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Use of enzymatic transesterified palm stearin-sunflower oil blends in the preparation of table margarine formulation

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Abstract

Palm stearin–sunflower oil (PS:SO) blends, formulated by mixing 40 to 80% palm stearin in increments of 10% (w/w), were subjected to transesterification catalysed by lipases from *Pseudomonas* sp. and *Rhizomucor miehei* (Lipozyme 1M 60). 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 difflaction (XRD) analyses. SMP results indicate that *Pseudomonas* lipase caused a bigger drop in SMP (33%) in the PS–SO (40:60) blend than the *R. miehei*-lipase-catalysed reaction blend (13%). The *Pseudomonas*-catalyzed blends of PS-SO, at 40:60 and 50:50 ratios, showed complete melting at 37 and 40°C, respectively, while the *R. miehei*-catalyzed PS–SO blend at 40:60 ratio had a residual SFC of 3.9% at 40°C. *Pseudomonas* lipase also successfully changed the polymorphic form(s) in the unreacted PS–SO mixture from a predominantly β form to a predominantly β' form in the transesterified blends. However, no changes in polymorphic forms were observed after transesterification with *R. miehei* lipase (as against to the unreacted PS–SO blends). These results suggest that the *Pseudomonas* lipase caused a greater randomization and diversification of fatty acids, particularly palmitic acids, in palm stearin with the unsaturated fatty acids from sunflower oil than did *R. miehei* lipase. Based on the physical characteristics, the *Pseudomonas*-catalyzed 40:60 and 50:50 PS:SO blends would be the two most suitable blends to be used as table margarine formulations.

1. Introduction

Transesterification of fully hydrogenated fats and liquid oils can produce fat blends with optimum physical characteristics. Randomization and rearrangement of the fatty acids in the glycerol molecule leads to modification of the triglyceride (TG) composition and consequently, of physical behaviour (Sreenivasan, 1978). Such methods allow the possibility of producing a 'tailor-made' product to suit a particular food. In the case of margarine production, the melting point, spreadability, shelf-life and nutritional properties of the natural fats and oils can be modified and custom-made. Furthermore, this method also provides a range of alternative raw material sources, thus improving commercial viability.

Lately, however, there has been growing consumer concern about formation of *trans* fatty acids during the hydrogenation process. *Trans* fatty acids of hydrogenated fat products have been implicated in causing adverse effects on the high density lipoprotein levels in blood (Mensink and Katan, 1990; Zock and Katan, 1992; Willett et al., 1993). This has generated an interest in the development of low and zero-*trans* solid fats in the food industry, and use of alternative processing methods, such as enzymatic transesterification, to generate viable sources of solvent-free, non-hydrogenated fat blends for table margarine formulations.

Palm stearin is the solid fraction obtained by controlled temperature fractionation. The liquid fraction is known as palm olein and is the more expensive of the two due to wider usage. It contains 1–2% myristic acid, 47–74% palmitic acid, 4–6% stearic acid, 16–37% oleic acid and 3–10% linoleic acid (Pantzaris, 1987). The main TGs include C_{46}, C_{48}, C_{50}, C_{52} and C_{54} (Pantzaris, 1987). With SMP range between 44 and 56°C, palm stearin is a very useful source of fully natural hard component for products such as margarine, shortening and other edible fats. However, at this level of

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saturation, palm stearin may not be able to impart the required plasticity and body to the end-product. This makes it necessary to enrich palm stearin, with a polyunsaturated oil such as sunflower oil that is basically too soft to impart the necessary plasticity to the end-product on its own and must therefore be modified.

This present study deals with the physical evaluation of transesterified fat products prepared from palm stearin enzymatically transesterified with sunflower oil in different proportions. 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:SO blend which produces the desired melting properties for use as table margarine.

2. Materials and methods

2.1. Materials

Refined, bleached and deodorised hard palm stearin (PS) (SMP 54.5°C) and sunflower oil (SO) were obtained from Ngo Chew Hong Oils and Fats (M) Sdn. Bhd. and a local supermarket (Malaysia), respectively. These were stored at 0–4°C. Prior to use, palm stearin was melted at 60°C in the oven. Amano Pharmaceutical Co. (Nagoya, Japan) donated the *Pseudomonas* sp. lipases (powder form) while *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.

2.2. Immobilization of lipase

One tenth of a gram of *Pseudomonas* lipase powder was dissolved in 100 µl of cold deionized water, followed by mixing with 0.25 g of Celite (Ghazali et al., 1995). 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. *R. miehei* lipase was used as is in its immobilized form.

