On the Formation of Granular Crystals in Fat Blends Containing Palm Oil

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The use of palm oil for plastic fats has encountered serious difficulty due to its crystallization properties, one of which is the formation of granular crystals. The granular crystals cause sandy taste and inhomogeneity of fat crystal morphology of end products. In the present study, we analyzed the formation process of these granular crystals. Chemical and physical analyses for triacylglycerol (TAG) compositions, polymorphism and melting points of the granular crystals led to the conclusion that the granular crystals are of the most stable polymorph, β_1, of POP (1,3-dipalmitoyl 2-oleoyl glycerol) with triple chainlength structure. This conclusion was supported by reproducing the formation process of the granular crystals in a model fat blend consisting of POP and rapeseed oil. The following characteristics of β_1 polymorph of POP contribute to the formation of granular crystals in fat blends containing palm oil: (i) β_1 reveals well-defined plate-like crystal morphology, which grows easily into the bulky granular form; (ii) the triple chainlength structure of POP has a tendency to segregate and does not form a solid solution together with other TAGs.

KEY WORDS: Chainlength structure, 1,3-dipalmitoyl 2-oleoyl glycerol, fat crystallization, granular crystal, melting point, palm oil, polymorphism, triacylglycerol.

Palm oil has been a prominent fat and oil resource for the food industry due to several advantageous properties, such as (i) high productivity, (ii) low price, (iii) high thermal and oxidative stability and (iv) plasticity at room temperature (1). The use of palm oil, however, for solid fats in foods has encountered serious difficulty because of the following two crystallization properties: (i) low rate of nucleation (2) and (ii) growth of granular crystals (3–9). Both properties are related to specific crystallization and polymorphism behavior of palm oil (7). The latter problem is particularly bothersome because crystals with diameters of 20 to 50 μm form easily during long storage, causing sandy mouth feel and inhomogeneity of fat crystal morphology of end products.

The crystallization behavior related to the formation of granular crystals has been analyzed by Kawamura (3,4) and Ishikawa (5). The influence of free fatty acid components and diacylglycerols, which are involved in palm oil itself, on the crystallization rate was examined by Jacobsberg (6) and Okiy (9). In all of these studies, palm oil has been thought to create its own polymorphic behavior like tripalmitin, *etc.* This approach may be misleading because palm oil is composed of seven major triacylglycerol (TAG) components. To fully understand the polymorphic crystallization of palm oil, phase behavior involving miscible and eutectic properties of various fat mixtures of these major components must be clarified.

In the present study, we analyzed the formation processes of granular crystals occurring in fat blends of rapeseed and palm oil. Chemical and physical analyses for the granular crystals showed that the most stable polymorph, β_1, of POP (1,3-dipalmitoyl 2-oleoyl glycerol) causes palm oil-originating granular crystals. This conclusion was confirmed by reproducing the formation process of the granular crystals in a model fat blend of POP and rapeseed oil. The present work is based on the polymorphic behavior of Sat-O-Sat (1,3-saturated acyl 2-oleoyl glycerol) TAGs that has been established recently by Sato *et al.* (10–12). In the Sat-O-Sat TAGs, the more stable polymorphs are of triple chainlength structure, whereas the less stable forms are of double chainlength. This specificity of the chainlength structure may partly give rise to the formation of the granular crystals.

MATERIALS AND METHODS

Fat blends. Two kinds of fat blends were prepared: palm oil/rapeseed oil at 4:6 in wt% (Blend A), and POP/rapeseed oil at 1:9 in wt% (Blend B). Blend A represents a real system, and Blend B is a model system that was used to reproduce the formation mechanism observed in Blend A. In the two blends, the concentration of the POP fraction was almost the same. Both blends were melted at 60°C and cooled to 30°C at the same rate, 3°C/min, and further cooled to 5°C. After this cooling, temperature fluctuation was applied to form the granular crystals; 20°C for 12 h and 15°C for 12 h. The granular crystals were picked up with needles and subjected to TAG composition analyses, as well as X-ray and differential scanning calorimetry (DSC) analyses. Polarized optical microscopy was also employed to observe the crystal morphology. POP (>99%) was purchased from Sigma Chemical Co. (St. Louis, MO). No further purification was done. Rapeseed oil and palm oil were prepared in our laboratory.

