POLYMORPHISM OF ANHYDROUS MILK FAT AS AFFECTED BY THE ADDITION OF PALM STEARINS

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ABSTRACT

Blends of anhydrous milk fat (AMF), soft palm stearin (SPOs) and hard palm stearin (HPOs) were prepared and analysed by differential scanning calorimetry (DSC) to determine their maximum melting peak temperature, Tp and melting enthalpy, ΔH and by X-ray diffractometry (XRD) to determine their polymorphism. Contour plots were produced showing the variation of Tp and ΔH with blend composition. The XRD data showed a predominance of β′ crystals and a small proportion of β crystals, both for pure AMF and SPOs, but β crystals were dominant in pure HPOs. By blending AMF with SPOs or HPOs, the polymorphic stability of β crystals increased, with the most pronounced effect being observed in the blends containing high HPOs. This was considered to be due to the enrichment of AMF with symmetrical triacylglycerols of SPOs or HPOs which reduced the chain-length diversity that was originally found in pure AMF. An exception was observed for the binary blend of 75% AMF with 25% HPOs, which indicated that the blend occurred only in β′ polymorphic form.

Keywords: milk fat, polymorphic, palm stearin, chemical composition, melting behaviour.

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INTRODUCTION

Blends of milk fat and other fats and oils are common in shortenings and margarines, where the milk fat is blended with vegetable oil. In chocolates, milk fat is blended with cocoa butter to improve the physical properties and sometimes to reduce the production cost. It is also a necessary part of milk chocolate, 3.0%-3.5% being legally required in most countries. Moreover palm stearin, a high melting point fraction from palm oil is among the fats that has a considerable potential to be used in margarine and shortening milk fat-based formulations (Nor Aini *et al*., 1995; Rodrigues and Gioielli, 2003). Palm stearin can be considered a good competitor to high- melting fat like beef tallow which historically was used in shortening production. It was reported that simple blending of milk fat with palm stearin at levels 30% to 50% could modify the melting characteristics of pure milk fat, making it more suitable for use as a hard stock in shortening and chocolate fat formulations (Nor Hayati *et al.*, 2002). One of the most important concerns in the blending of fats is the crystalline structure of modified blended fats which is complicated further by the phenomenon of polymorphism. Basically, triacyglycerols of fats occur in three polymorphic forms designated as α, β′ and β that can be distinguished by X-ray diffraction analysis. The polymorphic form is distinguished by its X-ray short spacings where the β′ form is reported to show short spacings at 3.8 and 4.2Å or at 4.27, 3.97 and 3.71Å while the β form shows a single spacing at 4.6 Å (Larsson, 1966; deMan, 1992).

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Milk fat like other fats exhibits polymorphism which results from its complex crystal structure. The β′ form is generally the most stable in milk fat (Timms, 1980), while the polymorphic crystal structure for palm stearin commonly occurs as a β and β′ mixture. However, a greater β tendency was found in palm stearin containing higher concentrations of trisaturated triacylglycerols (Yap *et al*., 1989). Thus, blends of milk fat with palm stearin might provide a beneficial effect on the polymorphic stability of milk fat which could make it more suitable for use in products such as pastry and chocolate where fat crystals should exist predominantly in the β form. The objective of this study was to investigate the polymorphic modification of milk fat and palm stearin blends as a function of their chemical composition.

MATERIAL AND METHODS

Materials

Commercial AMF (Cow & Calf brand, Kumpulan Barkath, Malaysia and Singapore), was purchased from a local supermarket. Refined, bleached, and deodorized (RBD) palm oil was supplied by Malaysian Palm Oil Board (Bangi, Malaysia). SPOs was obtained bydry fractionation of palm oil at 26.0 ± 0.5°C for 24 hr after removing the olein fraction by vacuum filtration. The SPOs was then dissolved in acetone, at 4:6 oil to solvent ratio (w/v) and held at the same temperature for 12 hr to obtain 10% by weight of HPOs. The HPOs was freed of solvent in a rotary evaporator under reduced pressure, followed by nitrogen purging at 60°C. Blends of AMF, SPOs and HPOs were prepared according to conventional three component mixture designs (Md Ali and Dimick, 1994) as described in *Table 2*. Each blend was prepared in duplicate.

