
<td>β₁</td>
</tr>
<tr>
<td>β₂</td>
</tr>
<tr>
<td>β₁</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 5. Thermal Properties of Polymorphism of SOS, SRS, and SLS.</th>
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<tbody>
<tr>
<td></td>
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<tr>
<td>Tₘ (°C)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>ΔHₘ (kJ/mol)</td>
</tr>
<tr>
<td>ΔSₘ (J/mol/K)</td>
</tr>
</tbody>
</table>

ᵃTₘ: temperature of melting; ΔHₘ: enthalpy of melting; ΔSₘ: entropy of melting.
polymorphic forms of SRS and SLS are shown in Table 5 together with those of SOS. The $\alpha$ and $\gamma$ forms present in the two TAGs showed the same molecular structures, as revealed in wide-angle XRD patterns of SRS and SLS shown in Figure 14. It is postulated that hydrogen bonding in the ricinoleoyl chains is so tight that the O$_{\perp}$ subcell is stabilized through the glycerol groups, probably making $\beta'$ the most stable in SRS (Figure 15a) (64). The hydrogen bonding in SRS may make the enthalpy and entropy values for melting of the $\beta'$ forms much higher than $\beta'$ forms of SOS.

Figure 16 shows SR-XRD patterns of SLS taken during a temperature variation from 50°C to 10°C, kept at 10°C for about 10 min, and heated rapidly to 50°C (65).

\textbf{Figure 14.} Wide-angle X-ray diffraction patterns of polymorphic forms of SRS and SLS (unit, nm).
The occurrence of $\alpha$ on cooling, transformation from $\alpha$ to $\gamma$, and melting of $\gamma$ on heating of SLS were clearly observed. In particular, rapid heating from 10°C to 50°C clearly showed the transformation from $\alpha$ to $\gamma$ at 20°C. No $\beta'$ or $\beta$ forms were detected during further incubation of SLS after melting of $\gamma$, or also after long incubation of $\gamma$ below its melting point. In SLS, the interactions among the linoleoyl chains at the sn-2 position, each of which has two cis-double bonds, may stabilize the $\gamma$ form, prohibiting the transformation into more stable forms of $\beta'$ or $\beta$. For this reason, the enthalpy and entropy values for melting of the $\gamma$ form of SLS are much larger than those of SOS and SRS (see Table 5). The transformation from $\gamma$ to $\beta'$ or $\beta$ in SOS is associated with an inclined chain arrangement with respect to the lamellar interface, which might be prohibited by the linoleoyl chain-chain interactions in SLS.

4.2.2. 1,3-Diunsaturated-2-Saturated Mixed Acid Triacylglycerols

The polymorphic behavior of symmetric diacid TAGs, 1,3-dioleoyl-2-stearoyl-sn-glycerol (OSO), 2-elaidoyl(OEO), and 2-vaccinoyl (OVO) glycerols was studied by Kodali et al. (66). On quenching from the melt, OEO and OVO formed a double-chain-length $\beta'$ form, whereas OSO formed the $\alpha$ form. At $-7^\circ$C, $\alpha$ of OSO quickly transformed to $\beta'$. Long-time incubation of OVO, OEO, and OSO transformed $\beta'$ form into $\beta$ form of the triple chain-length structure, in which the two oleoyl chain leaflets are segregated from the vaccinoyl, elaidoyl, and stearoyl chain leaflet. It can be assumed that the driving force to form the triple chain-length $\beta$
form of the three TAGs may be the fact that the saturated or trans-unsaturated acyl chains at the sn-2 position do not pack with the bent oleoyl chains at the sn-1 and sn-3 positions in the stable polymorphic forms. This mechanism is essentially the same as that present in the Sat.-Unsat.-Sat. TAGs.

The saturated-unsaturated mixed-acid TAGs involving trans-unsaturated acids have recently been examined with and without the effects of surfactant additives (67, 68). It is notable that $\beta'$ is most stable in PEP (1,3-dipalmitoyl-2-elaidoyl-sn-glycerol). On the other hand, SES (1,3-distearoyl-2-elaidoyl-sn-glycerol) has the most stable form of $\beta$. In contrast to the stabilization of $\beta$ in ESS and SEE, the most stable form of EPP and PEE is $\beta'$. The mechanisms for the stabilization of the $\beta'$ in PEP, EPP, and PEE remain unknown. It seems that the methyl end stacking mode may be a key factor, although further clarification is needed.

