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Effect of enzymatic transesterification on the fluidity of palm stearin-palm kernel olein mixtures

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In an effort to improve the physical and/or melting characteristics of solid fats, the enzymatic transesterification of palm stearin-palm kernel olein (40:60) in a solvent-free system was investigated. The enzymes used were Celite-bound lipases that include 1,3-specific lipases such as *Aspergillus niger*, *Alcaligenes* sp. and non-specific lipases such as *Pseudomonas* sp. and *Candida rugosa*. Commercial immobilized lipase from *Rhizomucor miehei* (Lipozyme 1M60) was also used. The efficacies of these enzymes for improving the melting behaviour of the oil mixtures were followed by slip melting point (SMP), solid fat content (SFC) and differential scanning calorimetry (DSC) analyses. Results indicated that enzymatic transesterification was able to produce fat mixtures with substantially lower melting points by repositioning the fatty acids of triglycerides in the higher melting range to form lower- or middle-melting components. *Pseudomonas* lipase-catalyzed mixtures produced the highest degree (152.2%) and rate ($50.0\,h^{-1}$) of transesterification followed by *R. miehei* lipase at 151.7% and $27.1\,h^{-1}$, respectively. The highest % FFA liberated was also from the reaction mixture catalysed by *Pseudomonas* (2.90%) and *R. miehei* (2.54%) lipases. The *Pseudomonas*-catalyzed mixture also produced the biggest drop in SMP (12.0°C) and the SFC results showed complete melting at 35°C. Our findings also suggest that the positional specificity of lipases may not play a significant role in producing a more fluid product. © 1998 Elsevier Science Ltd. All rights reserved.

INTRODUCTION

Palm stearin, a high melting point fraction from palm oil, can be used as a source of fully natural hard component in the manufacture of edible fat products such as margarine, shortening and pastry (Pantzaris, 1987) The physical characteristics of palm stearin differ significantly from those of palm oil. It contains a wider range of fatty acids than the former (Pantzaris, 1987). The high melting range of palm stearin (44–56°C) poses problems in the manufacture of edible fats as it confers low plasticity to the product. At room temperature (≈25°C), palm stearin behaves as a solid and lacks the spreadability needed in products like margarine and shortening. However, considerable potential exists if the melting point of palm stearin can be lowered as it allows for better utilisation of the fat in preparing edible fat products (Foglia *et al.*, 1993). Reactions between the solid palm stearin and other liquid oils have been suggested to obtain fat mixtures with better melting properties. Enzymatic transesterification between the solid palm stearin and other oils offers a suitable method for the modification of the physical and chemical properties of the former (Posorske *et al.*, 1987). The current interest in favouring enzyme-catalyzed reactions over those carried out by chemical catalysts also lies in the fact that enzymes are biodegradable and this reduces environmental loading. In comparison with chemical catalysts, enzymes are able to catalyze at mild temperatures and this will reduce the deterioration in the quality of the fats that often takes place at the elevated temperatures required for chemical catalysts. Some enzymes are also able to distinguish between the *sn*1, and/or *sn*3 positions on the triglyceride molecules (Posorske *et al.*, 1987). Such a property allows for a more directed rearrangement of the fatty acids in the triglyceride molecules and a wide variety of specific products with different compositions and properties can be prepared (Forssell and Poutanen, 1992). Fats prepared using the enzymatic method do not contain *trans* fatty acids which have been implicated in having adverse effects on

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the high density lipoprotein levels in blood (Mensink and Katan, 1990).

In this study, we report on the efficacy of various immobilized 1,3-specific lipases such as from *Aspergillus niger*, *Rhizomucor miehei*, and *Alcaligenus* sp. and non-specific lipases such as from *Pseudomonas* sp. and *Candida rugosa* in reducing the melting properties of the palm stearin-palm kernel olein (PS-PKO) (40:60) mixture in a solvent-free system. The work was carried out in an effort to produce a fat mixture with optimum melting characteristics for use in margarine production.

MATERIALS AND METHODS

Samples

Refined, bleached and deodorized hard palm stearin (PS) (SMP 54.5°C) and palm kernel olein (PKO) were obtained from Ngo Chew Hong Oils and Fat (M) Sdn. Bhd. and Southern Edible Oil Ind. Sdn. Bhd. Malaysia, respectively. The fats and oil were stored at −4°C. Prior to use, the palm stearin was melted at 60°C in an oven. Celite, used as a carrier for the lipases, was purchased from BDH Ltd., England.