Gas Chromatography Analysis

The fatty acid composition of the AMF, SPOs and HPOs were determined as fatty acid methyl esters (FAME). The molten fat (0.05 g) was dissolved in 1 ml of hexane. Sodium methoxide solution (0.2 ml; 2 M $NaOCH_3$ in anhydrous methanol) was added and the mixture was mixed for 1 min using a vortex mixer. After sedimentation of sodium glycerolate, 1 μl of the clear supernatant was injected into an SGE-BPX 70 polar silica column (60 cm x 0.32 μm) (Ontario, Canada) and analysed using a gas chromatograph (Shimadzu-17A, Kyoto, Japan), equipped with an FID detector and a C-R6A Chromatopac integrator. The oven temperature was programmed in two stages as follows: first, from 50°C to 180°C (8°C min^{-1}), and then from 180°C to 200°C (5°C min^{-1}). The flow rate of the carrier gas (helium) was 6.8 ml min^{-1}. Correction response factors were determined by analysis of an RM-5 standard mixture of FAME (Supelco-Cat. No: 4-7024, Tokyo, Japan).

High Performance Liquid Chromatography Analysis

The instrument was fitted with a model 501 single-pump system, a model 7125 Rheodyne injector and a Model 410 RI detector (Millipore Waters, USA). The columns were a Waters Novapax C18 (4 μm, 300 mm x 3.9 mm i.d.) (Millipore waters, USA) and a ODS 2 (5 μm, 150 mm x 4 mm) (GL Sc., Japan) which were connected in series. Triacylglycerols were eluted in chloroform (10%, w/v) and 4 μl of the sample were injected into the column which temperature was maintained at 45°C. The mobile phase consisted of acetone:acetonitrile (64:36, vol/vol) solvent mixture. Chromatograms were processed using Waters System Maxima 820 program manager software (Millipore Waters, USA). Identification and quantitation of triacylglycerols was carried out by calibration with a pure triacylglycerol mixture (Sigma Chemical Co., USA) of known composition (PORIM, 1995) and the results were expressed as peak area percentages.

Differential Scanning Calorimetry Analysis

The melting parameters (*i.e*. maximum melting peak temperature, Tp and melting entalphy, ΔH) of each sample were determined by a DSC (DSC-7 Series Perkin-Elmer, Norwalk, CT). About 4-5 mg of melted sample was placed in a DSC pan and hermetically sealed. The samples were first melted at 60°C-70°C for 30 min before cooling to 0°C for 90 min. The samples were subsequently stabilized at 26°C for two days, before being stored at 5°C for four days (Timms, 1980). Prior to measurement, the samples were again cooled at 0°C for 90 min. DSC melting curves were recorded at a heating rate of 20°C min^{-1} from -25°C to 70°C and their melting parameters subsequently determined. Data obtained were used to generate contour plots based on the Simplex Centroid Design using a Minitab (Version 14) statistical software on a personal computer.

X-Ray Diffraction Analysis

The polymorphic form of the fat crystals of the samples was determined using an FR 592 Diffractis X-ray generator (Delft, Holland) and Enraf Nonius model FR 552 Guinier camera (Delft, Holland). As in the DSC analysis, the same stabilization procedure was applied on each sample prior to analysis. The film used was Kodak 8162 (Cat 155) and analyses were carried out at 5°C. Samples were placed at 5°C with a single-compartment cell using a custom-made

temperature controlled holder maintained at 5°C by an external circulation thermostatic bath. The short spacings on the X-ray film were measured with an Enraf-Nonius Guinier viewer capable of reading to the nearest 0.001 nm under illuminated magnification. The nomenclature and assignment of polymorphic forms were based on those reported by Larsson (1966).