By now, we have discussed the polymorphism of mono-acid and mixed-acid TAGs. To summarize, Table 6 shows the number and types of polymorphic forms of principal TAGs.
5. FAT MIXTURES AND POLYMORPHISM

Fats are multicomponent in two ways: (1) a fat phase contains many different types of TAGs and (2) each TAG molecule involves different types of fatty acid moieties, namely, mixed-acid TAGs. Therefore, it is important to precisely analyze physical and chemical properties of the TAGs in multicomponent systems to understand thermal, structural, and rheological properties of the real food fat systems (69, 70). Particularly, one may note that the kinetic properties of the molecular compound-forming mixture phase are closely related to fat blending and interesterification in food technology (71, 72) and separation of liquid/solid fractions from natural oil resources (73, 74). As a first step in the investigation of multicomponent fat systems, the phase behavior of binary TAG mixture systems has been studied by many researchers (75–84).

The phase behavior of the binary TAG mixtures is classified into three cases: solid-solution, eutectic, and molecular compound formation, as introduced in Section 1. Peculiarities in the mixtures of the TAGs may be explained by the following:

1. The TAGs with similar chemical structures tend to form a solid-solution phase.
2. A eutectic phase is formed between TAGs whose molecular shapes are largely different.
3. Specific interactions result in the formation of a molecular compound as reviewed elsewhere (17, 54).
4. In addition, influences of polymorphism make the phase behavior more complicated.

5.1. Binary Mixtures of Saturated-Acid Triacylglycerols

Rossell (85) suggested that a eutectic phase with a limited region of solid solution was formed for the stable β form; yet, the solid solution phase was formed in the

<table>
<thead>
<tr>
<th>Triacylglycerol</th>
<th>Polymorphic Forms</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSS (tristearoyl-glycerol)</td>
<td>α, β', β</td>
</tr>
<tr>
<td>OOO (trioleoyl-glycerol)</td>
<td>α, β', β, β1, β2</td>
</tr>
<tr>
<td>EEE (trielaidoyl-glycerol)</td>
<td>α, β', β</td>
</tr>
<tr>
<td>PP14 (1,2-dipalmitoyl-3-myristoyl-sn-glycerol)</td>
<td>α, β', β'</td>
</tr>
<tr>
<td>PP10 (1,2-dipalmitoyl-3-decanoyl-sn-glycerol)</td>
<td>α, β', β, β1, β</td>
</tr>
<tr>
<td>CLC (1,3-dicaproyl-2-lauroyl-sn-glycerol)</td>
<td>α, β'</td>
</tr>
<tr>
<td>SOS (1,3-distearoyl-2-oleoyl-sn-glycerol)</td>
<td>α, γ, β', β2, β1</td>
</tr>
<tr>
<td>POP (1,3-dipalmitoyl-2-oleoyl-sn-glycerol)</td>
<td>α, γ, δ, β2, β1, β2, β1</td>
</tr>
<tr>
<td>BOB (1,3-dibehenoyl-2-oleoyl-sn-glycerol)</td>
<td>α, γ, β', β2, β1</td>
</tr>
<tr>
<td>SRS (1,3-distearyl-2-rycinooleoyl-sn-glycerol)</td>
<td>α, γ, β', β1</td>
</tr>
<tr>
<td>SLS (1,3-distearoyl-2-linooleoyl-sn-glycerol)</td>
<td>α, γ</td>
</tr>
<tr>
<td>OSO (1,3-dioleoyl-2-stearoyl-sn-glycerol)</td>
<td>α, β', β</td>
</tr>
</tbody>
</table>

TABLE 6. Occurrence of Polymorphic Forms in Representative Triacylglycerols.
metastable $\alpha$ and $\beta'$ in the mixtures of the saturated-acid TAGs. In the 1990s, this behavior was precisely analyzed by a time-resolved SR-XRD study for the PPP/SSS mixtures (78). The 50:50 mixture of PPP/SSS crystallized in $\alpha$ by quenching the mixed liquid. A single long-spacing peak was the evidence of the solid-solution of the mixture of $\alpha$. Upon heating, the $\alpha$ form transformed to $\beta'$ and subsequently to $\beta$. The miscible $\beta'$ form also appeared on cooling from the liquid phase. The miscibility was, however, disrupted when the $\beta'$ transformed to $\beta$ upon heating, as expressed in a splitting of the long spacing pattern.

Very recently, the phase behaviors of the other types of saturated mono-acid binary mixtures TAGs, LLL (trilauroyl-glycerol)/(trimyristoyl-glycerol), LLL/PPP, and LLL/SSS, were examined by a SAXS/WAXS simultaneous measurement of SR-XRD (86).

