Physical Properties of *Pseudomonas* and *Rhizomucor miehei* Lipase-Catalyzed Transesterified Blends of Palm Stearin:Palm Kernel Olein

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ABSTRACT: The physical properties of *Pseudomonas* and *Rhizomucor miehei* lipase-catalyzed transesterified blends of palm stearin:palm kernel olein (PS:PKO), ranging from 40% palm stearin to 80% palm stearin in 10% increments, were analyzed for their slip melting points (SMP), solid fat content (SFC), melting thermograms, and polymorphic forms. The *Pseudomonas* lipase caused a greater decrease in SMP (15°C) in the PS:PKO (40:60) blend than the *R. miehei* lipase (10.5°C). Generally, all transesterified blends had lower SMP than their unreacted blends. *Pseudomonas* lipase-catalyzed blends at 40:60 and 50:50 ratio also showed complete melting at 37°C and 40°C, respectively, whereas for the *R. miehei* lipase-catalyzed 40:60 blend, a residual SFC of 3.9% was observed at 40°C. Randomization of fatty acids by *Pseudomonas* lipase also led to a greater decrease in SFC than the rearrangement of fatty acids by *R. miehei* lipase. Differential scanning calorimetry results confirmed this observation. *Pseudomonas* lipase also successfully changed the polymorphic forms of the unreacted blends from a predominantly β form to that of an exclusively β′ form. Both β and β′ forms existed in the *R. miehei* lipase-catalyzed reaction blends, with β′ being the dominant form.
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KEY WORDS: DSC, lipase, palm kernel olein, palm stearin, polymorphic forms, *Pseudomonas, Rhizomucor miehei,* slip melting points, solid fat content, table margarines, transesterification.

Margarine was developed in a search for a butter substitute. Most margarines are formulated by using hydrogenation as a means to modify fats and oils, because it is a cheap process that is tried and true on an industrial scale. Although chemical and enzymatic interesterification can be used in the preparation of margarine, there has been no necessity or incentive for the use of these methods in modifying fats and oils. However, public awareness of the results of recently published works (1–3) on the effects of *trans* fatty acids on serum lipids may lead to significant changes in the near future. Studies by Mensink and Katan (1), by Zock and Katan (2), and by Willett *et al*. (3) have shown that *trans* fatty acids of hydrogenated fat products contribute to several health problems, including thrombogenesis, which leads to coronary heart disease. For this reason, interest in the production of low- and zero-*trans* margarine has grown. One distinct approach is transesterification, which involves randomization of the fatty acids in their triglyceride (TG) molecules.

Margarines are customarily composed of a basestock (often a soft-consistency fat/oil) and a hardstock. They are blended in judicious proportions to give the best possible consistency requirements for packing, handling and composition. Palm stearin, the cheaper high-melting fraction from palm oil, consists of myristic acid (1–2%), palmitic acid (47–74%), stearic acid (4–6%), oleic acid (10–37%), and linoleic acid (3–10%) (4). With a slip melting point (SMP) ranging between 44 and 56°C, palm stearin is a useful natural source of hardstock for margarine (5). Palm kernel olein, with an SMP of 26°C, is quite commonly used in margarine formulation. It is characterized by its high proportion of saturated fatty acids and is distinguished from other fats (except coconut oil) by its high content of lauric acid (40–50%) (6). Both palm stearin and palm kernel olein contain smaller amounts of saturated acids with 8, 10, 14, and 18 carbon atoms. Their unsaturated acids are minor and consist of oleic and linoleic acids (6). Due to its quick crystallization properties, palm kernel olein is known to give good creaming properties (5). Its high content of short-chain fatty acids gives rapid melt characteristics, and this gives a cool sensation when it melts in the mouth (5).

The physical properties of margarine are dictated by the solid fat content (SFC), particularly of the high-melting glycerides (HMG), because these triglycerides (TG) are thought to set the trend in polymorphic crystal behavior (7,8). Fat blends that melt completely at 37°C and have a sharp decrease (steep) in the SFC profile at around body temperature give good oral melt-down in the mouth. The fat crystals should preferably be in the β′ form, because these crystals do not impart the undesirable graininess characteristics that are often related to β crystals.

