Composition of Crystals of Palm Olein Formed at Room Temperature

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ABSTRACT: The composition of purified palm olein crystals formed at room temperature (25°C) was identified in this study. Two peaks were obtained when the crystals were analyzed by reverse-phase high-performance liquid chromatography (RP-HPLC). The retention times of these peaks suggested that they were not triglycerides. Gas–liquid chromatography of fatty acid methyl ester analysis of the crystals showed the presence of C16, C18:0, and C18:1 fatty acids. Further analysis by gas–liquid chromatography of carbon number, after collecting the fractions from RP–HPLC, concluded that the major peak A was 1,3-dipalmito-glycerol. The minor peak B was tentatively identified as 1-palmito-3-stearo-glycerol and/or 1-palmito-3-oleo-glycerol due to unavailability of respective standard glycerides. The differential scanning calorimetry thermogram of the crystals show that A and B were indeed the high melting glycerides, with melting and crystallization points of 70.4 and 53.8°C, respectively. *JAOCS 72,* 343–347 (1995).

KEY WORDS: Crystals, 1,3-dipalmito-glycerol, HMGs, 1-palmito-3-oleo-glycerol, 1-palmito-3-stearo-glycerol, palm olein.

The unique crystallization behavior of palm oil is caused by the composition of the triglycerides and related compounds, e.g., fatty acids and partial glycerides (1). However, the composition of palm olein crystals was somehow overlooked. Refined, bleached, and deodorized (RBD) palm olein crystallizes when stored at room temperature (about 25°C). Removal of the crystals may be possible if their identity or composition is known. A number of researchers have studied the crystallization behavior of palm oil based on the polymorphism of glycerides (1–4) and nucleation of triglyceride crystals (5,6). Okiy (4) conducted an investigation on the interaction of triglycerides and diglycerides of palm oil during crystallization and has shown that diglycerides have a deleterious effect on the quality of palm oil when used in situations where palm oil has to crystallize. However, Ng (6), who conducted a study on the kinetics of nucleation in a palm oil melt, found that, as the hard components from the palm oil are removed, the melting point shifts toward low temperature, suggesting that crystallization is due to the early nucleation of certain high-melting glycerides (HMGs). This study was conducted to identify the composition of the palm olein crystals formed at room temperature.

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MATERIALS AND METHODS

Malaysian unblended RBD palm olein (*Elaeis guineensis* var. tenera) was used. All chemicals used were either of analytical or high-performance liquid chromatographic (HPLC) grade. Standard methyl esters, diglycerides, and triglycerides were purchased from Sigma Chemical Co. (St. Louis, MO).

Consumer packs of RBD palm olein that contain crystals were purchased from a local store. The crystals were separated from the mother oil by centrifugation at 2,500 rpm for 2 min at room temperature. The crystals were then washed with hexane at a ratio of 1:2 (wt/vol) and centrifuged again at 2500 rpm for 2 min. Washing was done five times to remove any traces of the oil from the crystals.

The test methods used in this study were gas–liquid chromatography (GLC) for the analyses of triglyceride carbon number (CN) (7) and fatty acid methyl esters (FAME) (7) as used by the Palm Oil Research Institute of Malaysia (Kuala Lumpur, Malaysia) (8) and reverse-phase HPLC (RP-HPLC) for glyceride analysis. A Pye Unicam (Cambridge, United Kingdom) Series 204 gas chromatography system, equipped with flame-ionization detector (FID) and Hewlett-Packard (Palo Alto, CA) data integrator (Model 3380A) was used for both CN and FAME analyses. For CN analysis, the stationary phase was 3% OV-1 on Gas Chrom Q, 100/120 mesh size (Supelco, Bellefonte, PA), packed in a glass column (0.5 m × 30 mm i.d.). The nitrogen carrier gas flow rate was set at 80 mL/min, the detector and injector temperatures were at 400°C, and the oven temperature was programmed from 280 to 350°C at a rate of 5°C/min. For FAME analysis, a glass column (1.8 m × 2.5 mm i.d.), packed with 10% SP 2330 on 100:120 Supelcoport (Supelco), was used. The nitrogen carrier gas flow rate was set at 40 mL/min, the detector and injector temperatures were at 220°C and the isothermal column temperature was at 190°C. The RP-HPLC method used to determine the triglyceride composition was modified from Dong's and Dicesare's method (9). The HPLC system used was equipped with a Waters (Milford, MA) HPLC pump