As an example, the SR–XRD patterns of the LLL/PPP = 60/40 mixture taken during cooling and heating processes are shown in Figure 17. During cooling, it was clearly shown that the $\beta'$ form of LLL and the $\alpha$ form of PPP were crystallized. As for the LLL fraction, direct crystallization of $\beta'_{\text{LLL}}$ with a SAXS peak at 3.2 nm and WAXS peaks at 0.42 and 0.38 nm occurred without the crystallization of $\alpha_{\text{LLL}}$. Almost at the same time, $\alpha_{\text{PPP}}$ with a SAXS peak at 4.6 nm and a WAXS peak at

![Figure 17. Time-resolved synchrotron radiation X-ray diffraction patterns of concentration ratio LLL/PPP = 60/40. At left is the temperature change with time (unit: nm).](image-url)
0.42 nm appeared at 35.2°C. Upon heating, $\beta'_\text{LLL}$ transformed to $\beta_{\text{LLL}}$ at about 25°C, as identified by the SAXS peak at 3.1 nm and WAXS peaks at 0.46, 0.39, and 0.38 nm. On the other hand, $\alpha_{\text{PPP}}$ transformed to $\beta_{\text{PPP}}$ with a 4.0-nm SAXS peak at 35.2°C. The intensity of the (001) SAXS peak of $\beta_{\text{PPP}}$ (4.0 nm) started to increase soon after the melting of $\beta_{\text{LLL}}$ at 46.0°C. This suggests that the presence of $\beta_{\text{LLL}}$ might hinder the transformation from $\alpha_{\text{PPP}}$ to $\beta_{\text{PPP}}$. The results were obtained for the LLL concentrations from 50% to 90%. Hence, the immiscible phases were formed in the LLL/PPP mixture system for the three polymorphic forms. Figure 18 shows the phase behavior of the LLL/PPP mixtures, which is subdivided into the three regions:

1. In the LLL concentrations above 90%, the phase behaviors of the LLL/PPP mixtures were mainly governed by LLL.
2. In the LLL concentrations from 50% to 90%, the $\beta'$-$\beta$ transformation of the LLL fraction and the $\alpha$-$\beta$ transformation of the PPP fraction occurred separately. This indicates that phase separation occurred in the three polymorphic forms.
3. In the LLL concentrations below 50%, the LLL fraction was dissolved in the PPP fraction. Hence, the phase behaviors of the LLL/PPP mixtures were mainly governed by PPP.

Figure 19 shows the phase behavior of the polymorphic occurrence for the LLL/MMM mixtures obtained from DSC and SR X-ray scattering experiments. This diagram indicates the following three points:
1. $\beta'$ was formed in the mixture system, whereas $\alpha$ transformed directly to $\beta$ in LLL and MMM.
2. Miscible solid-solution phases were formed in the metastable $\alpha$ and $\beta'$ forms of the mixtures.
3. A eutectic phase was formed in the most stable $\beta$ form.

These three results are consistent with the results from the PPP/SSS system. Consequently, it can be concluded for the mixtures of LLL-MMM, LLL-PPP, LLL-SSS, MMM-PPP, and PPP-SSS that the TAG binary mixtures are miscible in metastable polymorphs of $\alpha$ and $\beta'$ forms when the difference in the number of carbon atoms of the fatty acid moieties, $\Delta n$, equals 2, whereas immiscible mixtures are found in all polymorphic forms when $\Delta n$ is larger than 2. Results obtained for these mixture systems may indicate a relationship between polymorphism and phase behavior of the binary mixtures of the saturated-acid TAGs in such a way that rotational freedom of hydrocarbon chains and entropy of methyl-end stacking are crucial factors determining the polymorph-dependent phase behavior.

As discussed in Section 1, hexagonal-packed $\alpha$ has the ability of the carbon atoms to rotate several degrees and form disordered conformations. Hydrocarbon chains of $\beta'$ and $\beta$ are all ordered conformations except for near methyl-ends, which have a little rotational freedom. When two types of saturated monoacid TAGs with different fatty acids are mixed, the molecules are arranged in a double chain-length structure because of the interactions among glycerol backbones. Thus, this crystal structure contains the methyl-end stacking gap. For the LLL/MMM mixtures, $\alpha$ and $\beta'$ polymorphs form solid-solution phases. These polymorphs contain disordered methyl-end groups so that they can accommodate a methyl-end

\[ \text{Figure 19. Phase behavior of the polymorphic occurrence for the LLL/MMM mixtures.} \]
stacking gap. In contrast, β polymorph has all-trans-hydrocarbon chains and these rigid chains cannot adjust themselves to their circumstance. Therefore, β polymorph shows a eutectic phase. As for the LLL/PPP and LLL/SSS mixtures, eutectic phases occur for all polymorphs. Because of large differences in carbon numbers for fatty acid chains between LLL and PPP (Δn = 4), and between LLL and SSS (Δn = 6), there are very large methyl-end stacking gaps in these crystals. Therefore, the increased entropy of methyl-end stacking becomes predominant and phase separation must be favored thermodynamically for all polymorphs.