2.3. Blend preparation

Liquefied palm stearin (PS) and sunflower oil (SO) 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 sunflower oil (PS:SO).

2.4. Transesterification

Transesterification was carried out as previously reported (Ghazali et al., 1995). Ten grams of PS–SO 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 described in the AOCS Method Cc. 3.25.

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 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, 1992). Levels of β' and β crystals in mixtures are estimated by the relative intensity of the short spacings at 4.2 and 4.6 Å.

3. Results and discussion

The use of palm stearin in table margarine is limited by its melting points (SMP: 44–56°C) and high solids at

30°C (Pantzaris, 1987). The minimum quantity that can be added to a standard table margarine is only 10% (Teah, 1982). To maximise the use of palm stearin, high levels of palm stearin (minimum 40%) were used in this work. The assumption made is that, if suitable table margarine formulation which is spreadable at room temperature can be obtained with a minimum of 40% palm stearin, then we should expect to obtain an even softer product with a lower % of palm stearin in the blend. Results (Table 1) showed that reductions in SMP of the blends occurred after transesterification with both lipases as compared to the unreacted blends. *Pseudomonas* lipase caused a bigger drop in SMP of 33.0% for the 40:60 blend as compared to only a 13.0% reduction in the same blend when *R. miehei* was used. Fig. 1, which plots the % change in the reduction of SMP caused by both the lipases on the PS:SO blends, clearly shows that the *Pseudomonas* lipase gave a much steeper slope than the *R. miehei* lipase, indicating that the *Pseudomonas* lipase caused a bigger rate of change and was more efficient in reducing the SMP of the blends than *R. miehei* lipase. Generally, when the % of sunflower oil decreased, the % reduction in SMP decreased for both the lipases. At higher levels of palm stearin in the blend, % change in SMP tended to converge to similar values. The decrease in SMP after transesterification by both the lipases indicates that palm stearin (IV 29.5) mixed with a liquid oil of lower saturated fatty acids (sunflower oil) was successful in yielding products with lower melting points than before.

The SFC profiles, as a function of temperature for the PS:SO blends before and after transesterification with *Pseudomonas* and *R. miehei* lipases, are shown in Fig. 2(a)–(c), respectively. A non-linear SFC profile was evident in the unreacted as well as reacted blends. In the unreacted blends (Fig. 2(a)), residual SFC values of 13.7, 18, 21.4, 25.5 and 31% were observed at 40°C for the 40:60, 50:50, 60:40, 70:30 and 80:20 blends, respectively. The largest decline in SFC occurred in the 20 to 25°C range in the unreacted blends. However, in the

Table 1
Slip melting points (SMP) of palm stearin–sunflower oil (PS:SO) blends before (control) and after transesterification with *Pseudomonas* and *R. miehei* lipases

% PS:SO (w/w)	Slip melting points (°C)		
	Control	*Pseudomonas* sp.	*R. miehei*
40:60	50.0	33.5	43.5
50:50	51.0	37.0	46.0
60:40	52.0	43.5	48.0
70:30	53.0	47.0	49.5
80:20	53.5	50.0	51.0

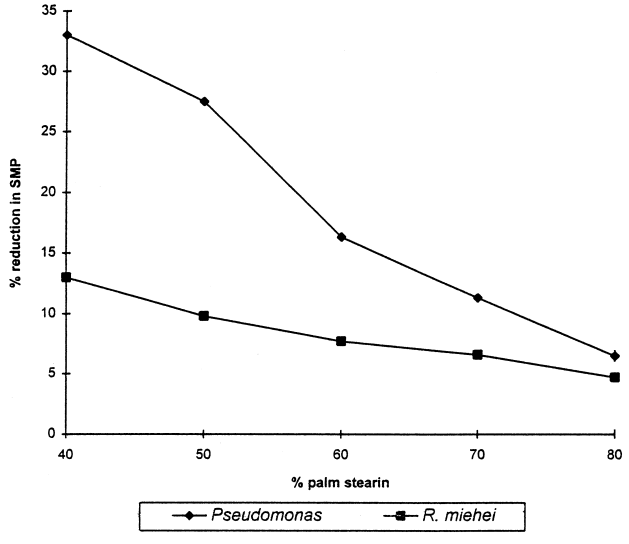

Fig. 1. Change in % reduction of SMP of palm stearin:sunflower oil (PS:SO) blends after transesterification with *Pseudomonas* and *R. miehei* lipases.