RESULTS AND DISCUSSION

Chemical Composition of Pure Fats

Table 1 shows the fatty acid and triacylglycerol compositions of AMF, SPOs and HPOs. In general, palmitic acid (C16:0) and oleic acid (C18:1) were the most abundant fatty acids in the three fats examined. The fatty acid profile for AMF was more diverse as compared to the other samples, with fatty acids ranging from C4:0 to C18:3. The wide fatty acid variety in AMF leads to a heterogeneous composition of triacylglycerols in AMF which are difficult to identify. However, identified triacylglycerols that were present in a significant amount compared to the others were PPB, PPS, PPO, PPL and PPP (P, palmitic acid; S, stearic acid; O, oleic acid; L, lauric acid) (Robinson and Mac Gibbon, 1998). The high levels of C16:0 (57.2%) and C18:1 (29.9%) fatty acids in SPOs, were related to high contents of POO (18.0%), POP (32.0%) and PPP (20.6%). The HPOs obtained by acetone fractionation of SPOs was further enriched in C16:0 (77.6%), which is mainly attributed to a high concentration of tripalmitin (PPP) of 51.0%, whereas C18:1 decreased to 10.4% due to a pronounced decrease in POP from 32.0% to 26.4% and POO from 18.0% to 3.0%.

TABLE 1. CHEMICAL COMPOSITION OF SELECTED FATS

	Anhydrous milk fat (AMF)	Soft palm stearin (SPOs)	Hard palm stearin (HPOs)
Fatty acids[a b]			
4:0	5.2 ± 0.4	-	-
C6:0	3.0 ± 0.2	-	-
C8:0	1.8 ± 0.1	-	-
C10:0	3.8 ± 0.1	-	-
C12:0	3.5 ± 0.1	0.2 ± 0.1	0.1 ± 0.0
C14:0	12.5 ± 0.1	1.3 ± 0.2	1.9 ± 0.1
C16:0	33.9 ± 0.3	57.2 ± 3.8	77.6 ± 2.2
C18:0	10.2 ± 0.1	3.8 ± 0.3	7.3 ± 0.6
C18:1	24.3 ± 0.5	29.9 ± 3.1	10.4 ± 1.0
C18:2	1.0 ± 0.0	7.2 ± 0.6	2.3 ± 0.3
C18:3	0.6 ± 0.1	0.3 ± 0.0	0.5 ± 0.1
Triacylglycerols[b]			
PLL		1.0	-
MLP		0.1	-
OLO		0.8	-
PLO		6.9	0.9
PLP		8.1	3.8
OOO		4.1	4.5
POO		18.0	3.0
POP		32.0	26.4
PPP		20.6	51.0
POS		4.9	2.9
PPS		3.5	7.4

Notes: [a] Values are reported as mean ± standard deviation (n=4). [b] Values reported in % weight of fat. P, palmitic acid; L, lauric acid; M, mauristic acid; O, oleic acid; S, stearic acid.

Melting Behaviour

In the present study, both Tp and ΔH data were used to generate contour plots in order to examine the miscibility of the blend components. More details on miscibility among AMF, SPOs and HPOs have been reported on our previous paper (Nor Hayati *et al.*, 2000) which was based on solid fat content (SFC) data. *Figure 1* displays contour plots for Tp (a) and ΔH (b) for the blend systems. Both contour plots having fairly linear lines without any pronounced depression area that could be attributed to interaction among fats in the blend system. Of the three fats examined, AMF showed the lowest ΔH of 76.1 J g^{-1} with the Tp of 33.7°C. Soft palm stearin exhibited a moderate melting behaviour with ΔH of 110.9 J g^{-1} and Tp of 49.6°C. As expected, HPOs demonstrated the highest ΔH of 174.9 J g^{-1} and the highest Tp of 61.5°C. Both melting parameters generally showed linear increases with increases in SPOs and HPOs proportions in the blend systems, without any suppressed value that could be related to eutectic interaction, indicating a good miscibility among the blend components.

Polymorphic Crystal Structure

Table 2 details the polymorphic modification of the blends. It was found that almost all blends contained a combination of β′ and β crystals.

