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Physical and chemical properties of a lipase-transesterified palm stearin/palm kernel olein blend and its isopropanol-solid and high melting triacylglycerol fractions

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Abstract

Palm stearin and palm kernel olein at 1:1 (w/w) ratio was transesterified using 1.0% w/w lipase from *Rhizomucor miehei* (Lipozyme IM60, Novo Nordisk). The transesterified blend, as well as its isopropanol-solid (fat solid fractionated using isopropanol at 5 °C) and high melting glycerides (HMG, fat solid fractionated using acetone at 10 °C) were studied. Low carbon number triacylglycerols (TG), ranging from C_{32} to C_{38}, and high carbon number TG (C_{48} and C_{50}) were used to produce TG ranging from C_{40} to C_{46}. Transesterification did not much alter the fatty acid (FA) composition of the mixture, but it increased the level of C12:0 (lauric acid) and reduced the C16:0 (palmitic acid) level in the isopropanol-solid and HMG fractions compared to the control. Transesterification also did not much alter the polymorphic forms of the mixture. However, the HMG fraction of the transesterified mixture had more tendency to form β′ crystals than the control. Slip melting point of transesterified fat was lower than the control. Heating thermograms of differential scanning calorimetry (DSC) illustrated that the high-melting TG peaks in the transesterified blend were reduced in size while the low-melting TG peaks were broadened, mainly due to the rearrangement of FA to form lower-melting TG. The temperature of the high-melting TG peaks of all samples showed a good correlation (correlation coefficient, $r = 0.9899$; $P < 0.05$) with PPP (P, palmitic acid) levels. Formations of low-melting TG also broadened the low-temperature (low-T) peak in the cooling curve of DSC and reduced the crystallisation temperature of the low-T peak. The high-temperature (high-T) peak of the cooling curves were more dominant in the isopropanol-solid and HMG fractions of both transesterified and control blends. The high-T peaks of the transesterified blend was broader and had a lower temperature than the control. Transesterified blends had lower solid fat contents (SFC) than the control at all temperatures. Production of more low-melting TG in the transesterified blend caused a sharp drop in SFC of the control which shifted from the range of 15–20 °C to 10–15 °C.

Keywords: Palm stearin/palm kernel olein; Transesterification; Solid fat content; Isopropanol-solid; High melting glycerides

1. Introduction

Hydrogenated fats are used in edible products, such as margarines, cooking fats and shortenings. However, it has been found that *trans* fatty acids (FA) of these hydrogenated fat products cause several health problems associated with coronary heart disease (Willett et al., 1992; Zocak & Katan, 1992). *Trans* FA-containing fat products showed less digestibility in rats (Ray & Bhattacharyya, 1996). Thus, there has been great interest in producing fat products that contain zero-*trans* FA. Palm stearin (PS), a natural hard component, is a good source of hard stock in these products. Mixtures of PS with a base stock (liquid oil) can then be modified to meet the product characteristics of margarine and shortenings. One of the most appropriate fat-modification methods is enzymatic transesterification. In enzymatic transesterification, the lipases perform catalysis under mild conditions and may also be more specific in their reactions (Ghazali, Hamidah, & Che Man, 1995; Ghazali, Maisarah, Yusof, & Yusoff 1995).

Different fat products require different crystal structures to produce their specific functionality. For instance, shortenings with β crystals aerate poorly but perform well in pie crust applications while margarines require β′ crystals to give smooth eating characteristics.

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The characteristics of the high melting glycerides (HMG) of a fat are important because the TG composition of HMG of the fat will dictate the polymorphic forms in which the fat crystallises and its future behaviour on storage (D'Souza, deMan, & deMan 1991; 1992).

In this study, PS and palm kernel olein (PKO) were enzymatically transesterified using a commercial 1.3 specific lipase (Lipozyme IM60, Novo Nordisk). The transesterified fat is a zero-*trans* blend that can be used as an alternative to the hydrogenated margarines, pastry or shortenings. The physical and chemical characteristics of the transesterified mixture, the fat solid fractionated by isopropanol and the acetone fractionated HMG were studied, in order to understand and predict the polymorphic behaviour of the transesterified blend.

2. Materials and methods

2.1. Materials

Refined, bleached and deodorised (RBD) PKO (slip point 23.4 °C) and PS (slip point 54.4 °C) were donated by Cargill Speciality Oils and Fats Pte. Ltd., Port Klang, Malaysia and purchased from Ngo Chew Hong Oils and Fats Pte. Ltd., Semenyih, Malaysia, respectively. A commercially immobilised *Rhizomucor miehei* lipase (Lipozyme IM60), which was obtained from Novo Nordisk Ind. (Copenhagen, Denmark), was used as the biocatalyst. The moisture content of the enzyme was *ca.* 2.5% w/w. All chemicals used were either of analytical or high-performance liquid chromatograph (HPLC) grade.

2.2. Sample preparation

PS was melted at 60 °C in oven prior to use. A mixture of PKO and PS at 1:1 ratio (by weight, a total weight of 150 g) was prepared in a 500-ml conical flask. The mixture was reacted with 1.0%w/w of Lipozyme IM60 lipase at 60 °C, and shaken at 200 rpm in an incubator. The reaction was stopped after 6 h by filtering out the enzyme, using a Whatman filter paper No. 4 (Ghazali, Maisarah, 1995; Ghazali, Hamidah, & Che Man, 1995; Lai, Ghazali, & Chong, 1998a;b). Similar procedures were done for the control, with the enzyme omitted.

