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Physical properties of lipase-catalyzed transesterified blends of palm stearin and anhydrous milk fat

O.M. Lai[a], H.M. Ghazali[a,*], France Cho[a], C.L. Chong[b]

[a]*Department of Biotechnology, Faculty of Food Science and Biotechnology, Universiti Putra Malaysia, UPM 43400 Serdang, Selangor, D.E., Malaysia*

[b]*Palm Oil Research Institute of Malaysia, Bandar Baru Bangi, 43650 Selangor D.E., Malaysia*

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Abstract

Palm stearin (PS) and anhydrous milk fat (AMF) blends, formulated by mixing 40 to 80% PS in increments of 10% (w/w), were subjected to transesterification catalyzed by lipases from *Pseudomonas* sp. and *Rhizomucor miehei* (Lipozyme IM60). The physical properties of the transesterified products were evaluated by slip melting point (SMP), differential scanning calorimetry (DSC), solid fat content (SFC) and X-ray diffraction (XRD) analyses. SMP results indicated that the *Pseudomonas* sp. lipase caused a bigger drop in SMP (15.6%) for a PS:AMF (40:60) blend compared to 12.5% reduction when *R. miehei* lipase was used. The same blend, when reacted with either of the lipases, had a residual SFC of 7.1% at 40°C. Generally, for all other ratios of PS:AMF blends, the % of reduction in SMP was higher in the *Pseudomonas* lipase-catalysed blends compared to the *R. miehei* lipase-catalysed blends. *Pseudomonas* lipase also successfully changed the polymorphic form of unreacted PS:AMF blends from a mixture of β and β' crystals to a predominantly β' mixture following transesterification. On the other hand, transesterification with *R. miehei* lipase resulted in a product that is β' dominating © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Transesterification; Palm stearin; Anhydrous milk fat; *Rhizomucor miehei*; *Pseudomonas*; Slip melting points; Solid fat content; Polymorphic forms; DSC; Table margarines

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1. Introduction

Transesterification of a hard fat such as palm stearin with natural oils can produce a low *trans* fat blend with desired characteristics. Rearrangement of acyl residues in TG has provided fats and oils with new physical properties (Hustedt, 1976; Lanning, 1985; Sreenivasan, 1978). Transesterification can be controlled by selection of the composition and source of raw material as well as the processing parameters.

When evaluating the physical properties of margarine and shortening blends, it is necessary to consider the crystal habit or tendency of parent oils or fats used and the effect on the crystal form of the product on storage. The β polymorph is more stable than the β', and the rate of transformation of β' to β differs for individual oil types. The β' form is desired in most margarine and shortenings because of the smooth texture it gives to the product. Palm oil is a β' oil but a margarine composed of a mixture of palm stearin, palm olein and palm oil will, on storage for about 2 weeks, become hard and brittle, a state associated with β structure (Young, 1985). To produce a fat with enhanced β' stability, it is necessary to avoid a preponderance of one triglyceride (TG) or closely similar TG (Young, 1985). For this reason, variation of fatty acid (FA) chain length, by, for example, inclusion of a lauric acid oil or sunflower oil, or by using 10–20% of a C16-containing fat, such as palm oil, is often recommended. The excessive presence of one TG can be mitigated by transesterification, and β' stability can also be enhanced by the use of additives such as tristearate and diglyceride (DG) (Young).

The physical properties of margarine are dictated by the solid fat content (SFC), particularly of the high melting glycerides (HMG), as these TG are thought to set the trend in the polymorphic crystal behaviour (D'Souza, de Man & de Man, 1991; 1992). A fat blend which melts completely at 37°C and has a steep SFC profile gives good oral melt down in the mouth. There is also a general relationship between the SFC and hardness

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[*] Corresponding author. Tel.: + 603-9486101, ext. 3420; fax: + 603-9423552.

E-mail address: hasanah@fsb.upm.edu.my (H.M. Ghazali).

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of margarine. The most desirable hardness range is found between 15 and 30% solid fat (D'Souza et al., 1991).

Differential scanning calorimetry (DSC) heating curves also give valuable information about the melting and solidification properties of a fat, including melting points. As the iodine value of a blend of fat decreases, the peak areas obtained from DSC curves are displaced toward high melting temperatures. The melting point of a fat can be taken as the point where the melting curve finally intersects with the baseline. DSC can also be used to determine crystallisation curves. It can show at what temperature the crystals start to form.