5.2. Binary Mixtures of Saturated- Unsaturated Mixed-Acid Triacylglycerols

In the 1960s, Rossell reported (85) that the binary mixtures of saturated monoacid TAGs and unsaturated monoacid TAGs form an immiscible phases, whereas Moran has suggested (87) that a molecular compound is formed in some binary mixtures of saturated-unsaturated mixed-acid TAGs. It was early 1990s when the formation of molecular compounds was observed in various mixtures of saturated-unsaturated diacid TAGs. Moreover, a miscible mixture phase was discovered in the mixtures of SOS-SLS. Recent studies of various types of the TAG binary mixtures clarified the formation of the molecular compound crystals at the 50/50 concentration ratio: 1,3-distearoyl-2-oleoyl-sn-glycerol/1,2-distearooyl-3-oleoyl-rac-glycerol (SOS/SSO) (77), SOS/1,3-dioleoyl-2-stearoyl-sn-glycerol (SOS/OOS) (79), 1,3-dipalmitoyl-2-oleoyl-sn-glycerol/1,2-dipalmitoyl-3-oleoyl-rac-glycerol (POP/PPO) (81), and POP/1,3-dioleoyl-2-palmitoyl-sn-glycerol (POP/OPO) (82). These properties are related to molecular-level understandings of the chain-chain interactions occurring in biomembrane lipids containing the saturated-unsaturated mixed acid moieties (16, 23, 88–91). Table 7 summarizes the phase behavior of binary mixtures of saturated-unsaturated diacid and triacid TAGs.

A molecular compound of β form, βc, was formed at the 1:1 concentration ratio of the binary mixtures of PPO-PPO, and SSO-SOS, giving rise to two monotectic

<table>
<thead>
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<th>Phase Behavior</th>
<th>Mixture Systems</th>
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<tbody>
<tr>
<td>Miscible</td>
<td>SSS-SSE</td>
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<tr>
<td></td>
<td>POS-SOS</td>
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<tr>
<td></td>
<td>SOS-SLS</td>
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<tr>
<td>Immiscible</td>
<td>POP-PPP</td>
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<td>Eutectic</td>
<td>POP-PPO</td>
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<td>Molecular compound forming</td>
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<tr>
<td></td>
<td>POP-OPO</td>
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<tr>
<td></td>
<td>SOS-OSO</td>
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</tbody>
</table>

Abbreviations: S: stearoyl, P: palmitoyl, O: oleoyl, L: linoleoy, E: elaidoyl.
TABLE 8. Thermal Properties of Polymorphism of Molecular Compound of PPO-POP and SSO-SOS, Together with PPO and SSO.

<table>
<thead>
<tr>
<th></th>
<th>PPO-POP = 50-50</th>
<th>PPO</th>
<th>SSO</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_m$ ($^\circ$C)</td>
<td>$\alpha_c$</td>
<td>$\beta'_c$</td>
<td>$\beta_c$</td>
</tr>
<tr>
<td></td>
<td>15.5</td>
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<td>31.2</td>
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<td>$\Delta H_m$ (kJ/mol)</td>
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<td>97</td>
</tr>
<tr>
<td>$\Delta S_m$ (J/mol/K)</td>
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<td>319</td>
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<td>Lining spacing (nm)</td>
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<td>4.2</td>
<td>4.1</td>
</tr>
<tr>
<td>Chain length</td>
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<td>double triple</td>
<td>triple triple</td>
</tr>
<tr>
<td></td>
<td>SSO-SOS = 50-50</td>
<td>SSO</td>
<td></td>
</tr>
<tr>
<td>$T_m$ ($^\circ$C)</td>
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<td>34.0</td>
<td>40.6</td>
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<tr>
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<td>$\Delta S_m$ (J/mol/K)</td>
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<td>440.5</td>
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<tr>
<td>Chain length</td>
<td>double double</td>
<td>double triple</td>
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</tr>
</tbody>
</table>

n.a.: not available.

phases of the component TAG and the compound in juxtapositional. Table 8 shows the physical properties of the polymorphs of PPO/POP compound and SSO/SOS together with PPO and SSO. The $\alpha$ and $\beta'$ forms of PPO and SSO are triple-chain-length structure, and all the polymorphic forms of POP and SOS except for $\alpha$ (SOS) or $\alpha$ and $\beta'$ forms (POP) are triple-chain-length structure. However, the three polymorphs of the molecular compounds of PPO-POP and SSO-SOS are a double chain-length structure. Figure 20 shows the phase diagram of the stable

Figure 20. Phase behavior in the SOS/SSO mixtures.
\(\beta\) form and metastable \(\alpha\) forms of the SOS/SSO mixtures examined by DSC and XRD experiments (92). The most stable forms of SOS and SSO are \(\beta_1\) and \(\alpha\), respectively. The phase diagrams of \(\alpha\), \(\beta_0\), and \(\beta\) polymorphs in the binary mixture of PPO-POP are already explained elsewhere (54, 81). Therefore, the formation of the molecular compound, which is accompanied with the conversion from triple- to double-chain-length structure, is a common feature of the binary mixtures of Sat.-Oleic-Sat. TAGs and Sat.-Sat.-Oleic TAGs. Figure 21 illustrates structure models of SOS, SSO, and SOS-SOS molecular compounds, in which one leaflet is formed of palmitoyl or stearoyl chains and the other leaflet contains the mixture of palmitoyl and oleoyl chains or stearoyl and oleoyl chains.