(Model 501), Waters differential refractometer (Model 410), Waters system interface module, a column oven, a computer, and a printer. A single commercially packed (250 mm × 4.0 mm) RP-18 (Merck, Darmstadt, Germany) column with a particle size of 5 μm was used. The mobile phase was a mixture of acetone/acetonitrile (63.5:36.5), and the flow rate was 1 mL/min. The injection volume was 10 μL. Glycerides fractions were collected according to their retention times prior to GLC analyses. All the analyses were done in replicates of four, and the results are expressed as mean weight percentages. Peaks were identified by injecting standard triglycerides and diglycerides individually and by matching retention time.

A Perkin-Elmer Model DSC-7 differential scanning calorimeter (DSC) (Norwalk, CT) was used for the thermal analysis of the crystals. An empty aluminum sample pan sealed with a lid was used as the reference. Samples of approximately 1.5 mg were sealed in sample pans and placed in the instrument's sample chamber. The temperature was programmed from 30 to 80°C at a rate of 10°C/min for heating and −10°C/min for cooling. Before cooling, the sample was first melted in the DSC by commencing the temperature program and held at 80°C for more than 15 min to destroy crystal nuclei before each scan.

RESULTS AND DISCUSSION

The RP-HPLC chromatograms of typical palm olein (control), concentrated crystals in oil and purified crystals are shown in Figures 1, 2, and 3, respectively. Figures 1 and 2 show that there were changes in the height of peaks A, B, palmitic–oleic–oleic (POO) and palmitic–oleic–palmitic (POP). The concentrations of A, B, and POP were greater,

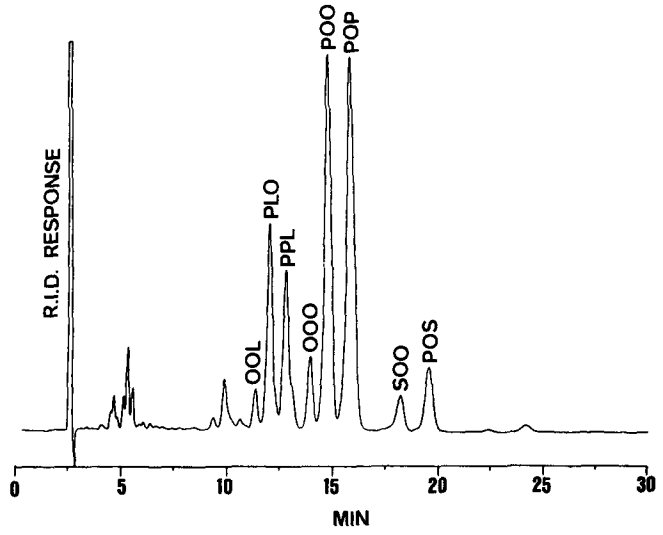

FIG. 1. Reverse-phase high-performance liquid chromatography chromatogram of typical palm olein: POO, palmitic–oleic–oleic; POP, palmitic–oleic–palmitic; OOL, oleic–oleic–linoleic; PLO, palmitic–linoleic–oleic; PPL, palmitic–palmitic–linoleic; OOO, oleic–oleic–oleic. SOO, stearic–oleic–oleic; POS, palmitic–oleic–stearic; R.I.D., refractive index detector.

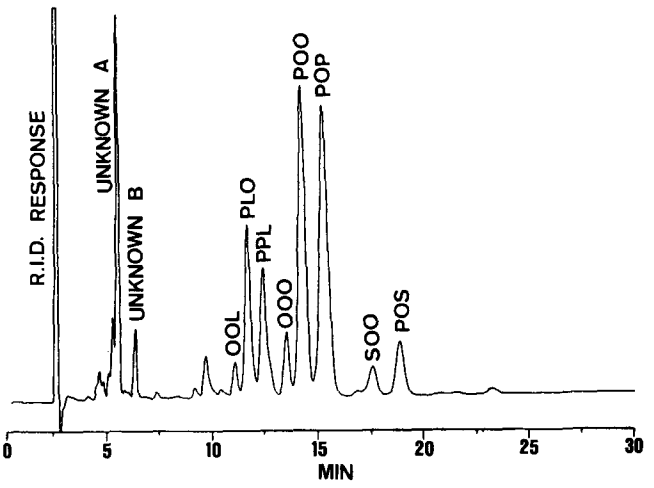

FIG. 2. Reverse-phase high-performance liquid chromatography chromatogram of concentrated palm olein crystals. See Figure 1 for abbreviations.

