X-RAY DIFFRACTION

ANALYSIS OF PRODUCTS OBTAINED BY DRY FRACTIONATION OF PALM OIL

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Palm oil was dry fractionated by four different methods, namely (1) cooling at 30°C and (2) at 23°C for 18 hours with no agitation, (3) cooling at 30°C for 18 hours with agitation and (4) at 22°C for 3 hours with agitation. The yields of stearin were 16%, 30%, 26% and 32% respectively. Filtration rates were good when fractionation took place with no agitation but were poorer with agitation. X-ray analysis showed the stearins from methods 1, 2 and 4 to be in the beta-prime form while that from method 3 was in the beta form. These polymorphic forms were stable when the stearins were stored up to 12 days. When the stearins from methods 1 and 2 were tempered at 40° and 45°C the polymorphic form changed to the beta form. When melted and then crystallized at 0°C all the stearins crystallized in the beta-prime form. A hydrogenated palm oil crystallized in the alpha form under these conditions.

The short spacing of the beta-prime form of palm oil and palm products is unique in that it shows two distinct lines of strong intensity at 0.43 and 0.41 nm as opposed to one at 0.42 nm reported in other fats. The softening points of the stearins correlated with the C48 triglyceride content but the dropping points did not.

INTRODUCTION

Palm oil contains both low and high melting triglycerides, which can be readily fractionated by controlled cooling methods. Two main processes for fractionating palm oil are in commercial use: the dry process which uses direct filtration of the crystals, and the detergent process which uses an aqueous detergent solution to separate the crystals from the olein by centrifugation. Dry fractionation is at present the more popular method. The products that result from dry fractionation are palm stearin and palm olein. On a second fractionation of the olein, a palm mid-fraction, a value added product, is obtained. Depending on the conditions during the fractionation process the resulting stearin can be graded into three types: hard, medium and soft (Deffense,1985). The amount of olein included in the fat crystals determines the grade of stearin. Size and shape of crystals in turn determines the amount of olein inclusion. Fat crystals can exist in several polymorphic forms. The three main forms are alpha, beta-prime and beta. The chain packings of the subcells are respectively: hexagonal, orthorhombic and triclinic (Hoerr and Paulicka, 1968; Lutton, 1950; Lutton,1972). Each polymorphic form has its particular X-ray short spacings. There is some controversy about the nomenclature of the sub- and pseudo-forms of the polymorphic forms (Hagermann,1988). The generally accepted definitions for the three main crystal forms of saturated mono acid triglycerides are those proposed by Larsson (1966) and Hoerr and Paulicka (1968). They are as follows: alpha-form, crystallizing from the melt and having a single X-ray short spacing at 0.415 nm; beta-prime form, exhibiting two strong X-ray short spacings at 0.42 and 0.38 nm; and beta form, exhibiting three strong X-ray short spacings at 0.46, 0.39 and 0.37 nm. Natural fats, and especially hydrogenated fats, are complex mixtures of triglycerides, and the X-ray short spacings of their crystals may differ slightly from those of the mono acid triglycerides. Cocoa butter is unique as up to 6 different polymorphic forms have been reported (Timms,1984). Several authors have reviewed the polymorphism of triglycerides (Hagermann,1988; Timms,1984; Chapman,1962; Larsson,1972).

Yap *et al.* (1989a) have studied the polymorphic forms of palm oil, palm stearin and hydrogenated palm oil upon cooling and temperature cycling. They found that hydrogenated palm oil was very stable in the beta-prime form, followed in stability by palm oil and then palm stearin, which was only moderately stable in the beta-prime form.

In margarine and shortening the crystal structure is of great importance. For good performance the crystals should be in the beta-prime form (Chrysam,1985). The beta-prime crystals are small and contribute to smoothness and creaming ability. The beta crystals can grow into large agglomerates that result in a sandy mouth-feel, and in the case of shortening in poor aeration of cakes.