It should be noted in Figure 20 that \(\beta_c\) form is a congruent-type molecular compound, and its melting point is lower than the most stable forms of SOS and SSO. This raises interesting questions, such as how the molecular compound could be structurally stabilized, and how its crystallization is kinetically favored. The latter question is important, because the supercooling value with respect to \(\beta_c\) is lowest compared with the supercooling values of \(\beta_1\) of SOS and \(\beta_0\) of SSO. Nevertheless, \(\beta_c\) is crystallized when the mixture liquid is cooled below its \(T_m\). Moreover, an in situ XRD study showed that the rate of crystallization of \(\beta_c\) is remarkably higher than \(\beta_1\) of SOS and \(\beta_0\) of SSO. When the molecular compound is formed, steric hindrance between saturated and oleic acid chains may be caused. Supposing that the double-chain-length structure is formed in a liquid phase (93, 94), we assume that formation energy of a crystal nucleus of the double-chain-length
structure may be minimized, compared with those having the triple chain-length structure, and the nucleation frequency of the double-chain-length molecular compound crystals may become higher. In this regard, a small-angle diffraction pattern peak at 4.5 nm without the presence of a wide-angle diffraction pattern occurred, long before the crystallization of $\beta_c$ from the molten sample of SSO/SOS = 1/3 when the mixture liquid was cooled to 37°C (92). This indicates the presence of the smectic liquid crystalline phase, which may be a precursor of the nucleation of $\beta_c$.

The binary mixture systems of SOS-OSO (79) and POP-OPO (83) were examined by DSC, XRD, and FT-IR, giving rise to two monotectic phases of POP (SOS)/compound and compound/OPO(OSO) in juxtaposition.

A new result was obtained for the mixture of SLS/SOS in which a solid-solution mixture was observed in the $\alpha$ and $\gamma$ forms in all concentration ranges: the double chain length in $\alpha$ phase and the triple chain length in $\gamma$, as shown in Figure 22 (92). The miscible $\gamma$ form did not transform to the $\beta'$ form, when the mixtures were subjected to simple cooling from high-temperature liquid to low-temperature solid phase. Incubation of $\gamma$ around its $T_m$ also did not result in transformation to the other polymorph; instead the miscible phase was retained. The $\alpha$-melt-mediated transformation, however, caused the disruption of the solid-solution phase, and immiscible phases of $\gamma_{SLS}$ and $\beta'_{SOS}$ were formed in concentration ranges of SLS below 30%. By contrast, in SLS concentration ranges above 30%, the $\alpha$-melt-mediated transformation caused the crystallization of only the $\gamma$ form, and $\beta'$ or $\beta$ of SOS did not appear. The structural model of solid-solution phases of $\alpha$ and $\gamma$ forms and the eutectic phase of the $\gamma$ of SLS and $\beta'$ of SOS are shown in Figure 23 (92).
The $\alpha$ form of the SLS/SOS mixture is stacked in the double-chain-length structure, in which the stearoyl and unsaturated (oleoyl and linoleoyl) chains are packed in the same leaflets (Figure 23a). Further thermodynamic equilibration induced the transformation from the double-chain-length $\alpha$ form to the triple-chain-length $\gamma$ form. In this transformation, chain segregation occurs during the $\alpha$-$\gamma$ transformation in the solid state, because the steric hindrance between the stearoyl and linoleoyl chains is limited. In the $\gamma$ form, the stearoyl and unsaturated chains are packed in the different leaflets (Figure 23b). Because of olefinic interactions between oleoyl and linoleoyl chains, coexistence between oleoyl and linoleoyl chains in the oleic/linoleic acid leaflet takes place in the $\gamma$ form having the triple-chain-length structure. This interaction makes the $\gamma$ form the most stable polymorph of the SLS/SOS mixture, and the transformation form $\gamma$ to $\beta'$ or $\beta_2$ forms does not occur in the mixture during simple cooling and heating processes. Disappearance of the miscible $\gamma$ form of the mixture can be achieved by melt-mediated transformation from the miscible $\alpha$ and $\gamma$ forms to immiscible $\beta'$ and $\beta$ forms in the SOS fraction (Figure 23c).

6. POLYMORPHISM OF NATURAL FATS

Most natural fats are composed of many different kinds of acylglycerols whose fatty acid compositions are diverse, and the acyl positions esterified at the glycerol groups are complicated. This situation makes the polymorphism of natural fats very complicated.