The primary objective of this study was to produce a suitable blend for the formulation of table margarine that is spreadable at room temperature and has all the inherent qualities of a "good" table margarine, yet contains low or zero

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trans fatty acids. Therefore, in this work, palm stearin:palm kernel olein (PS:PKO) blends were restructured by transesterification with 1,3-specific (*Rhizomucor miehei*) and nonspecific (*Pseudomonas* sp.) lipases, and their physical properties, such as SMP, SFC, melting thermograms, and polymorphic forms, were examined to assist in the proper choice of a PS:PKO blend with optimum melting characteristics for use in table margarines.

EXPERIMENTAL PROCEDURES

Materials. Refined, bleached, and deodorized hard palm stearin (PS) (SMP 54.5°C), which contained 1.14% PLL (dilinoleo-palmitin), 0.89% OOL (dioleo-linolein), 4.70% POL (palmito-oleolinolein), 7.40% PLP (dipalmito-linolein), 2.25% unknown, 1.95% OOO (triolein), 11.56% POO (dioleo-palmitin), 29.50% POP (dipalmito-olein), 29.32% PPP (tripalmitin), 1.77% SOO (dioleo-stearin), 4.20% POS (palmito-oleostearin), and 5.26% SOS (distearo-olein), was obtained from Ngo Chew Hong Oils and Fat (M) Pve. Ltd., and its triglyceride profile is shown in Figure 1A. Palm kernel olein (PKO) was obtained from Southern Edible Oil Ind. Pve. Ltd., and its triglyceride profile is shown in Figure 1B. Due to unavailability of standards, the TG of PKO were not determined. The fats were stored at 0–4°C. Prior to use, palm stearin was melted at 60°C in an oven. Amano Pharmaceutical Co. (Nagoya, Japan) donated the *Pseudomonas* sp. lipase (powder form), whereas *R. miehei* lipase (Lipozyme 1M60) was obtained in the immobilized form (moisture content: 2–3%) from Novo Nordisk Ind. (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.

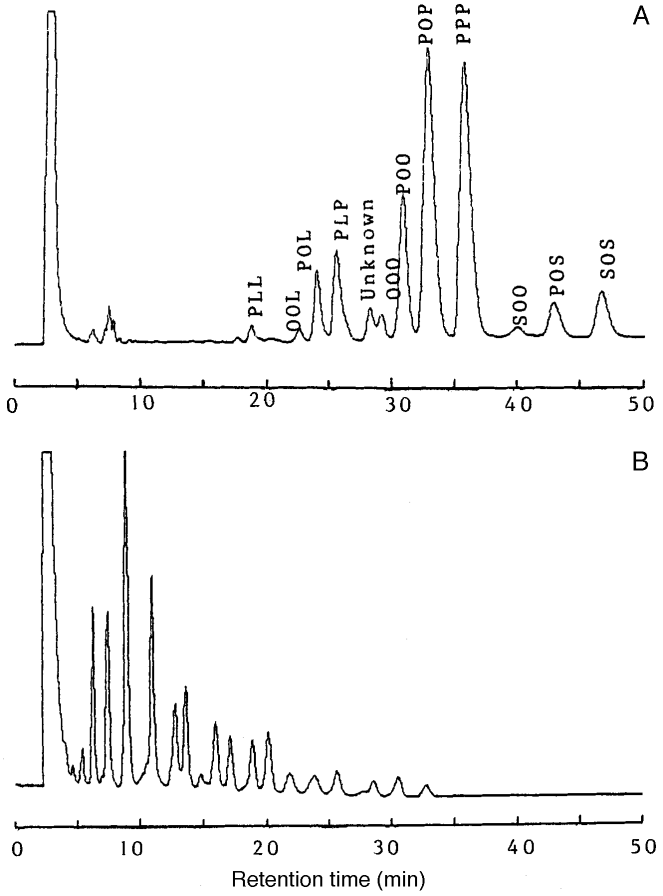

FIG. 1. Triglyceride profile of (A) palm stearin and (B) palm kernel olein (P = palmitic acid; L = linoleic acid; O = oleic acid; S = stearic acid).