Amano Pharmaceutical Co. (Nagoya, Japan) donated the *Aspergillus niger*, *Pseudomonas* sp. and *Candida rugosa* lipases. *Rhizomucor miehei* (Lipozyme 1M60) lipase was obtained in the immobilized form (granule size: 0.2 0.6 mm, moisture content: 2–3%) from Novo Nordisk Industries (Copenhagen, Denmark) while Meito-Sangyo Co. Ltd. (Japan) donated the *Alcaligenes* sp. lipase.

Immobilization of lipase

0.1 g of lipase powder was dissolved in 100 µl of cold deionized water, followed by mixing with 0.25 g of Celite (Ghazali *et al.*, 1995a). The preparation was lyophilized for 4 h at −43°C with an Alpha 1–4 Christ LDC-1 (B. Braun) freeze-dryer apparatus prior to the transesterification process.

Transesterification

Transesterification was carried out as previously reported (Ghazali *et al.*, 1995a). Prior to transesterification, all the lipases used, except for *R. miehei* lipase, were immobilized as previously reported (Ghazali *et al.*, 1995a). Ten grains of PS-PKO (40:60) mixtures were reacted with 0.1 g equivalent of immobilized lipases at 60°C, 200 rev per min for 8 h, for all lipases, except for mixtures with *R. miehei* and *Alcaligenes* lipases which were reacted for 6 h. After the transesterification reaction, 1 ml of the reaction mixture was withdrawn and the triglyceride (TG) composition determined by HPLC (Shimadzu Co., Japan) using a commercially packed RP-18 column (250×4 mm) with 5 µm particle size (E. Merck, Darmstadt, Germany). TG were eluted with acetone:acetonitrile (60:40) at 1 ml min^{-1} flow rate. Percent TG remaining (%TGR) is the total concentration of TG after reaction has occurred compared to the unreacted mixture (Ghazali *et al.*, 1995a). The degree of transesterification is defined as the change in concentration of TG, that is increased in value, $[TGI_t]$ at reaction time t with respect to the value at the start of the reaction, $[TGI_0]$. The rate of transesterification was calculated as shown below:

$$X(h^{-1}) = \frac{\text{initial velocity (\%/h)}}{\text{enzyme activity (\%)}} \quad (1)$$

where initial velocity is $([TGI_t]-[TGI_0])/t$ at the linear range of reaction, and enzyme activity is the actual activity of the lyophilized immobilized enzyme used. The enzyme activity is expressed as the % of TG hydrolyzed (total TG minus remaining TG), determined here, rather than as the activity specified by the manufacturer.

Hydrolytic activity

The amount of FFA present was determined according to the method of Cocks and van Rede (1966). At the end of the incubation period, 100 ml of ethanol:diethyl ether (1:1) was added to 4 g of mixture and then titrated with 0.05 N NaOH to a phenolphthalein end-point. The degree of hydrolysis is expressed as the % of FFA liberated and was corrected for the presence of the acids in controls. Duplicate runs were carried out for each sample.

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 thermostatted baths. The SFC was measured within the temperature range of 5–40°C.

Thermal properties by DSC analysis

The instrument used was a Perkin–Elmer DSC-7 (Norwalk, CT). Samples weighing from 3–15 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 80°C per min to 0°C and held for 1 min before heating the samples to 70°C again at 10°C per min for the melting thermograms.

Slip melting point (SMP)

This was determined by the method as described in the AOCS, Method Cc. 3.25.

RESULTS AND DISCUSSION

Table 1 shows the degree of hydrolysis (% FFA), degree and rate of transesterification and enzyme activity of the PS:PKO (40:60) mixtures using different lipases. Based on the degree and rate of transesterification, *Pseudomonas* lipase-catalyzed reactions produced the highest degree of transesterification (152.2%), followed by *R. miehei* (151.7%) with transesterification rates of 50.0 h^{-1} and 27.1 h^{-1}, respectively. The highest % FFA obtained was also from the reaction mixture catalyzed by *Pseudomonas* (2.90%) and *R. miehei* (2.54%) lipases. These results show that the transesterification reactions do occur in parallel with hydrolysis. As *Pseudomonas* and *R. miehei* lipases differ in terms of specificity, this result may confirm the findings of Ghazali *et al.* (1995*a*) in their work on palm olein, which reported no clear correlation between the enzymes' positional specificity and the products formed. As for *A. niger* and *Alcaligenes*-catalyzed reactions, the degrees of transesterification (139.6% and 144.7%, respectively) were moderate.

