Triacylglycerols Responsible for the Onset of Nucleation During Clouding of Palm Olein

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ABSTRACT: This paper discusses the results of an investigation to identify triacylglycerols that induce clouding of refined bleached deodorized (RBD) palm olein, which occurred within 24 h after fractionation. The experiments were conducted in a jacketed glass vessel in which the liquid sample was cooled from 70 to 23°C at a predetermined rate. Clouding began at around 28.5°C. The presence of three different types of saturated triglycerides, namely tripalmitin, dipalmitoyl-myristoyl-glycerol and dipalmitoyl-stearoyl-*rac*-glycerol, is critical in the formation of nuclei and thus clouding of the RBD palm olein. This conclusion is based on the significant increase in the relative concentration of these components in the nuclei as compared to the mother oil.
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KEY WORDS: Clouding, nucleation, palm olein, triglycerides.

Palm oil, which consists mainly of triacylglycerols of palmitic and oleic acid, is semisolid at room temperature. Its composition and properties, however, can be modified by various processing techniques to produce a series of different products that can be tailored for specific uses. Among the major products of palm oil is a variety of oils and fats that are produced through dry fractionation, which separates palm oil into a liquid palm olein and a solid palm stearin by means of crystallization.

Crystallization of triglycerides is complex and characterized by the existence of multiple crystalline states. Because of its commercial importance, intensive studies on this subject have been carried out for over a century (1). The conditions under which crystallization is performed have a profound effect on the shape, size, structure, and composition of the resulting crystals. Among the critical factors that have been identified are the source of oil (2), the final crystallization temperature, and cooling rate (3). According to Taylor (4), slight variations in the fatty acid composition of the oil seem to have a greater effect on the type of crystals obtained than do other factors. Amer *et. al.* (5) investigated the crystallization of milk fat and found that the crystallization temperature affects the formation of polymorphs, crystal size, the composition of the solid fat and its physical properties. The effect of cooling rate on the crystallization of fats also was investigated by Deffence (6) who showed that cooling rates significantly affect the physical properties of the crystalline fraction, especially at higher temperatures. By increasing the cooling rate, the yield and the drop point of the solid fraction decreased while crystal size increased.

Crystallization can be divided into four steps: nucleation, growth, agglomeration, and attrition. Primary nucleation can be described as an ordering process of triacylglycerol (TAG) molecules in solution. As soon as clusters of TAG molecules in the solution have grown to a certain critical size, intermolecular forces within the TAG cluster overcome the forces of the TAG molecules in the surrounding solution. Consequently, the clusters become stable and form crystal nuclei. According to Kawamura (7), TAG molecules combine to form clusters by collision with each other and/or with foreign particles in the supercooled melt of palm oil. When the change in the activation energy exceeds the critical level for nucleation, crystal growth begins. Crystal growth consists of surface nucleation and transport of TAG molecules to adhere to surface nuclei or spherulites. TAG daughter crystals are constantly nucleated on the surface of the parent spherulites and finally cover them to link the adjacent crystals. Watanabe *et al.* (8) conducted a series of experiments to identify the main TAG in stearin crystals, formed from rapeseed oil blended with palm oil, and identified 1,3-dipalmitoyl-2-oleoyl-glycerol as the main TAG constituent. In a separate study, Swe *et al.* (9) investigated the composition of crystals that formed in palm olein after prolonged storage at room temperature, a commonly encountered phenomenon. These investigators deduced that the cloud consisted of two diglycerides (DAG), one of which was 1,3-dipalmitin. The other DAG was not identified.