Take for example, milkfat that consists of TAGs, diacylglycerols (DAGs), monoacylglycerols (MAGs), free fatty acids, phospholipids, sterols, and other polar lipids (95–97). As for TAGs, milkfat is made of about 400 different TAGs containing various kinds of saturated and unsaturated fatty acids whose carbon numbers range from 2 to 24. Because of this fact, milkfat has a wide range of melting temperature from about $-30^\circ$C to $40^\circ$C, and three polymorphic forms of $\alpha$, $\beta'$, and $\beta$ reveal complicated chain-length structures and occurrence behavior that are affected by thermal treatment. Another example is cocoa butter (CB) in which stearic, palmitic, and oleic acids account for about 80 % out of its total fatty acids. This property causes sharp melting behavior of CB. However, polymorphism of cocoa butter is complicated, and its origins are still unanswered.

Figure 23. Structure models of the polymorphic forms of the SOS/SLS mixtures.
It is difficult to simply define the polymorphism of natural fats composed of multiple TAGs because of the following two reasons:

1. The polymorphic nature of the multicomponent TAG systems is related to phase behavior that is affected by molecular interactions among the component TAGs. The fat crystals in a miscible phase may exhibit simple polymorphic properties. By contrast, the immiscible eutectic phase may show complicated polymorphic properties as a superposition of the polymorphic forms of the component TAGs. Furthermore, if the molecular compound is formed by specific TAG components, the polymorphic behavior becomes complicated, as shown for the case of POP-OPO (see Section 5.2). Therefore, knowing the phase behavior of the principal TAG components is a prerequisite for precise understanding of the polymorphism of natural fats.

2. The phase behavior of the mixed TAG system is influenced by polymorphism. For example, a miscible phase is formed in α and β phases, but it transforms into a eutectic phase in β, as revealed in the SSS-PPP mixture. Then, the polymorphic occurrence is largely affected by cooling rate and temperature fluctuation, and it is therefore necessary to observe the polymorphic properties of the natural fats by varying the rate of cooling or by fluctuating the temperature (so-called tempering).

In this section, the polymorphic properties of natural fats are briefly discussed by highlighting milkfat, cocoa butter, and palm oil fractions based on recent research into the effects of external factors on the polymorphic crystallization such as shear stress, ultrasound stimulation, and addition of food emulsifiers.

### 6.1. Milkfat

Crystallization of milkfat is an important process for fractionation of its contents and production of butter, whipped cream, and ice cream. As the quality of these products strongly depends on polymorphism of milkfat, physical chemical properties of milkfat have been studied by many researchers (98).

As mentioned above, milkfat is characterized as a very complicated mixture of TAGs, and thereby it is almost impossible to clarify how every TAG component crystallizes in a cooperative way with the other TAG components. Instead, milkfat is fractionated in accordance with different melting ranges to obtain three major fractions: high melting fraction (HMF), medium melting fraction (MMF), and low melting fraction (LMF). Marangoni and Lencki concluded that HMF and MMF are fully miscible in the solid state, and mixtures of LMF with HMF and MMF showed monotectic property with nature of partial solid solution (70).

As for the polymorphism of milkfat, α and β phases frequently appear, and β form appears under special conditions when HMF and milkfat are stored for long duration (99–101). In regard to the effects of thermal treatment and emulsification on the polymorphic crystallization of milkfat, Lopez et al. recently performed synchrotron radiation X-ray diffraction and DSC studies, using anhydrous milkfat.
(AMF) as a bulk fat system and milkfat globule of cream as an emulsion system (102–106).

No difference in the polymorphic occurrence was observed between cream and AMF when the samples were rapidly cooled from 50°C to −8°C: α form first appeared and β' form appeared during subsequent heating after the melting of α form. On the other hand, crystallization by slow cooling (<0.15°C/min) caused remarkable differences between the emulsion and bulk systems. As summarized in Table 9, β' form first crystallized and β' and α forms coexisted until the end of cooling in the bulk AMF. By contrast, α form first crystallized and β' form started to crystallize during further cooling. In the heating process after the crystallization, α first melted and then β' form melted in both samples.

The chain-length structures largely differed between the bulk AMF and cream samples. Table 9 also shows four different crystals formed in the AMF bulk samples, and four crystals in cream. Quite interestingly, the lamellar spacing values are all different from each other, and double-chain-length and triple-chain-length structures are coexisted. The occurrence domains of the four crystals in AMF during the slow cooling are shown in Figure 24 that shows relative intensity of small-angle X-ray diffraction peaks of the four crystals observed in the bulk AFM and DSC thermopeaks taken during the slow cooling. It is clearly shown that a large exothermic peak around 22°C, a large exothermic peak around 13°C, and a small peak at 4°C are caused by the crystallization of β' form, α form, and β' form, respectively.