while POO was less, than those present in the mother oil. The presence of triglycerides other than peaks A and B was due to the presence of residual palm olein in the concentrated crystals. After the crystals were further purified by washing with hexane, there were only two major peaks, A and B, present (Fig. 3). It also shows that the retention times of these peaks were much shorter, compared to those of triglycerides of palm olein, which suggested that A and B are probably diglycerides. The relative concentrations of A and B were 83.65 ± 2.26% and 16.35 ± 2.26%, respectively.

After a series of spike tests were carried out on HPLC with standard glycerides, such as 1,2-dipalmito glycerol (Fig. 4), 1,3-dipalmito glycerol (Fig. 5), tripalmito glycerol (Fig. 6), and 1,2-dilauro-3-myristo glycerol (Fig. 7), only 1,3-dipalmito-glycerol boosted the height of peak A. From this, it was deduced that A is 1,3-dipalmitin. This finding was sup-

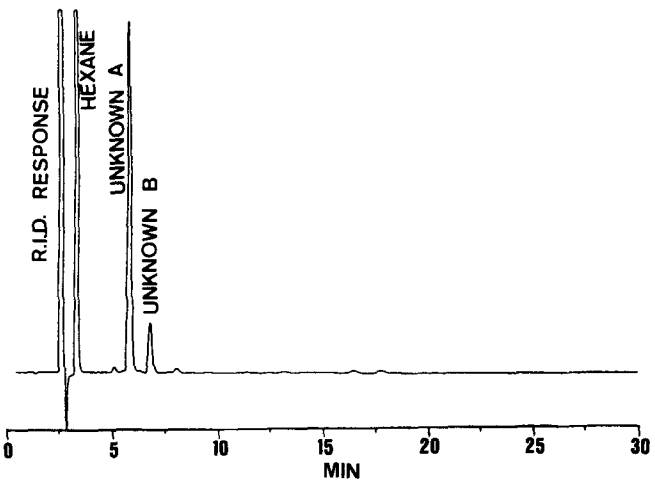

FIG. 3. Reverse-phase high-performance liquid chromatography chromatogram of purified palm olein crystals. See Figure 1 for abbreviation.

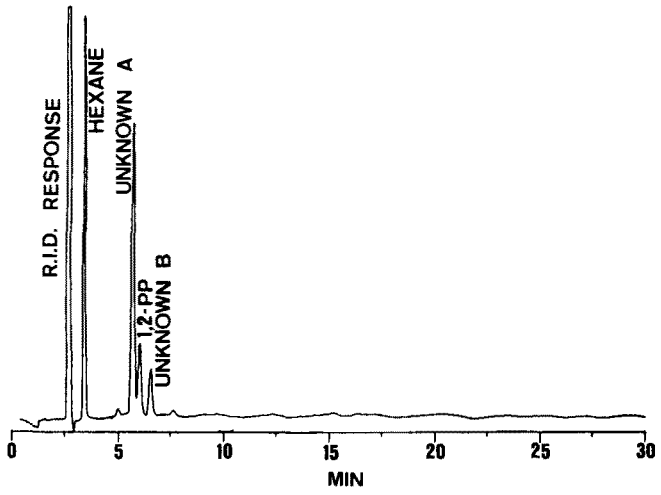

FIG. 4. Spike Test 1: 1,2-dipalmitin (1,2-PP) was added to purified crystals. See Figure 1 for abbreviation.