The present study examined the physical properties of transesterified fat products prepared from palm stearin enzymatically transesterified with anhydrous milkfat in different proportions. The study aims to maximise the use of palm stearin, which is the cheaper fraction obtained from fractionation of palm oil. AMF was used in the study to impart a buttery flavour to the end product. Non-specific (*Pseudomonas* sp.) and 1,3-specific (*R. miehei*) lipases were used in a solvent-free transesterification process. The SMP, SFC, melting thermograms and polymorphic form(s) were analysed to assist in the proper choice of PS:AMF which produces the desired melting properties for use, in or as standard table margarine or shortening.

2. Materials and methods

2.1. Materials

Refined, bleached and deodorised hard palm stearin (PS) (SMP 54.5°C) and anhydrous milkfat (AMF) were obtained from Ngo Chew Hong Oils and Fats (M) Sdn. Bhd. and Promac Enterprise (M) Pte. Ltd., respectively. These were stored at 4°C. Prior to use, palm stearin and anhydrous milk fat were melted at 60°C in the oven. Amano Pharmaceutical Co. Ltd. (Nagoya, Japan) donated the *Pseudomonas* sp. lipases (powder form) while *R. miehei* lipase (Lipozyme IM60) was obtained in the immobilized form (moisture content: 2–3%) from Novo Nordisk (Copenhagen, Denmark). Celite, used as a carrier for the immobilization of the *Pseudomonas* lipase, was purchased from BDH Ltd, England. All other chemicals used were of analytical or HPLC grade.

2.2. Immobilization of lipase

Pseudomonas lipase powder (0.1 g) was dissolved in 100 μl of cold deionized water, followed by mixing with 0.25 g of Celite (Ghazali, Hamidah & Che Man, 1995). The preparation was lyophilized for 4 h at −43°C with an Alpha 1-4 Christ LDC-1 (B. Braun, Melsungen, Germany) freeze dryer prior to the transesterification process. *R. miehei* lipase was used as is in its immobilized form.

2.3. Blend preparations

Liquefied palm stearin (PS) and anhydrous milk fat (AMF) were mixed in proportions ranging from 40 to 80% palm stearin, in 10% increments (w/w). Five blends were prepared: 40:60, 50:50, 60:40, 70:30 and 80:20, identified by the mass ratio of palm stearin to anhydrous milk fat (PS:AMF).

2.4. Transesterification

Transesterification was carried out as previously reported (Ghazali et al., 1995). Ten grams of PS:AMF blends were reacted with 0.1 g equivalent of an immobilized lipase at 60°C and 200 rev/min for 8 h for the *Pseudomonas* lipase and 6 h for *R. miehei* lipase.

2.5. Solid fat content

A Bruker Wideline Pulse NMR (Karlsruhe, Germany), using the direct measurement procedure, was employed for the solid fat content (SFC) measurements. Nine tubes were used for each sample. Each sample 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 melting, chilling and holding of the samples were carried out in pre-equilibrated thermostated baths. The SFC was measured within the temperature ranges of 5–40°C.

2.6. Thermal properties by DSC analysis

The instrument used was a Perkin–Elmer DSC-7 (Norwalk, CT). Samples weighing from 3 to 15 mg, sealed in an aluminium pan, were heated to 70°C for 15 min to ensure that no residual nuclei remained. The samples were then cooled from melt (70°C) at 5°C/min to −30°C and held for 15 min before heating the samples to 70°C again at 5°C/min for the melting thermograms.

2.7. Slip melting point (SMP)

This was determined by the method as described in the AOCS Method Cc. 3.25 (1989).

2.8. X-ray diffraction (XRD) analysis

The camera used was an Enraf Nonius model FR 592 (Delft, The Netherlands). The instrument was fitted with a fine focus copper X-ray tube. The sample holders were flat stainless-steel plates each with a rectangular hole.

Samples were melted to 70°C and tempered at 25°C for 30 min. Short spacings on the X-ray film were measured with a Guiner viewer (Enraf Nonius). The short spacings of the β' form are at 4.2 and 3.8 Å and that of the β form is at 4.6 Å (de Man, Xu, Chen & de Man, 1993). Levels of β' and β crystals in mixtures were estimated by the relative intensity of the short spacings at 4.2 and 4.6Å. The XRD analysis was done within a period of a week from the time of transesterification and kept at 4°C prior to analysis.

