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CRYSTALLISATION BEHAVIOUR OF PALM OLEINS

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The tendency for palm oleins to cloud when subjected to cold temperatures has long been a problem for the palm oil industry. In the present study, the crystallisation behaviour of palm oleins was evaluated by means of differential scanning calorimetry (DSC), X-ray diffraction and nuclear magnetic resonance (NMR) spectrometry. While the ratio of saturated to unsaturated triacylglycerols in the olein was an important criterion affecting crystallisation at low temperatures, the content of diacylglycerols particularly 1,3 dipalmitoylglycerol, was also important in the crystallisation behaviour.

INTRODUCTION

Palm olein is widely used throughout the world as a cooking oil. However, its usage is affected by it tendency to crystallise at low temperatures. This crystallisation causes the formation of clouds, sometimes observed as white sediments at the bottom of the bottle containing the olein. The presence of these crystals is often seen as a defect by consumers although there is no deterioration in oil quality.

A similar type of problem has also been observed with unsaturated oils such as canola oil (Liu *et al.*, 1993, Liu *et al.*, 1994, Przbylski *et al.*, 1993) and sunflower oil (Rivarola *et al.*, 1985, Rivarola *et al.*, 1988). The composition of the sediments in these oils have been identified as wax esters (Daun *et al.*, 1991; Liu *et al.*, 1994). The crystals in palm olein have not been fully identified although studies by Swe *et al.*, 1994 showed that the POP and the POS triacylglycerols were high in the cloud material obtained upon storing palm olein for 15-16hr at 12.5°C.

The importance of studying crystallisation tendencies in palm olein is to provide

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information on the cold stability of the oil and to be able to predict and control the appearance of cloudiness under different temperature conditions. From experience, it has been observed that palm oleins tend to crystallise when subjected to temperature fluctuations between 5°C and 28°C. Such conditions are quite often experienced in sub-tropical and temperate climates or in supermarkets.

In the present study, the crystallisation behaviour of palm oleins of varying iodine values was investigated. The composition of the crystals obtained during storage of palm olein between 10°C and 28°C, was also analysed by GLC and HPLC while crystals structure and their melting behaviour were determined by X-ray diffraction and differential scanning calorimetry (DSC).

MATERIALS & METHODS

Refined Palm Olein from Malaysian Refiners

Some 35 palm olein samples were analysed for their fatty acid composition, triglyceride content and their crystallisation behaviour. Detailed examination of the crystals obtained upon storage were carried out on five palm olein samples from Lam Soon Sdn Bhd, Petaling Jaya and Soctek Sdn Bhd, Johor.

Test on Stability

A 200g sample pre-heated at 70°C, was weighed into a 250ml beaker. Prior to the stability test, the samples were heated to 70°C for 30 min to remove previous thermal history. The samples were then subjected to a stability test for three months. This test consisted of storing the samples at 28°C in an incubator. After 24 hrs, the samples were transfered to a 10°C incubator where they were stored for another 24 hrs. The temperature cycle was repeated for three months. A duplicate set of samples was prepared and filtered after the first sign of crystallisation. Filtration was carried out under vacuum with a Buchner funnel using Whatman filter paper (No. 542) and a Schleicher Schull Rundfilter. These early crystals were designated as crystal 1, while those obtained after three months of thermal treatment were designated crystal 2. The crystals were subjected to X-ray diffraction studies, fatty acid and triacylglycerol composition analysis and melting and crystallisation thermograms by DSC analysis.

Effect of Added Diacylglycerols on the Crystallisation of Palm Olein

Triacylglycerol from palm olein (IV 58.1 and 62.8) were purified using the procedure of Quinlin and Weiser (1958). The purified samples were used for preparing mixtures containing diacylglycerols in various proportions ranging from 0.5 to 10% (expressed as a percentage of total glycerides). The crystallisation time for the samples to form crystals at 5°C was investigated.

Analyses (fatty acids and triacylglycerol composition; crystallisation behaviour)

Fatty acid composition was analysed using the ISO Methods (ISO 5508 and ISO 5509). The triacylglycerol composition was determined using a procedure similar to that of AOCS Ce5b-89. Crystallisation behaviour of the palm oleins were examined by determining nucleation according to Wong (1991) and their crystallisation time at 5°C.