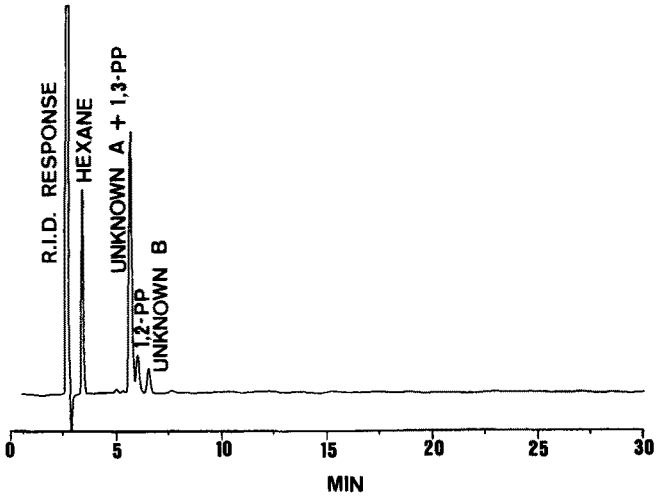

FIG. 5. Spike Test 2: 1,3-dipalmitin (1,3-PP) was added to purified crystals. See Figures 1 and 4 for abbreviations.

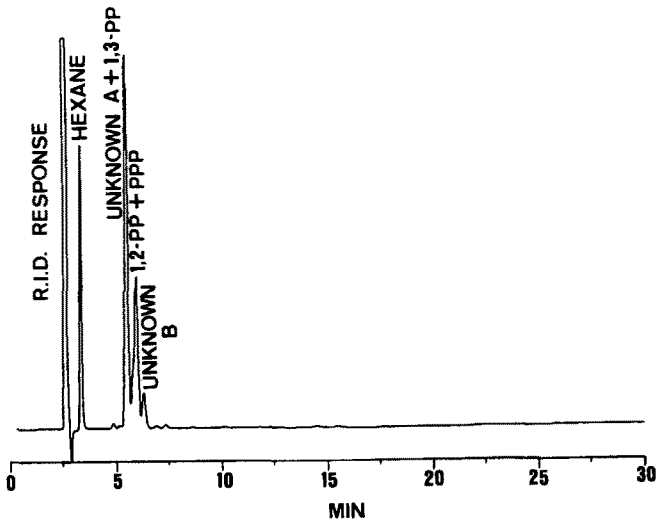

FIG. 6. Spike Test 3: tripalmitin (PPP) was added to purified crystals. See Figures 1, 4, and 5 for abbreviations.

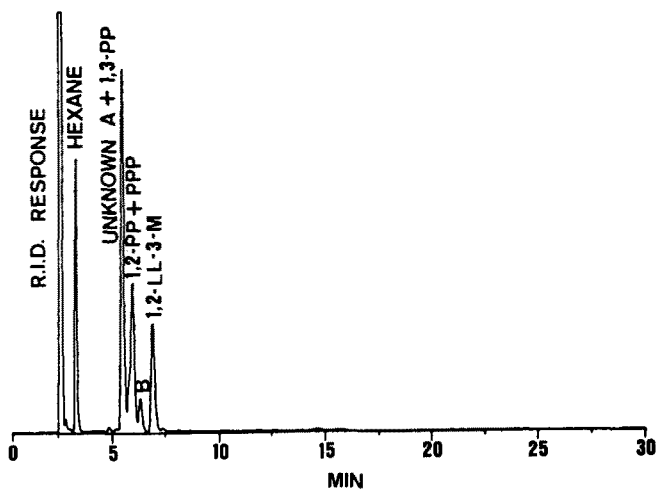

FIG. 7. Spike Test 4: 1,2-dilaurin (LL), 3-myristin (3-M) was added to purified crystals. See Figures 1, 4, 5, and 6 for abbreviations.

ported by the fact that the diglyceride could not be dissolved in hexane due to its poor solubility (10). However, B could not be identified due to unavailability of standards.

The purified crystals were further analyzed by GLC for their fatty acid composition. Figures 8 and 9 show the fatty acid profile of palm olein (control) and crystals, respectively. The crystals were composed of palmitic (P) acid 89.90 ± 1.08%, oleic (O) acid 7.13 ± 0.47%, and stearic (S) acid 3.17 ± 1.35%. These findings suggest that the crystals are composed mainly of the diglycerides of palmitic–palmitic with