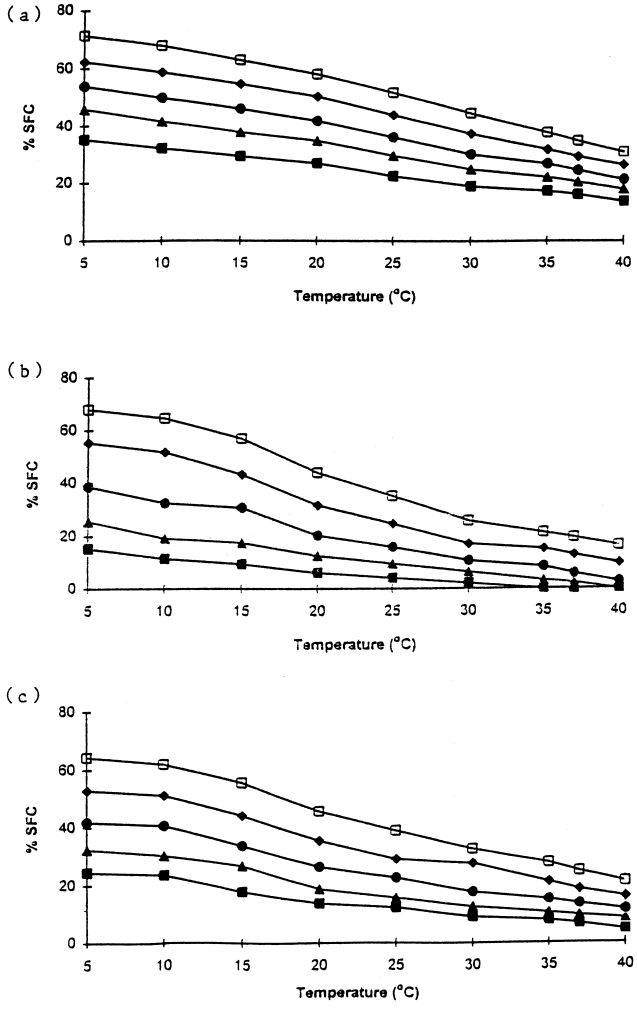

Fig. 2. Solid fat content (SFC) of palm stearin-sunflower oil (PS:SO) blends before (a) and after transesterification with *Pseudomonas* (b) and *R. miehei* (c) lipases. (PS:SO (w/w); 40:60 (–■–) 50:50 (–▲–) 60:40 (–●–) 70:30 (–◆–) 80:20 (–□–).

Pseudomonas and *R. miehei* lipase-catalyzed blends of PS:SO, the sharp decline in SFC at 20 to 25°C in the unreacted blends shifted to 15 to 20°C. This could be due to a larger proportion of TGs that liquefy in this temperature range. This shift to a lower temperature range could also explain the lower SMP obtained (Table 1) after transesterification as compared to the unreacted blends. However, as the proportion of sunflower oil increased, the sharp drop in the 15 to 20°C range in the transesterified blends became less promi-