Pure components. The diffraction data shows a strong short spacing at 3.79Å and 4.22 Å, indicating mainly β′ polymorph for both pure AMF (code A) and pure SPOs (code E) together with a very weak short spacing at ~4.6 Å, indicating a small amount of β polymorph. These results are in agreement with the data previously reported by Rouseau *et al.* (1996) and Sabariah *et al.* (1998) for AMF and Y*ap et al.* (1989) for SPOs. The presence of β polymorph in AMF is related to the small amount of high melting trisaturated triacylglycerols while the β′ polymorph was associated with the abundance of asymmetrical triacylglycerols (Timms, 1984; D'Souza, 1990). *Table 1* confirms that AMF is comprised of fatty acids with varying chain-length which reflects a fat rich in asymmetrical triacylglygerols. Conversely, HPOs (code I) showed a strong short spacing at 4.56 Å, indicating the presence of predominantly β polymorph. POP and PPP are the most abundant triacyglycerols in SPOs and HPOs (*Table 1*), and both are β tending triacylglycerols (Timms, 1984; Yap *et a.l.*, 1989). The greater β tending property of HPOs can be explained by its higher concentration of PPP. However, this study showed that β′ crystals were still dominant in SPOs despite the fact that POP and PPP were present at the highest concentration. This is probably explained by the significant level of asymmetrical triacylglycerols present such as POO, PLO, POS and PPS which are known to be extremely stabilized in the β′ polymorphic form (Timms, 1984). As a result, POP cannot reach its stable β

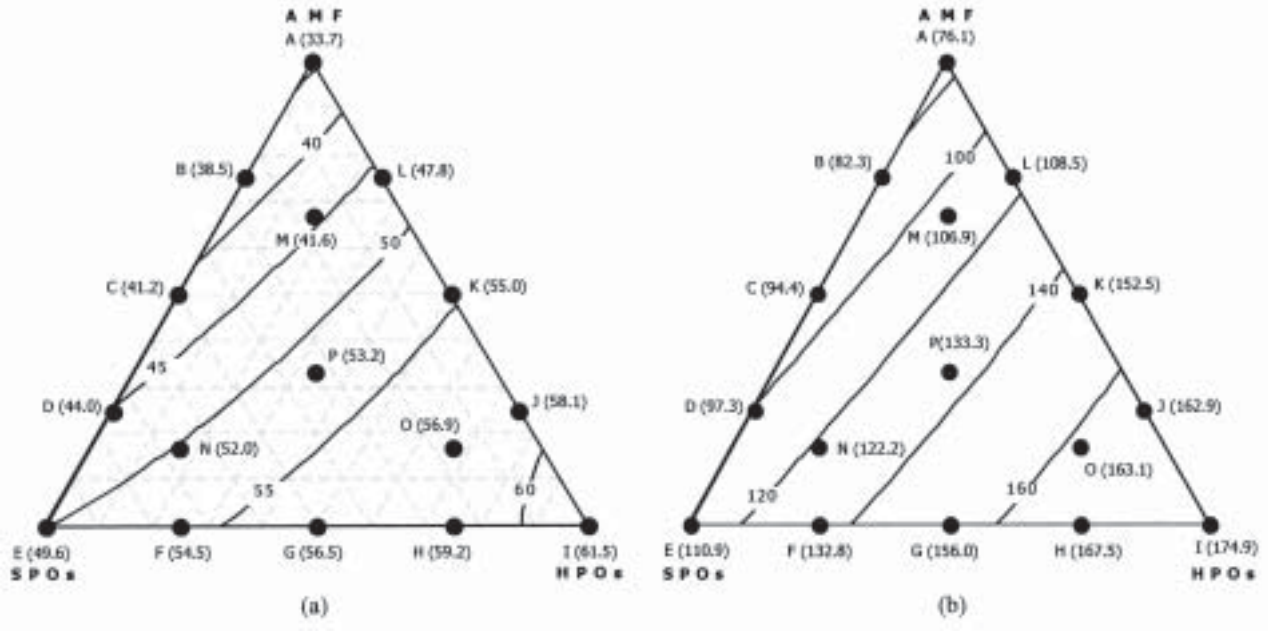

Figure 1. Contour plots of maximum melting peak temperature (°C) (a) and melting entalphy (J g^{-1}) (b) for AMF:SPOs:HPOs blends as measured by differential scanning calorimeter. Values in ( ) are given as means of duplicate samples.