It is assumed that the crystallization behavior of milkfat is different between emulsion and bulk, and the lack of nucleation centers in the emulsion droplets may delay the nucleation, making less stable α form nucleated in the first. The occurrence of multiple forms of double-chain-length and triple-chain-length structures may be caused by segregated crystallization of multicomponent TAGs exhibiting complicated mixing behavior, but its details are open to future study.

### 6.2. Cocoa Butter

Cocoa butter is the most popular fat used for confectionery. CB consists of three major TAGs, POP, POS, and SOS, and other minor components (107, 108). The three TAGs determine the polymorphic nature of CB that exhibits six polymorphs, Form I through Form VI in accordance with the nomenclature given by Wille and
Lutton (109). This section employs this nomenclature, although the other nomenclatures such as $\beta_{\text{III}}$ and $\beta_{\text{V}}$, are used in other researchers (110). As Form V functionally works for chocolate, crystallization of CB in Form V, and preservation of this polymorph during long storage are the prerequisites for quality control of the end products. For this purpose, a tempering method including cooling from a molten state, reheating, and recooling has widely been applied (107, 108). The other technique is to use seed crystals of BOB $\beta_2$ whose polymorphic structure is identical to that of Form V of CB and whose melting point is higher than Form V of CB (107). The BOB $\beta_2$ seed crystals can be put in molten chocolate during a simple cooling process without tempering to obtain Form V of CB.

Recently, interesting work has been done to examine the effects of shear stress (19, 110) and ultrasound irradiation on the polymorphic crystallization of CB (111).
As for the effects of the shear stress, it was shown by a Synchrotron radiation X-ray diffraction study that transformations from metastable to more stable forms, especially to Form V, were accelerated by high shear stress (110). Figure 25 shows the time variation of relative intensities of X-ray diffraction peaks of CB crystals formed after cooling from 50°C to 18°C at a rate of 3°C/min. In the case of no shear, Form III appeared at first after the temperature reached at 18°C, and then Form IV crystallized at the expense of Form III. On the other hand, applying the shear stress at 1440 s⁻¹ caused accelerated transformation from Form III to Form V, without the occurrence of Form IV. The same result was observed with lower shear rates (19), and the persistence time of Form III was reduced as the shear rate was increased. Mazaanti et al also observed that the orientation of CB crystals are aligned with the shear flow (110). These results indicated that temperature and shear treatments are the tools for tailoring the desired polymorphic structures of fats.

It was observed that ultrasound stimulation (ultrasonication) also accelerated the crystallization of the more stable polymorphs of CB (111). A fundamental study of the effects of ultrasonication on the polymorphic crystallization of PPP and LLL showed that several factors, such as pressure effect, shear flow, cavitation, and thermal energy caused by absorption of attenuated ultrasound wave, may play concurrent effects of ultrasonication. As a result, there are optimal conditions for temperature and duration of ultrasonication to increase the rate of crystallization and the occurrence of the more stable polymorphs (20). This effect was also observed in CB (111).

Figure 26 shows wide-angle X-ray diffraction profiles of CB with ultrasonication of three durations and without ultrasonication taken after cooling at 20°C from
60°C. Ultrasound (200 kHz, 300 W) was stimulated to a 250-mL sample of CB at 32.3°C during cooling before crystallization. Form II occurred without ultrasonication, whereas Form V was observed when ultrasonication was done for 3 seconds. Further ultrasonication for 9 seconds formed a mixture of Form II and Form V, and only Form II was observed by the ultrasonication for 15 seconds. It is assumed that there are conflicting effects by ultrasonication: promotion of nucleation by pressure effect and retardation of nucleation by thermal energy caused by absorption of attenuated ultrasound wave. The former effect may prevail at the ultrasonication for 3 seconds. The temperature rise, however, of the sample caused by absorption of attenuated ultrasound wave was 2°C for 9 seconds and 3.9°C for 15 seconds, and the latter effect may result in the case of cooling from above the melting point of CB.

6.3. Palm Oil

Palm oil is a common fat and oil resource for many industrial uses. For example, of food applications, it is used as cooking oil, margarine, shortening, and in confectionery products (73, 112). Palm oil has several advantageous properties such as high productivity and high thermal and oxidative stability and plasticity at room temperature. However, the crystallization properties of palm oil are disadvantageous because of a low rate of nucleation and crystal growth of granular crystals (113–114). The granular crystals are easily formed during long storage, causing sandy taste and inhomogeneity of fat crystal morphology of the end products (8, 115–117). Although many TAGs, DAGs, free fatty acids, and so on, are involved in it, palm oil exhibits two polymorphic forms, α and β′ under normal cooling rate, and β form also appears at a very slow crystallization rate. The addition of food
Emulsifiers into palm oil has been an efficient external factor to modify the polymorphism crystallization of palm oil.