TAG analysis with high-performance liquid chromatography (HPLC). TAGs were analyzed by Shukla's method (13). The HPLC columns consisted of two 150 mm × 4.6 mm i.d. in series. They were packed with 3 μm C_{18} bonded-phase particles. The columns were maintained at 40°C by a column oven. The analyses were carried out by mixing acetonitrile (ACN) and tetrahydrofuran (THF) (70:30, vol/vol) at 0.6 mL/min, the usual flow rate. The laser light-scattering detector was used to identify the separated TAGs.

Positional isomers (for example, 2-oleoyl 1,3-dipalmitoyl glycerol and 3-oleoyl 1,2-dipalmitoyl glycerol) could not be distinguished with Shukla's method. Therefore, they were separated with an argentation column (14). The column size was 250 mm × 4.6 mm i.d. and was packed with 5 μm LiChrosorb Si60 argentated silica. Benzene was used as the mobile phase at the flow rate of 0.5 mL/min. A refractive index detector was used.

Polymorphic analyses. X-ray diffractometry (XRD) (Rigaku, Tokyo, Japan) (CuK α: $\lambda = 0.1542$ nm) was employed to measure short (wide angle) and long spacings (small angle). The measurements were carried out at

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0–5°C to prevent the crystals from melting. Melting points were measured with DSC (Mettler, Greifensee, Switzerland, TA3000 system); heating rate was 10°C/min from −50°C to 70°C.

RESULTS

Blend A. Figure 1 shows the granular crystals with diameters of around 30 μm. Their spherulitic morphology is the same as reported before (3–5). The TAG composition of the granular crystal fraction is shown in Table 1. Because of its importance, the P_2O (dipalmitoyl monooleoyl glycerol) fraction was further analyzed to be POP:90.1% and PPO:9.9%. From this, it was determined that the granular crystal fraction contains POP at 47.9%. This is much higher than the overall POP content, 12%, in Blend A.

The XRD pattern of the granular crystals is shown in Figure 2. The strongest peak at 0.462 nm indicates that this form is in the $T_{//}$ subcell (15) of the β modification. The XRD pattern with a strong peak at 0.374 nm compared to 0.386 nm proves that this is the most stable β_1 form of POP (10). An additional peak of 0.423 nm, which is of pseudo-β'_2 of POP (10) may occur during the XRD measurement procedure because of freezing of liquid oil adhering to the granular crystals.

The XRD long-spacing pattern is shown in Figure 3. The peaks of 6.30 nm and 3.10 nm are (001) and (002) spectra of long spacing of the β_1 form of POP (10), respectively and prove the triple chainlength structure of this polymorph. The peak at 4.26 nm represents (001) of the pseudo-β'_2 form of the double chainlength structure (10), which is caused by freezing during the XRD measurement as described above.

Figure 4 shows a DSC heating thermogram of the granular crystal fraction. There is a sharp melting peak with onset temperature of 23.0°C and peak-top temperature of 26.8°C. This melting point is lower than that of pure POP (36.7°C, onset). This difference is due to mixing with low-melting oil fraction, as will be proved in Blend B.

To summarize, temperature fluctuation after crystallization from the melt phase of Blend A formed the granular crystals whose major TAG is POP, at a concentration of 47.9%, of the most stable β_1 modification. To further demonstrate this, the crystallization procedure with Blend B is described below.