Changes in the SMP and SFC of the PS:PKO (40:60) mixtures catalyzed by five different lipases are shown in Table 2. The SMP for PS and PKO before transesterification were 54.5° and 25.0°C, respectively, while that of the non-transesterified PS-PKO (40:60) mixture was 47.5°C. From Table 2, mixtures catalyzed by *Pseudomonas* lipase showed the greatest decrease in the SMP with a reduction of 12°C when compared with the non-transesterified mixture. Figure 1 shows the triglyceride (TG) profiles of PS:PKO (40:60) mixtures before (Fig. 1a) and after transesterification (Fig. 1b–f) with various lipases. In relative concentration, several TGs were found to increase while others decreased. Although it was not possible to identify the TGs of PKO and only several TGs of PS could be identified, it is clear that the concentration of the higher melting TG (POP and PPP), particularly in the *Pseudomonas* catalyzed mixture (Fig. 1e), were reduced after transesterification, thus creating a substantially softer product. *R. miehei*, *Alcaligenes* sp. and *A. niger* lipase catalyzed mixtures also showed slightly lower SMPs with reductions of 4.5°C, 3.5°C and 3.0°C, respectively, while catalysis using *C. rugosa* lipase resulted in only a slight change in SMP (1.0°C) as compared with the control.

Changes in the TG profiles of mixed oil substrates after enzymatic transesterification reaction have also been reported by Forssell *et al.* (1992) and Foglia *et al.* (1993). Such changes were often accompanied by changes in the SMP and SFC of the fats and oil blends (Ghazali *et al.*, 1995b). In our studies, enzymatic transesterification was also shown to reduce the SFC for all the lipases used throughout the temperature range investigated. Generally, SFC of the fat mixture is responsible for many of the characteristics of a product, including its general appearance, ease of packing, spreadability, oil exudation and organoleptic properties. The SFC of the PS:PKO (40:60) mixtures catalyzed by *Pseudomonas* sp. lipase was reduced to 0°C at 35°C,

Table 1. Degree of hydrolysis (% FFA), degree and rate of transesterification and enzyme activity of palm stearin-palm kernel olein (40:60) mixtures using different lipases

Source of lipases	FFA (%)	Degree of transesterification (%)	Rate of transesterification (h^{-1})	Activity (% TG hydrolyzed)
A. niger	2.13	139.6	11.3	13.5
R. miehei	2.54	151.7	27.1	19.0
Alcaligenes	2.10	144.7	5.5	5.5
Pseudomonas	2.90	152.2	50.0	15.0
C. rugosa	2.16	112.2	9.5	9.5

Table 2. Slip melting point (SMP) and solid fat content (SFC) of the transesterified palm stearin-palm kernel olein (40:60) mixtures before (control) and after transesterification with *A. niger*, *R. miehei*, *Alcaligenes*, *Pseudomonas* sp. and *C. rugosa* lipases

Lipases	Control	*A. niger*	*R. miehei*	*Alcaligenes* sp.	*Pseudomonas* sp.	*C. rugosa*
SFC (°C)						
5	75.8	71.5	69.7	71.8	68.1	73.2
10	66.6	60.6	58.5	62.2	58.6	63.6
15	52.8	45.8	42.6	48.1	41.7	49.4
20	30.3	26.3	24.5	27.0	23.2	28.0
25	19.0	17.3	15.7	16.1	13.1	18.3
30	14.7	12.9	10.2	11.2	4.2	13.9
35	12.0	9.2	5.5	6.9	0.0	11.4
37	10.7	9.7	4.2	4.9	0.0	10.3
40	9.2	8.6	1.6	4.1	0.0	8.0
SMP (°C)	47.5	44.5	43.0	44.0	35.5	46.5