3. Results and discussion

The study was initiated in an effort to maximize the use of palm stearin, the cheaper and more solid fraction of palm oil. Currently, the quantity of PS that is commonly incorporated in a standard table margarine is only 10% (Teah, 1982). To maximize the use of palm stearin, a minimum of 40% was tried. It was reasoned that if a suitable table margarine formulation, which is spreadable at room temperature, could be made with this level of PS, then softer products could be made with lower levels.

Table 1 shows the results of the SMP of the transesterified PS and AMF blends, ranging from 40 to 80% PS (w/w). For all blends, a reduction in SMP was observed after transesterification with *Pseudomonas* and *R. miehei* lipases compared to the unreacted blends (Table 1). *Pseudomonas* lipase caused a bigger drop (15.6%) in SMP for the PS:AMF (40:60) blend as compared to a 12.5% reduction when *R. miehei* lipase was used. The results clearly indicated that *Pseudomonas* lipase is a slightly better biocatalyst in randomizing the FA in the TG molecule of palm stearin than *R. miehei* lipase. The decrease in SMP after transesterification by both lipases indicated that palm stearin (I.V. 29.5) mixed with AMF was successful in yielding products with lower melting points than before. A bigger reduction in SMP would be obtained if PS was transesterified with more liquid oils such as with sunflower oil (Lai, Ghazali & Chong, 1998a) and palm kernel olein (Lai, Ghazali & Chong, 1998b). However, in this work, AMF (SMP: 35°C), which also contained a rather high amount of saturated FA, was incorporated to give a buttery flavour to the end product.

The SFC profiles, as a function of temperature for the PS:AMF blends before and after transesterification with *Pseudomonas* and *R. miehei* lipases, are shown in Fig. 1. As shown, the unreacted mixture of PS:AMF (40:60) had a SFC of 77.8% at 5°C while the SFC at the same temperature for the reacted blend was 62.2%. At 40°C, the same PS:AMF blend still had 15.7% solids. Following reaction with either *Pseudomonas* or *R. miehei* lipases, the blend had a SFC of 7.1% at 40°C, an amount of solid fat that would leave a greasy mouthfeel. Similar rates of decline of SFC by both lipases were probably caused by similar degrees of randomization of the FA molecules by both lipases. From the results, 0% solids could be achieved by lowering the quantity of palm stearin in the blend.

Table 1
Slip melting points (SMP) of palm stearin:anhydrous milkfat (PS:AMF) blends before (control) and after transesterification with *Pseudomonas* and *R. miehei* lipases

% PS:AMF (w/w)	Slip melting points (°C)		
	Control	*Pseudomonas* sp. (%)[a]	*R. miehei* (%)
40:60	48.0	40.5 (15.6)*	42.0 (12.5)
50:50	50.5	42.0 (16.8)	44.0 (12.9)
60:40	51.5	43.0 (16.5)	45.0 (12.6)
70:30	53.0	44.5 (16.0)	46.0 (13.2)
80:20	54.0	46.0 (14.8)	48.0 (11.1)

[a] The values in parentheses are the % of SMP reduction.