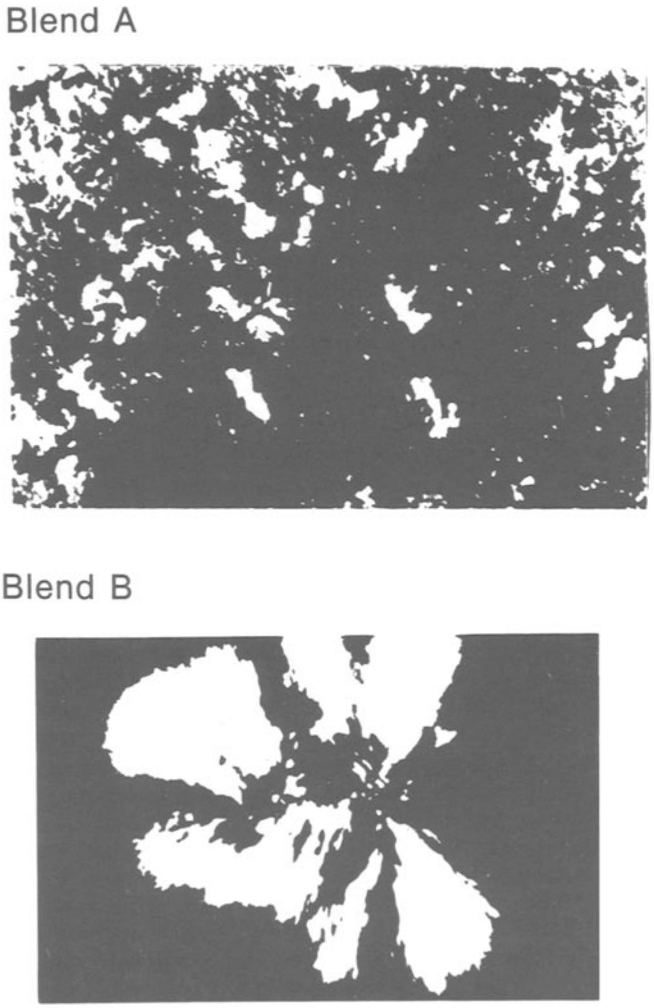

FIG. 1. Granular crystals in palm oil/rapeseed oil blend (Blend A) and POP/rapeseed oil blend (Blend B). Scale bar, 50 μm.

TABLE 1

Major TAG[a] Composition of Granular Crystals

TAGs	Blend A (%)	Blend B (%)
LLO	2.8	6.9
LnOO	0.0	5.8
LOO	8.7	19.1
OOO	13.0	25.2
POO	6.9	1.8
POP	47.9	27.4
PPO	5.3	0.0
PPP	1.0	0.0
POS	4.5	0.0

[a] TAG, triacylglycerol.

Blend B. A granular crystal formed in Blend B with a size of 200 μm is shown in Figure 1. In comparison with Blend A, the granular crystals in Blend B are larger in size, smaller in number and form large clusters. This is due to the fact that Blend B contained the pure POP material, and the crystallization process is not disturbed by impurities. Hence, the limited number of the POP crystals in Blend B grows in size. In Blend A, impurities or homologous TAGs may accelerate heterogeneous nucleation. As a result, many relatively small granular crystals may be formed. Table 1 shows the concentration of POP in the granular crystals in Blend B to be 27.4%. This value is smaller than that in Blend A. The granular crystals in Blend B contain a higher content of liquid oil, which decreased the POP concentration. The TAG composition of the liquid oil portion of the granular crystals, such as triolein (OOO) and LOO (monolinoleoyl dioleoyl glycerol), is almost the same as that in rapeseed oil. Therefore, the content of OOO is not increased, and OOO does not form the granular crystals together with POP. Figures 2 and 3 show X-ray diffraction short- and long-spacing spectra of granular crystals in Blend B. The strong short-spacing peaks at 0.462 nm and 0.374 nm and the long-spacing peaks at 6.30 nm (001) and 3.10 nm (002) prove

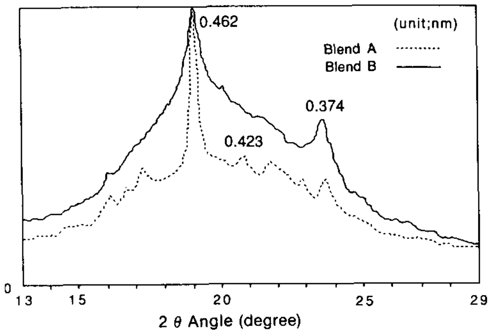

FIG. 2. X-ray diffraction short-spacing pattern of granular crystals in Blend A (dash) and Blend B (line).