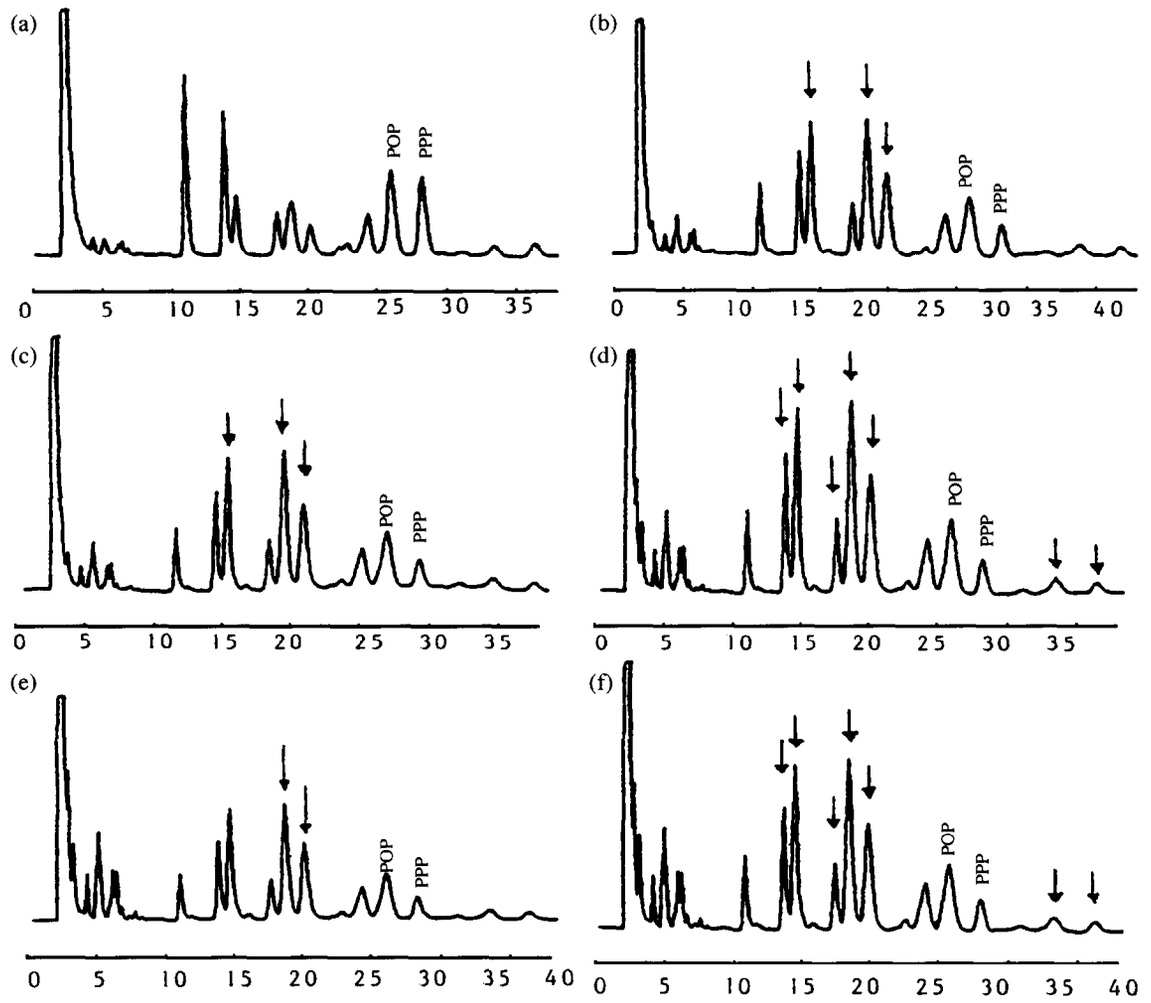

Fig. 1. Triglyceride profiles of palm stearin-palm kernel olein (40:60) mixtures before (a) and after transesterification with *A. niger* (b), *R. miehei* (c), *Alcaligenes* (d), *Pseudomonas* (e) and *C. rugosa* (f). P and O denote palmitic and oleic acids, respectively. Triglycerides represented by arrows indicate increase in triglyceride concentrations.

indicating a possible usage in margarine and shortening production which would give a good oral melt-down as the melting point of margarine below body temperature would not give a taste of waxiness or greasiness in the mouth (Rasid *et al.*, 1996). On the other hand, the SFC for PS:PKO (40:60) mixtures catalyzed by *R. miehei* was 1.6% at 40°C. The other lipase-catalyzed mixtures of PS:PKO (40:60) showed only slight reductions in the SFC as compared with the control. As can be seen in Fig. 1, despite the reduction of several TGs in the mixtures, the harder TGs of palm stearin were still very much in evidence in the transesterified mixtures.

Figure 2 shows the DSC scans for the mixtures of PS:PKO (40:60) catalyzed with different lipases. In control (unreacted) mixtures (Fig. 2a), four endotherms, namely peaks A, B, C and D and three exotherms, X_B, X_C and X_D at temperatures of 12.2, 34.1 and 44.8°C, respectively, were observed. However, following transesterification, with lipases, peak D was either reduced in size (Fig. 2b and f) or disappeared (Fig. 2c, d and e). Endotherm D represents the higher melting TG, hence the decrease in peak D size is consistent with the lower SMP obtained for each of the lipases. In the PS:PKO (40:60) mixtures catalyzed by *A. niger* lipase (Fig. 2b), the exotherm, X_B broadened and became less pronounced while endotherms B and C became more prominent. The broadening of exotherm X_B and increase in endotherm B suggest that the crystalline form of TG responsible for peak A is transformed by a simple rearrangement to the slightly higher melting form represented by peak B. Mixtures transesterified by *R. miehei* (Fig. 2c) and *Alcaligenes* (Fig. 2d) lipases have similar thermograms to the control except that peaks D have disappeared while peak C increased in size. The most likely TGs, making up the high melting components (peak D), would be mainly POP, PPP and POS. The disappearance of endotherm D could be due to the 1,3-specific positional rearrangement of the fatty acids of the high melting component by *R. miehei* and *Alcaligenes* to form lower and middle melting components. In the case of PS:PKO (40:60) mixtures catalyzed by *Pseudomonas* sp. lipase (Fig. 2e), endotherm A was found to decrease in size, exotherm X_B broadened, a new exothermic peak E appeared while endotherm B increased