2.3. Hydrolytic activity

One hundred ml of acetone/ethanol (1:1 v/v) was added to 4.0 g of the transesterified blend. The mixture was then titrated with 0.01 N NaOH to a phenolphthalein end-point. The degree of hydrolysis by Lipozyme IM60 lipase was expressed as the percentage of free fatty acid (FFA) liberated and was corrected for the presence of the acids in the control (Lai et al., 1998b).

2.4. Removal of FFA

The FFA was removed from the transesterified blend (Long, Ghazali, Ariff, & Brucke, 1997). The melted transesterified blend was placed in a 250-ml conical flask and 20-ml of acetone:ethanol (1:1 v/v) was added. The mixture was shaken slowly to dissolve the sample and titrated with 0.1 N NaOH to a phenolphthalein end-point. The titrated sample was diluted with 5 ml hot water (about 80 °C) and transferred into a 100 ml separating funnel. After shaking and standing for several minutes, the bottom layer (aqueous phase) containing the FFA was discarded. The top layer, containing oil, was transferred into a McCartney bottle and dried overnight at 80 °C in a vacuum oven. The absence of FFA was confirmed by thin layer chromatography with a solvent system of petroleum ether, diethyl ether and formic acid (210:90:0.4 v/v/v), and viewed in iodine vapour.

2.5. Extraction of isopropanol-solid and HMG

The transesterified blend obtained was separated into three equal portions. The isopropanol-solid and HMG were extracted from the first and second portions, respectively, and the last portion was retained as the original transesterified blend. The isopropanol-solid was extracted with isopropanol at 5 °C without solubilising the fat. The extraction process was modified from previous studies (D'Souza et al., 1991; deMan, D'Souza, deMan, & Blackman, 1992). The transesterified blend was first dispersed using a mechanical pestle and 20 g was transferred into a 250-ml bottle with cap. Isopropanol (120 ml) was then added and the fat crystals were dispersed by shaking the bottle vigorously, followed by agitation for 10 min with a vortex mixer. The mixture was allowed to stand for 24 h at 5 °C, after which two distinct layers appeared. The top layer, which consisted mainly of liquid oil and isopropanol, was removed by filtration using a 0.45-μm nylon filter paper, while the lower layer, which contained the solid fat, isopropanol and some liquid oil, was resuspended in 120 ml isopropanol. This procedure was repeated twice and the final suspension was centrifuged at 5000 rpm for 10 min at 5 °C. The isopropanol-solid was collected after removal of the supernatant. The solid fractionated was then dried in a vacuum oven at 30 °C overnight. The yield of isopropanol-solid was then weighed.

HMG was prepared as described by D'Souza et al. (1992). The transesterified blend was first melted at 60 °C and 20 g of the sample was added to 400 ml of acetone and shaken vigorously. The mixture was then kept at 10 °C overnight to allow the crystallisation to occur. The HMG crystals were separated by filtering the mixture through a 0.45-μm nylon filter paper. The yield was dried in a vacuum oven overnight at 30 °C and the

weight was then recorded. Control sample was treated in a similar manner. The samples (original fat, isopropanol-solid and HMG fractions of the transesterified and control blend) were then analysed for their TG and fatty acid (FA) compositions, iodine value (IV), polymorphic forms, melting profiles, slip melting point (SMP) and solid fat content (SFC).

2.6. TG profile

The TG profiles of the samples were determined by a Shimadzu LC-10 AD HPLC, equipped with a RID-6A refractive index detector (Kyoto, Japan) with a commercially packed RP-18 column (250×4 mm) with particle size of 5 μm (E. Merck, Darmstadt, Germany). The TG were eluted using acetone/acetonitrile (63.5:36.5 v/v) as the mobile phase. The flow rate was 1 ml/min. The degree of transesterification is defined as the change of peak area of the TG that increased in percentage value at reaction time t, [TGI_t] with respect to the value at the start of the reaction, [TGI_0] minus 100%. The rate of transesterification, X, was calculated as below (Ghazali, Hamidah et al., 1995; Lai et al., 1998b):

$$X(\mathrm{h}^{-1}) = \frac{\text{Initial velocity, \%/h}}{\text{Enzyme activity, \%}} \quad (1)$$

where initial rate is ([TGI_t]–[TGI_0])/t, in the linear range of reaction and enzyme activity, is total TG minus remaining TG. Remaining TG is the total concentration of TG after transesterification reaction compared to the unreacted control. The HPLC chromatograms peaks were identified by comparing with the standards.

2.7. FA composition

All samples were melted in oven prior to use and 50 mg of each sample was weighed out; 0.8 ml of petroleum ether and 0.2 ml of sodium methoxide were added. The mixtures were shaken for few seconds by a vortex mixer and left to stand for 5 min (Timms, 1978). 0.3 μl of the solution were injected into a gas chromatograph model GC-17A (Shimadzu Co., Kyoto, Japan) with a flame-ionisation detector. A polar capillary column, model BPX70 (0.32 mm internal diameter, 30 m length, 0.25 μm film thickness), was used (SGE Australia, Pty. Ltd., Ringwood, Australia). Oven temperature, initially at 115 °C, was increased to 180 °C at a rate of 8 °C/min, and temperatures of both detector and injector were kept at 240 °C through out the analysis. The flow rate of the helium carrier gas was 50 ml/min.

