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ORIGINAL PAPER

# Formation of Granular Crystals in Margarine with Excess Amount of Palm Oil

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**Abstract** Crystallization behavior of palm oil and tripalmitin (PPP) in a model margarine system was investigated. The model margarine was held in a programmable oven, heated to 5 °C for 12 h and then 20 °C for a further 12 h. After 3 weeks, the model margarine was evaluated by polarization microscopy. Granular crystals were observed in the margarine containing an excess amount of palm oil and PPP. The concentration of higher-melting fatty acids was higher in the crystals relative to the surroundings. Likewise, the presence of lower-melting fatty acids was lower in the crystals. The polymorphic structure of the margarine with excess palm oil and PPP was determined to be all $\beta'$ by X-RD spectra. The result suggested that the agglomeration of higher-melting point triglycerides (TAGs), such as PPP in this study, led to the formation of granular crystals in the margarine without $\beta$ polymorphic emergence.

**Keywords** Polymorphism · Granular crystals · Tripalmitin (PPP) · Crystallization behavior · Agglomeration · Triacylglycerol

## Introduction

The physical properties of margarine and shortening such as consistency and plasticity are determined largely by triacylglycerol (TAG) composition and crystallization behavior. Depending upon the end use of the product, crystallization behavior is critical. However, certain TAG compositions exhibit undesirable crystallization behavior, such as the growth of granular crystals in margarine which impair the softness and spreadability of the margarine. Thus, control of granular crystal formation in high fat formulations is a very important point in the food industry.

Palm oil is one of the major fat sources in the world, due to its heat stability, physical characteristics, availability, and price competitiveness. However, in certain applications, palm oil is also known to exhibit undesirable crystallization behavior. The occurrence of granular crystal formation in palm oil has been investigated extensively. Ishikawa et al. [1] studied granular crystals present in palm oil and concluded that the slow crystallization rate of palm oil leading to the $\beta$ polymorph might be one reason for the formation of granular crystals in the margarine.

Miura et al. studied the composition of granular crystals in margarine produced with an excess of palm oil, showing that 1,3-dipalmitoyl-2-oleoyl-glycerol (POP) was one of the major TAGs present in the granular crystals [2]. The crystallization behavior of POP and 1-palmitoyl-2, 3-dioleoyl-glycerol (POO), which are the major TAGs found in palm oil, and their mixture was then investigated. They found that POP and POO were immiscible with each other, resulting in the formation of POP crystals surrounded by POO. Miura et al. suggested that granular crystals are formed as the higher-melting point TAGs are agglomerated, followed by transformation to the more stable $\beta$-crystal polymorph. Sato et al. studied the crystallization behavior of POP and POO mixtures, and they showed the immiscible monotectic properties of POP and POO [3].

Miura et al. did not, however, show whether other higher-melting TAGs, such as tripalmitin (PPP) affect the growth of granular crystals in margarine.

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In the present study, palm oil and tripalmitin (PPP) were used in the production of a model margarine and the crystallization behavior investigated.

## Material And Methods

### Preparation of the Model Margarine

Table 1 shows the major ingredients used in the production of the model margarine. Preparation of the model margarine was carried out following the procedure of Hui [4]. All of the oils were melted and mixed at 60 °C, followed by the addition of emulsifiers under gentle agitation. The pasteurized aqueous phase was also held at 60 °C and was added to the oil phase with agitation to form an emulsion. A high-pressure pump fed the emulsion to a tubular-swept surface-heat exchanger (Schröder GMBH&Co Germany) in which the emulsion was cooled and well kneaded to develop rapid crystal nucleation. The shaft rotation speed was kept at 200 rpm, and the temperature at the product inlet and outlet were 60 and 10 °C, respectively [4]. The margarine was then passed through a pin-machine (Schröder GMBH&Co Germany) to promote further crystallization. The temperature at the product outlet of the pin-machine was 12–14 °C. The final product was poured into a container.

### The Temperature Cycle

The model margarine was stored in a programmable oven in which the temperature was held at 5 °C for 12 h and 20 °C for another 12 h [2]. This temperature program was repeated each day. Each week, the model margarine was examined under a polarization microscope.

### Observation Using The Polarization Microscope

About 50 mg of margarine was placed on a cooled glass-plate followed and covered with a cover glass. The cover glass was manipulated using a wooden-needle until the sample layer was thin enough to observe the crystals. A Microphot-FX polarization microscope (Nikon, Tokyo, Japan) was used.

