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Enzymatic transesterification of palm stearin: anhydrous milk fat mixtures using 1,3-specific and non-specific lipases

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Abstract

The physical characteristics of a palm stearin:anhydrous milkfat (40:60) mixture, enzymatically transesterified in a solvent-free system, was investigated by monitoring changes in the slip melting point (SMP), solid fat content (SFC) and melting characteristics. The enzymes used were 1,3-specific lipases from *Aspergillus niger, Rhizomucor miehei, Rhizopus javanicus, Rhizopus niveus, Alcaligenes* sp. and non-specific lipases from *Pseudomonas* sp. and *Candida rugosa*. Results indicated that *Pseudomonas* lipase-catalyzed mixtures produced the highest degree of transesterification (33.9%) and rate of transesterification (50.0/h), followed by *R. miehei* lipase at 32.3% and 27.1/h. The highest % free fatty acid (FFA) liberated was also from the reaction mixture catalyzed by *Pseudomonas* (2.6 1%) lipase followed by *Alcaligenes* (2.56%) and *R. miehei* (2.88%) lipases. The SMP of all the transesterified PS:AMF mixtures underwent only slight reductions, ranging from 0.5 to 2.5°C with reactions catalyzed by *Pseudomonas* and *R. miehei* lipases, producing the biggest drop in SMP values.

Keywords: Transesterification; Palm stearin; Anhydrous milkfat; 1,3-Specific; Non-specific; Slip melting points; Solid fat content; DSC; Table margarines

1. Introduction

Palm stearin is the solid fraction obtained by controlled temperature fractionation of palm oil. The liquid fraction is known as palm olein and is the more expensive of the two due to wider usage. The physical characteristics of palm stearin differ significantly from those of palm olein (Pantzaris, 1987). 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 triglycerides (TG) include C_{46}, C_{48}, C_{50}, C_{52} and C_{54}. Unfortunately, the high degree of saturation (SMP: 44–56°C) of palm stearin poses problems in the manufacture of edible fats such as margarine and shortening as it confers low plasticity to the end product, thus limiting the commercial exploitation of the material.

A great deal of work has been done in utilizing palm stearin by transesterification and by simple blending with liquid oils to obtain fat mixtures with better melting properties (Ghazali, Hamidah & Che Man, 1995; Graille, Pina, Moutet & Muderhwa,1992; Lai, Ghazali & Chong, 1998a, b). Palm stearin, when enzymatically transesterified with another vegetable oil (linseed, mustard or cotton seed oils) produced products having different slip points from the unreacted blends (Bhattacharyya, Bhattacharyya, Basu & Sil, 1989). Enzymatically transesterified palm stearin and palm kernel olein mixtures (40:60), catalyzed using *Pseudomonas* lipase, melts completely at 37°C (Lai et al., 1998b) while palm stearin:sunflower oil mixtures at 40:60 ratio catalyzed by the same lipase, produced a decrease in SMP of 13.5°C compared to the unreacted mixture (Lai et al. 1998a). These results indicated the possibility of using enzymatic transesterification as a means of producing a more fluid product.

Margarine is customarily composed of a base stock and a hardstock that are blended in judicious proportions to give the best possible consistency requirements for composition, packing and handling. The physical properties of margarine are dictated by the SFC, particularly of the high melting glycerides (HMG), because these TG are thought to set the trend in the polymorphic crystal behaviour.

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The present study examined the possibility of using enzymatic transesterification as a means of changing the physical properties of palm stearin:anhydrous milkfat mixtures to formulate a suitable table margarine that is spreadable at room temperature. AMF was used to impart a buttery flavour to the end product. The efficacy of various immobilized 1,3-specific lipases, such as those from *A. niger, M. javanicus, R. miehei* (Lipozyme 1M60), *R. javanicus, R. niveus* and *Alcaligenes* and non-specific lipases, such as those from *Pseudomonas* and *C. rugosa* on the transesterification of PS:AMF (40:60) mixture, was evaluated with respect to FFA content, degree and rate of transesterification, SMP, SFC and DSC properties.