2.8. IV

The IV of the samples were determined as described in the AOCS Method Cd 1-25 (AOCS, 1990). The sample (0.400 g) was diluted in 15 ml of carbon tetrachloride and 25 ml of Wijs' solution were added. After storing at room temperature in the dark for 1 h, the mixtures were reacted with 20 ml of 1 N potassium iodide and then titrated with 0.1 N sodium thiosulphate solution.

Table 1
Triacylglycerol (TG) composition (% peak area) and iodine value (IV) in the original fat, isopropanol-solid and high melting glycerides (HMG) of the control and transesterified blends

TG (in carbon number)	TG composition (% peak area)					
	Control			Transesterified		
	Original blend	Isopropanol-solid	HMG	Original blend	Isopropanol-solid	HMG
C_{28}	0.18	0.02	0.05	0.24	0.09	0.01
C_{30}	0.88	0.17	0.06	1.29	0.55	0.12
C_{32}	3.96	0.78	0.41	2.78	1.14	0.29
C_{34}	6.27	1.31	0.56	4.48	2.23	0.50
C_{36}	9.48	2.26	1.05	8.94	3.39	0.88
C_{38}	6.80	2.01	0.74	4.63	3.05	0.72
C_{40}	6.89	2.40	1.03	9.59	5.20	1.79
C_{42}	1.45	1.12	0.71	2.33	2.65	1.60
C_{44}	6.24	1.45	0.47	8.36	3.79	3.24
C_{46}	2.85	0.78	0.36	5.53	2.54	0.89
C_{48}	19.5	59.2	66.7	16.5	43.6	57.9
C_{50}	19.4	19.9	21.3	17.2	17.5	20.6
C_{52}	11.4	3.28	1.44	11.56	4.03	3.14
C_{54}	5.36	5.31	1.27	6.24	7.34	7.83
% Trisaturated TG	55.0	81.3	91.3	47.4	66.7	73.1
% Monounsaturated TG	26.6	14.9	14.2	35.5	21.3	17.9
% Diunsaturated TG	11.50	2.43	0.70	11.9	3.89	1.37
% Polyunsaturated TG	6.94	5.19	4.71	5.15	8.03	7.62
IV, g of I_2/100 g of oil	28.9	8.37	6.84	30.55	9.25	7.05

2.9. Polymorphic forms

X-Ray diffraction analysis was used to determine the polymorphic forms of the transesterified and control mixtures, as well as their isopropanol-solid and HMG, as described by AOCS Method Cj 2-95 (AOCS, 1990). An Enraf Nonius Model FR592 (Delft, The Netherlands) camera was used. The instrument was fitted with a fine-focus copper X-ray tube. The samples were analysed at room temperature.

2.10. Melting and cooling characteristics and SMP

The melting and cooling profiles of the samples were analysed using a differential scanning calorimetry (DSC) model, Perkin-Elmer DSC-7 (Norwalk, CT). Samples, weighing 8–10 mg, and sealed in aluminium pans, were first heated at 80 °C for 15 min to ensure no residual nuclei remained, and then cooled to −40 °C at a rate of −10 °C/min. The samples were then held at −40 °C for 15 min before being heated to 80 °C at a heating rate of 5 °C/min. The melting and crystallisation temperatures of the samples were recorded as the temperatures of endothermic peaks of the melting thermograms and the bottom of exothermic peaks of the cooling thermograms, respectively. SMP of the samples were determined according to the AOCS Method Cc 3.25 (AOCS, 1990).

2.11. SFC

The indirect parallel method (PORIM, 1995) was used for this analysis (PORIM Test Method p. 4.9). Each sample (3–4 g) was tempered at 70 °C for 30 min, followed by chilling at 0 °C for 90 min, and then kept at the desired temperatures for 30 min prior to measurements. The solid contents of the samples were recorded at different temperatures ranging from 5 to 60 °C, with 5 °C intervals, by using a Bruker Minispec pulse-NMR Analyser Model No.120 (Karlsruhe, Germany). Duplicate runs were carried out for each sample.

Duplicate runs were carried out for each sample in all analyses, as well as in the transesterification process.

3. Results and discussion

3.1. General

Results of this study showed that the enzyme activity (based on total TG minus TG remaining after 6 h), degree of hydrolysis (% FFA) and the amount of TG remaining at the end of the transesterification process were 7.3%h^{-1}, 2.1% and 92.7%, respectively. The degree of transesterification for the fat blend was 18.9%, while the rate of transesterification was 16.4 h^{-1}. These were relatively low compared to Lai et al.'s work (1998b) on PS/sunflower oil (40:60) blend.

The isopropanol-solid extracted in this study consisted of all the TG that did not dissolve in isopropanol at 5 °C. They were mainly TG with carbon numbers ranging from C_{34} to C_{54} (Table 1). Meanwhile, the HMG fraction consisted of TG that did not dissolve in acetone at 10 °C, which were mainly C_{48}, C_{50} and some C_{52} and C_{54} TG (Table 1). The yield of the isopropanol-solid and HMG of transesterified blend were 50.2 and 16.8%, respectively; and 58.4 and 23.6%, respectively, for the control. For both transesterified and control blends, the isopropanol-solid fat yields were higher than the HMG's, because the HMG represented only a part of the isopropanol-solid. The yields of both isopropanol-solid and HMG were higher for the control blend than the transesterified blend, probably because, during transesterification, the FA of the higher melting TG were rearranged to produce TG with lower melting points, and hence, the levels of the isopropanol-solid and HMG in the transesterified fat were reduced.

