Partition Coefficient of Diglycerides in Crystallization of Palm Oil

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ABSTRACT: Crystallization of palm oil with and without solvent was carried out over a temperature range of 10–25°C. The yields of olein, the diglyceride contents, and compositions of the stearin and olein phases were determined by thin-layer and gas–liquid chromatography. The three major diglycerides, analyzed as C_{32}, C_{34}, and C_{36}, are mainly dipalmitoyl glycerol, palmitoyloleoyl glycerol, and dioleoyl glycerol. In crystallization without solvent, C_{32} (PP) had a strong affinity for the stearin fraction and C_{36} diglycerides concentrated in the olein phase. The partition coefficient of diglycerides between the olein and stearin phases was temperature-dependent and was influenced by the type of solvent used. Although solvent enhances the diglyceride partition into the olein phase, partitioning is more effective at low temperatures and with acetone as the solvent for fractionation.
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KEY WORDS: Diglycerides, dry fractionation, fractionation, olein, palm oil, partition coefficient, solvent fractionation, stearin, winterization.

Palm oil is a complex mixture of glycerides but can be fractionated into solid stearin and liquid olein fractions of various compositions. The solid fraction consists mainly of high-melting triglycerides, whereas the liquid fraction is dominated by low-melting components. The three main processes being used currently in the palm oil industry are dry (winterization), detergent, and solvent fractionations. The stearin crystals are separated from the liquid olein by means of conventional filtration. In the detergent process, surfactants, such as sodium lauryl sulfate, are used to create a suspension of stearin crystals in the aqueous phase. In solvent fractionation, an organic solvent, such as hexane, acetone, or isopropanol, is used to prepare a solution with an appropriate oil concentration.

Palm oil is unique in that it contains a high percentage of diglycerides (averaging 6.3%), which mainly consists of palmitoyloleoyl glycerol (PO), dipalmitoyl glycerol (PP), and dioleoyl glycerol (OO)(1). During crystallization of the oil, the diglycerides may distribute differently between the two phases when in equilibrium.

The presence of diglycerides affects crystallization behavior of the oil (2,3). For instance, Loncin (3) showed that diglycerides can form eutetic mixtures with triglycerides and reduce the proportion of stearin obtained. On the other hand, diglycerides may have a seeding effect and cause crystallization to start earlier than in oils without diglycerides. Diglycerides in palm olein may also be partly responsible for the fine crystalline deposits that may develop during prolonged storage (4).

Thus, it is important to know how diglycerides distribute between the two phases because the presence of diglycerides may affect the properties of the olein and stearin fractions. In this work, we determined the partition coefficient of the diglycerides in palm oil for both dry and solvent fractionations.

MATERIALS AND METHODS

Fractionation. Refined palm oil from Pandamaran Oil Products Sdn. Bhd. (Selangor, Malaysia) was used in the experiments. The fatty acid and triglyceride compositions were determined by standard methods (5,6).

Palm oil (50 g) was melted at 70°C before being cooled at a rate of 0.5°C/min to its desired fractionation temperature with a Haake P20 programmable controller (Karlsruhe, Germany). The sample was held at the fractionation temperature for 18 h to allow for complete crystallization. Phase separation of the liquid olein fraction and stearin crystals was achieved by vacuum filtering in a cold room. Fractionation temperatures spanned the range of 13–25°C.

Palm oil was dissolved in solvent to form a final concentration of 1:1 (wt/vol). Fractionation was then carried out as described previously. The solvents used were hexane and acetone. For hexane solution, the fractionation was carried out at 10–25°C and the temperature range for acetone solution was 10–20°C. After phase separation, the solvent in the olein phase was removed by rotary evaporation, and the olein yield was determined.

Analyses. The diglycerides were analyzed in terms of 1,2 and 1,3 isomers by thin-layer chromatography after the standard procedure described by Amenta (7). Glass plates (20 × 20 cm) were coated with 0.5-mm thick boric acid-impregnated silica gel 60 G (Merck, Darmstadt, Germany). The

plates were air-dried overnight and activated at 110°C for 2 h prior to use. Samples of oil were dissolved in chloroform at 5% concentration. Standard solutions of 1,2 and 1,3 PP at 1% concentration were used for identification and calibration. A calibration graph was prepared with 1, 10, 15, 20, and 25 μL of the standard solution. A 50-μL test solution was applied to the plate, which was then placed in a chamber and chromatographed in chloroform/acetone (96:4, vol/vol). The plates were then dried and placed in a chamber with iodine crystals. When the glyceride bands were clearly seen, the plate was removed, and areas of equal size were drawn around the test components with a pencil. The silica gel within each area was scraped off. Quantitative analysis of the lipids was carried out based on the ability to reduce acid dichromate $Cr_2O_7^{2-}$ to Cr^{3+} (8). The standard deviation of the test (five replicates) was 0.65 for a mean value of 5.3% diglyceride. The test was used to help confirm the 1,2 isomers obtained by the gas–liquid chromatography (GLC) method.