### Fatty Acid Analysis

The fatty acid compositions of the granular crystals and surrounding materials were determined according to AOCS official Ce 1c-89 [5]. A GLC using HP5890 (Agilent Technologies, Inc., Palo Alto, CA, USA) with SP-2560 column (Supelco, Inc., Bellefonte, PA, USA) and equipped with a FID operated at 300 °C was employed. The injector port temperature was held at 250 °C, and analyses performed by ramping from 180 to 200 °C at 2 °C/min after an initial holding time of 45 min at 180 °C.

### Polymorphic analysis

X-RD (Rint-Ultima 2000, CuK $\alpha$:$\lambda$ = 1.54 Å; Rigaku, Tokyo) was carried out to measure the wide angle diffraction pattern ($2\theta$ = 18°–25°) and to obtain the short spacing of the crystal polymorphs formed in the model margarine. The sample was held at 5 °C during the X-RD measurement to prevent the crystals from melting.

## Results And Discussion

### Formation Of The Granular Crystals In Margarine With An Excess Of Palm Oil

Granular crystals were observed in the temperature-cycled margarine under the polarization microscope after 3 days to 3 weeks (Fig. 1). Figure 1a shows the control margarine. No large crystals were observed. Figure 1b and c show large crystals after 3 weeks. The margarine shown in Fig. 1b contained 1.5 times more palm oil than the control, and the margarine shown in Fig. 1c contained twice the

**Table 1** The composition of the model margarine

| Ingredients | Ratio (%) | | |
|---|---|---|---|
| | Control | 1.5 times the amount of palm oil | Twice the amount of palm oil |
| Partially hydrogenated soybean oil | 35.0 | 27.5 | 20.0 |
| Palm oil | 15.0 | 22.5 | 30.0 |
| Soybean oil | 20.0 | 20.0 | 20.0 |
| Emulsifiers | 0.7 | 0.7 | 0.7 |
| Sodium chloride | 1.2 | 1.2 | 1.2 |
| Spray-dried whey | 0.5 | 0.5 | 0.5 |
| Water | 27.6 | 27.6 | 27.6 |
| Total | 100.0 | 100.0 | 100.0 |