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 (9). The preparation was lyophilized for 4 h at −43°C with an Alpha 1-4 Christ LDC-1 (B. Braun) freeze dryer prior to the transesterification process. *Rhizomucor miehei* lipase was used as supplied, in its immobilized form.

Blend preparations. Liquefied palm stearin (PS) and palm kernel olein (PKO) 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 palm kernel olein (PS:PKO).

Transesterification. Transesterification was carried out as previously reported (9). Ten grams of each PS-PKO blend was 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.

Solid fat content. A Bruker Wideline Pulsed NMR (Karlsruhe, Germany) was employed in the direct measurement mode to measure the solid fat content (SFC). 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 measurement. The melting, chilling, and holding of the samples were carried out in pre-equilibrated thermostated baths. The SFC was measured within a temperature range of 5–40°C.

Thermal properties by DSC analysis. The instrument used was a Perkin Elmer DSC-7 (Norwalk, CT). Samples, weighing between 3 and 15 mg and sealed in aluminum pans, 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 being heated to 70°C again at 5°C/min for the melting thermograms.

Slip melting point (SMP). SMP was determined as described in AOCS Method Cc. 3.25. Capillary tubes with a 1-cm high column of fat were chilled at 10 ± 1°C for 16 h before being immersed in a beaker of boiled distilled water. The water bath was stirred and heated, and the temperature was noted where the column of fat rises in the tube.

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 with rectangular holes. 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

(8). Levels of β′ and β crystals in mixtures were estimated by the relative intensity of the short spacings at 4.2 and 4.6Å.

RESULTS AND DISCUSSION

Transesterified PS and PKO blends, ranging from 40% to 80% PS (w/w) in 10% increments, were evaluated for their SMP (Table 1) and SFC (Fig. 2). The minimum quantity of PS that is usually added to a standard table margarine is 10% (10). To maximize the use of PS, high levels of this fat (minimum 40%) were used in this work. The assumption is that if a suitable table margarine formulation that is spreadable at room temperature can be obtained with a minimum of 40% PS, then we should expect to obtain softer products at lower percentages of PS in the blend. For all PS:PKO blends at different ratios, a reduction in SMP was observed after transes-