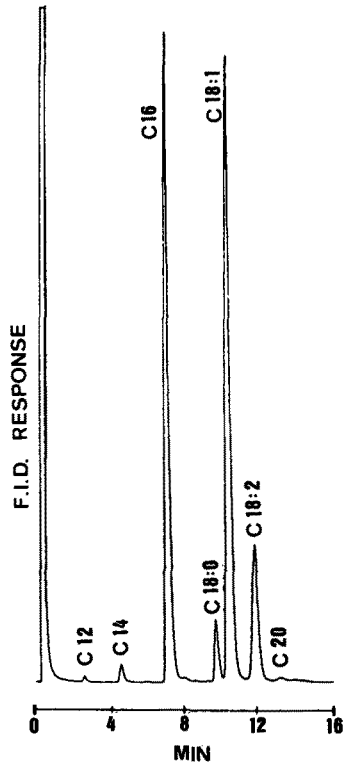

FIG. 8. Fatty acid profile of typical palm olein. F.I.D., flame-ionization detection. C12, lauric; C14, myristic; C16, palmitic; C18:0, stearic; C18:1, oleic; C18:2, linoleic.

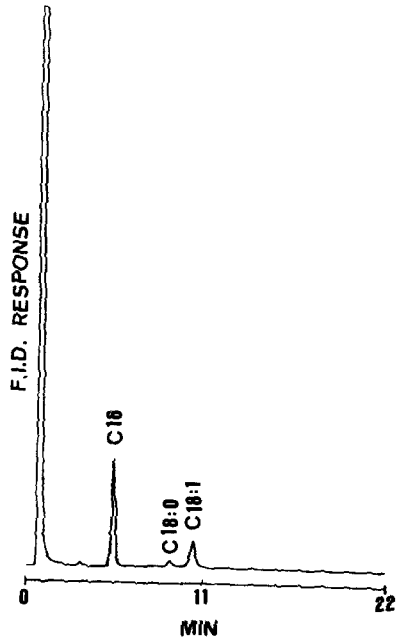

FIG. 9. Fatty acid profile of purified palm olein crystals. See Figure 8 for abbreviations.

small amounts of palmitic–stearic and palmitic–oleic acid combinations. Glyceride A and B fractions were further collected to check for purity by HPLC according to their retention times, and then they were rechromatographed (Figs. 10 and 11). Each fraction was then analyzed on GLC for their CN. A had a retention time between that of C34 and C36 (Fig. 12), using palm kernel olein as secondary standard to provide a range of CN peaks of the triglycerides. This finding shows that A was not a triglyceride. B could not be subjected to CN analysis because the fraction collected was insufficient in size. From the evidence obtained, one can conclude that crystals of palm olein are mainly composed of 1,3-dipalmitoglycerol (83.65 ± 2.26%). Also, based on fatty acid composition of C16:0, C18:0, and C18:1 for the crystals by GLC–FAME, that minor peak B could be composed of 1-palmito-3-stearo-glycerol and/or 1-palmito-3-oleo-glycerol (16.35 ± 2.26%).

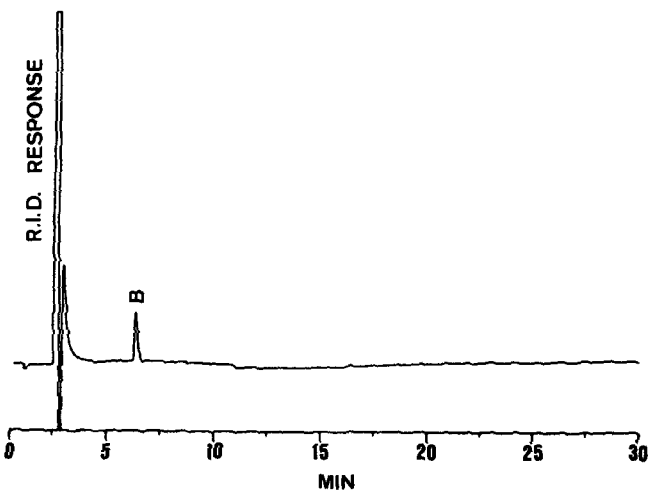

FIG. 11. Fraction collected for unknown B: rechromatographed to check the purity. See Figure 1 for abbreviation.