Polyglycerol fatty acid esters are biograded surfactants that are used widely in industries such as food, cosmetics, toiletries, and pharmaceuticals (118). Advantageous properties of the PGFEs are derived from the easy modification of their hydrophobicity and hydrophilicity by changing the degree of polymerization of glycerol and esterification with fatty acid moieties and by modifying the chemical structures of fatty acid moieties.

It was observed that the addition of polyglycerine fatty acid ester to palm oil affected the polymorphic crystallization and morphological properties of palm oil (119). In particular, polyglycerol behenic acid ester showed a remarkable effect. Optical microscopy observation confirmed that palm oil crystals with the addition of 1 wt.% of polyglycerol behenic acid esters were smaller and the number of palm oil crystals larger than without the additives, as shown in Figure 27. This indicated that the polyglycerol behenic acid ester promoted nucleation and inhibited crystal growth of palm oil. X-ray diffraction patterns of palm oil without the additives revealed that palm oil crystallized in the $\alpha$ form after rapid quenching of melted palm oil at 10°C. During the heating process from 10°C to 45°C, the $\alpha$ form transformed to the $\beta'$ form around 15°C, and the $\beta'$ form changed to the $\beta$ form around 40°C. The X-ray diffraction patterns of palm oil with the addition of polyglycerol

![Figure 27](http://www.mrw.interscience.wiley.com/biofp)

**Figure 27.** Optical micrographs of isothermal crystallization of palm oil with and without an additive (1 wt.% of polyglycerine behenic acid ester). (A) 60 min at 20°C without the additive; (B) 60 min at 20°C with the additive; (C) 60 min at 27°C without additive; (D) 60 min at 27°C with the additive. (This figure is available in full color at http://www.mrw.interscience.wiley.com/biofp.)
behenic acid esters showed that palm oil crystallized in the $\beta'$ form at 10°C, and it did not transform to the $\beta$ form during the heating process.

It is generally considered that the nucleation rates of $\alpha$ and $\beta'$ are largely different, with $\alpha$ crystallizing much more rapidly than $\beta'$ and $\beta$. The size of the crystal is associated with the crystal form; $\beta$ form tends to produce granular crystals, and $\beta'$ is recommended for producing small crystals. Figure 27 shows palm oil with polyglycerol behenic acid esters added, the $\beta'$ form is preferentially crystallized, and the crystal does not grow to a granular crystal. One reason for this may be that polyglycerol behenic acid esters, which promote the nucleation of palm oil, have higher melting points than that of palm oil; namely, the emulsifier as an additive may crystallize faster than palm oil, inducing heterogeneous nucleation of palm oil as template.

7. SUMMARY

This chapter described polymorphic properties of principal TAGs and natural fats based on recent research work to clarify fundamental aspects of polymorphism of fats and oils. The authors hope that the basic understanding of the polymorphism of the principal TAGs would be useful to elucidate rather complicated polymorphic properties of natural fats and oils that contain TAGs with very heterogeneous fatty acid compositions.

It may be worth noting the following subjects, which are nowadays still open to question and therefore should be worked out in future.

1. Polymorphism of diacid and triacid TAGs

Little knowledge of polymorphism of diacid and triacid TAGs have been obtained, because these TAGs have high relevance to natural fats and oils. The main reasons for this must be difficulty in preparing pure materials, which may provide convincing results. It is highly interesting and important to work on diacid and triacid TAGs with an emphasis on the effects of chirality on polymorphism that has rarely been known.

2. Polymorphic structures of saturated-unsaturated mixed-acid TAGs

Although much effort has been done to unveil precise structures of saturated-unsaturated mixed-acid TAGs, no atomic-level structure analysis has been successful because of difficulty to obtain high-quality single crystals suitable for X-ray analysis. In particular, SOS and POP are the most representative TAGs that are major components of cocoa butter and palm oil. It is expected that structures data of the stable $\beta$ forms of SOS and POP may give key ideas to resolve fat bloom phenomena in confectionery fats. Molecular simulations have been done (120, 121), but the experimental results so far obtained are the XRD patterns and FT-IR spectra using powder and single crystals, which do not provide detailed molecular structures, in particular, about olefinic conformation and interfacial structures of oleic acid and saturated acid leaflets.
3. Phase behavior of TAG mixtures

As the natural fats and oils are mixtures of different TAGs, their polymorphism is influenced by the phase behavior of the mixture phases. In order to mimic natural fats and oils, the mixture phases of diacid TAGs were studied, as reviewed in Section 4. However, we more need basic research of the mixtures of TAGs whose fatty acid compositions are heterogeneous, e.g., the mixtures of between saturated diacid TAGs of saturated diacid TAGs and unsaturated diacid TAGs. These studies may be significant in understanding the polymorphism of palm oil and its fractions (palm stearin, palm olein, etc.), or milkfat and its fractions (high-melting fraction, etc.).

REFERENCES

40. In ref. 17, pp. 368–369.