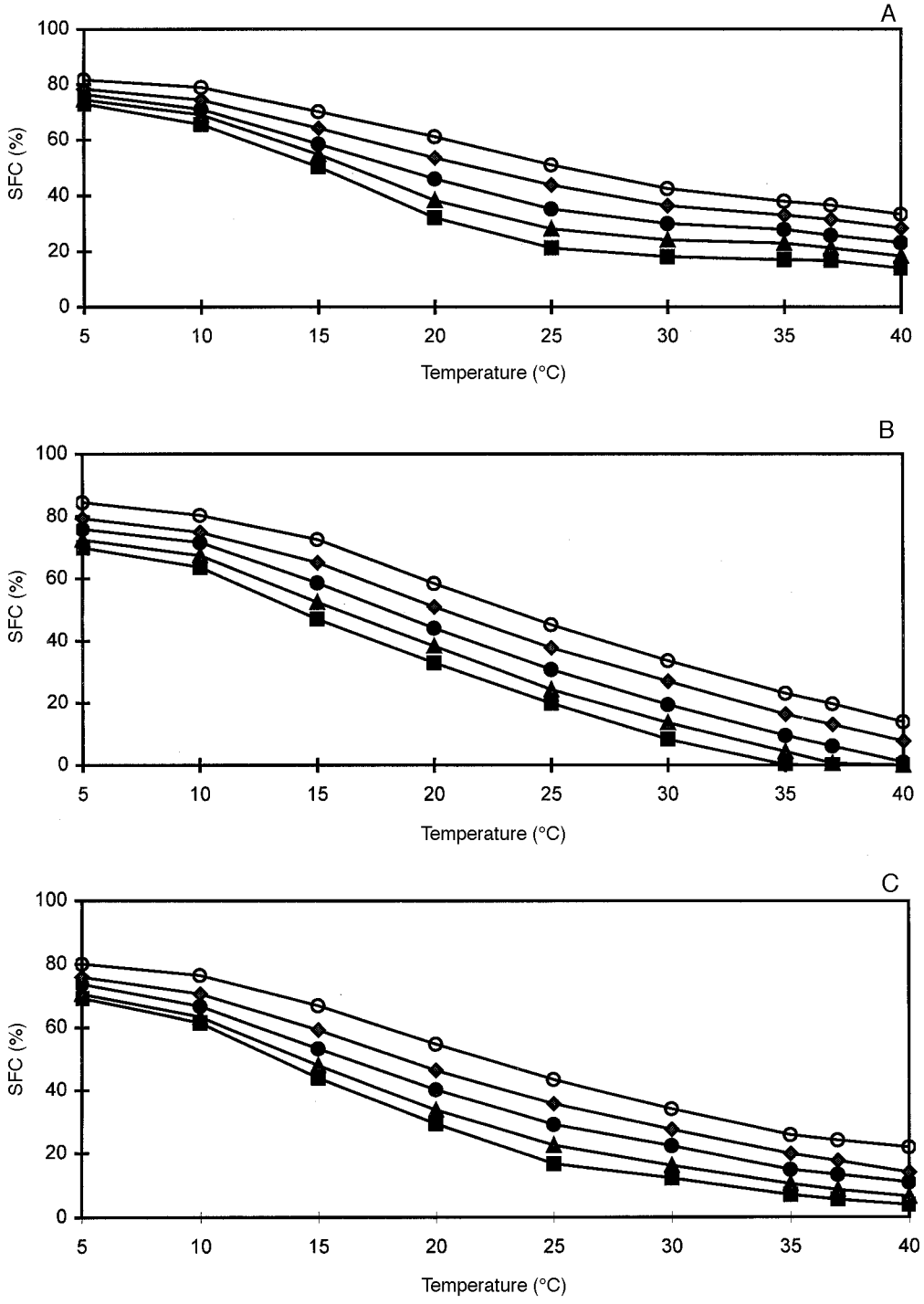

FIG. 2. Solid fat content (SFC) of palm stearin:palm kernel olein (PS:PKO) blends before (A) and after transesterification with *Pseudomonas* (B) and *Rhizomucor miehei* (C) lipases [PS:PKO (w/w); 40:60 (■), 50:50 (▲), 60:40 (●), 70:30 (◆), 80:20 (○)].

TABLE 1
Slip Melting Points of Palm Stearin:Palm Kernel Olein (PS:PKO) Blends Before (control) and After Transesterification with *Pseudomonas* and *Rhizomucor miehei* Lipases

% PS:PKO (w/w)	Slip Melting Points (°C)		
	Control	*Pseudomonas* sp.	*R. miehei*
40:60	49.0	34.0	38.5
50:50	51.0	36.0	41.0
60:40	52.0	38.5	43.0
70:30	53.0	41.0	44.0
80:20	54.5	42.0	50.5

terification with *Pseudomonas* and *R. miehei* lipases, compared to the unreacted blends (Table 1). Figure 3 shows the change in the % reduction of SMP after transesterification with both lipases. The slightly steeper profile obtained with the *Pseudomonas* lipase indicates that this enzyme is a better catalyst in randomizing the fatty acids in the TG molecules of PS than *R. miehei* lipase, which gave a slightly flatter profile. Reductions of 15.0°C and 10.5°C in SMP were observed when the PS:PKO (40:60) blend was transesterified with *Pseudomonas* and *R. miehei* lipases, respectively. As expected, the SMP increased with an increase in the percentage of PS from 40 to 80% in the blends.