2. Materials and methods

2.1. Materials

Refined, bleached and deodorised hard palm stearin (PS) (SMP 54.5°C) and anhydrous milk fat (AMF) were obtained from Ngo Chew Hong Oils and Fat (M) Pte. Ltd. and Promac Enterprise (M) Pte. Ltd., respectively. These were stored at 4°C. Prior to use, the fats were melted at 60°C in the oven. Amano Pharmaceuticals Co. Ltd. (Nagoya, Japan) donated *A. niger, M javanicus, R. javanicus, R. niveus, C. rugosa* and *Pseudomonas* sp. lipases (powder form) while *R. miehei* lipase (Lipozyme IM 60) was obtained in the immobilised form (granule size 0.2–0.6 mm; moisture content, 2–3%) from Novo Nordisk (Copenhagen, Denmark). *Alcaligenes* sp. lipase (Lipase PL) was purchased from Meito-Sangyo Co. Ltd of Japan. Celite, used as a carrier for immobilisation of the lipases, was purchased from BDH Ltd. (Poole, England). All other chemicals used were of analytical or HPLC grade.

2.2. Immobilization of lipase

Lipase powder (0.1 g) 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, Melsungen, Germany) freeze dryer prior to the transesterification process. *R. miehei* lipase was used in its immobilized form.

2.3. Transesterification

Transesterification was carried out as previously reported (Ghazali et al., 1995). Ten grams of PS:AMF (40:60) mixtures were reacted with 0.1 g equivalent of immobilized lipases at 60°C, 200 rev/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) and conditions according to Lai, Ghazali and Chong (1998c). Percentage TG remaining (%TGR) is the total concentration of TG after reaction has occurred, compared to the unreacted mixture (Ghazali et al., 1995). The degree of transesterification is defined as the change in concentration of TG that increased in value, $[TGI_t]$ at reaction time t with respect to the value at the start of the reaction, $[TGI_0]$ minus 100%. The rate of transesterification was calculated as shown below:

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

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 (Lai et al., 1998c). 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.

2.4. Hydrolytic activity

The amount of FFA present was determined according to the method of Cocks and van Rede (1966). At the end of the transesterification reaction, 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.

2.5. Solid fat content

A Bruker Wideline Pulse NMR (Karlsruhe, Germany) experiment, using the direct measurement procedure for the determination of solid fat content (SFC), was done according to the method described in Lai et al., (1998c).

2.6. Thermal properties by DSC analysis

The instrument used for the determination of the melting profiles was a Perkin–Elmer DSC-7 (Norwalk, CT). The conditions for the experiment were according to the method described in Lai et al., (1998c).

2.7. Slip melting point (SMP)

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

3. Results & discussion

Table 1 shows the degree of hydrolysis (% FFA), degree and rate of transesterification and enzyme activity when PS:AMF (40:60) was reacted using different lipases. Lipases from different sources, exhibiting either 1,3-specificity or non-specific, were used in this study. Based on the rate of transesterification, the use of bacterial *Pseudomonas* lipase resulted in the fastest rate of reaction (50.0/h) while intermediate rates were obtained for the lipases from *R. miehei* (27.1/h) and *A. niger* (11.3/h). The values obtained showed that the rate catalyzed by *Pseudomonas* lipase was 1.8 and 4.4 times faster than the rates of *R. miehei* and *A. niger* lipases, respectively. The reaction rates for other lipases were between 5.3 and 9.1 times less than that of *Pseudomonas* lipase.

In the transesterified mixtures of PS:AMF (Table 1), the degree of transesterification was the highest for the reaction catalyzed by *Pseudomonas* (33.9%) and *R. miehei* (32.3%) lipases. Although the reaction mixtures catalyzed by the lipases from *Alcaligenes* (2.56%) and *C. rugosa* (2.10%) had % FFA higher or similar to that of R. *miehei's* (2.18%), the degrees of transesterification, were, however, lower at 12.2 and 8.0%, respectively. The trend for *Pseudomonas* lipase seemed consistent where this enzyme exhibited the highest degree of transesterification and % FFA, indicating that it is a highly active preparation. Differences in the degree of transesterification between the lipases could be due to differences in the ease of inactivation of certain enzymes in the immobilization process and the differences in the suitability of the support used for that lipase (Mustranta, Forssell & Poutanen, 1993). According to Mustranta et al. (1993) lyophilisation lowers the activity of the immobilised lipase of *C. rugosa* but not of *A. niger* or *P. fluorescens*. Based on the FFA results, further refining of the blend is required if a desirable end product is to be obtained.