3.2. Chemical properties

Fig. 1 illustrates the TG profiles of the control (Fig. 1A) and the transesterified blends (Fig. 1B) after 6 h of reaction. TG peaks (Fig. 1B) with arrows indicating TG that have increased in concentration after the reaction. The increase in concentrations of several TG species in the transesterified blend compared to the control indicates that transesterification has taken place. As shown in the figure, the concentration of the medium chain TG such as LaLaO, LaLaM, LaOP, LaPP and MOM (La, O, M and P representing lauric, oleic, myristic and palmitic acid, respectively) showed substantial increases after the reaction. The concentrations of high melting TG such as POP and PPP decreased. The results obtained were similar to previous findings (Ghazali, Maisarah, et al., 1995; Lai et al., 1998b; Zainal & Yusoff, 1999).

Table 1 shows the TG composition (as carbon number) of the original fat, isopropanol-solid and HMG of the transesterified blend, as well as those of the control blend. The blending of PS and PKO gave a fat mixture, composed of almost all TG species that could be found in palm oil fruit. The TG types ranged from C_{28} to C_{54}. The concentrations of TG, with carbon numbers ranging between C_{40} and C_{46} in the transesterified blend, increased, and TG with higher (C_{48} and C_{50}) and lower (C_{32}–C_{38}) carbon numbers decreased compared to the control blend. The net change of TG was due to the rearrangement of the FA from the lower and higher melting TG to form more TG that had melting points in between.

During the extraction of isopropanol-solid and HMG, most of the TG with carbon numbers below C_{48}, in both blends, were removed. For the HMG, the two

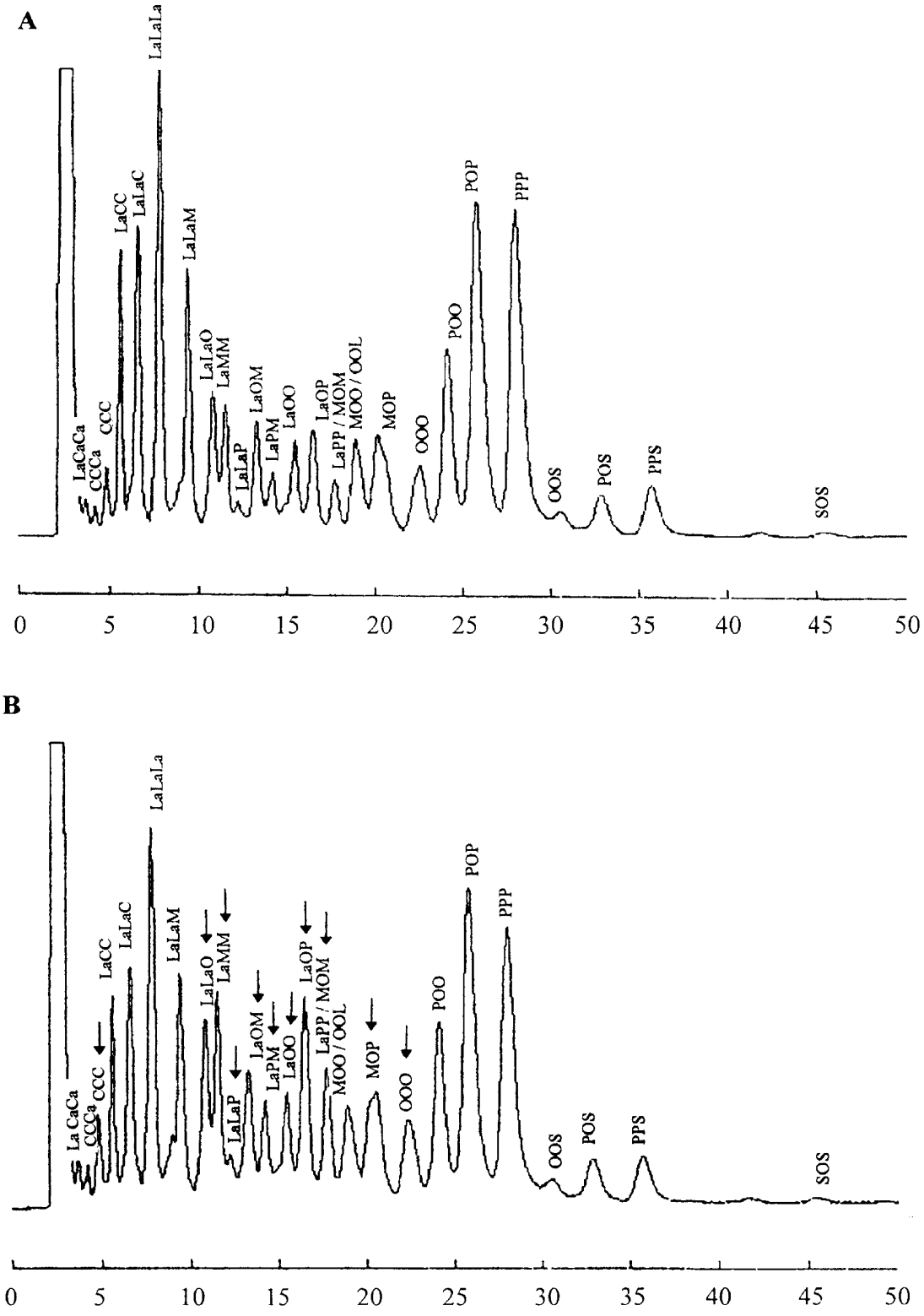

Fig. 1. HPLC chromatograms of palm stearin/palm kernel olein (1:1 w/w) mixture before (A) and after (B) transesterification. Triacylglycerols represented by arrows indicate the triacylglycerols that increased in concentration after 6 h of reaction (Ca, caprylic; La, lauric; C, capric; M, myristic; P, palmitic; O, oleic; L, linoleic and S, stearic acid).

main TG that remained were C_{48} and C_{50}, which represented mainly PPP and POP, respectively. It is noted that the levels of these TG (C_{48} and C_{50}) were lower in the isopropanol-solid and HMG of the transesterified blend than the control as some have been hydrolysed or transformed into low carbon number TG.