Figure 4 shows the TG profiles of PS:PKO (40:60) mixtures before and after transesterification with *Pseudomonas* and *R. miehei* lipases. The relative concentrations of several TG increased, while others decreased. Although it was not possible to identify the TG of PKO, and only several of the TG of PS could be identified, it is clear that TG were synthesized (as indicated by the arrows in Fig. 4B and C) that constituted only minor components in the initial mixture (Fig. 4A). The concentrations of the higher-melting TG (POP and PPP), particularly in the *Pseudomonas*-catalyzed (Fig. 4B) mixture, were reduced after transesterification, thus creating a substantially softer product (Table 1). Generally, the SMP of all blends catalyzed by *Pseudomonas* lipase were lower than those in blends catalyzed by *R. miehei* lipase. We concluded that palm stearin (IV 29.5), when enzymatically transesterified with an oil of shorter saturated fatty acids (e.g., PKO), was successful in yielding products with satisfactory melting points, with the lipase from *Pseudomonas* being the better catalyst.

The SFC of the fat is responsible for many of a margarine's characteristics, including general appearance, ease of packing, organoleptic properties (flavor release, coolness, and thickness), ease of spreading, and oil exudation (11). Unreacted PS:PKO (40:60) had a residual SFC of 14.0% and was not completely melted at 40°C. The largest decline in SFC occurred in the 15–20°C range (Fig. 2A); it could be due to the larger number of TG that liquefy and solubilize in this temperature range. This result is similar to the work of Rousseau *et al.* (12), who reported a large decline in SFC in the above temperature range for chemically interesterified butterfat and canola oil blends. However, when the proportion of PKO increased, the decline in SFC in the above temperature range remained fairly constant. However, when the proportion of

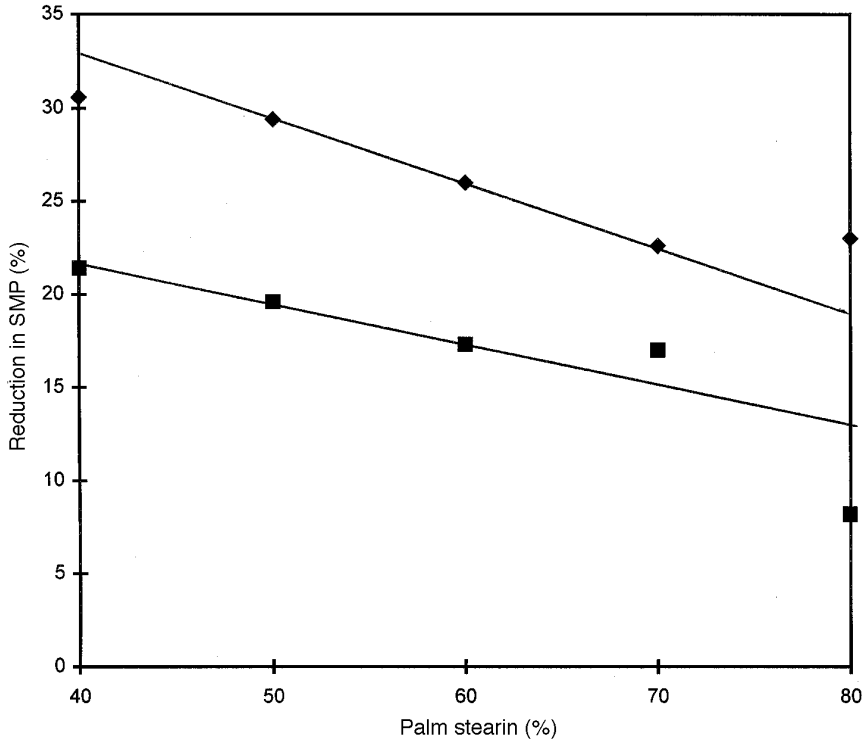

FIG. 3. Change in the percentage reduction in slip melting points (SMP) of PS:PKO blends after transesterification with *Pseudomonas* and *Rhizomucor miehei* lipases. ◆, *Pseudomonas*; ■, *Rhizomucor miehei*. See Figure 2 for abbreviations.