In many fractionation plants, certain batches of palm olein, which have the required iodine value (IV) of 56, will form clouds much quicker than other batches. Often, clouding occurs within 24 h after fractionation. This phenomenon can be observed for one olein fraction and not another, even though both fractions originate from the same refined, bleached, and deodorized (RBD) palm oil. The behavior of the olein sample that forms a cloud within 24 h mimics the RBD palm oil from which it is obtained in that, within a few hours at 30°C, it produces a two-phase mixture of solid and liquid. Clouding of such palm olein continuously causes problems to refiners because this oil

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TABLE 1
Triacylglycerols That Are Present in RBD[a] Palm Oil and Palm Olein as Determined by High-Performance Liquid Chromatography

Identification	Triacylglycerol
LLO	Dilinoleoyl-oleoyl-glycerol
PLL	Dilinoleoyl-palmitoyl-glycerol
MLP	Linoleoyl-myristoyl-palmitoyl-glycerol
OLO	Dioleoyl-linoleoyl-glycerol
PLO	Linoleoyl-palmitoyl-oleoyl-glycerol
PLP	Dipalmitoyl-linoleoyl-glycerol
MPP	Dipalmitoyl-myristoyl-glycerol
OOO	Triolein
POO	Dioleoyl-palmitoyl-glycerol
POP	Dipalmitoyl-oleoyl-glycerol
PPP	Tripalmitin
SOO	Dioleoyl-stearoyl-glycerol
POS	Palmitoyl-oleoyl-stearoyl-glycerol
PPS	Dipalmitoyl-stearoyl-glycerol
SOS	Distearoyl-oleoyl-glycerol
PSS	Distearoyl-palmitoyl-glycerol

[a]Refined, bleached, and deodorized.

has to be rejected even before it is sent to end users. In view of the importance of crystallization behavior, this project was undertaken to identify the TAG responsible for initiation of the crystallization process and thus the clouding of palm olein.

MATERIALS AND METHODS

RBD palm oil and palm olein were obtained from local refineries. The various triglycerides present in the samples are given in Table 1. Compositional details for the samples are given in Table 2. Because of the similar clouding behavior of the palm olein sample and the RBD palm oil, all crystallization experiments were performed with RBD palm oil. This approach was adopted because it would be difficult to identify the TAG responsible for nucleation if samples of olein were so low in certain TAG that crystallization results may become inconclusive. However, the relationship between the results obtained with palm oil and with palm olein will be discussed. The standards for identification of TAG peaks from high-performance liquid chromatography chromatograms were obtained from Sigma Chemical Co. (St. Louis, MO). For a typical experiment, about 100 mL of sample was charged into a 200-mL jacketed glass vessel, connected to a programmable water-circulating bath (Model RC6 CP; Lauda Dr. R. Wobser GmBH and Co. KG, Lauda-Konigshofen, Germany). The sample was heated to and maintained at 70°C for about 10 min to destroy any nuclei that might be present. The run was begun by cooling the sample from 70°C, at a rate of 0.5°C/min, to an intermediate temperature of 30°C, and finally to 20°C at a rate of 1°C/min. For each run, samples of about 5 mL were taken in duplicate at temperatures of 29, 28.5, and, from then on, at every interval of 1°C drop until a temperature of 23°C was reached. These temperatures were selected to cover the entire range from nucleation to crystal growth. Samples were centrifuged at 3000 rpm for 3 min to separate the liquid and solid fractions. Each solid fraction was separated by decantation, washed with 20 mL acetone at about 20°C, and centrifuged at 3000 rpm for 3 min. The washing process was repeated three times. During washing, only the liquid phase that is entrapped in the solid crystal matrix is washed away, and no TAG from the solid fraction is removed, particularly at this low temperature. The identification of TAG in the solid fraction was performed with a Waters high-performance liquid chromatograph Model 510, equipped with a differential refractometer Model 410 as the detector (Millipore Corporation, Milford, MA). The TAG were separated on a Merck Lichrosphere RP-18 Column (250 mm × 4 mm, particle size 5 µm) (Darmstadt, Germany). During analysis, the column was maintained at 45°C. The mobile phase was acetone/acetonitrile in a ratio of 63:37 (vol/vol) at a flow rate of 1.0 mL/min. For each

TABLE 2
Analysis of Different Batches of RBD Palm Oil, Clouding Palm Olein, Nonclouding Palm Olein, and Super Palm Olein by High-Performance Liquid Chromatography[a]