Table 2
Fatty acid (FA) composition (% peak area) of the original fat, isopropanol-solid and high melting glycerides (HMG) of the control and transesterified blends

FA	FA composition (% peak area)					
	Control			Transesterified		
	Original blend	Isopropanol-solid	HMG	Original blend	Isopropanol-solid	HMG
C6:0	Trace	–	–	Trace	–	–
C8:0	4.04	Trace	Trace	3.71	1.78	Trace
C10:0	4.09	1.19	Trace	3.75	2.10	0.98
C12:0	43.2	14.5	9.51	41.4	27.4	14.6
C14:0	10.6	7.34	6.05	10.1	9.61	8.01
C16:0	27.9	72.1	79.7	27.9	52.5	72.5
C18:0	1.07	2.19	2.07	2.11	1.77	2.04
C18:1	7.63	3.29	2.66	8.65	4.42	2.42
C18:2	1.48	Trace	Trace	2.35	0.85	Trace
% Unsaturated FA, U	9.11	3.29	2.66	11.0	5.27	2.42
% Saturated FA, S	90.9	96.7	97.3	88.0	94.7	97.6
Ratio U/S	0.10	0.03	0.03	0.12	0.06	0.02

Table 3
Polymorphic forms, slip melting point (SMP), melting and crystallisation temperatures of palm stearin (PS), palm kernel olein (PKO), the original fat, isopropanol-solid and high melting glycerides (HMG) of the control and transesterified blends

	Polymorphic forms	SMP, °C	Melting temperature, °C			Crystallisation temperature, °C	
			Peak I	Peak II	Peak III	Peak IV	Peak V
PS	β	54.4	8.63	39.08	55.03	24.89	−3.14
PKO	–	23.4	−15.05	19.27	–	−6.04	−17.64
Control							
Original blend	β′ > > β	49.3	14.43	–	49.72	18.13	−11.84
Isopropanol-solid	β > β′	58.8	21.20	–	59.87	28.76	−2.17
HMG	β > > β′	60.1	–	–	62.28	30.70	−2.17
Transesterified							
Original blend	β′ > > β	47.0	13.47	32.80	47.78	17.16	−13.77
Isopropanol-solid	β > β	55.8	15.88	41.50	56.48	26.83	6.53
HMG	β′ > > > β	59.0	–	–	61.80	28.76	–

In Table 1, the IV of the transesterified blend and its control were found to be similar. The monounsaturated TG were the main contributors to unsaturation in all samples. The existence of more monounsaturated TG in the transesterified blend was mainly caused by the increase in LaOP, LaOM and LaLaO TG, with percentage increases of 94.0%, 27.5% and 19.7%, respectively (Fig. 1). The percentage of the polyunsaturated TG in the transesterified blend was reduced slightly after the reaction (Table 1). The IV of the isopropanol-solid and HMG, of both transesterified and control blends, decreased compared to their respective original fat (isopropanol-solid and HMG of transesterified blend decreased by 36.8 and 48.8%, respectively. For the control blend, it decreased by 58.5 and 80.6%, respectively). Both the isopropanol-solid and HMG of the transesterified blend showed a smaller reduction of IV compared to the isopropanol-solid and HMG of the control blend.

The FA composition of transesterified and control blends, as well as their respective isopropanol-solid and HMG fractions are shown in Table 2. For all samples, C12:0 and C16:0 were the main FA found and are contributed by PKO and PS, respectively. For both transesterified and control blends, the level of C12:0 decreased while C16:0 increased in the isopropanol-solid and HMG fractions. This is mainly due to the removal of C12:0-containing TG during the extractions. Transesterification did not alter much of the composition of C12:0 and C16:0 in the original fat but it increased the level of C12:0 and reduced the C16:0 level in the isopropanol-solid and the HMG. In general, the ratios of unsaturated FA over the saturated FA (U/S) of the transesterified and control blends were similar.

3.3. Polymorphic Forms

Table 3 details the polymorphic forms of PS, the transesterified and control blends, as well as their isopropanol-solid and HMG fractions. All mixtures consisted of combinations of β and β′ crystals. Incorporation of PKO into PS increased the diversity of the