polymorphic form in the SPOs system. Furthermore, these results confirmed the melting behaviour of pure AMF and SPOs is mainly determined by the melting of β′ fat crystals. On the other hand, the melting behaviour of pure HPOs was clearly dominated by the melting of β fat crystals.

Binary blends. The X-ray diffraction patterns for blends of AMF with SPOs or HPOs showed a pronounced modification of polymorphic behaviour. It is interesting to find that replacement of 25% AMF with SPOs (code B) led to a β dominated blend, despite β′ domination for both pure AMF and SPOs. This phenomenon was also observed by Timms (1979) in blends of milk fat and beef tallow. It was reported that the β crystals, which were present at low levels in the pure fats, increased in the blends and showed a maximum at 60%-80% beef tallow levels. Timms (1980) claimed that this phenomenon could be associated with the presence of the low melting fraction of milk fat in the blend. It was suggested that after four days of storage at 5°C, the low melting fraction may have existed in liquid form and could accelerate the transition of the higher melting triacylglycerol into its most stable polymorphic form, by recrystallizing in the liquid phase (Timms, 1980). In blend B, the transformation of high melting triacylglycerols (mainly POP and PPP from SPOs) to their most stable form (β) is believed to be similarly accelerated by the low melting fraction of AMF. However, with higher ratios of SPOs (code C and D), the levels of β crystals was reduced in the blends, resulting in blends containing β and β′ crystals in the same proportions. This suggests that higher ratios of SPOs in these blends reflected a lower proportion of the low melting milk fat fraction which reduced the transformation of β′ to β polymorpic crystals.

Binary blends of AMF and HPOs showed a different polymorphic behaviour. At 25% HPOs (code L), the blend showed an unusual polymorphic behaviour, with strong short spacings at 3.79 and 4.22Å being observed, indicating only β′ formation. This shows that the β stability of HPOs was dominated by the high concentration of AMF (75%), which provided triacylglycerols with varying chain-length to keep the blend in the β′ form. Furthermore, aggregation of the small amount of β crystals in the β′ crystal network is suspected to have occurred in this blend as only β′ crystals were observed. It is also suggested that the melting point of the HPOs triacylglycerols (formerly β stable) was greatly reduced by the presence of the lower-melting AMF triacylglycerols for them to be stable only in the β′ form with lower melting point. According to Timms (1984), triacylglycerols display a lower melting point when they exist in the β′ modification rather than β modification. At levels of HPOs above 25%, the blends showed a strong short spacing at ~4.6Å indicating that the β crystal form predominated. This revealed that the increase in HPOs content led to an increase in the content of β crystals.

Ternary blends. Ternary blends of AMF:SPOs:HPOs showed simpler polymorphic crystal structures (*Table 2*). Blend M (4:1:1) containing ~66% AMF showed a high level of β′ crystals and, the β′ crystal form predominated in blend N (1:4:1) though to a lesser extent than in blend M. A stronger β′ tendency in blend M is a reflection of the greater variety of fatty acid chain-length of AMF as shown in *Table 1*. According to Larsson (1994) and Rouseau *et al.* (1996), variation in chain-length results in a more disordered packing near the methyl-end regions, leading to less chance of a tightly knit crystal lattice. Because β crystals have the most ordered structure, a decrease in the degree of variation in chain-length increased the likelihood of their formation. On the other hand, the reappearance of β crystals in the blends P (1:1:1) and O (1:1:4) is due to a lack of variety in chain length, corresponding to the increase of HPOs levels (~33%-66%). A stronger tendency to β crystallinity for blend O was undoubtedly due to its high concentration of PPP contributed by ~66% HPOs in the blend.