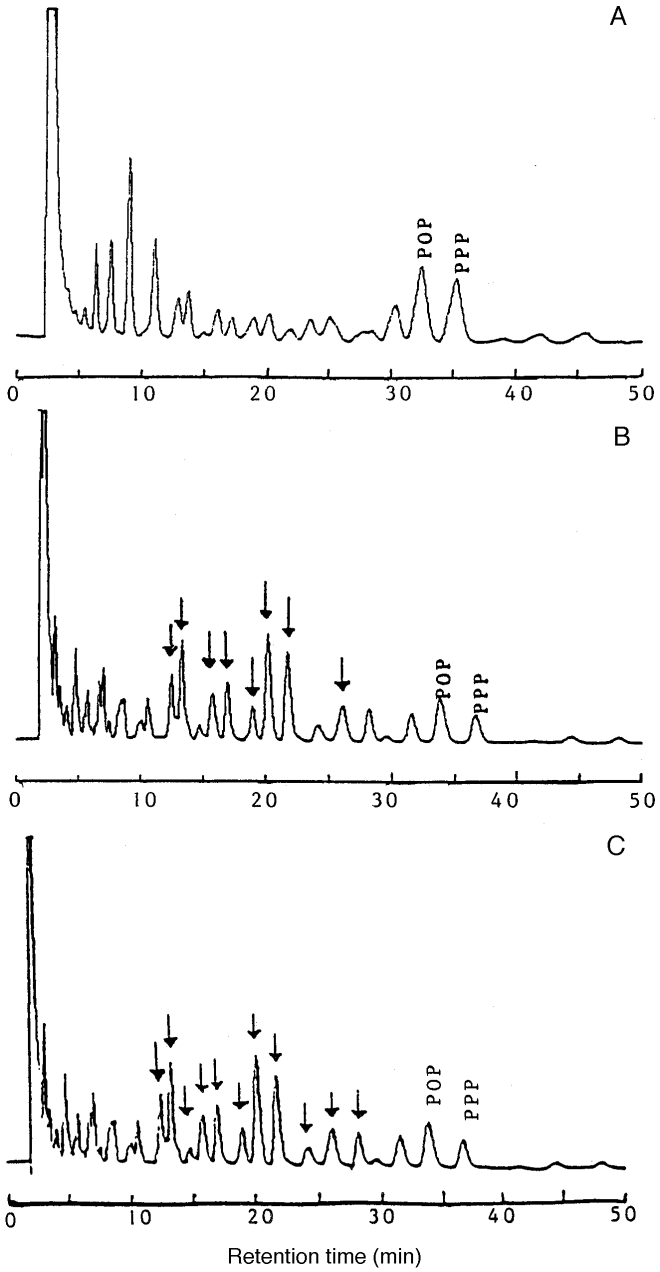

FIG. 4. High-performance liquid chromatography triglyceride profile of PS:PKO blends at 40:60 ratio before (A) and after transesterification with *Pseudomonas* (B) and *Rhizomucor miehei* (C) lipases. P, palmitic acid; O, oleic acid. Arrows indicate triglycerides whose concentrations increased. See Figure 2 for abbreviations.

canola oil was increased, the sharp drop became more prominent. Our work showed a less pronounced drop, most probably because the fatty acids in these raw materials were not as diverse as the fatty acids in butterfat and canola oil blends. The SFC profiles of the *Pseudomonas* (Fig. 2B) and *R. miehei* (Fig. 2C) lipase-catalyzed blends were similar to those of the unreacted blends (Fig. 2A) but at lower SFC. The larger decline in SFC in the 15–25°C range influences the coolness of the spread in the mouth. The bigger the difference, the greater the coolness effect (9). When the PS:PKO blends at 40:60 and 50:50 ratio were transesterified with *Pseudomonas* lipase, complete melting was obtained at 35°C and 40°C, respectively (Fig. 2B). *Rhizomucor miehei* lipase-catalyzed blends, however, do not show complete melting at both of these temperatures (Fig. 2C). A 3.9% SFC was observed at 40°C in *R. miehei* lipase-catalyzed 40:60 blend. Randomization of fatty acids by *Pseudomonas* seems to have led to a greater decrease in SFC than the 1,3-selective rearrangement of fatty acids by *R. miehei* lipase.