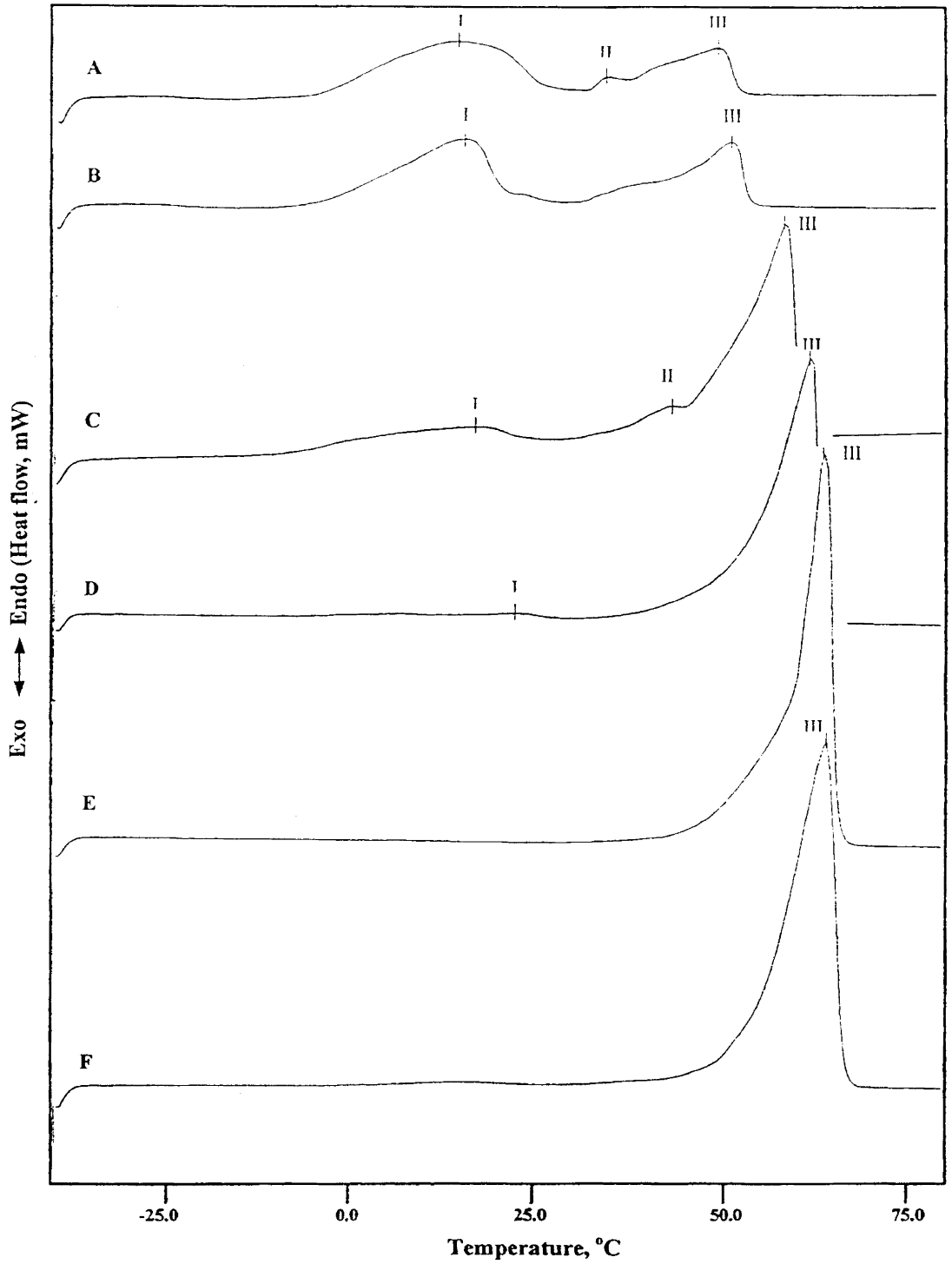

Fig. 2. Differential scanning calorimetry (DSC) heating thermograms of the palm stearin/palm kernel olein (1:1 w/w) mixture for transesterified original fat (A), control original fat (B), isopropanol-solid of transesterified blend (C), isopropanol-solid of control blend (D), HMG of transesterified blend (E) and HMG of control blend (F) at heating rates of 5 °C/min. All of the thermograms are shown on the same scale.

TG and FA pools in the blend and thus altered the crystal forms of PS. Transesterification did not much alter the polymorphic behaviour of PS/PKO mixture, except to generate a little more β′ crystals with a weaker band at 4.60Å (data not shown).

The HMG of the transesterified fat had more β′ crystals than the control. High levels of PPP (65.5%) and POP (10.9%) in the HMG of the control were responsible for the formation of more β crystals. D'Souza et al. (1991) reported that the HMG of margarines, that