Table 2 shows the changes in the SMP and SFC of the PS:AMF mixture when enzymatically transesterified. The SMP for palm stearin and AMF before transesterification, were 54.5 and 35°C, respectively, while that of the non-transesterified PS:AMF (40:60) mixture was 44.5°C. The SMP of PS:AMF mixtures, transesterified by all the lipases, showed only slight reductions, ranging from 0.5 to 2.5°C. Reactions catalyzed by *Pseudomonas* and *R. miehei* lipases resulted in the highest decline in SMP values. The slight reduction in SMP indicated that the transesterified products were still relatively hard and not much different from the unreacted mixture. The

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

Sources of lipase	FFA (%)	Degree of transesterification (%)	Rate of transesterification (h^1)	Activity (% TG hydrolyzed)
A. niger[a]	1.79	10.3	11.3	13.5
M. javanicus[a]	0.43	10.4	8.8	7.0
R. miehei[a]	2.18	32.3	27.1	19.0
R.javanicus[a]	0.78	9.5	8.2	9.0
R. niveus[a]	1.58	6.8	6.0	6.0
Alcaligenes[a]	2.56	12.2	5.5	5.5
Pseudomonas[b]	2.61	33.9	50.0	15.0
C. rugosa[b]	2.10	8.0	9.5	9.5

[a] Denotes 1,3-specific lipases.
[b] Denotes non-specific lipases.

Table 2
Slip melting point (SMP) and solid fat content (SFC) of transesterified palm stearin:anhydrous milkfat (PS:AMF) mixtures at 40:60 ratio using different lipases

Temperature (°C)	SFC (%)								
	Control	*A. niger*	*M. javanicus*	*R. miehei*	*R. javanicus*	*R. niveus*	*Alcaligenes*	*Pseudomonas*	*C. rugosa*
5	62.7	56.9	56.4	57.0	51.4	54.8	59.6	61.7	58.4
10	60.7	5O.3	53.0	49.3	45.5	47.8	52.8	54.8	54.7
15	52.7	45.6	48.7	43.7	41.1	43.9	48.3	48.4	49.3
20	38.5	33.1	34.2	30.9	30.8	31.1	32.3	31.7	34.8
25	31.2	26.6	28.3	24.8	25.8	26.4	26.5	24.4	29.0
30	23.4	19.5	20.9	17.5	19.9	20.0	19.0	16.6	21.6
35	15.6	12.9	13.9	11.1	13.4	13.8	12.8	9.6	14.4
37	13.7	10.8	12.1	8.6	11.5	11.3	9.9	6.7	12.8
40	11.1	7.3	9.8	4.2	8.9	8.5	7.0	1.9	10.0
SMP	44.5	42.5	44.0	42.0	44.0	43.0	42.5	42.0	43.5

SFC values obtained also show that *Pseudomonas* lipase-catalyzed PS:AMF mixture was the softest with 1.9% SFC at 40°C while the other lipases had % SFC ranging from 4.2 to 11.1% at 40°C. The SFC of the unreacted mixture at 40°C was 11.1%.

In a similar work on PS:SO mixtures (Lai et al, 1998a), all lipases used to catalyze the mixture effected only a slight decline in the SMP (range: 0.5–3.0°C) except for *Pseudomonas* lipase, which reduced the SMP of the mixture by 13.5°C, consistent with its high degree of transesterification (77.3%). The SFC results in the same study reported that *Pseudomonas* lipase-catalyzed PS:SO mixtures showed complete melting at 30°C. The relatively high degree of transesterification catalyzed by *Pseudomonas* lipase could indicate the preference of *Pseudomonas* lipase for long chain FA (Reyes & Hill, 1994). Also, the slight reduction in SMP of PS:AMF mixtures, when *Pseudomonas* lipase was used, could be due to the fact that both PS and AMF contain high amounts of saturated FA.