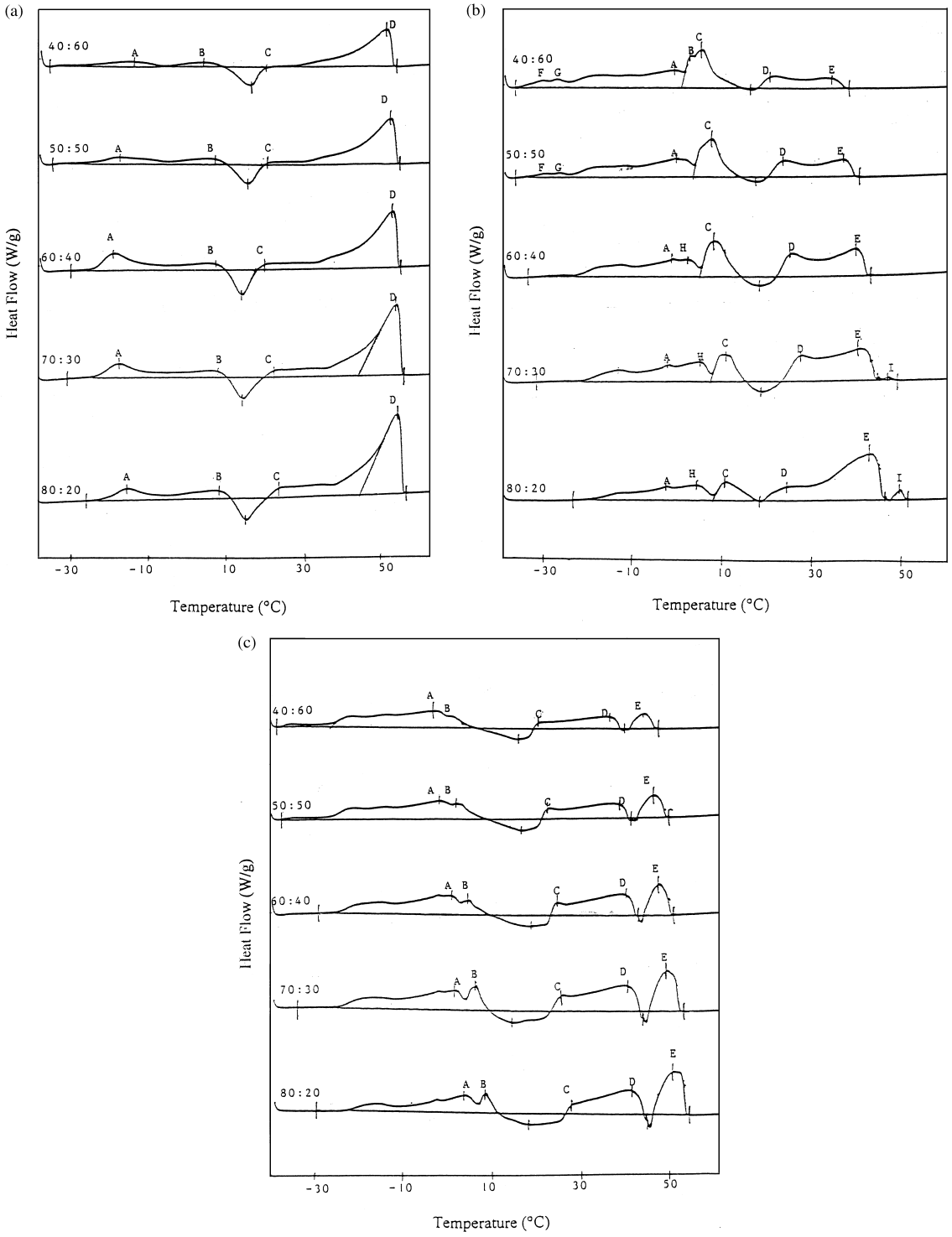

Fig. 3. DSC heating thermograms of palm stearin-sunflower oil (PS:SO) blends before (a) and after transesterification with *Pseudomonas* (b) and *R. miehei* (c) lipases at heating rates of 5°C/min. Pretreatment: cooled from 70°C to −30°C at 5°C/min. Heating programme was started after 15 min at −30°C.

nent. This was similar to the work reported by Rousseau et al. (1996) which shows a less pronounced drop in SFC when the proportion of canola oil was increased in the chemically interesterified butterfat-canola oil blends. *Pseudomonas* lipase-catalyzed PS:SO blends at 40:60 and 50:50 ratio, showed complete melting at 37 and 40°C, respectively. In the *R. miehei* lipase-catalyzed PS:SO blend (Fig. 2c), a residual SFC of 4.5% was still evident in its 40:60 blend, showing that the *Pseudomonas* lipase was more successful in lowering the melting properties of the PS:SO blends than *R. miehei* lipase. The bigger declines in SFC upon transesterification with *Pseudomonas* and *R. miehei* lipases are due probably to the replacement of saturated fatty acids with the lower melting unsaturated fatty acids in the palm stearin TG, which contain high levels of tripalmitin. SFC of the fat phase in margarine is responsible for many of its characteristics. Fat/oil blends that show complete melting at 37 and 40°C will not leave any waxy taste in the mouth.