In non-tropical countries, margarines and shortenings consist mainly of hydrogenated oils made from soybean, canola and/or sunflower. When the triglycerides of the fat crystals are made up mainly of fatty acids which all have the same chain length, the fat usually crystallizes in the beta form. Canola and sunflower oils contain approximately 4 - 5%, and soybean oil 10 - 11% of 16-carbon fatty acids, with the remaining fatty acid having 18-carbons. The crystals of these hydrogenated oils are therefore relatively high in 18-carbon fatty acids. Depending on the degree of hydrogenation, these fats will tend to crystallize in the beta form (Hernqvist,1988; Lee *et al.*,1984). Palm oil products, which contains 44% or more of 16-carbon fatty acids, are often incorporated into these materials to diversify the fatty acid chain length of the crystals and therefore promote the beta-prime crystallinity of the product (Chrysam,1985; Yap *et al.*,1989b).

This study is concerned with the polymorphic forms and X-ray diffraction patterns of palm stearins obtained during fractionation of palm oil by several methods. The physical and

chemical characteristics of the palm stearins were also determined.

MATERIALS AND METHODS

Refined and bleached palm oil was obtained from Lam Soon Bhd, Petaling Jaya, Malaysia. The palm oil was heated to 80°C and cooled by the following four methods:

Method 1 100 g of palm oil initially at 80°C was left in a 30°C incubator overnight (18 hours) undisturbed.

Method 2 100 g of palm oil at 80°C was left in a 23°C incubator overnight undisturbed.

Method 3 500 g of palm oil at 80°C was placed in a 30°C thermostated water bath for 18 hours. The oil was agitated by an S-shaped stirrer at 24 rpm.

Method 4 500 g of palm oil at 80°C was placed in a 30°C thermostated water bath for 30 minutes. The bath was then cooled to 22°C at a rate of approximately 1°C/min. The oil was agitated as in Method 3. Total residence time was 3 hours.

Filtration was carried out under vacuum with a Buchner funnel and Whatman No. 1 filter paper.

The softening point of the samples (without further treatment) and the dropping points of melted fats were determined with the Mettler FP3 automatic apparatus (Mertens, *et al.*, 1972).

The Solid Fat Content (SFC) of the crystallized mixtures was estimated by pulsed nuclear magnetic resonance (pNMR) with the Bruker PC 20 Minispec. The instrument was calibrated with three factory-supplied standards.

DSC-melting and crystallization characteristics were determined using a DuPont Thermal Analyzer Model 900. Each sample was heated from 20° to 80°C at 5°C/min and was then cooled from 80° to 20°C at the same rate. The DSC melting temperature was taken as the temperature of the melting peak, while the crystallization temperature was taken as the temperature at the start of the exothermal deflection.

Fatty acid composition was determined by transesterification of the fat with sodium methoxide. The resulting methyl esters were injected into a Shimadzu Gas Chromatograph with a 2 m column packed with 10% SP-2330. Operating temperature was 170°C.

Triglyceride composition was determined by GC with a 0.5 m glass column of 4 mm ID packed with 3% OV 1. Temperature programming was carried out from 280° to 350°C at 5°C/min.

X-ray diffraction patterns were established by means of a 601 Diffractor X-ray generator and a Guinier X-ray diffraction camera model FR552 (Enraf Nonius, Delft, The Netherlands). The diffraction lines on the X-ray film were measured with a Guinier optical viewer and converted to short spacings by using the instrument constant supplied by the manufacturer. The spacings were visually judged to be of strong, medium, weak or very weak intensity.