For the study on crystallisation time at 5°C, samples were heated at 70°C for 1 hr, and allowed to cool to room temperature for 1 hr after which they were transferred to a waterbath set at 5°C.

Thermal Analysis by Differential Scanning Calorimetry (DSC)

DSC analyses of the samples were performed with a Perkin Elmer 7 DSC (Norwalk, USA) instrument. The sample was heated to 80°C before being weighed (10 mg) into an aluminium pan which was then sealed using a sample pan crimper. The previous thermal history of the sample was erased by heating the sample to 80°C in the DSC instrument and holding it for 10 minutes. The sample was then cooled to -30°C at a rate of 40°C/min. The sample was re-heated at 10°C/min to 80°C for the melting curve. It was then held at 80°C for 10 mins and cooled at 10°C/min to -30°C for the crystallisation curve.

Crystal Polymorphism by X-ray Diffraction

X-ray analysis of the crystals obtained from filtration of the crystallised palm oleins was conducted to identify the polymorphic form of the crystals using a X-ray diffractometer Enrag Nonius FR 590 (Delft, Holland). X-ray patterns were recorded on a photographic film with a Guinier camera and the diffraction bands measured with the Enraf Nonius viewer.

RESULTS & DISCUSSION

Table 1 shows the general crystallisation behaviour of palm olein at 5°C to 20°C. Oleins with a high IV are expected to be able to resist crystallisation. This is true for most oleins, but exceptions were found as shown in *Table 2*. The nucleation test shows that in general, palm oleins cloud in less than 1 hr, some between 1 to 6 hrs while the rest remained stable beyond 6 hrs. In general, the IV increases with the stability of the olein. However, one can find that there are oleins with high IVs and yet crystallise easily while others with lower IVs have better stability. A high ratio of the two major triacylglycerols, POP and POO in palm olein, is also detrimental to the physical stability of the olein.

TABLE 1. PALM OLEIN'S RESISTANCE TO CRYSTALLISATION AT 5-20°C
(Reproduced with kind permission from Idris *et al.* 1993)

Temperature °C	IV 60	IV 63	IV 65
5	3h	< 1d	< 1d
10	> 5h	> 1d	1d
15	> 5h	1d	4d
20	> 7d	37d	35d

Note: h-hours, d-days

TABLE 2. CRYSTALLISATION BEHAVIOUR OF PALM OLEIN

Nucleation Test (11°C)	IV	POP/POO Ratio
< 1hr (11 samples)	59.3 ± 3.0	0.89 ± 0.24
1 to 6 hrs (9 samples)	61.4 ± 2.9	0.83 ± 0.22
>6 hrs (13 samples)	63.6 ± 2.8	0.56 ± 0.20

In order to understand the crystallisation behaviour of palm olein, samples were stored under a temperature cycle of between 28°C and 10°C. Crystals were monitored visually. When sufficient crystals were formed, they were filtered and the fatty acid and triacylglycerol compositions were analysed (*Tables 3* and *4*). The iodine values of these early crystals (crystal 1) were low (53.9) as reflected by their more saturated composition. In comparison, the crystals obtained after 3 months of storage (crystal 2) showed a higher IV than crystal 1. This is due to the presence of a higher palmitic acid content in crystal 1 compared to crystal 2. Thus, the palmitic acid content of crystal 1 was 41.9%, but only 38.5% in crystal 2. The oleic acid content of both crystals was significantly lower than that in palm olein. The composition of the major triacylglycerols of palm olein was as follows:- POO:33.9%, POP:19.4%, PLO:14.9% and PLP:10.5%. Other triacylglycerols were also present but in minor proportions. Except for the slight variability in SOO, the composition of triacylglycerols in the crystal 1 and 2 and palm olein remained relatively constant. Differences in fatty acid composition were not reflected in the triacylglycerols but only in the diacylglycerol as shown in *Table 5*. Upon washing with isopropanol, the triacylglycerols were reduced to 42.1% of the glycerides in the crystals, the remaining being diacylglycerols. When these triglycerides were normalised to 100% as shown in *Table 4*, a higher SOO content was evident.