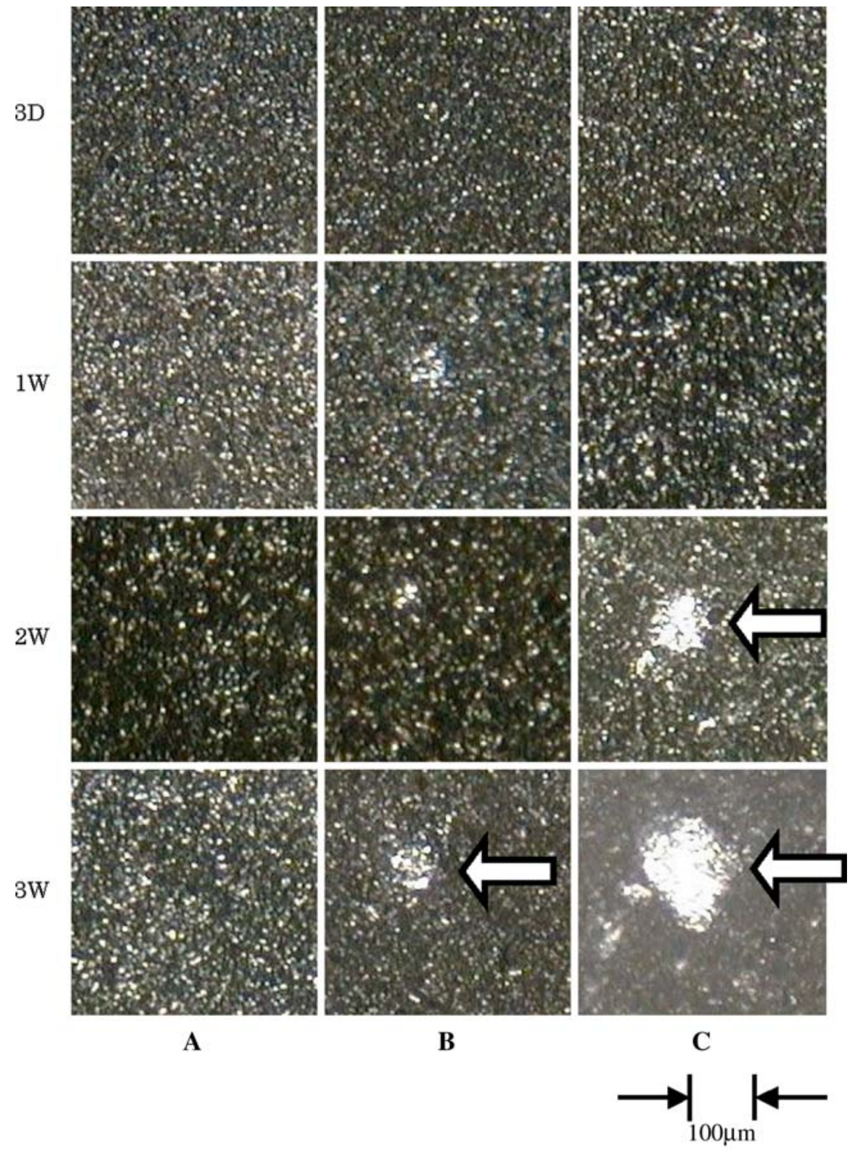


**Fig. 1** Granular crystals observed by polarization microscope in the model margarine under temperature fluctuation cycle after 3 days (*3D*), 1 week (*1W*), 2 weeks (*2W*), and 3 weeks (*3W*). **a** Control, **b** 1.5 times palm oil, **c** 2.0 times palm oil

amount of palm oil of the control (Table 1). It was obvious that higher the concentration of palm oil, the larger the crystals that were formed. The size of the crystals exceeded 100 μm in margarine with twice the palm oil of the control (Fig. 1c). This result suggested that palm oil accelerated the growth of granular crystals in the margarine.

## Formation Of The Granular Crystals In Margarine With PPP

The above result indicated the possibility of TAGs in palm oil accelerating the growth of granular crystals. Since PPP was the third major TAG in palm oil, and the amount was about 7% (Table 2) [6–10], we assumed that PPP was one of TAGs that accelerated the crystallization. In order to prove this point, PPP was added into the margarine at levels equivalent to those with excess palm oil as above.

Granular crystals were observed in the temperature-cycled margarine with PPP after 3 days to 3 weeks (Fig. 2). Figure 2a shows the control margarine. Again, no large crystals were observed in the control. Figure 2b, c and d show large crystals after 3 weeks. Figure 2d indicates the most rapid appearance of crystals among samples. The margarine shown in Fig. 2b contained 1.5 times the PPP, and the margarine shown in Fig. 2c and d contained twice and fourfold the levels of PPP as compared to the control. It was obvious that higher the amount of PPP, the larger the crystals that are formed. The size of the crystals in the fourfold PPP margarine exceeded 100 μm (Fig. 2d).

This result indicated that PPP also accelerated the growth of granular crystals in the margarine.

**Table 2** Melting point of polymorphs of major TAGs in palm oil

| Molecular species | Ratio (%) | Melting point (°C) | | | |
|---|---|---|---|---|---|
| | | α | β′ | β or β2 | β1 |
| POP | 23.7 | 15.2 | 30.3 | 35.1 | 36.7 |
| POO/OPO | 21.5 | –18.3 | 11.7 | 15.8 | 21.9 |
| PPP | 7.2 | 44.7 | 56.6 | 66.4 | – |
| PPO | 6.9 | 18.5 | 35.2 | – | – |