	RBD palm oil (batch number)				Clouding palm olein (batch number)				Nonclouding palm olein (batch number)				Super olein
Triacylglycerol	1	2	3	4	1	2	3	4	1	2	3	4	
PPP	5.63	5.59	5.97	5.63	0.47	0.53	0.49	0.52	0.32	0.24	0.33	0.23	n.d.
MPP	0.47	0.51	0.60	0.50	0.18	0.19	0.19	0.18	0.13	0.12	0.11	0.10	n.d.
PPS	1.12	1.07	1.15	1.09	0.15	0.14	0.15	0.14	0.08	n.d.	n.d.	0.06	n.d.
PSS	0.60	0.60	0.63	0.60	0.61	0.64	0.59	0.64	0.64	0.63	0.63	0.64	0.51
LLO	0.31	0.37	0.35	0.36	0.53	0.53	0.51	0.50	0.54	0.49	0.39	0.52	0.62
PLL	2.21	1.95	1.43	1.93	3.06	2.90	2.90	2.91	3.03	2.88	2.62	2.99	3.58
MLP	0.59	0.31	0.33	0.31	0.76	0.66	0.73	0.71	0.78	0.75	0.57	0/71	0.86
OLO	1.85	1.84	1.73	1.81	1.82	1.89	1.84	2.00	1.94	1.94	1.98	1.94	2.31
PLO	10.57	10.48	10.36	10.49	11.81	11.76	11.42	11.38	11.77	11.66	11.66	11.90	13.88
PLP	10.11	10.07	9.94	10.04	11.20	11.27	10.92	10.85	11.24	10.98	10.79	10.97	11.60
OOO	3.97	4.13	4.11	4.10	4.02	4.09	4.13	4.29	4.15	4.36	4.39	4.26	4.83
POO	24.31	24.65	24.71	24.65	25.79	26.05	26.21	26.68	26.31	26.96	26.95	26.49	31.06
POP	31.22	31.26	31.78	31.31	31.34	31.49	31.32	31.21	31.08	31.00	31.22	31.08	23.51
SOO	2.07	2.09	1.97	2.05	2.68	2.34	2.77	2.50	2.47	2.57	2.75	2.57	3.05
POS	4.89	4.91	4.86	4.95	5.74	5.34	5.68	5.32	5.37	5.22	5.23	5.36	3.47
SOS	0.15	0.17	0.09	0.18	0.17	0.16	0.18	0.18	0.15	0.20	0.19	0.18	0.23

[a]Abbreviation: n.d., not detected. For other abbreviations see Table 1.

analysis, the sample was first diluted with acetone to form a 5% (w/w) solution, and 5 μL of this solution was injected onto the column.

RESULTS AND DISCUSSION

As the oil sample was cooled from 30°C at a rate of 1°C/min, minute white spots began to appear in the liquid at 29°C. On further cooling, a cloud formed at about 28.5°C, which signaled the nucleation stage. Accordingly, the temperature interval used for sampling the RBD oil covered the range of nuclei formation to crystal growth. The chromatograms for the different solid fractions, taken at various temperatures, are given in Figure 1. The relative concentration of the different TAG components changes as the temperature is reduced, indicating the significance of the presence of the different TAG