Diglyceride identification and analysis. GLC by a method similar to that described by Bruschweiler *et al.* (9) was used to identify the diglycerides. Samples were derivatized at 80°C with 1 mL of *bis*-trimethyl silyl trifluoroacetamide and 0.5 mL trimethylchlorosilane for 40 min. The derivatized samples (0.5 μL) were injected directly into the column. A Hewlett-Packard (Palo Alto, CA) 5890 Series II gas chromatograph was used. The column was a fused-silica polyimide of 12 m × 0.53 mm i.d. with a 5% phenyl equivalent siloxane-carborane crosslinked stationary phase. Chromatographic conditions were: 360°C flame detector temperature, oven programming from 50 to 300°C at 10°C/min followed by 5°C/min from 300 to 350°C and holding at 350°C for 10 min. The He carrier gas flow rate was 7.30 mL/min. Peak areas and retention times were determined with a Hewlett-Packard Vectra Q5/65 microprocessor.

Melting thermogram by differential scanning calorimetry (DSC). The DSC melting thermogram of the palm oil sample was determined with a Perkin Elmer (Norwalk, CN) 7 instrument. The sample was completely melted at 80°C before being weighed (10 mg) into an aluminum pan, which was then sealed in a sample pan crimper. The previous thermal history of the sample was erased by heating to 80°C in the DSC instrument. The sample was then cooled to −30°C at a rate of 40°C/min. The melting thermogram was determined from −30 to 80°C at a temperature programming rate of 10°C/min.

RESULTS AND DISCUSSION

The palm oil used in this study has an iodine value of 53.0, and palmitic, oleic, and linoleic acids comprised 43.7, 40.6, and 10.5%, respectively. The triglyceride composition consists mainly of C_{50} (42.1%), C_{52} (40.0%), C_{48} (8.1%), and C_{54} (8.5%). These values are typical of palm oil produced in Malaysia (10). When cooled, palm oil yields two products known as olein and stearin. The yield of these products depend upon the type of fractionation process, cooling rates, and

TABLE 1
Diglycerides in Palm Oil Fractions After Dry Fractionation

Temperature (°C)	Yield (%)	Diglyceride (DG) (wt%)				
		C_{32}	C_{34}	C_{36}	1,2 DG	1,3 DG
			Olein fraction			
13	40.1	1.1	5.6	2.1	2.4	6.4
15	50.1	1.3	5.4	2.3	2.4	6.7
16	55.1	1.2	5.4	2.1	2.3	6.4
17	64.5	0.4	4.4	3.1	3.0	5.0
18	67.9	0.4	4.0	3.2	2.1	5.6
20	71.3	0.4	4.0	3.0	2.1	5.3
22	71.0	0.5	4.4	2.9	2.7	5.1
25	73.7	0.6	4.0	3.0	2.8	4.9
SD[a]	1.2	0.02	0.03	0.05	0.04	0.06
			Stearin fraction			
13	59.9	1.7	2.9	1.0	1.1	4.6
15	49.9	2.0	2.3	0.8	1.0	4.0
16	44.3	2.2	2.3	0.7	1.0	4.2
17	35.5	1.5	2.6	1.4	1.6	3.9
18	32.1	1.6	2.4	1.4	1.0	4.5
20	28.7	1.6	2.7	1.6	1.1	4.8
22	29.0	1.6	2.4	1.3	1.1	4.2
25	26.3	1.4	2.5	1.4	1.1	4.2
SD[a]	1.2	0.05	0.04	0.02	0.03	0.08

[a]Based on triplicate fractionation at 20°C

temperature of fractionation (T_f). Tables 1–3 show the olein and stearin yields and the diglyceride contents of each fraction. In general, the yield of olein decreased as the T_f was lowered (Fig. 1). Below a certain temperature, however, a drastic drop in yield was observed. This break in the yield was dependent on the fractionation process used. For instance, in dry fractionation, the break occurred below 18°C. This can be explained by Figure 2, which reveals the presence of two crystallization exotherms of high-melting and low-melting (L_m) crystals. When the T_f falls close to the L_m exotherm, crystallization of the L_m crystals will take place, thereby causing a sudden reduction in olein yield. In the solvent process, a linear relationship was observed throughout the T_f range