*P* palmitic acid, *O* oleic acid

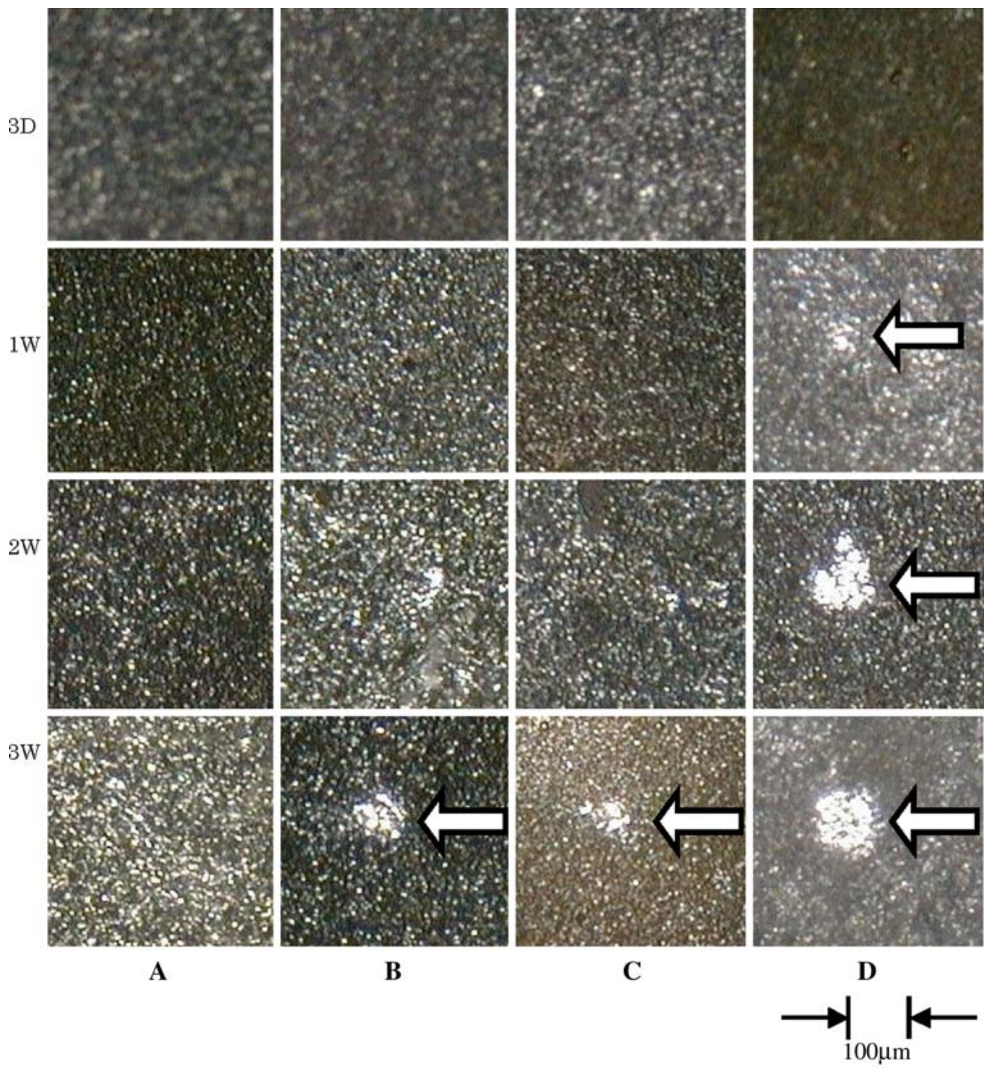


**Fig. 2** Granular crystals observed by polarization microscope in the model margarine under the temperature fluctuation cycle after 3 days (*3D*), 1 week (*1W*), 2 weeks (*2W*), and 3 weeks (*3W*). **a** Control (1.0% PPP is included), **b** additional 0.5% PPP was added, **c** additional 1.0% PPP was added, **d** Additional 3.0% PPP was added

## Fatty Acid Composition Of Granular Crystals

Table 3 shows the fatty acid compositions of the granular crystals with excess PPP as well as the surrounding material. Stearic acid levels in the crystals was 6 times that of the surrounding material. Palmitic acid did not increase significantly, even though PPP was added to the margarine. Table 4 shows the fatty acid composition of the three oils involved in this study. From Table 4, it is assumed that there is at least 3% tristearin in partially hydrogenated soybean oil, as the content of TAGs in unhydrogenated soybean oil with three C-18 fatty acids is greater than 50%. Tristearin in the β′ polymorph has a needle crystal habit, which is expected to promote aggregation. These results suggested that even though PPP initiated the localization of higher melting TAGs, it did not localize or agglomerate extensively with itself. Rather, localization of TAGs with stearic acid was initiated by PPP. Even if PPP actually does localize in crystal nuclei, such a phenomenon was too small to observe in this study. Not until the nuclei grew

**Table 3** Fatty acid composition of granular crystal in margarine with excess PPP

| Fatty acid | Crystal (%) | Surroundings (%) |
|---|---|---|
| Palmitic acid | 18.4 | 17.3 |
| Stearic acid | 36.7 | 6.2 |
| Oleic acid | 19.7 | 28.5 |
| Linoleic acid | 23.0 | 32.2 |