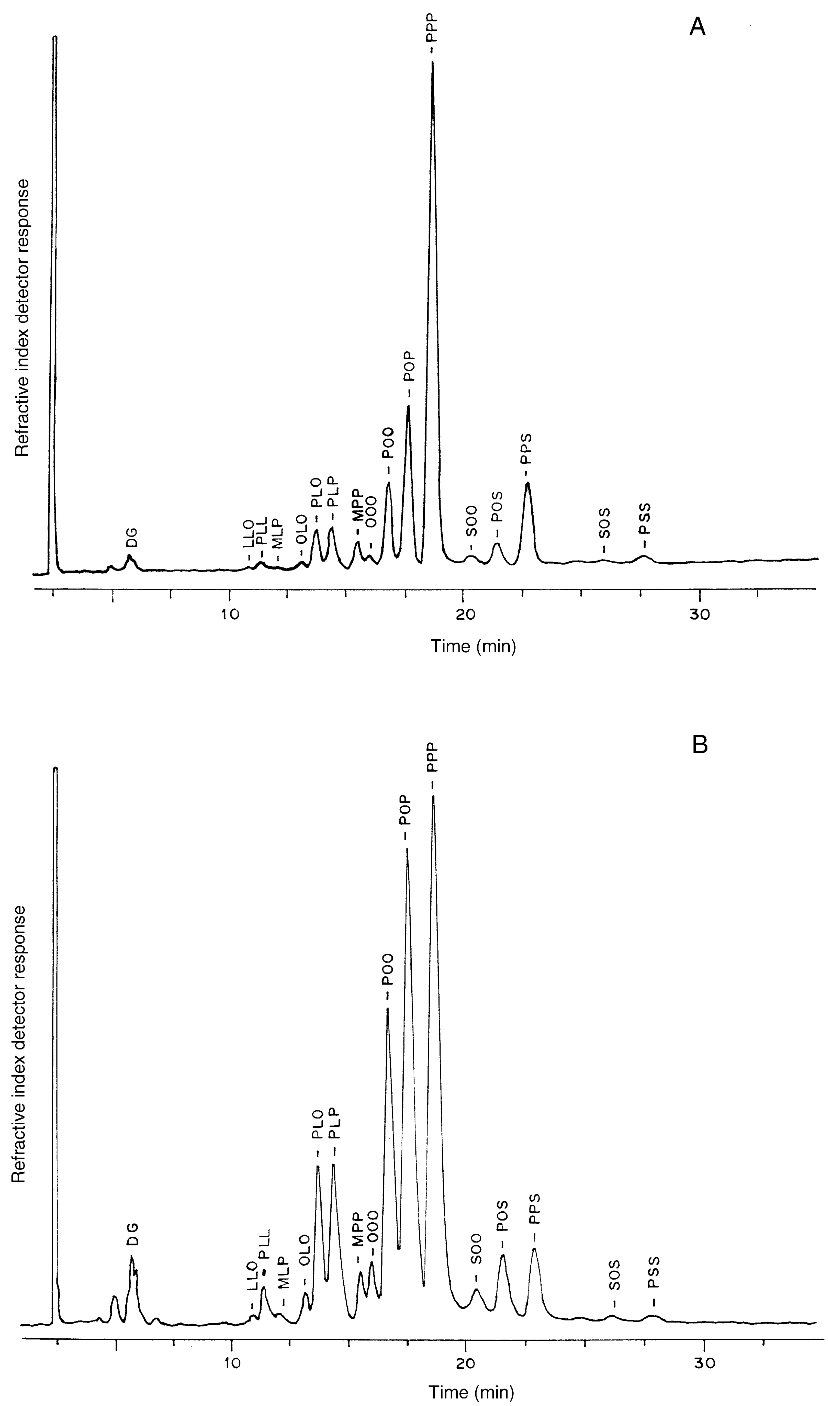

FIG. 1. High-performance liquid chromatograms of the solid phase at different stages of crystal formation: (A) 29°C; (B) 27°C; (C) 25°C; (D) 24°C. For abbreviations and names see Table 1.