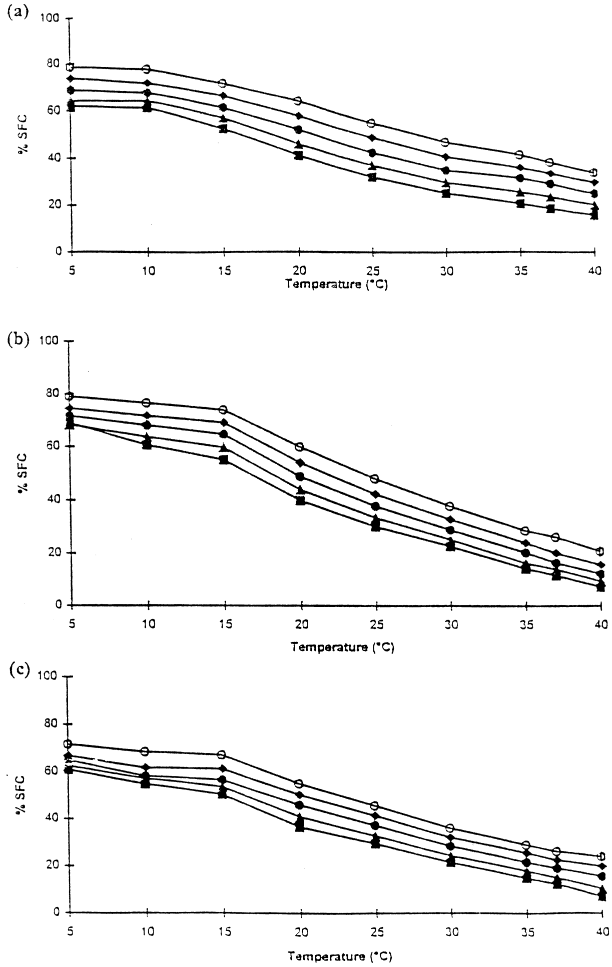

Fig. 1. Solid fat content (SFC) of palm stearin:anhydrous milkfat (PS:AMF) blends (a) before and after transesterification with *Pseudomonas* (b) and *R. miehei* (c) lipases [PS:AMF (w/w); 40:60 ■, 50:50 ▲, 60:40 ●, 70:30 ◆, 80:20 ○].