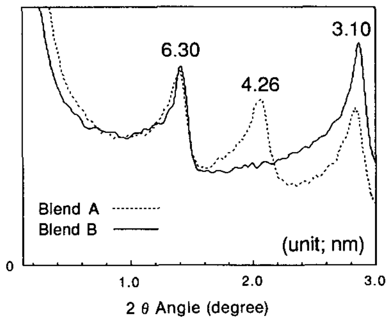

FIG. 3. Small-angle X-ray diffraction long-spacing pattern of granular crystals in Blend A (dash) and Blend B (line).

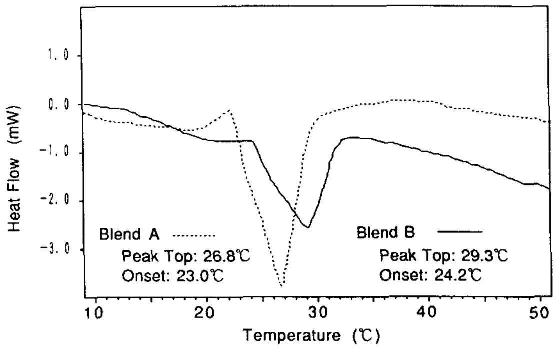

FIG. 4. Differential scanning calorimetry heating thermogram of granular crystals in Blend A (dash) and Blend B (line).

the β_1 form of POP. In Figure 4, the DSC melting curve shows a peak-top temperature of 24.0°C. This melting behavior is in good agreement to Blend A.

DISCUSSION

The granular crystals have often been thought to be a stable modification of palm oil itself (2–4,6–9). However, this concept is rather inaccurate because (i) palm oil is a mixture of many TAGs whose polymorphic behavior differs from one component to another, and (ii) a systematic study of the multiple phase behavior of major TAG components of palm oil has not been completed. Ishikawa *et al.* (5) claimed that the granular crystals in palm oil are the β form of POP, based on chemical and structural analyses. This conclusion, however, is still insufficient, because there are two β forms of POP whose crystal morphologies are quite different (10). The metastable form, β_2 reveals an irregular shape and dispersible crystal morphology, whereas the more stable β_1 crystals are of well-defined slender rhombic shape (12). The solubility of β_1 is lower than that of β_2. Due to the Ostwald step rule (16), the metastable β_2 form first crystallizes after rapid cooling (11). After stabilization, β_2 transforms to β_1, changing the crystal morphology. Hence, differentiation of the two β forms is of great importance.

The present study has clarified that the granular crystals in Blend A, containing palm oil and rapeseed oil, are equal to the granular crystals of POP. This was supported by the study of Blend B, which contained only POP and rapeseed oil. The occurrence of β_1 of POP has two specific implications: (i) β_1 reveals well-defined plate-like crystals of typical triclinic crystal structure; (ii) the triple-chainlength structure of POP β_1 has a tendency to segregate from other fats and does not form a solid solution with other TAGs. For example, Moran (17) suggests the formation of continuous solid-solution in POP and POO in the double-chainlength structure. However, such a mixture system may not be realized in the granular portions observed in the present experiments.

Higher content of liquid oil of the granular crystals formed in Blend B is due to cluster formation of the granular crystals. Liquid oil is thus included between the granular crystals.

A detailed formation process of the granular crystals in the cooling curve is still uncertain. The problem is when the granular crystals are actually formed, before or during the temperature fluctuation. We infer that β_2 crystals are first nucleated after cooling, and $\beta_2 \rightarrow \beta_1$ conversion may be accelerated by the temperature fluctuation. In Blend A, we presume that the presence of pseudo-β'_2 is due to freezing of liquid oil that adheres to the granular crystals, but its presence may contribute to incomplete conversion to β_1. To confirm this, further observation is needed. It may be stressed, from present work, that possible techniques to prevent the formation of the granular crystals may be developed, by paying attention to the nucleation and growth of β_1 crystals. Their formation is similar to fat bloom in cocoa-butter chocolate, because the polymorph of the bloomed portion of cocoa butter, Form VI, corresponds to β_1 of SOS, POP and POS (18).

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[Received March 20, 1992; accepted September 14, 1992]