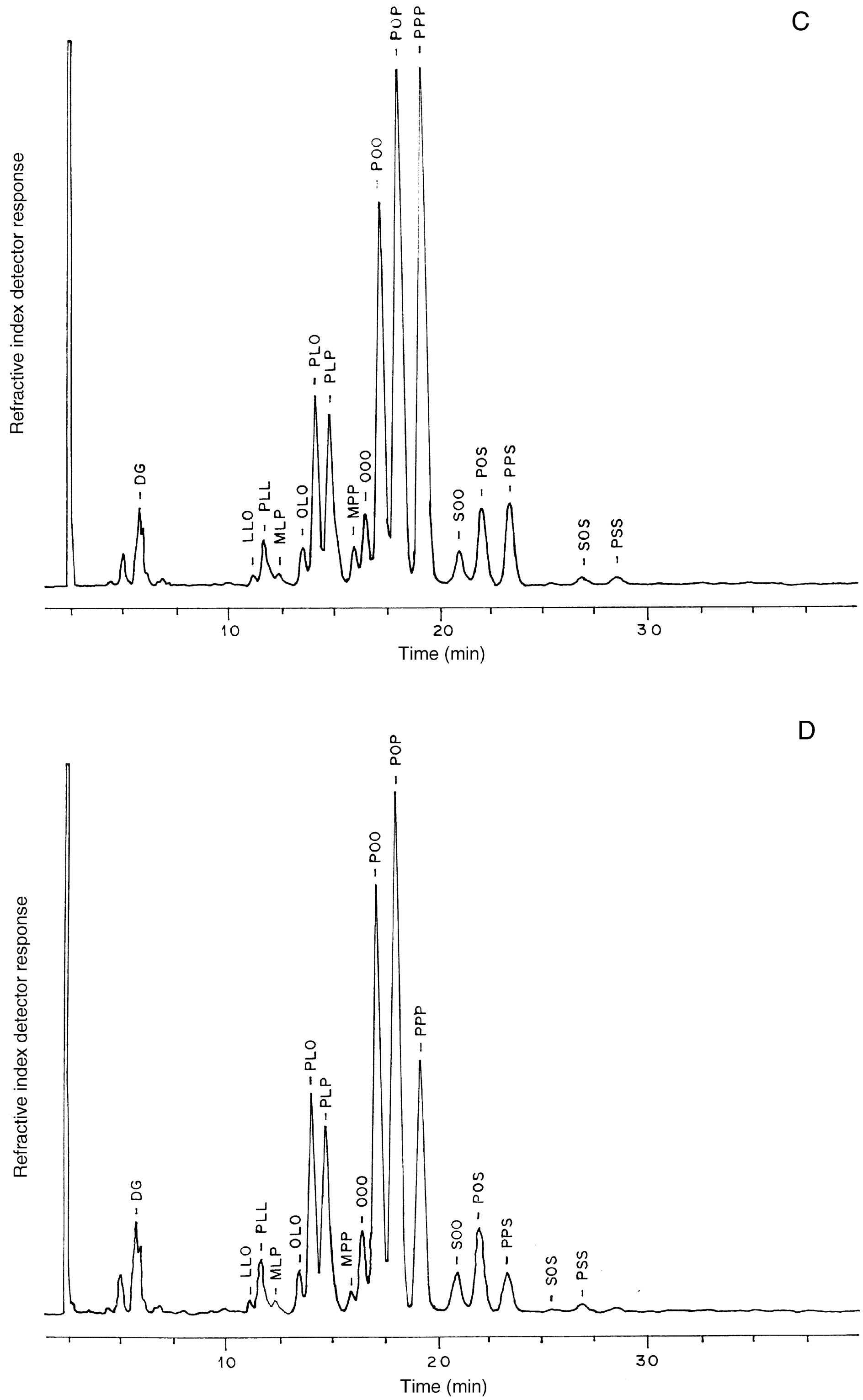

FIG. 1. (continued)

species from nucleation up to crystal growth. Detailed analyses of these chromatograms showed that the concentration of three saturated triglycerides, namely; tripalmitin (PPP), dipalmitoyl-myristoyl-glycerol (MPP) and dipalmitoyl-stearoyl-glycerol (PPS), were higher in the solid crystal than in the mother liquor. Figure 2 shows plots of percentage concentration of TAG in the crystal relative to the starting RBD palm oil against temperature. The relative concentrations of these three components continue to decrease with decreasing temperature. At the nucleation stage, PPP showed the highest