The melting thermograms of the PS:SO blends before and after transesterification with *Pseudomonas* and *R. miehei* lipases are given in Fig. 3(a)–(c), respectively. Transesterification with both the lipases produced more prominent changes in the melting profile (Fig. 3(b)–(c)) while simple blending (Fig. 3(a)) alone only resulted in a dilution effect. In the unreacted blends (Fig. 3(a)), as the amount of palm stearin increased, peak D, representative of the higher melting triglycerides (HMG), increased in size. However, in all the reaction blends catalysed by *Pseudomonas* lipase, when the % of palm stearin was increased, a few observations could be noted: (a) peaks F and G, representative of the lower melting glycerides (LMG) disappeared; (b) peaks A and D melted to form peaks H and C, respectively; (c) peak D decreased in size to form peak E which became more pronounced and (e) formation of peak I occurred in the 70:20 blend, which increased in size in the 80:20 blend, indicative of a firmer product. In the reaction blends catalysed by *R. miehei* lipase, when the % of palm stearin was increased, peak E consisting of the higher melting triglycerides, increased in size, suggesting a harder product being formed. Transesterification has, thus, created a much altered thermogram from that of the unreacted blends. Transesterification with both the lipases caused more smaller peaks to be formed between the temperature ranges of 0 to 30°C as compared to the unreacted blends. The smaller peaks are composed of low-and middle-melting glycerides. The existence of the low-and middle-melting TGs could also explain the lower SFCs obtained after transesterification. This result is similar to that reported by Rousseau et al. (1996) which show that chemical interesterification of butterfat and canola oil produced more noteworthy changes in the DSC melting profile than simple blending alone. Generally, the final melting peak temperature for the blends catalysed by *Pseudomonas* lipase were lower than those catalysed by *R. miehei* lipase. Changes in the melting profiles were possibly due to greater randomization of fatty acids by the *Pseudomonas* lipase, which consequently led to a greater decrease in the HMG than the changes caused by *R. miehei* lipase.

Edible oils go through a series of increasingly organized crystal phases with cooling until a final stable crystal form is achieved. The crystal types formed define the textural and functional properties of most fat-based products (O'Brien, 1996). Table 2 shows the polymorphic form(s) of the PS:SO blends before and after transesterification with *Pseudomonas* and *R. miehei* lipases. In the unreacted blends, both β and β' forms existed with the β form representing the dominant crystal formation. After transesterification with *Pseudomonas* lipase, β' polymorphs became the dominant crystal form although both β and β' crystal forms existed. This may be due to the randomization and rearrangement that occurred during the transesterification process. The β' form is desired for favourable molecular packing of the fatty acid chains of a solid fat used for the production of margarine or table spread (Wiedermann, 1978). Wiedermann (1978) has grouped some common fats according to crystal habits. In general, he found that the more diverse the TG structure of the high melting portion of a fat, the lower the β-forming tendencies. Therefore, oils such as sunflower oil are most likely to undergo this transformation because the palmitic acid contents are very low and the solid components consist of a series of closely related homologues (Wiedermann, 1978). Thus, sunflower oil margarines can benefit from the incorporation of palm stearin, which has a higher amount of palmitic acid. This could be a plausible explanation for the existence of the dominant β' polymorphic form observed after transesterification with *Pseudomonas* lipase. *R. miehei* lipase however, was not so successful in producing β' as the dominant crystal form. One probable reason could be that the enzyme was not as efficient as *Pseudomonas* lipase in diversifying the palmitic acid in the TG molecule with the unsaturated fatty acids of sunflower oil. The high levels of β-tending TGs, such as tripalmitin, PPP (C_{48}) and POP (C_{50}) in palm stearin (de Man and de Man, 1995), were

Table 2
Polymorphic forms of palm stearin–sunflower oil (PS:SO) blends before (control) and after transesterification with *Pseudomonas* and *R. miehei* lipases

% PS:SO (w/w)	Polymorphic forms		
	Control	*Pseudomonas* sp.	*R. miehei*
40:60	$\beta > > \beta'$	$\beta' > > \beta$	$\beta > > \beta'$
50:50	$\beta > > \beta'$	$\beta' > > \beta$	$\beta > > \beta'$
60:40	$\beta > > \beta'$	$\beta' > > \beta$	$\beta > > \beta'$
70:30	$\beta > \beta'$	$\beta' > > \beta$	$\beta > \beta'$
80:20	$\beta > \beta'$	$\beta' > > \beta$	$\beta > \beta'$

not efficiently randomized by *R. miehei* lipase, causing the β form to predominate in the blends.

Based on the SMP, SFC, DSC and XRD results, the *Pseudomonas* lipase-catalyzed blends of PS:SO at 40:60 and 50:50 ratio are the two most suitable blends to be used for the formulation of a table margarine that is spreadable at room temperature. Both blends showed complete melting at 37 and 40°C, respectively, and exhibited β' as the dominant crystal form.

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