**Table 4** The fatty acid composition of oils

| Fatty acid species | Ratio (%) | | |
|---|---|---|---|
| | Partially hydrogenated soybean oil | Palm oil | Soybean oil |
| C16 | 12.3 | 44.0 | 10.6 |
| C18 | 7.7 | 4.4 | 4.0 |
| C18:1t | 17.7 | 0.5 | 0.0 |
| C18:1c | 24.2 | 39.5 | 24.1 |
| C18:2c | 31.6 | 8.8 | 52.3 |
| C18:3c | 3.5 | 0.1 | 5.9 |
| others | 2.9 | 2.8 | 3.1 |
| Total | 100.0 | 100.0 | 100.0 |

with stearic acid was the crystal visible. At the same time, oleic acid and linoleic acid levels were observed to decrease in the crystals. This is not surprising, as it is generally known that TAG species with longer and more saturated fatty acids have higher melting points (Table 5) [11–15]. The above results point to the possibility that the localization of higher-melting TAGs such as POP, SOS and SSS lead to the formation of granular crystals.

Figure 3 shows X-ray diffraction profiles of the margarine with 3.0% PPP, which is 2.0 that of palm oil. The polymorph of the margarine with excess palm oil and with excess PPP was determined to be all $\beta'$ by X-RD spectra.

**Table 5** Higher melting point TAG species with long saturated fatty acid

| Molecular species | Melting point at most stable polymorph (°C) |
|---|---|
| OOO | 5.5 |
| OPO | 21.9 |
| POP | 36.7 |
| PPP | 66.4 |
| OSO | 25.0 |
| SOS | 43.0 |
| SSS | 73.5 |

*P* palmitic acid, *O* oleic acid, *S* stearic acid

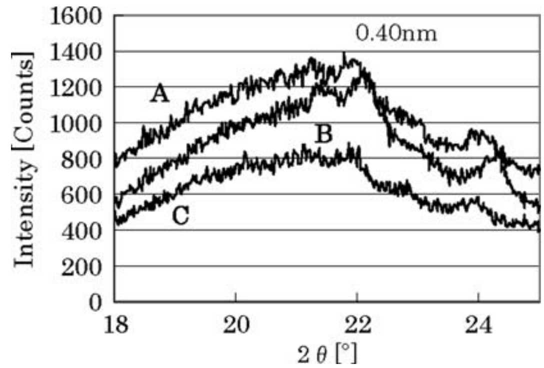


**Fig. 3** X-RD spectra of model margarine after 4 weeks. **a** Control (1.0% PPP is included), **b** additional 3.0% PPP was added, **c** 2.0 times the quantity of palm oil

Therefore, the higher-melting fatty acid agglomerates occurred without any change of polymorph in the first stage. No polymorphic transition from $\beta'$ to $\beta$ was observed after one month. This result differed from the study by Miura et al. in which $\beta$ polymorph occurred in POP/POO mixtures. It is likely that the presence of various TAG species in margarine prevents a rapid polymorph transition in the present study.

In this study, granular crystal formation in the margarine produced with excess amounts of palm oil or PPP was investigated. The results suggested that the agglomeration of higher-melting TAGs such as PPP, led to the formation of granular crystals in margarine as crystal nuclei. Once nucleation occurs, other higher melting TAGs, such as POP, SOS or SSS become components of the growing crystals and resulting agglomerates. At same time, the crystal polymorph of the margarine was $\beta'$. This phenomenon suggests that the agglomeration of higher-melting point TAGs is the cause of the formation of granular crystals. This is consistent with the results and conclusion obtained by Miura et al.

These results show that higher-melting TAGs are one of the key factors for the formation of granular crystals in margarine. Another factor may be the effect of emulsifiers [16]. Though this investigation of this reference was conducted on bulk oil, a similar effect might occur in margarine. In addition, it is assumed that monoacylglycerols and diacylglycerols have a delayed effect on crystallization [17]. A major objective of the fats and oil industry is to determine the relevant key factors and to understand their impact on crystallization. Based on the results of this study, investigating the effect of various TAGs helps to predict which TAGs should be avoided. In this way, satisfactory quality parameters, such as the consistency and plasticity of fat and oil products, can be maintained.

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