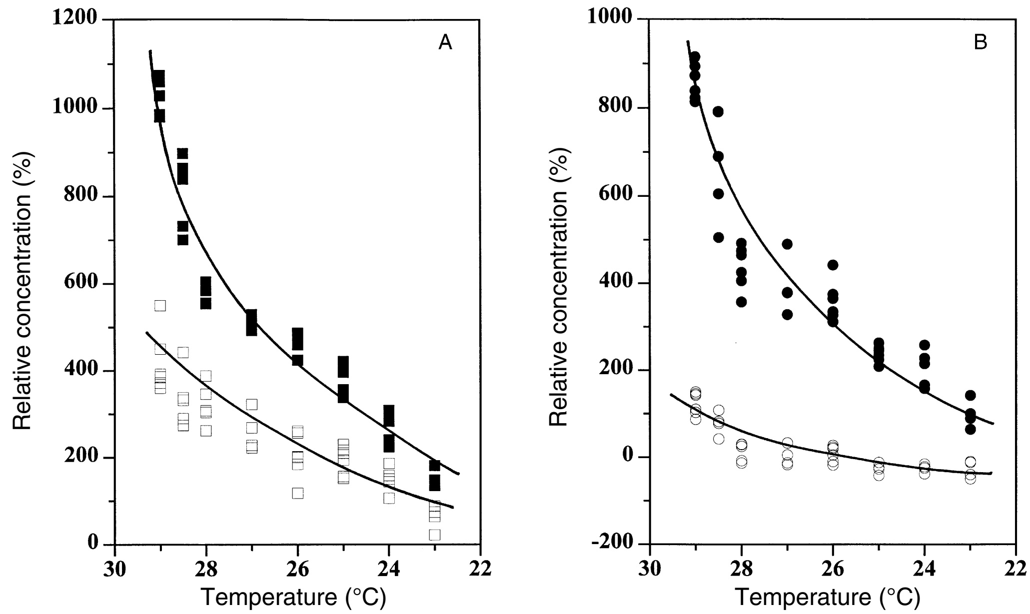

FIG. 2. Relative concentrations of the saturated triglycerides at different stages of crystal formation: tripalmitin (■), dipalmitoyl-stearoyl-glycerol (□), dipalmitoyl-myristoyl-glycerol (●), distearoyl-palmitoyl-glycerol (○).

relative increase (1073%), compared with the starting amount in the palm oil, followed by PPS (873%) and MPP (360%). The concentration of these components in the final crystal, however, was similar to that of palm oil, an increase of only about 30%. The higher concentration of these components in the solid crystals, especially at the point of clouding, can be attributed to their importance in nucleation. As the nuclei form, other TAG components can attach themselves to the nuclei during the growth step, as shown by the decreasing percentage of PPP, MPP, and PPS with temperature. It can be argued that the higher concentration of these TAG in the solid crystal is expected because of the high melting points of 67, 62.9 and 59°C, respectively, for PPP, PPS, and MPP. However, this argument can be ruled out because the concentration of another saturated triglyceride, distearoyl-palmitoyl-glycerol (PSS), which has a melting point of 66°C, remained relatively constant in both nuclei and completely formed crystals. As shown in Figure 2, this value was close to that in the palm oil. This observation supports further the conclusion regarding the importance of the former TAG for the nucleation stage of the crystallization process.

As indicated earlier, experiments were conducted with samples of RBD palm oil and not with palm olein that showed clouding within 24 h. There are several reasons why this approach was used. First, the crystallization behavior of this palm olein sample was similar to that of the RBD palm oil. Accordingly, the observed results from the latter should parallel the behavior of the olein sample. Second, the concentrations of several TAG in palm olein are relatively low. Thus it is difficult to draw conclusions with confidence from this material; the errors in the analysis may be large enough to create variations in the observed trends. Table 2 also compares the composition of the different TAG in a palm olein fraction that clouds with one that does not cloud. We see that the concentrations of the TAG PPP, MPP, and PPS are lower in the olein fraction that did not cloud. Typically, for nonclouding olein, PPP is about 50% and MPP about 60% lower than that found in the clouding olein sample. PPS is almost nonexistent in the nonclouding sample. This observation is in agreement with the foregoing hypothesis that the presence of these components, even in small amounts, enhances the crystallization process and therefore clouding of palm olein. To substantiate this conclusion, an analysis of a super olein sample (SOL) with IV 60 is also included in Table 2. The cloud point for this sample, as determined by the AOCS test method Cc6-25 (10), is 5°C compared to 9.2 and 8.7°C, respectively, for the clouding and nonclouding olein samples. As expected, the higher content of unsaturated TAG, such as dioleoyl-palmitoyl-glycerol (POO), in the SOL (31% as compared to about 26% in the other nonclouding sample) improves its resistance to clouding. Moreover, as shown in Table 2, the absence of the three saturated TAG mentioned earlier also enhanced its stability against clouding.