RESULTS AND DISCUSSION

Table 1 shows a summary of the fractionation procedures and *Table 2* shows the physical characteristics of the solid fractions. In cooling methods 1 and 2 *(Table 1)*, with no agitation, the crystals had settled at the bottom of the container, resulting in an easily filtering stearin. The solids content of palm oil, as determined by pNMR, before filtration was 7.1% and 15.7% in methods 1 and 2 respectively, while the yield of stearin was about twice as much (16% and 30% respectively), indicating that the stearin consisted of about 50% solid fat. The fatty acid composition *(Table 3)* showed that method 1 stearin contained a high percentage of palmitic acid (59%) of which approximately half was contained in tripalmitin (the C48 content was 27.5%: *Table 4*). In method 2 cooling was done at a lower temperature than

TABLE 1. FRACTIONATION CONDITIONS

Method No.	Intermediate Temperature °C	Intermediate Time hr	Final Temperature °C	Holding time at final Temperature hr	Approximate cooling rate °C/min	Agitation
1	-	-	30	18	0.5	No
2	-	-	23	18	0.5	No
3	-	-	30	18	4.0	Yes
4	30	0.5	22	3	1.0	Yes

TABLE 2. PHYSICAL CHARACTERISTICS OF SOLID FRACTIONS

Cooling Method No.	Yield %	Filtration rate	SFC at time of filtration %	Softening point °C	Dropping point °C[a]	DSC melting °C	DSC crystallization °C
1	16	Good	7.1	55.1	55.3	53.0	33.0
2	30	Good	15.7	46.4	50.4	49.0	30.5
3	26	Poor	7.1	53.3	53.4	56.1	32.5
4	32	Medium	7.0	48.2	50.1	49.8	30.0

[a]Dropping point of unfractionated palm oil: 38.7°C

TABLE 3. FATTY ACID COMPOSITION OF STEARINS AND PALM OIL(%)

Fatty Acid	Stearins 1	Stearins 2	Stearins 3	Stearins 4	Palm Oil
12:0	0.2	0.2	0.2	0.2	0.3
14:0	1.2	1.2	1.2	1.2	1.1
16:0	59.0	51.2	55.7	53.8	44.1
16:1	0.1	0.1	0.1	0.2	0.3
18:0	4.9	4.5	4.5	4.7	4.2
18:1	27.8	34.3	30.6	32.1	39.8
18:2	6.1	7.8	7.0	7.5	9.5
18:3/20:0	0.5	0.5	0.5	0.5	0.5
20:1	0.1	0.1	0.1	0.1	0.1
22:0	0.1	0.1	0.1	0.1	0.2

TABLE 4. TRIGLYCERIDE COMPOSITION OF STEARINS AND PALM OIL (%)

Carbon No.	Stearins 1	Stearins 2	Stearins 3	Stearins 4	Palm Oil
46	1.5	1.1	1.5	1.4	0.6
48	27.5	16.1	23.0	19.4	7.8
50	39.1	41.1	41.4	42.6	39.5
52	26.0	33.0	28.1	29.9	40.0
54	5.5	7.6	6.1	6.6	11.4
56	-	0.3	-	0.2	0.2

in method 1, and the stearin contained less palmitic acid (51.2%) and therefore less tripalmitin (16.1%) than the stearin of method 1. In methods 3 and 4, which employed agitation (24 rpm), the filtration rate was poor. The solid formation before filtration in method 3 was the same as in method 1 (7.1%: *Table 2*), but the yield of stearin was much higher (26%) although method 1 and method 3 involved cooling to the same temperature (30°C). The palmitic acid content of the stearin from method 3 was slightly less than that from method 1 and so was the tripalmitin (C48) content *(Table 4)*. Agitation during cooling resulted in more entrapment of olein than occurred with no agitation.

In method 4 the palm oil was cooled to 22°C under agitation but had a crystallization time of only 3 hours compared with 18 hours for the other methods *(Table 1)* and contained only 7% solids at the time of filtration *(Table 2)*. The filtration rate was better than in method 3 but poorer than in method 2 in which final crystallization temperature was 1°C higher. Judging from the fatty acid and triglyceride compositions, the tripalmitin content was higher in the stearin from method 4 than in that from method 2.