The DSC melting curve of the palm olein crystals (Fig. 13) showed a single sharp endothermic peak, starting at 62.5°C, with a maximum at 67.9°C. The samples completely melted at 70.4°C with a heat of fusion of 129.84 J/g. The melting point of the crystals matched the β-a polymorph of Shannon *et al.* (11) and were nearest to that of Howe and Malkin (12) (Table 1). According to Shannon *et al.* (11), the β-a polymorph was transformed into the thermodynamically stable β-b polymorph near its melting point. The β-b form could also be directly formed by crystallization at higher temperatures and from less polar solvents relative to the conditions applied in the production of the β-a polymorph.

In the crystallization process, crystals were probably in the β-a polymorphic form due to the recrystallization from the

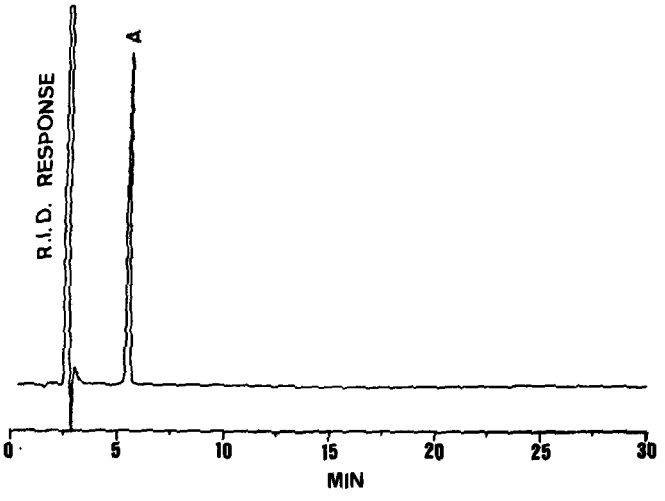

FIG. 10. Fraction collected for unknown A: rechromatographed to check the purity. See Figure 1 for abbreviations.

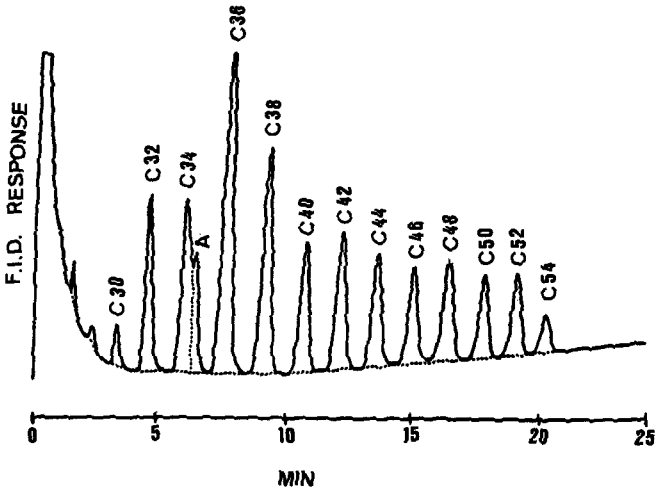

FIG. 12. Carbon number chromatogram of Fraction A from palm olein crystals added to palm kernel olein. See Figure 8 for abbreviation.

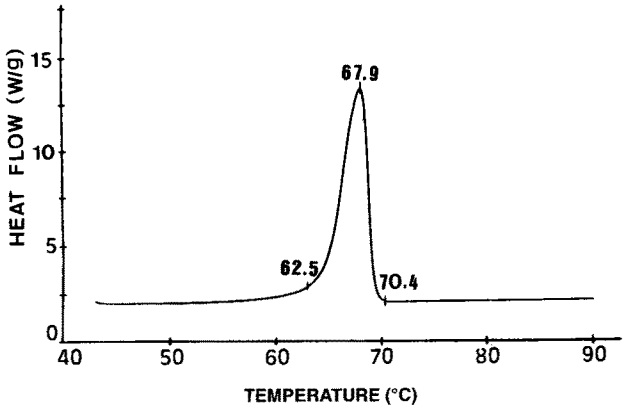

FIG. 13. Differential scanning calorimetry melting curve of purified palm olein crystals.