Figure 5 shows the melting thermograms of PS:PKO blends before and after transesterification with *Pseudomonas* and *R. miehei* lipases, respectively. In the unreacted (control) blends, five endotherms (A–E) and one exotherm (X_E) were observed (Fig. 5A). As the amount of palm stearin in the blends was increased, the final peak melting temperature, E, increased from 49.9°C in the 40:60 blend to 54.3°C in the 80:20 blend (Fig. 5A). Peak E, which represents the HMG, also increased in size with an increase in PS content, indicating that a firmer product is being formed. This is confirmed by the SMP results (Table 1). Increasing the percentage of PS in the blends caused peak A, which represents the low-melting glycerides (LMG), to disappear gradually. Peak C underwent extensive crystallization to form peak D, denoting conversion of LMG to HMG. Reaction blends catalyzed by *Pseudomonas* lipase (Fig. 5B) had lower final melting temperatures than those catalyzed by *R. miehei* lipase (Fig. 5C), which is consistent with SMP values. The LMG of peak A also disappeared, while peak D became more distinguishable with increasing percentage of PS (Fig. 5B and C). In Figure 5B, the triglycerides of peak B seem to have been transformed by simple rearrangement to form a slightly higher-melting form, represented by peak C, whereas in Figure 5C, the increase in endotherm D suggests the transformation of peak C to peak D (HMG). The appearance of peak F at 60:40 blends catalyzed by *R. miehei,* which increased in size with increasing percentage of palm stearin, is also consistent with the higher SMP obtained.

Table 2 shows the polymorphic forms of the various blends before and after transesterification with both lipases. In the unreacted blends, both β and β′ polymorphs existed, although the β form dominated. D'Souza *et al.* (7) have shown that margarines formulated by blending an interesterified palm stearin hardstock and palm kernel oil are not β′ stable. Where the solid fat component of margarine consists of high levels

TABLE 2
Polymorphic Forms of PS:PKO Blends Before (control) and After Transesterification with *Pseudomonas* and *Rhizomucor miehei* Lipases

% PS:PKO[a] (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

[a]See Table 1 for abbreviations.