X-ray diffraction analysis *(Table 5)* indicated that the stearins from methods 1,2 and 4 were in the beta-prime form, while the stearin from method 3 was in the beta form. The presence of the beta-prime polymorphic form was judged by the X-ray short spacings around 0.42 nm and of the beta polymorphic form by the short spacing at 0.46 nm. Differences between method 1 and method 3 are agitation and the amount of the sample, but the latter may be ignored. The agitation might have caused the occurrence of small-sized crystalline particles, presumably of beta-prime, which then transformed rapidly to beta. The rate of beta-prime-to-beta transition is much higher in small crystals than in large crystals. Mixtures of beta-prime and beta forms were estimated by the relative intensity of these two short spacings. The short spacings at 0.38 (beta-prime), 0.39 and 0.37 nm (beta) were ignored since they are very close together and difficult to differentiate. The beta-prime short spacings in the 0.42 nm region consisted of two distinct sharp lines of equal and strong intensity at 0.430 and 0.415 nm. These two separate lines were also observed by Yap *et al.* (1989) in palm products. In other beta-prime fats there is only one line in the 0.42 nm region. The significance of the other short spacings of minor intensity has not been established.

Softening points *(Table 2)* followed the same trend as the palmitic acid and C48 contents of the stearins – they increased with increasing tripalmitin content. Tripalmitin is the highest melting component in palm oil. Dropping points did not follow this trend. DSC-melting curves showed that the peak temperature of method 3 stearin was the highest (56.1°C: *Table 2*). This agreed with the observation that this stearin was predominantly in the beta form. The beta forms of triglycerides exhibit the highest melting points.

DSC-crystallization temperatures varied less than DSC-melting peak temperatures (*Table 2*), probably because the samples all crystallized initially in the same polymorphic form.

The polymorphic forms of the stearins after 12 days of storage are shown in *Table 6*. The crystal habit of stearins 1,2 and 4 did not change after storage. Stearin 3 showed a small amount of the beta-prime form after storage. This stearin contained a relatively large amount of olein, which may have crystallized upon storage, causing the additional weak beta-prime spacing. The occurrence of this secondary crystallization of the stearins is further supported by the observation that the oleins obtained from all methods did partially crystallize upon storage at room temperature.

Tempering of stearins 1 and 2 at 40° and 45°C caused a polymorphic transition from beta-prime to beta *(Table 7)*. When the stearins and the original palm oil were melted and then crystallized at 0°C, all except stearin 1 crystallized in the beta-prime form *(Table 8)*. Stearin 1 showed some beta crystallinity and it contained the largest amount of tripalmitin (C48: *Table 4*). Tripalmitin is a beta-tending triglyceride.

In order to demonstrate the X-ray diffraction

TABLE 5. X-RAY DIFFRACTION PATTERNS AND POLYMORPHIC FORMS OF STEARINS OBTAINED BY FOUR DIFFERENT METHODS FROM PALM OIL – ON THE DAY OF FILTRATION

	Stearins			
	1	2	3	4
Short Spacings (nm)			0.535VW 0.518VW 0.457M 0.453M 0.441VW	
	0.429S 0.414S 0.398M	0.431S 0.415S		0.431S 0.416S 0.405M
	0.381S	0.382S	0.389S 0.380M 0.365M 0.252VW	0.378S
Long Spacings (nm)	4.347S 2.975S 1.400M	4.391S 2.975S 1.400M	4.414S 3.027S 1.426M	4.530S 3.170S 1.427M
Polymorphic form	β'	β'	β	β'

TABLE 6. X-RAY DIFFRACTION PATTERNS AND POLYMORPHIC FORMS OF STEARINS OBTAINED BY FOUR DIFFERENT METHODS – AFTER 12 DAYS' STORAGE AT ROOM TEMPERATURE

	Stearins			
	1	2	3	4
Short Spacings (nm)			0.531VW 0.456S 0.437VW	
	0.429S 0.415S 0.398W	0.432S 0.417S	0.417VW	0.434S 0.418S 0.402W
	0.379S	0.379S	0.389S 0.379S 0.366W	0.383S
Long Spacings (nm)	4.391S 2.975S 1.405M	4.483S 2.975S 1.409M	4.483S 2.975S 1.405M	4.414S 2.975S 1.425M
Polymorphic form	β'	β'	$\beta >> \beta'$	β'