An interesting feature of palm olein and its crystals, is the diacylglycerol content and

TABLE 3. FATTY ACID COMPOSITION OF PALM OLEIN AND THEIR CRYSTALS

Samples[a]	(wt %)						
	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3	IV
Olein	1.0 (0.11)[b]	34.1 (0.86)	3.7 (0.25)	47.1 (0.65)	12.9 (0.73)	0.3 (0.04)	63.4 (1.43)
Crystal 1	1.0 (0.04)	41.9 (4.01)	4.2 (0.15)	40.9 (3.18)	10.6 (1.16)	0.2 (0.19)	53.9 (4.77)
Crystal 2	1.1 (0.05)	38.5 (1.91)	4.1 (0.17)	43.6 (1.29)	11.6 (0.71)	0.2 (0.12)	58.1 (2.44)

[a]Mean of 5 samples
[b]Values in parenthesis are standard deviation

composition (*Table 5*). While the palm olein had a diacylglycerol contents of 6.6-8.2%, both crystal 1 and crystal 2 showed significantly higher diacylglycerol contents ranging from 10.4 to 15%. These diacylglycerols were enriched in 1,2 and 1,3 dipalmitoylglycerol (PP). Total PP in crystal 1 was in the range of 7.4 to 10.7%, equivalent to 56 to 79% of the total diacylglycerol content. A slightly lower percentage was found in crystal 2.

When crystals 2 were washed with isopropanol to remove the entrapped olein, the results (*Table 5*) indicated a very high concentration of diacylglycerol (57.9%) with PP being the highest, (47.6%), followed by palmitoyloleoylglycerol (PO), (7.6%) and dioleoylglycerol (OO), (2.7%). In the initial crystallisation of the palm oleins, the PP tended to precipitate first while the more unsaturated diacylglycerols remained in the olein phase. With prolonged storage, the PP was 'diluted' by the more unsaturated PO and OO diacylglycerols. This was confirmed by the increase in PO and OO contents in crystal 2.

The DSC heating and cooling thermograms of the olein crystals revealed two peaks. The heating thermogram (*Figure 1*) showed a low-melting component (3°C), which melted completely at 13°C and a high-melting component (61.9°C) which melted completely at 65°C. Upon cooling from the melt, the palm olein crystals showed a large exotherm at 52.7°C and a small exotherm at -16°C. The high melting component could be due to the mixture of diacylglycerols (in particular PP) and other high melting triacylglycerols.

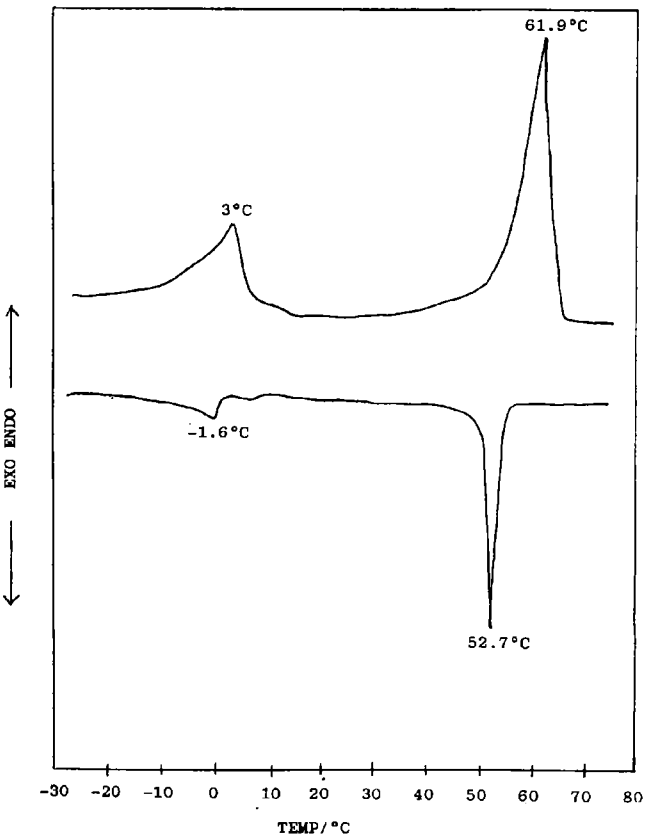

Figure 1. DSC thermograms of crystals of palm olein.