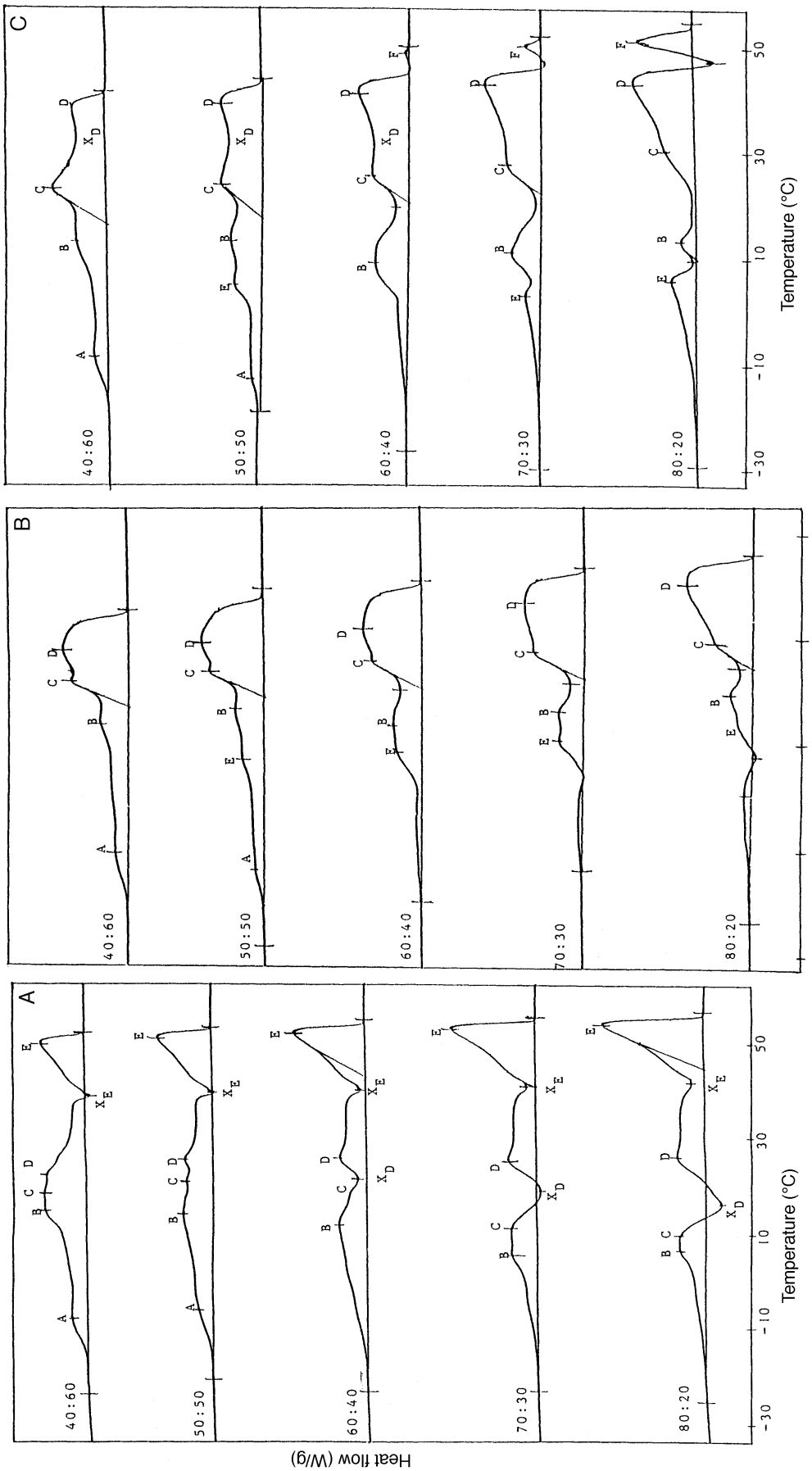

FIG. 5. Differential scanning calorimetry heating thermograms of PS:PKO blends before (A) and after transesterification with *Pseudomonas* (B) and *Rhizomucor miehei* (C) lipases at heating rates of 5°C/min. Pretreatment: cooled from 70°C to −30°C at 5°C/min. Heating program was started after 15 min at −30°C. See Figure 2 for abbreviations.

(>50%) of C_{54} TG or C_{48} TG, the products are usually in the β form (7,8). The levels of C_{48} TG, a β former consisting mainly of tripalmitin, in the HMG of palm stearin ranged between 10.2 and 42.7% (12). Palm stearin also contains a high level of C_{50} TG, ranging between 39.4 and 42.2% (12, 13). POP, a C_{50} TG, is a β-tending TG (13). The relatively high level of these TG in the HMG of palm stearin, and the fact that PKO is also a β-type oil (11), could explain the tendency to crystallize in the β form in the unreacted blends. However, after transesterification with *Pseudomonas* lipase, the blends were exclusively in the β′ form (Table 2). Simple physical blending, as seen in the unreacted blends, was not successful in changing polymorphic nature. The only explanation could be that the randomization of fatty acids of PS with PKO fatty acids by *Pseudomonas* lipase could have led to the diversification of the fatty acids in the resulting TG blends. Diversification of fatty acid chainlength in the solids and HMG promotes β′ stability (13). A plausible explanation for the existence of the β′ form after transesterification could be that the palmitic acid in palm stearin has been replaced with the lauric acids from PKO. Because of the high content of palmitic acid in palm stearin, high levels of lauric acid could be incorporated. The incorporation of PKO in the PS:PKO blends catalyzed by *R. miehei*, however, did not produce blends exclusively in the preferable β′ form. Both β and β′ existed, but β′ was the more dominant form present. Many edible oil products contain various combinations of β- and β′-tending components. The ratio of β and β′ crystals helps to determine the dominant crystal habits, but the HMG portion of a solidified fat product usually influences the fat to assume its dominant crystal form (14).

Based on SMP, SFC, DSC and XRD results, *Pseudomonas*-catalyzed blends of PS:PKO at 40:60 and 50:50 ratio are suitable for the formulation of table margarine. These blends showed complete melting at 37°C and 40°C, respectively, and exhibited β′ crystals, which are desirable in the production of margarine.

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