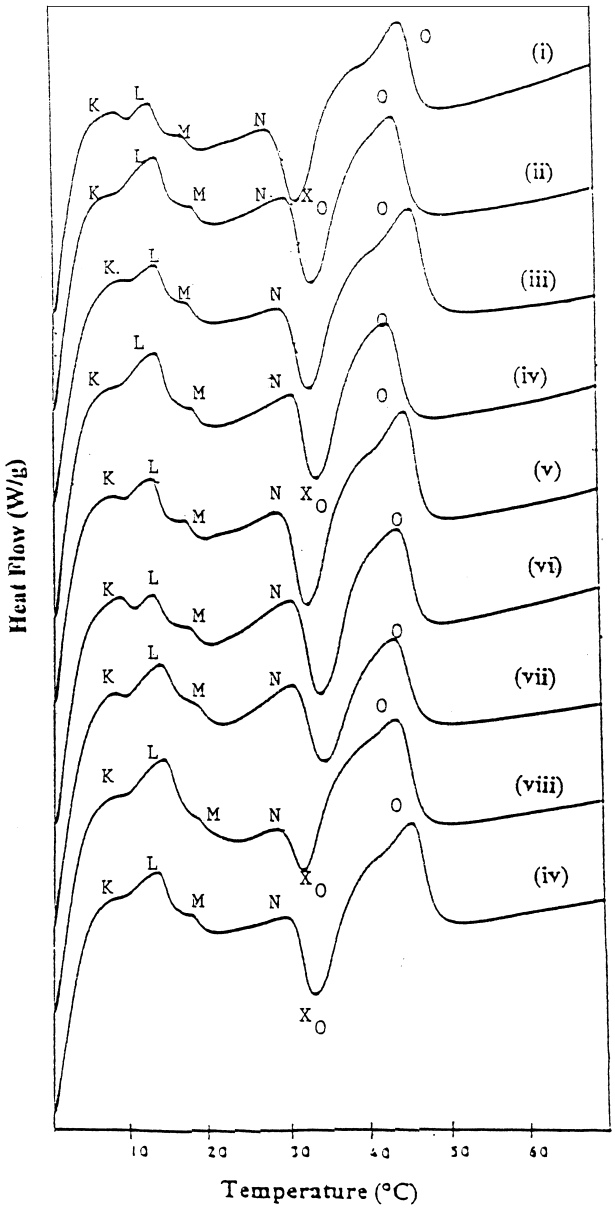

Fig. 1. Melting profiles of palm stearin:anhydrous milkfat (40:60) mixtures (i) before and after transesterification with lipases from (ii) *A. niger* (iii) *M. javanicus* (iv) *R. miehei* (v) *R. javanicus* (vi) *R. niveus* (vii) *Alcaligenes* (viii) *Pseudomonas* sp. and (ix) *C. rugosa* at heating rates of 10°C/min. Pretreatment: cooled from 70 to 0°C at 80°C/min; heating programme started after 1 min at 0°C.

The heating thermograms of PS:AMF mixtures using the 8 lipases are shown in Fig. 1. (ii–ix) while that for the control mixture is given in Fig. 1(i). In the control mixture, five endotherms and one exotherm, X_0 were observed. All the transesterified mixtures of PS:AMF have rather similar thermograms and were not distinguishable into groups. This is expected as the SMP obtained for all the PS:AMF mixtures fell within a close range of 42–44°C, being 0.5–2.5°C lower than the SMP of the control mixture (Table 2). The PS:AMF mixtures catalyzed by *R. miehei* and *Pseudomonas* lipases had the lowest SMP (42°C) and the highest % FFA (2.2 and 2.6%, respectively) and degree of transesterification (32.3 and 33.9%, respectively) while the rest of the mixtures showed lower % FFA (range: 0.4–1.8%) and degree of transesterification (6.8–10.4%) (Table 1). However, for samples transesterified with *Alcaligenes* and *C. rugosa* lipases, although the % FFA is higher than or similar to that exhibited by *R. miehei* lipase (2.2%), the degree of transesterifcation still remained low at 12.2 and 8.0%, respectively (Table 1). From these observations, it can be concluded that a high rate of hydrolysis and degree of transesterification did not necessarily facilitate lower SMP in the samples. The differences in the TG formed play a more important role; e.g. if more higher melting TG were to be formed, the fat would be harder. The role played by the positional specificity of the lipases does not seem obvious here.

Results of SFC, SMP and DSC suggest that *Pseudomonas* and *R. miehei* lipases would be the two most noteworthy enzymes to be used for the preparation of a more fluid product. However, although both these lipases produced the largest decline in SMP, the resulting formulation may still be considerably hard for the production of a table margarine. The SMP and SFC of this mixture can be still further reduced by lowering the % of palm stearin or by incorporating a more liquid oil into the blend. The formulation may find usage as an industrial shortening or pastry margarine which requires higher solids content in the blends.

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