TABLE 4. TRIACYLGLYCEROL COMPOSITION OF PALM OLEIN AND THEIR CRYSTALS

Sample[a]	Triacylglycerols[d] (Area %)												
	OLL	PLL	MLP	OLO	PLO	PLP	OOO	POO	POP	PPP/SOO	POS	PPS	SOS
Olein	0.8 (0.05)[b]	3.3 (0.70)	0.7 (0.11)	2.9 (0.09)	14.9 (0.68)	10.5 (0.44)	5.9 (0.26)	33.9 (1.23)	19.4 (2.30)	3.4 (0.50)	3.4 (0.5)	0.2 (0.11)	0.3 (0.13)
Crystal 1	0.7 (0.11)	3.4 (0.66)	0.6 (0.19)	2.7 (0.45)	14.8 (0.98)	10.4 (0.35)	5.8 (0.27)	34.1 (1.09)	19.7 (2.97)	4.0 (0.11)	3.5 (0.63)	0.1 (0.09)	0.2 (0.08)
Crystal 2	0.8 (0.05)	3.5 (0.32)	0.7 (0.18)	2.8 (0.17)	14.9 (0.67)	10.5 (0.54)	5.9 (0.31)	34.2 (1.47)	19.1 (2.31)	3.9 (0.30)	3.3 (0.53)	0.1 (0.04)	0.45 (0.35)
Crystal 2[c]	0.6	2.2	0.4	3.9	14.2	12.3	6.4	33.3	20.1	5.1	1.6	-	-

[a]Mean of 5 samples
[b]Values in parenthesis are standard deviations
[c]Washed with isopropanol
[d]Triacylglycerols, the abbreviations indicate the acylgroup as follows:-
O : Oleic, L : Linoleic, P : Palmitic,
M : Myristic, S : Stearic

TABLE 5. DIACYLGLYCEROLS (DG) COMPOSITION (AREA %) OF PALM OLEIN AND THEIR CRYSTALS

Sample[a]	1,2 OO	1,3 OO	1,2 PO	1,3 PO	1,3 PP	1,2 PP	Total PP	Total PO	Total OO	Total DG
Olein	0.7 (0.05)[b]	1.1 (0.19)	1.2 (0.09)	2.9 (0.21)	1.4 (0.15)	0.1 (0.04)	1.5 (0.18)	4.1 (0.30)	1.8 (0.19)	7.4 (0.61)
Crystal 1	0.6 (0.09)	1.2 (0.13)	0.7 (0.50)	2.3 (1.11)	8.4 (1.24)	0.4 (0.05)	8.8 (1.27)	3.0 (1.55)	1.8 (0.11)	13.6 (1.89)
Crystal 2	0.6 (0.07)	1.4 (0.04)	1.2 (0.08)	3.1 (0.23)	5.5 (1.42)	0.7 (0.25)	6.2 (1.61)	4.3 (0.19)	2.0 (0.11)	12.5 (1.70)
Crystal 2[c]	1.1	1.6	0.3	7.3	42.2	5.4	47.6	7.6	2.7	57.9

[a]Based on 5 samples
[b]Values in parenthesis are standard deviations
[c]Washed with isopropanol

At 28°C, palm oleins with iodine values higher than 60, did not crystallise. However when the oil was subjected to a temperature fluctuation between 28°C and 10°C, crystallisation occured rapidly, usually after 1 to 2 cycles of changes in temperature. The palm olein gave some solids on cooling to 10°C. When the solidified olein was allowed to be warmed up to 28°C, most of the triacylglycerols re-melted, leaving behind the higher melting diacylglycerol, dipalmitin. Further cycling caused some of the lower melting diacylglycerols (such as PO and OO) to precipitate out as well.