The observations made in this work suggest that the nucleation of palm olein during prolonged storage is different from the nucleation of palm olein that occurs within 24 h. Swe *et al.* (9), who investigated clouding during prolonged storage at 12.5°C, suggested that one glyceride species contributing to nucleation was the diglyceride 1,3-dipalmitin. They came to this conclusion by observing the presence of a significant amount of this diglyceride in the olein fraction after clouding occurred. The diglyceride was not detected in the mother liquor prior to storage. During prolonged storage and exposure to light, triglycerides would hydrolyze to form fatty acids and diglycerides, including 1,3-dipalmitin. The typical concentration of dipalmitin in RBD palm oil is less than 0.5%. No explanation was given by the investigators on the role of the diglyceride in clouding. As shown in Figure 1, the diglyceride (identified as DG on chromatogram) also was detected in the crystals that formed in our clouding palm oil. However, its relative concentration increased from nucleation up to the crystal growth stage, as did the TAG components other than the three saturated TAG, that is, PPP, MPP and PPS. Thus it is apparent that the diglyceride is not responsible for the nucleation in this particular case.

The source of PPP, MPP, and PPS in the olein fraction that was fractionated from RBD palm oil is not clear. However, we think that the processing steps may have contaminated the sample with these TAG. In usual practice, products from dry fractionation are passed through a membrane filter to separate crystals that have formed. A fraction of the olein flow is reversed at times to clean the membrane from accumulated crystals. It is likely that, during this process, the olein becomes contaminated with the crystals, which explains the presence of the three saturated triglycerides that are identified as the source of nuclei formation.

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REFERENCES

1. Kellens, M., New Development in the Fractionation of Palm Oil, *Proceedings of PORIM International Palm Oil Congress,* Palm Oil Research Institute of Malaysia, Bangi, Selangor, 1993, pp. 128–140.
2. Badings, H.T., J.E. Schaap, C. DeJong, and H.G. Hagerdoorn, An Analytical Study of Fractions Obtained by Stepwise Cooling of Melted Milk Fat, *Milchwissenschaft. 38*:150–156 (1983).
3. Grall, D.S., and R.W. Hartel, Kinetics of Butter Crystallization, *J. Am. Oil Chem. Soc. 69*:741–747 (1992).
4. Taylor, A.M., The Crystallisation and Dry Fractionation of Malaysian Palm Oil, *Oleagineux 3*:73–79 (1976).
5. Amer, M.A., D.B. Kupranycz, and B.E. Baker, Physical and Chemical Characteristics of Butterfat Fractions Obtained by Crystallization from Molten Fat, *J. Am. Oil Chem. Soc. 62*:1551–1557 (1985).
6. Deffense, E., Fractionation of Palm Oil, *Ibid. 62*:376–386 (1985).
7. Kawamura, K., The DSC Thermal Analysis of Crystallization Behavior in Palm Oil, *Ibid. 56*:753–758 (1979).
8. Watanabe, A., I. Tashima, N. Matsuzaki, J. Kurashige, and K. Sato, The Formation of Granular Crystals in Fat Blends Containing Palm Oil, *Ibid. 69*:1077–1080 (1992).
9. Swe, P.Z., Y.B. Che Man, H.M. Ghazali, and L.S. Wei, Identification of Major Triglycerides Causing the Clouding of Palm Olein, *Ibid. 71*:1141–1144 (1994).
10. *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 4th edn., American Oil Chemists' Society, Champaign, 1989, Cc6-25.

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