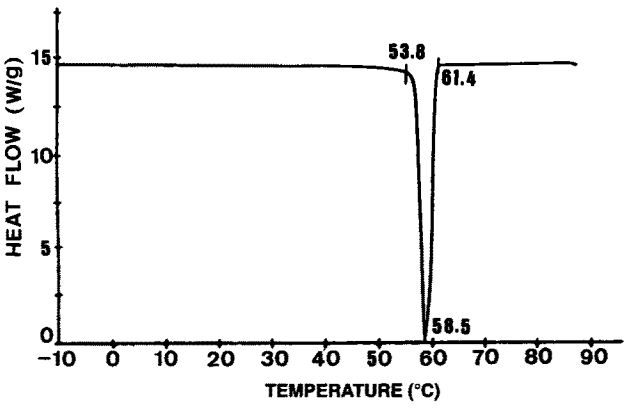

FIG. 14. Differential scanning calorimetry crystallization curve of purified palm olein crystals.

TABLE 1
Comparison of Melting Points (°C) of 1,3-Dipalmitin from the Literature and the Crystals from This Study

	Polymorphic form	
	β-a	β-b
Baur *et al.* (1949) (Ref. 13)	71.8	72.9
Crowe and Smyth (1950) (Ref. 14)	71.7	72.8
Howe and Malkin (1951) (Ref. 12)	71.5	72.5
Shannon *et al.* (1992) (Ref. 11)	70.0	72.0
This study	70.4	

complete melt. According to Baur *et al.* (13), 1,3-dipalmitin has a β-a polymorph when it is crystallized from the melt. It is also probable that crystals are in the β-b form due to the higher temperature (room temperature) used in the crystallization process. However, along the purification process, hexane solvent was used to separate the crystals from oil. Due to the use of solvent, β-a and β-b produced a single peak. When both β-a and β-b were crystallized from a solvent, they both were reported to have the same melting point (11). However, if the β-a polymorph was alternatively formed by solidification of the melt, it was found to melt about 2°C below the melting temperature of the corresponding β-b polymorphic form. This latter supposition was confirmed earlier by Crowe and Smyth (14), but Crossley *et al.* (15) reported that 1,3-distearin, crystallized from the melt and from solvent, melt at the same temperature.

Figure 14 shows the crystallization curve of palm olein crystals. Only one sharp exothermic peak was observed at 58.5°C, which indicates rapid crystal formation due to spontaneous crystallization of the sample. The crystallization occurred at 61.4°C, and complete crystal formation was observed at 53.8°C. The sample crystallized at a heat of crystallization of −129.24 J/g. The nature of the single sharp endothermic and exothermic peaks reflect the high purity of the experimental samples. The DSC findings show that the crystals were made up of HMGs. Due to their high-melting nature, these components in palm olein crystallize during storage at room temperature.

REFERENCES

1. Jacobsberg, B., and O.C. Ho, *J. Am. Oil Chem. Soc. 53*:609 (1976).
2. Kawamura, K., *Ibid. 56*:753 (1979).
3. Kawamura, K., *Ibid. 57*:48 (1980).
4. Okiy, D.A., *Oleagineux 33*:625 (1978).
5. van Putte, K.P.A.M., and B.H. Bakker, *J. Am. Oil Chem. Soc. 64*:1138 (1987).
6. Ng, W.L., *Ibid. 67*:879 (1990).
7. *IUPAC Standard Methods for the Analysis of Oils, Fats and Derivatives*, 6th edn., Pergamon Press, New York, 1979.
8. PORIM (Palm Oil Institute of Malaysia), *PORIM Test Methods*, Ministry of Primary Industries, Malaysia, 1988.
9. Dong, M.W., and J.L. Dicesare, *J. Am. Oil Chem. Soc. 60*:788 (1983).
10. Timms, R.E., R.J. Hamilton and K.G. Berger, *Ibid. 58*:1 (1981).
11. Shannon, R.J., J. Fenerty and R.J. Hamilton, *J. Sci. Food Agric. 60*:405 (1992).
12. Howe, R.J., and T. Malkin, *J. Chem. Soc.*, 2663 (1951).
13. Baur, F.J., F.L. Jackson, D.G. Kolp and E.S. Lutton, *J. Sci. Food Agric. 71*:3363 (1949).
14. Crowe, R.W., and C.P. Smyth, *J. Am. Chem. Soc. 72*:5281 (1950).
15. Crossley, A., I.P. Freeman, B.J.F. Hudson and J.P. Pierce, *J. Chem. Soc.*, 760 (1959).

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