X-ray diffraction patterns showed that the palm olein were of the β form. Temperature cycling or fluctuations tended to induce the formation of β crystals from β' crystals. The high melting β crystals were relatively more stable than its β' form and hence remained in the melt even when the temperature returned to 28°C.

The effect of diacylglycerols on crystallisation behaviour of palm olein may be seen in *Tables 6* and *7*. The addition of diacylglycerols to palm olein had a detrimental effect on the crystallisation stability of the olein. This was evident with 1,2 and 1,3 dipalmitoylglycerol (PP) with the 1,3 isomer being more effective in enhancing crystallisation in palm olein. The dioleoylglycerol (OO) and palmitoyloleoyl–glycerol (PO) did not show the same crystallisation effect on the palm olein. However, the PO in the high IV palm olein appeared to retard the crystallisation of the olein. This behaviour was not very obvious in the low IV olein. In the case of OO, its effect appeared insignificant.

The palm diacylglycerols (PDG) which consisted of a mixture of PP, PO and OO had a strong enhancing effect on the crystallisation of palm olein. This was evident from the significant reduction in crystallisation time from 35 min to 12.5 min and 2 min, when 2% PDG and 5% PDG respectively were added to palm olein. The effect was particularly strong in high IV oleins compared to the lower IV oleins. Since diacylglycerols are known to inhibit crystallisation in oils such as salfat, palm oil and hydrogenated rapaseed oil, the effect shown here was all the more surprising. This effect seen in a high IV, more liquid olein was probably due to the early crystallisation of the high-melting diacylglycerol inducing the subsequent crystallisation of the triacylglycerols of the palm olein. The onset of crystallisation of palm diacylglycerols (PDG) was 32.0°C while that for palm olein was 3°C. The crystallisation for pure 1,2 PP and 1,3 PP took place at 50°C and 65°C respectively.

TABLE 6 : EFFECT OF ADDED DIACYLGLYCEROLS (DG) ON THE CRYSTALLISATION OF PALM OLEIN (IV 58.1) AT 5°C

Percentage of DG	Crystallisation Time (mins)						
	PDG	1,2 PP	1,3 PP	1,2 PO	1,3 PO	1,2 OO	1,3 OO
0	3.9	3.9	3.9	3.9	3.9	3.9	3.9
0.5	1.9	1.7	1.2	2.5	2.5	2.7	2.3
1	1.9	0.8	0.8	2.6	2.3	2.7	2.3
1.5	1.9	0.7	rt	2.8	2.3	3.0	2.4
2	1.9	0.7	rt	3.0	2.3	3.0	2.8
2.5	1.9	0.6	rt	3.0	2.0	3.0	3.3
5	1.4						
7.5	1.4						
10.0	1.4						

LSD (P >0.05)0.7
PDG : diacylglycerol mixtures extracted from palm oil
rt : crystallise at room temperature

TABLE 7: EFFECT OF ADDED DIACYLGLYCEROLS (DG) ON THE CRYSTALLISATION OF PALM OLEIN (IV 62.8) AT 5°C

Percentage of DG	Crystallisation Time (mins)						
	PDG	1,2 PP	1,3 PP	1,2 PO	1,3 PO	1,2 OO	1,3 OO
0	35.0	35.0	35.0	35.0	35.0	35.0	35.0
0.5	36.0	8.5	1.9	37.5	38.0	29.5	29.5
1	33.5	2.1	rt	37.5	38.0	29.5	29.5
1.5	17.5	1.4	rt	37.5	38.5	29.5	29.5
2	12.5	1.4	rt	40.5	42.0	29.5	29.5
2.5	9.0	1.4	rt	45.0	45.0	29.5	29.5
5	2.0						
7.5	1.3						
10.0	1.3						

LSD (P >0.05)1.3
rt : crystallise at room temperature

CONCLUSION

The study showed the importance of diacylglycerols in influencing the crystallisation tendency of palm olein. Different diacylglycerols exhibited different effects on the crystallisation behaviour. The dipalmitoylglycerol (PP) was most effective as an inducer of crystallisation in palm olein. The triacylglycerol composition of the palm olein was also important, in particular a low ratio of POP/POO ensured that the olein remained clear.

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