TABLE 7. X-RAY DIFFRACTION PATTERNS AND POLYMORPHIC FORMS OF STEARINS OBTAINED BY THE FIRST TWO METHODS FROM PALM OIL – AFTER A TEMPERING TREATMENT OVERNIGHT

	Stearins			
	1		2	
Tempering temperature (°C)	**45**	**40**	**45**	**40**
Short Spacings (nm)	0.522W	0.522VW	0.525VW	0.528VW
				0.516VW
	0.454S	0.453S	0.456S	0.457S
				0.450S
	0.441W		0.446M	0.441VW
		0.416VW		
		0.401VW		0.409VW
				0.401W
	0.385S	0.384S	0.384M	0.386M
				0.377M
		0.372S	0.370M	0.372S
	0.359M	0.359M		0.360W
	0.252VW	0.252W	0.252VW	0.250W
Long Spacings (nm)	4.303S	4.303S	4.347S	4.303S
	2.975S	2.975S	3.059S	2.975S
	1.405M	1.357M	1.361S	1.405M
Polymorphic form	β	$\beta>>>\beta'$	β	β

TABLE 8. X-RAY DIFFRACTION PATTERNS AND POLYMORPHIC FORMS OF SOLID FRACTIONS OBTAINED BY THE FOUR DIFFERENT METHODS FROM PALM OIL. FRACTIONS WERE MELTED AT 80°C AND COOLED IMMEDIATELY AT 5°C

	Stearins				Palm oil	Fully hydrogenated palm oil
	1	2	3	4		
Short spacings (nm)	0.452M					
	0.431S	0.430S	0.429S	0.429S	0.431S	
	0.414S	0.412S	0.415S	0.412S	0.416S	0.412S
	0.381S	0.382S	0.381S	0.380S	0.385S	
Long Spacings (nm)	4.303S	4.414M	4.198M	4.303M	4.303M	4.414VW
	2.925S	2.975S	2.975S	2.975S	2.975S	2.975S
	1.409S	1.427W	1.405M	1.423W	1.423M	1.646W
Polymorphic form	$\beta'>>\beta$	β'	β'	β'	β'	α

pattern of the alpha polymorph, a fully hydrogenated palm oil was melted and crystallized at 5°C. The result is shown in *Table 8*. There was only one diffraction line at 0.412 nm.

Long spacings were also recorded (*Tables 5* to *8*) and were similar for the beta and beta-prime forms. Only one of the intermediate spacings of hydrogenated palm oil was different (1.6 nm instead of 1.4 nm for the other fats). Long spacings for pure long-chain triglycerides have been recorded by Bailey (1950) and fall into the region of 4.2 to 4.7 nm for SPP (stearic-palmitic-palmitic) depending on the polymorphic form. Timms (1984) reported those for StStSt (tristearin), which ranged from 4.5 to 5.1 nm. Long spacings depend not only on the polymorphic form but also on the triglyceride composition, which makes the significance of the long spacing more difficult to interpret in mixed triglycerides.

All the observed spacings are recorded here, rather than only the major ones. It is hoped that further interpretation may be added in the future when the significance of all the spacings is better understood.

CONCLUSIONS

Continuous agitation (24 rpm) over a prolonged period of time during crystallization results in poor filterability of the stearin. Formation of beta crystals in the fractionation process is undesirable.

Storage of stearin at room temperature does not change the polymorphic form of the crystals. Tempering of the stearins at 40° and 45°C does change the polymorphic form of the crystals. The higher the temperature the more complete is the transition to the beta form. The beta-prime polymorphic form of palm stearin or palm oil is peculiar in that it exhibits two strong short spacings close together in the 0.42 nm region – usually around 0.432 and 0.415 nm.

More research is needed on process parameters in the dry fractionation of palm oil especially if a palm-mid fraction of consistent quality is required. Special attention should be paid to the rate of cooling, rate of agitation or intermittent agitation, seeding versus no seeding, and tempering during crystallization.

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