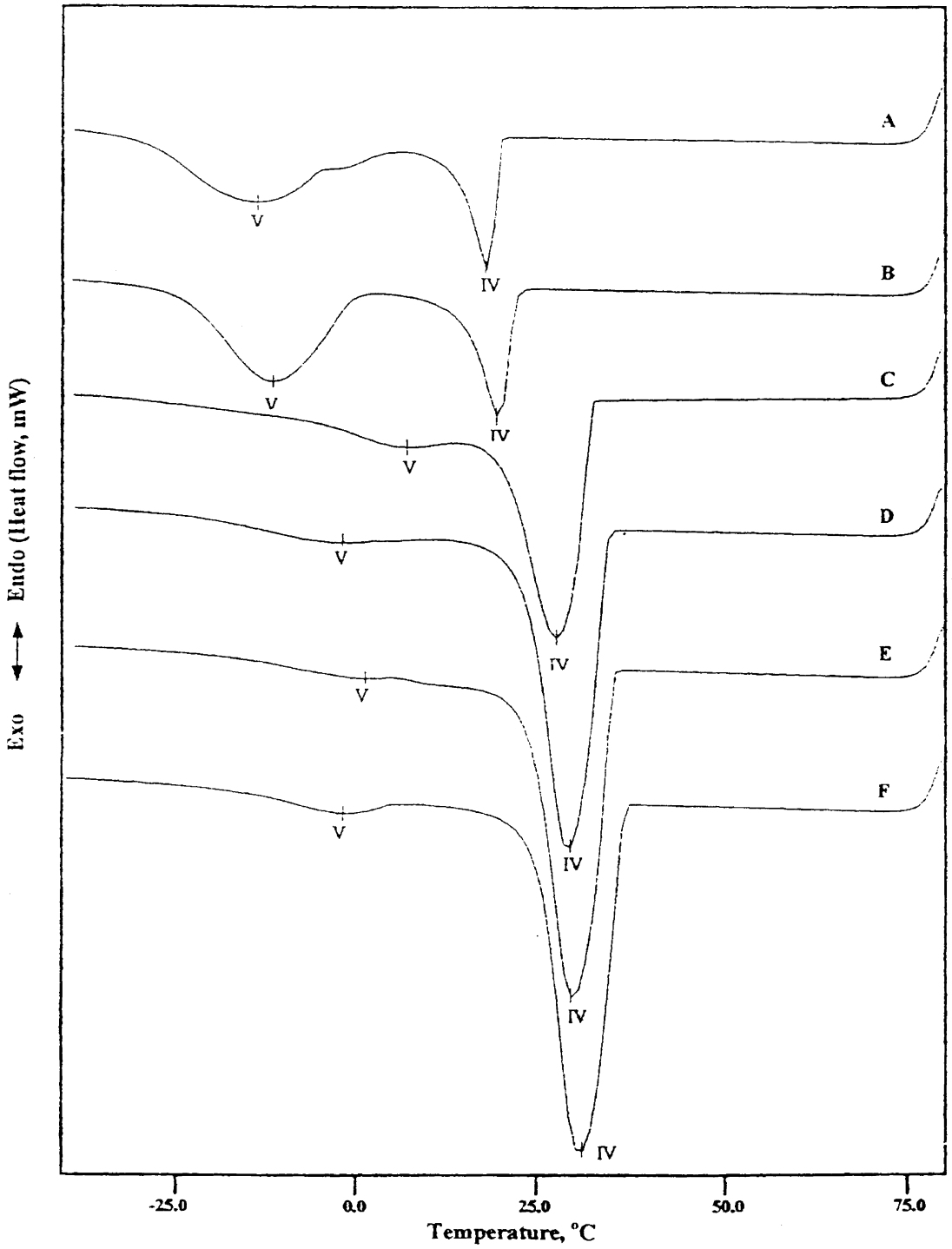

Fig. 3. Differential scanning calorimetry (DSC) cooling thermograms of palm stearin/palm kernel olein (1:1 w/w) mixture for transesterified original fat (A), control original fat (B), isopropanol-solid of transesterified blend (C), isopropanol-solid of control blend (D), HMG of transesterified blend (E) and HMG of control blend (F) at cooling rates of −10 °C/min. All of the thermograms are shown on the same scale.

consisted of high levels (> 50%) of C_{48} or C_{54} TG, were normally in the β form. POP is also a β-tending TG. Transesterification reduced PPP and POP levels in the HMG (45.8 and 9.9%, respectively), and hence it contained more β′ crystals than the control. Unlike the HMG of the control blend, the HMG of the transesterified blend also consisted of relatively higher levels of C_{40} to C_{46} TG, which contributed to a greater diversity in the FA pool in HMG, thus promoting β′ crystals formation.