TABLE 2
Diglycerides in Palm Oil Fractions After Acetone Fractionation

Temperature (°C)	Yield (%)	Diglyceride (DG) (wt%)				
		C_{32}	C_{34}	C_{36}	1,2 DG	1,3 DG
			Olein fraction			
10	77.8	0.9	4.2	2.7	1.9	6.0
15	80.8	0.9	4.1	2.7	1.8	5.9
18	82.8	0.8	4.2	2.5	1.8	5.7
20	85.4	0.5	4.0	2.9	2.7	4.0
SD[a]	0.54	0.02	0.04	0.03	0.04	0.09
			Stearin fraction			
10	22.2	0.8	2.4	1.4	1.1	3.5
15	19.2	0.7	2.1	1.3	1.2	2.9
18	17.2	0.6	2.1	1.4	1.1	2.9
20	14.6	0.4	2.2	1.8	2.0	2.3
SD[a]	0.43	0.09	0.06	0.06	0.06	0.14

[a]Based on triplicate fractionation at 20°C.

TABLE 3
Diglycerides in Palm Oil Fractions After Hexane Fractionation

Temperature (°C)	Yield (%)	Diglyceride (DG) (wt%)				
		C_{32}	C_{34}	C_{36}	1,2 DG	1,3 DG
		Olein fraction				
10	80.2	0.4	4.0	2.7	1.6	5.5
15	85.6	0.7	3.8	2.6	2.0	5.2
17	86.5	0.8	3.8	2.5	2.0	5.1
19	86.1	0.8	4.0	2.7	1.7	5.8
23	93.8	0.8	3.6	2.6	1.9	5.2
25	94.5	0.8	3.8	2.6	1.9	5.3
SD[a]	0.83	0.03	0.04	0.05	0.03	0.05
		Stearin fraction				
10	19.7	1.7	3.1	1.7	0.6	5.9
15	14.4	1.1	3.1	1.9	0.7	5.3
17	13.5	0.6	1.9	1.2	1.2	2.6
19	13.9	0.6	1.8	1.2	0.6	2.9
23	6.2	0.6	2.6	1.6	1.5	3.3
25	5.5	0.6	2.2	1.4	1.2	3.0
SD	0.66	0.02	0.04	0.03	0.04	0.09

[a]Based on triplicate fractionation at 19°C

studied. Discontinuity in olein yield was reported by other workers (11,12) at lower temperatures, for example, hexane solution at 0°C and acetone solution at 10°C (11).

Partition coefficient (KD) for individual diglycerides. Diglycerides in palm oils were analyzed by GLC in terms of carbon number. The fatty acid compositions of the diglycerides showed that oleic acid (46.6%) and palmitic acid (35.9%) were the main components. Linoleic acid (12.8%) and stearic acid (3.7%) were minor components. Based on that information, C_{32} was mainly composed of PP, whereas C_{34} and C_{36} were mainly composed of PO and OO, respectively. These diglycerides were present in the two phases that were in equilibrium at the completion of crystallization. Diglyceride distribution between the two phases can be described by its partition coefficient, KD, which is defined as the ratio of the concentration of diglycerides in the olein and stearin phases as given in Equation 1:
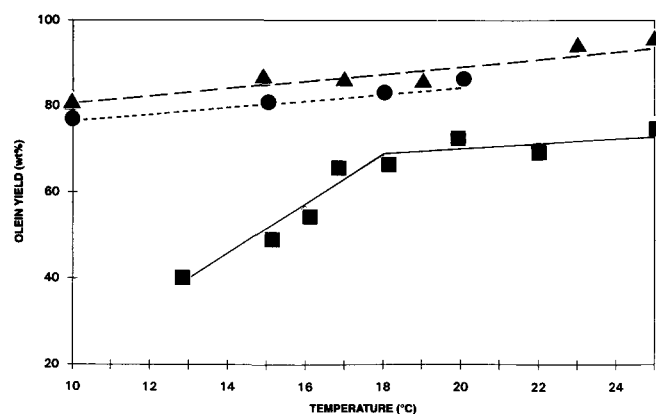
$$KD = [Dg]_o/[Dg]_s \qquad [1]$$