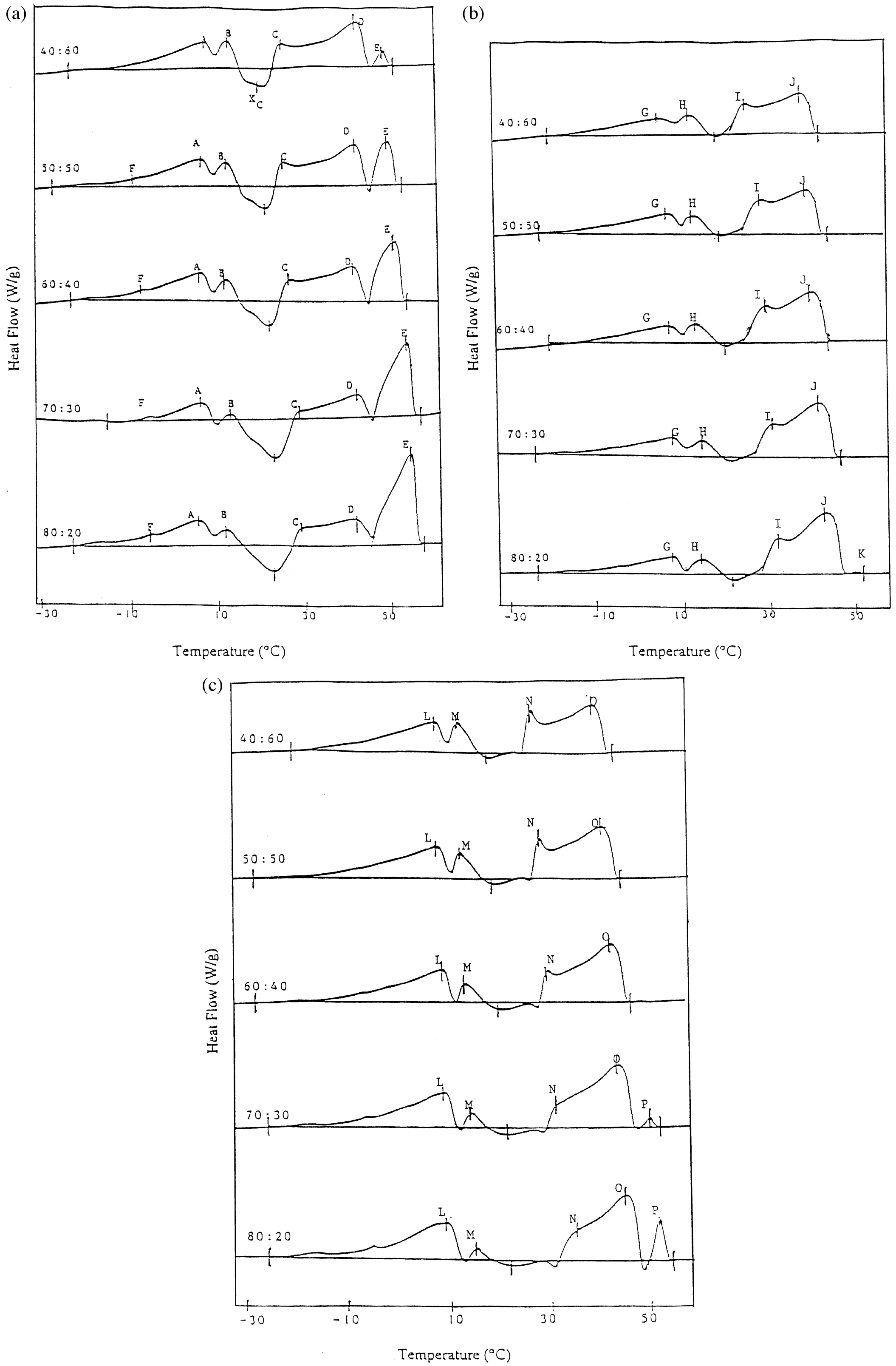

Fig. 2. (a) Melting thermograms of palm stearin:anhydrous milkfat (PS:AMF) blends before transesterification (control) at heating rates of 5°C/min. Pretreatment: cooled from 70 to −30°C at 5°C/min. Heating program was started after 15 min at −30°C. (b) Melting thermograms of palm stearin:anhydrous milkfat (PS:AMF) blends after transesterification with *Pseudomonas* lipase at heating rates of 5°C/min. Pretreatment: cooled from 70 to −30°C at 5°C/min. Heating program was started after 15 min at −30°C. (c) Melting thermograms of palm stearin:anhydrous milkfat (PS:AMF) blends after transesterification with *Rhizomucor miehei* lipase at heating rates of 5°C/min. Pretreatment: cooled from 70 to −30°C at 5°C/min. Heating program was started after 15 min at −30°C.

Fig. 2a–c shows the melting thermograms of PS:AMF blends before and after catalysis with *Pseudomonas* and *R. miehei* lipases. In the unreacted mixture (Fig. 2a), peak D became less pronounced while peak E, which represents the high melting glycerides (HMG), became more so as the proportion of palm stearin in the mixture was increased. This indicates the formation of a firmer product as confirmed by the SMP results. The PS:AMF mixtures catalyzed by *Pseudomonas* lipase (Fig. 2b) had lower final melting temperatures than those catalyzed by *R. miehei* lipase (Fig. 2c). In the reaction mixtures catalyzed by *Pseudomonas* lipase, four endotherms, namely G, H, I and J and one exotherm, X_I were observed. With increasing proportion of PS in the reaction mixtures, the final peak J became more pronounced due to the higher heat of melting of the HMG formed during transesterification. In the 80:20 PS:AMF mixture, a higher melting peak K was noticeable (Fig. 2b). This is probably due to a higher HMG content when the amount of palm stearin in the mixture was higher (Fig. 2a). Catalysis with *R. miehei* lipase caused the peaks to become more pronounced. The appearance of the final melting peak P in the *R. miehei*-catalyzed mixture (Fig. 2c) was observed at PS:AMF of 70:30 which increased in size with a corresponding increase in PS. Peak P could be due to residual unreacted TG (Fig. 2a). Peak N also became more distinguishable while peak O increased in size, suggesting that the middle-melting component of peak N had formed a mixed crystal structure with those of peak O.

The polymorphic crystal structures of PS:AMF before and after catalysis with *Pseudomonas* and *R. miehei* lipases are given in Table 2. In the unreacted mixture, both β and β′ forms existed in equal proportions. However, on transesterification with *Pseudomonas* lipase, only β′ crystals were formed while after catalysis with *R. miehei* lipase, both β′ and β forms were present with β′ dominating. Crystallization of fats is always complicated and is influenced by many factors, but crystallisation of milk fat is even more intricate because of its complex composition. Milk fat contains more than 40 different FA (Timms, 1979). Due to its large number of FA, a huge number of TG are present (Deffense, 1993). Diffraction data by Timms indicated that MF occurs mainly in the β′ form with traces of β polymorph. The presence of β characteristics is attributed to the presence of a small amount of high melting trisaturated TG (Timms). Thus, it can be assumed from the results that *Pseudomonas* lipase was more efficient in randomizing the FA than *R. miehei* lipase was.

Based on SMP, SFC, DSC and XRD results, *Pseudomonas*-catalyzed PS:AMF blends at 40:60 ratio may find possible usage as an industrial shortening or pastry margarine which requires higher solids content in the blend.

Table 2
Polymorphic forms of palm stearin:anhydrous milkfat (PS:AMF) blends before (control) and after transesterification with *Pseudomonas* and *R. miehei* lipases

% PS:AMF (w/w)	Polymorphic forms		
	Control	*Pseudomonas* sp.	*R. miehei*
40:60	β = β′	β′	β′ > > > β
50:50	β = β′	β′	β′ > > > β
60:40	β = β′	β′	β′ > > β
70:30	β = β′	β′	β′ > > β
80:20	β = β′	β′	β′ > > β

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