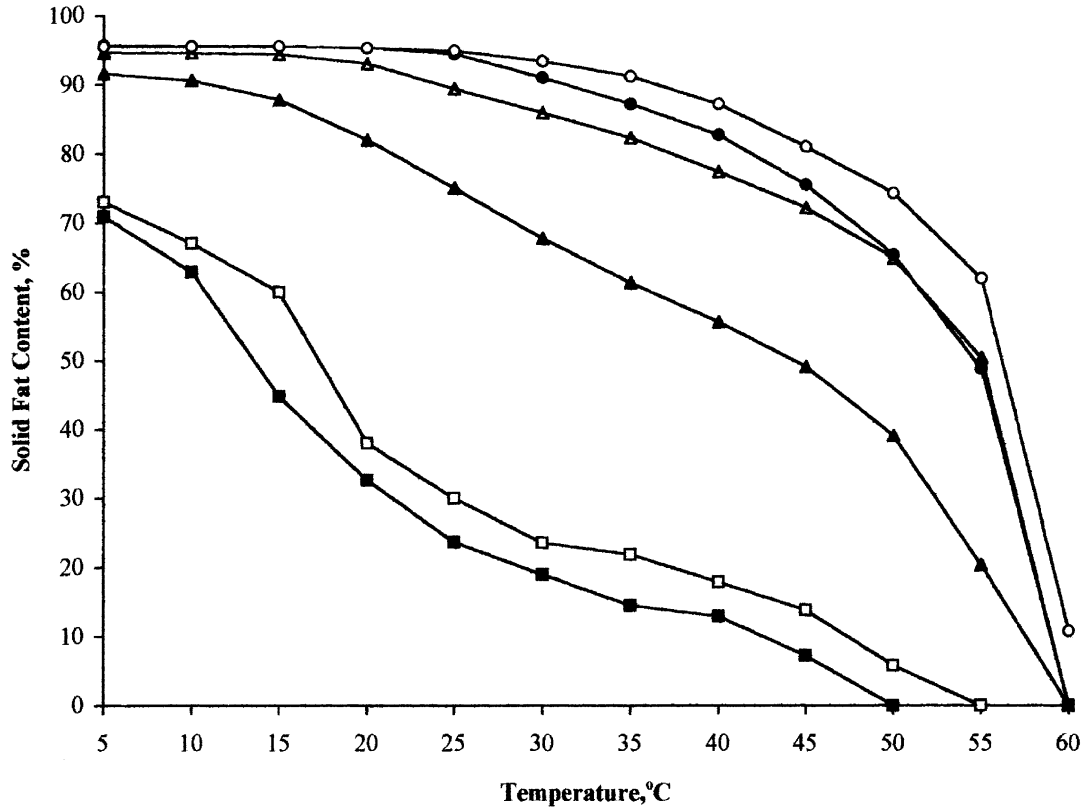

Fig. 4. Changes in solid fat content (SFC) of palm stearin/palm kernel olein (1:1 w/w) mixture for transesterified original fat (■), control original fat (□), isopropanol-solid of transesterified blend (▲), isopropanol-solid of control fat (△), HMG of transesterified and HMG of control blend (○) as a function of temperature.

3.4. Melting and cooling profiles and SMP

Fig. 2 illustrates the DSC melting thermograms of the transesterified and control blends, as well as their respective isopropanol-solid and HMG (Fig. 2A–F). The melting temperatures, crystallisation temperatures and SMP for each sample are cited in Table 3. In Fig. 2B (control blend), the control blend showed two endothermic peaks (Peaks I and III). After transesterification, the size of Peak III was reduced (Fig. 2A). Since Peak III represents the higher-melting TG of the blends, reduction in peak size resulted in a lower SMP in the transesterified blend (47.0 °C; Table 3), compared to the control (49.3 °C). The results supported earlier results which showed rearrangement and acyl exchange of the transesterified blend to form lower- and middle-melting TG species. Formation of more lower- and middle-melting TG caused Peak I of the transesterified blend to broaden compared to the control. Removal of liquid phase caused Peak I in the isopropanol-solid (Fig. 2D) and HMG (Fig. 2F) of the control blend to become indistinguishable. Peaks I and II also markedly decreased in size in the isopropanol-solid of the transesterified blend (Fig. 2C) and disappeared completely in the HMG fraction (Fig. 2E). Peak III consisted of high-melting TG that were most likely to be PPP, POP and SOS (S, stearic acid). There was a good correlation ($r = 0.9899$; $P < 0.05$) between the concentrations of PPP (the main TG in Peak III) in all samples with the melting temperatures, suggesting that PPP largely contributed to the melting temperatures of Peak III. Transesterification reduced the level of PPP in the PS/PKO blend by 22.4% compared to the control and, therefore, resulted in the decrease of melting points of the original fat, isopropanol-solid and HMG, compared to those of the controls. SMP for all samples were lower but close to the melting temperatures of Peak III (Table 3).

The cooling curves of the samples are shown in Fig. 3, and their respective crystallisation temperatures in Table 3. Both transesterified (Fig. 3A) and control (Fig. 3B) blends show two major exotherms: a sharp high-temperature or high-T peak (Peak IV) and a broad low-temperature exotherm or low-T peak (Peak V). The low-T and high-T peaks represent the low melting TG and high melting TG component of the fats, respectively. Transesterification introduced more low melting TG and this broadened the low-T peak (Fig. 3A) and reduced the crystallisation temperature. For both mixtures, extraction into isopropanol-solid and HMG fractions caused the low-T peak to decrease in size. The low-T peak of isopropanol-solid (Fig. 3C) of the transesterified blend was still slightly perceptible compared to

the control (Fig. 3D), which disappeared. The high-T peak became more dominant in the isopropanol-solid and HMG of both the transesterified and control blends (Fig. 3C–F). In a similar study, Watanabe et al. (1992) reported that the high-T peak in palm oil products is well correlated to the PPP content. Similar observations were noted in this study, whereby the high-T peak became sharper and the peak height increased with an increase in the PPP content of the samples. The high-T peak also shifted to the right to give higher crystallisation temperature when more of the liquid phase was removed from the fat blends. The temperature of the high-T peak agreed well ($r = -0.9891$; $P < 0.05$) with IV. The more saturated the fat, the higher the temperature of high-T peak. High-T peak of the original transesterified blend was broader and at a lower temperature (17.2 °C) than the control blend (18.2 °C) as a result of transesterification, which reduced the high-melting TG, especially PPP.

3.5. SFC

Fig. 4 shows the changes in the SFC profiles of the fat samples as a function of temperature ranging between 5 and 60 °C. The transesterified blend had a lower SFC than the control for all temperatures, with both the profiles mimicking each other. A decrease in PPP may be responsible for the reduction of SFC levels. The sharp drop in SFC of the control shifted from 15–20 to 10–15 °C in the transesterified blend. This is due to the production of more low-melting TG that melted in this temperature range (Lai et al., 1998a; Lai, Ghazali, & Chong, 1998c; Zainal & Yusoff, 1999). The SFC of the HMG in both blends melted almost completely at 60 °C.

From the results, the transesterified PS/PKO blend was found to be quite suitable for the formulation of shortenings. However, for margarine formulations, the amount of the hard stock (PS) may need to be reduced, as the blend did not melt completely at 37 °C. The results of this study provided a better understanding of the modification of fats to produce products that meet their functional requirements.

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