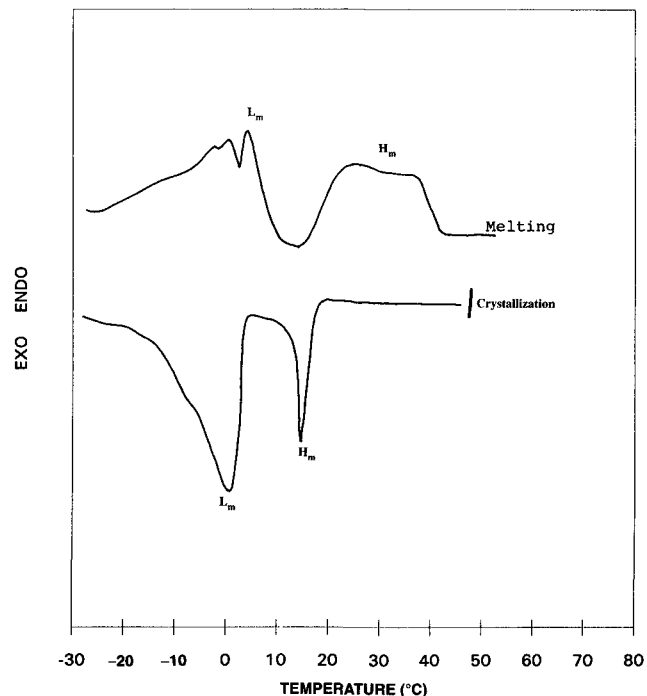**FIG. 1.** Yield of palm olein vs. fractionation temperatures. ▲, Hexane; ●, acetone; and ■, dry.

FIG. 2. Differential scanning calorimetry thermograms of palm oil. L_m, low-melting; H_m, high-melting.

where [Dg] denotes diglyceride concentration, and the o and s denote the olein and the stearin phase, respectively. The relationship between KD and temperature of fractionation is shown from van't Hoff's equation (Ref. 13):

$$\frac{d \ln KD}{d \, 1/T} = \frac{-\Delta H}{R} \qquad [2]$$

where ΔH is the reaction enthalpy at temperature (T).

Tables 1–3 show the diglycerides in palm olein and stearin obtained from dry and from solvent fractionation. The 1,3 isomers are predominant in all samples. C_{34} forms the major diglyceride component, followed by C_{36} and C_{32}. In dry fractionated palm oleins and stearins, C_{34} ranged from 4.0–5.6 and 2.3–2.9%, respectively. In solvent fractionated oleins, the C_{34} component was slightly lower, being 3.6–4.0% in hexane and 4.0–4.2% in acetone. The way the diglycerides partition between the olein and stearin phases is better evaluated in terms of their KD values.

KD values at different temperatures were determined with respect to C_{32}, C_{34}, and C_{36} diacylglycerols for dry and solvent fractionations. The logarithms of KD values against $10^3/T$ are shown in Figure 3 where T is the absolute temperature. The results show that, in general, KD for dry fractionation was lower than that of the solvent process. Among the diglycerides, the value of KD was the lowest for C_{32}, which was below the equipartition line (KD = 1). KD was the high-
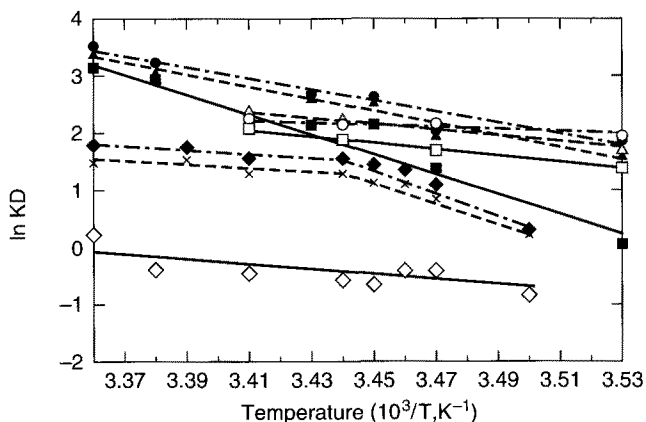
FIG. 3. ln partition coefficient (KD) of component palm oil diglycerides vs. fractionation temperature. Grey square, C_{32} dry; grey triangle, C_{34} dry; grey circle, C_{36} dry; ■, C_{32} hexane; ▲, C_{34} hexane; ●, C_{36} hexane; □, C_{32} acetone; △, C_{34} acetone; and ○, C_{36} acetone.
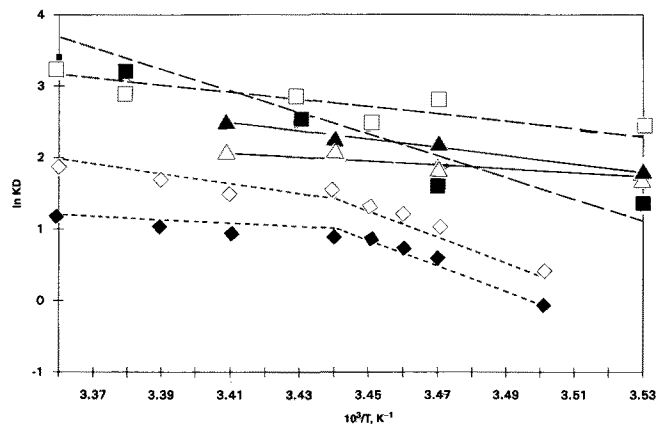