CONCLUSION

A β′ stability of the fat blend is of value in margarine and shortening and this could be readily found in pure milk fat. Otherwise, β stability is useful in pastry fats which provide microscopic layers that are necessary for good crumb and flaky structure and which are also workable at high temperature. In chocolate making, the objective of tempering is to obtain most of the fat crystals to be stabilized in the β polymorph. Undesirably, the use of milk fat in chocolate formulation leads to softening effect whereby the tempering temperature has to be lowered to cause the formation of suitable crystals. This could explain why chocolates containing milk fat are relatively softer. This study showed that by blending milk fat with palm stearins, the resultant fat blends were generally found to have a greater tendency to be stabilized in β polymorphic form rather than β′. The effect is likely due to the increase in the melting point of the blends. Thus, the blend is expected to provide a better functionality for use in chocolate formulation in which the softness of the chocolate fat blend might be decreased.

TABLE 2. X-RAY DIFFRACTION PATTERNS OF ANHYDROUS MILK FAT (AMF), SOFT PALM STEARIN (SPOs), HARD PALM STEARIN (HPOs) AND THEIR BLENDS AFTER STABILIZATION AT 26°C FOR TWO DAYS AND 5°C FOR FOUR DAYS

Blend code	Short spacing (Å)										Polymorphic
(AMF:SPOs:HPOs)	4.5	4.4	4.3	4.2	4.1	4.0	3.9	3.8	3.7	3.6	form[a]
A (1:0:0)	4.56vw	-	4.37m	4.22s	-	4.07m	-	-	3.79s	3.69vw	β′ >>> β
B (3:1:0)	4.58s	4.43vw	4.30w	-	4.15w	-	-	-	3.79s	-	β >> β′
C (1:1:0)	4.56m	4.43vw	4.30m	-	4.15m	-	3.98vw	-	3.79s	-	β = β′
D (1:3:0)	4.58s	4.43vw	4.30m	-	4.15s	-	3.98vw	-	3.79s	3.67vw	β = β′
E (0:1:0)	4.53vw	-	4.34s	4.25w	4.18s	4.00w	-	3.89s	3.76s	-	β′ >>> β
F (0:3:1)	4.56w	4.43vw	4.32m	4.24s	4.18s	4.08m	3.90m	3.82w	3.74s	-	β′ >> β
G (0:1:1)	4.56w	4.43vw	4.32m	4.24s	4.18s	4.08m	3.90m	3.82w	3.74s	-	β′ > > β
H (0:1:3)	4.56s	4.43vw	4.32w	4.24m	4.18m	4.08w	3.90vw	3.82m	3.74s	3.68m	β > β′
I (0:0:1)	4.56s	4.44vw	4.33w	4.25w	4.18m	4.09w	-	3.83m	3.75s	-	β > β′
J (1:0:3)	4.56s	4.43vw	4.32m	4.24s	4.18s	4.08s	3.90vw	3.82vw	3.74s	-	β = β′
K (1:0:1)	4.56vw	-	4.34m	-	4.18s	4.09m	-	-	3.76s	3.69vw	β′ >>> β
L (3:0:1)	-	-	4.37m	4.22s	4.07m	-	-	-	3.79s	3.69vw	β′
M (4:1:1)	4.56vw	-	4.37m	4.22s	-	4.07m	-	-	3.79s	3.69vw	β′ >>> β
N (1:4:1)	4.56m	4.43vw	4.32m	4.24s	4.18s	4.08s	3.90w	3.82w	3.74s	-	β′ > β
O (1:1:4)	4.58s	4.43w	4.31vw	-	4.15vw	4.00vw	-	3.83s	-	3.68s	β >>> β′
P (1:1:1)	4.56s	4.43w	4.32w	4.24w	4.18w	4.08w	-	3.82m	3.74m	3.68m	β >> β′

Note: [a] Intensity of diffraction spacings is indicated by s, strong; m, medium; w, weak and vw, very weak.

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