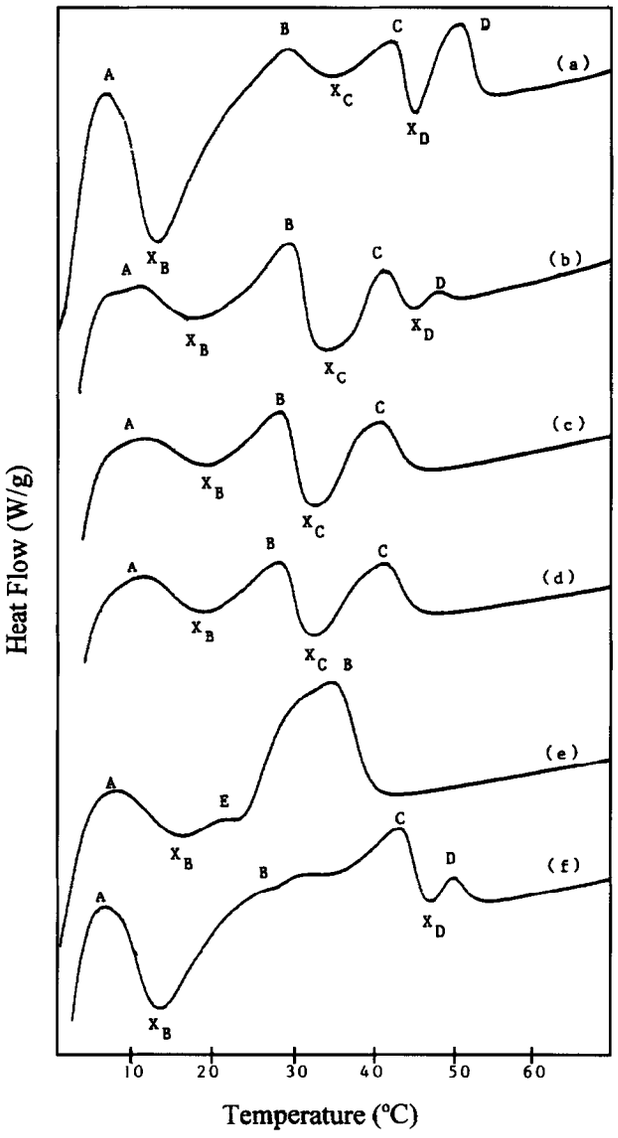

Fig. 2. DSC heating thermograms of PS-PKO (40:60) mixtures before (a) and after transesterification with lipases from *A. niger* (b), *R. miehei* (c), *Alcaligenes* sp. (d), *Pseudomonas* sp. (e) and *C. rugosa* (f) at heating rates of 10°C per min. Pretreatment: cooled from 70°C to 0°C at 80°C per min. Heating programme was started after 1 min at 0°C.

in size and became dominant. The broadening of exotherm X_B and the formation and increase in sizes of peaks E and B suggest that the crystalline form of peak A is transformed into a melt of mixed crystal structures from which the high melting fraction can crystallize into a crystal form which gives rise to peak E. The disappearance of the high melting peaks C and D is consistent with the lower SMP obtained (35.5°C).

Results obtained indicate that transesterification of PS:PKO (40:60) mixtures with enzymes can reduce the melting point of the mixtures and thus produce a more fluid product for specific food applications. The characteristics of the mixtures produced can be controlled by the proportions of oils used in the mixture and the selection of appropriate lipases. *Pseudomonas* and *R. miehei*-catalyzed mixtures also showed the highest reductions in SMP and SFC and these lipases would be the two most noteworthy lipases to be selected for the preparation of a more fluid product in our future work. Our results also indicate that the specificity of lipases may not play a major role in lowering the melting point of the PS:PKO (40:60) mixtures.

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