FIG. 4. ln partition coefficient (KD) of 1,2 and 1,3 diglycerides (DG) vs. fractionation temperature. ◆, 1,3 DG (dry); ◇, 1,2 DG (dry); ■, 1,3 DG (hexane); □, 1,2 DG (hexane); grey triangle, 1,3 DG (acetone); and △, 1,2 DG (acetone).

est for C_{36}. This implies that C_{32} had higher affinity for the stearin phase, whereas C_{36} was concentrated in the olein phase.

A solvent changes the preferred distribution of C_{32} from stearin to the olein phase. As for C_{34} and C_{36}, solvent enhances their distributions in the olein fraction to different degrees. From the van't Hoff graphs (Fig. 3), the enthalpies of diglyceride partitioning during solvent fractionation are obtained from the gradients and summarized in Table 4. The values were significantly higher in hexane solution than in acetone. This was true for all diglycerides analyzed. The high enthalpy of partition in hexane solution implied that less diglycerides partition into the olein phase in hexane solution than in acetone solution. The most saturated diglyceride (PP) showed the highest enthalpy. This was followed by C_{34} and C_{36}. Thus, C_{32} showed less tendency to partition into the olein phase than did C_{34} and C_{36}. The differences in the enthalpies of the diglycerides in hexane and acetone solutions were in line with a higher solubility of the diglyceride in acetone. For instance, the relative solubilities of PO and PP at 10°C were 14 and 0.2 in acetone, compared with the exceedingly low values of 0.6 and 0.007 in hexane (14).

Partition coefficients for 1,2 and 1,3 isomers. The logarithms of KD values for 1,2 and 1,3 isomers were plotted against T_f (Fig. 4). As for individual diglycerides, the KD val-

ues for 1,2 and 1,3 isomers were again higher for hexane and acetone solutions than for dry fractionation. These results suggest that the diglycerides have higher affinity for the olein phase. The 1,2 and 1,3 isomers from the dry fractionation process showed preferential partition in favor of the olein phase. This preferred partition was enhanced by the presence of either acetone or hexane. This could well be attributed to increased solubilities as a result of the solvent's presence. However, it was also observed that in dry fractionation, with the exception of C_{32}, all other diglycerides, including the isomers, showed the presence of a kink in the curve at low temperatures. This kink was also observed in Figure 1, where the olein yield suddenly dropped at 18°C.

The KD values for the partitioning of diglycerides between the two phases were temperature-dependent and influenced by the type of solvent used. Apart from enhancing the solubility of the diglycerides, the organic solvent also reduced the viscosity of the oil and, therefore, facilitated better phase separation. This reduced the amount of olein that was retained in the stearin phase. Hexane solution had a higher temperature coefficient in the van't Hoff's plot of ln KD vs. temperature.

In dry fractionation, the 1,2 isomer had a higher preference for the olein phase than the 1,3 isomer. This preferential retention in the olein phase was less significant as temperature was reduced.

Of the three types of diglycerides, C_{32} showed the highest affinity for the stearin phase in dry fractionation, and C_{36} was strongly retained in the olein phase. At low temperatures in hexane solution, there was a higher tendency for the diglycerides to be retained in the stearin phase as compared with acetone solution. This larger temperature coefficient observed in hexane solution implies that the diglycerides can be separated at low temperatures into the stearin phase. More information about the way in which diglycerides partition between the olein and stearin phases will help our understanding of the properties and behavior of palm oil fractions.

TABLE 4
Enthalpy of Partition (kJ mol^{-1}) for Diglycerides in Palm Oil Fractionation

Process	Diglyceride[a]		
	C_{32}	C_{34}	C_{36}
Dry	78 ± 16	32 ± 7	30 ± 7
Acetone	48 ± 3	43 ± 2	23 ± 4
Hexane	140 ± 23	80 ± 11	76 ± 12

[a]Enthalpy values with their standard errors.

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