Plastic fats from sal, mango and palm oil by lipase catalyzed interesterification

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Abstract Speciality plastic fats with no *trans* fatty acids suitable for use in bakery and as vanaspati substitute were prepared by interesterification of blends of palm stearin (PSt) with sal and mango fats using Lipozyme TLIM lipase as catalyst. The blends containing PSt/sal or PSt/mango showed short melting range and hence are not suitable as bakery shortenings. Lipase catalysed interesterification extended the plasticity or melting range of all the blends. The blends containing higher proportion of PSt with sal fat (50/50) were harder having high solids at and above body temperature and hence cannot be used as bakery shortenings. The blends with PSt/sal (30–40/60–70) after interesterification showed melting profiles similar to those of commercial hydrogenated bakery fats. Similarly, the blends containing PSt/mango (30–40/60–70) after interesterification also showed melting profiles similar to those of commercial hydrogenated shortenings. The slip melting point and solidification characteristics also confirm the plastic nature of these samples. The improvement in plasticity after interesterification is due to formation of higher melting as well as lower melting triglycerides during lipase catalysed interesterification.

Keywords Zero *trans* fats · Bakery fats · Vanaspati · Lipase catalyzed interesterification · Plastic fat

Introduction

Vanaspati, a hydrogenated fat, is normally being used for bakery, culinary purposes and traditional sweet preparation

in India and many South East Asian countries. However, hydrogenated fats contain large amount of *trans* fatty acids, which have been reported to be the risk factors involved in cardiovascular diseases (Etherton 1995). *Trans* fatty acids and their effect on human health have received much attention over the past three decades (List 2004) and have been reported to raise levels of low density lipoproteins (LDL) and lower the levels of high density lipoproteins (HDL) in humans. In view of such deleterious effects of hydrogenated fat, the demand for speciality plastic fats with zero *trans* fatty acids is increasing. The interesterification process is alternative process to hydrogenation to obtain *trans* free plastic fats (Adhikari et al. 1981; Khatoon and Bhattacharya 1985). The interesterification modifies the physical properties of oils/fats by rearranging the distribution of fatty acid into a random pattern on glycerol backbone without changing chemical composition. There are two types of interesterification processes namely chemical and lipase catalyzed. In terms of substrate selectivity, lipases are classified into three groups, like sn-1,3 regiospecific, non-specific and fatty acid specific. The 1,3-specific lipase catalysed interesterification is used to obtain fats with desired composition (Daniel et al. 2001; Zarringhalami et al. 2010). Structured lipid was synthesized using perilla and soybean oil for higher incorporation of alpha linolenic acid by enzymatic esterification (Mitra et al. 2010). Non-specific lipase catalysed interesterification is similar to random chemical interesterification (Boleslaw et al. 2004). Random interesterification was used to prepare low- or zero *trans* plastic fats from blend of liquid oils and hard fats (Mayamol et al. 2009; Reshma et al. 2008; De Martini Soares et al. 2009; Farmani et al. 2007; Khatoon 2000; Norizzah et al. 2004). It was reported that randomized menhaden oil was more stable than the original oil, whereas randomized seal blubber oil was more vulnerable

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to oxidation compared to its counterpart, due to differences in distribution of polyunsaturated fatty acids after lipase esterification (Wang and Shahidi 2011).

The *trans* free bakery shortenings were prepared from tallow and sunflower oil blends in various ratios by chemical interesterification (Rodriguez et al. 2001). Preparation of plastic shortenings from vegetable fats and oils by chemical, enzymatic interesterification and fractionation followed by blending is reported (Wang and Shahidi 2011; Zahra and Alemzadeh 2011; Khatoon and Reddy 2005; Wai et al. 2007; Reddy and Jeyarani 2001). It is known that most of the vegetable shortenings are in the β' form, where as those of the animal fat shortenings are in the β form (De Man et al. 1992). Fats with β' form are preferred for plastic shortenings as the crystal tend to be small, uniform and impart smooth texture to the product, where as the β form gives sandiness and graininess to the products. A variety of hard fats are reported and found that palm hard fat is the most stable β' form (Sabine and Claude 2003). The purpose of the present study is to prepare *trans* free plastic fats from palm stearin, mango and sal fats by using lipase catalyzed interesterification, suitable for use in bakery and confectionery to replace hydrogenated fats. Palm stearin is a by-product of palm oil industry and also improves smooth consistency required for shortening. Mango and sal fats are non-traditional fats having good potential in India and could be upgraded by preparing value added products from them, as they are harder in consistency suitable for use as bakery fat.

Material and methods

Crude palm oil was purchased from M/s Palm Tech. India Ltd., Mysore, Karnataka, India. Crude mango kernel (*Mangifera indica*) and sal (*Shorea robusta*) fats were procured from M/s K.N. oil industries, Mahasamund, M.P., India. Crude oils were refined and bleached in laboratory (Hodgson, 1996). Bakery shortenings designed for puff pastry, biscuits and cake were procured from M/s Hindustan Lever Ltd., Mumbai, India. Lipozyme TLIM was procured from Novozymes, Bangalore, India. Fatty acid methyl ester and triglyceride standards were procured from M/s Sigma Chemicals, St. Louis, Mo, USA.

Fractionation and blending of palm oil Refined palm oil was heated to about 50 °C, cooled gradually with stirring to 25 °C and held at this temperature for 4 h, the partially crystallized mass was filtered to separate palm stearin (50% yield) fraction (PSt), which is used in this study. PSt was blended with refined mango and sal fats in various proportions to carry out the interesterification.

Intesterification The interesterification reaction was carried out with 100 g purified oil blend at 50 °C with continuous stirring by magnetic stirrer using 10% by weight of oil of Lipozyme TLIM from *Thermomyces lanuginosa*. The reaction was carried out for time intervals of 2, 4, 6 and 8 h. The interesterified fat was filtered and the enzyme was washed with hexane. The product was refined using calculated amount

Fig. 1 Differential Scanning Calorimetric melting endotherms of (a) sal fat/PSt blends and their interesterified samples; 1, 2, 3=Sal/PSt (70:30, 60:40, 50:50 resp.); 4, 5, 6=Sal/PSt (70:30, 50:50, 60:40

resp.) after interesterification; (b) 1, 2=Mango/PSt (70:30) blend before and after interesterification respectively

Fig. 2 Melting profiles of palm stearin (PSt) blends (BL) with sal and mango (Mg) fats; (a) before and (b) after interesterification

of alkali to neutralize FFA, which were generated during the reaction. The product was filtered and de-solventized under vacuum.

Fatty acid composition The fatty acid methyl esters (FAME) were prepared using KOH/MeOH by AOCS method Ce 2–66 (AOCS 2002). FAME were analysed by gas liquid chromatography (model GC-15A, Simadzu corporation, Kyoto, Japan) equipped with data processor (Model CR-4A, Simadzu corporation, Kyoto, Japan) and FID detector. The column used was 3 m long $\times$ 3.3 mm i.d., stainless steel coated with 15% DEGS (Diethylene Glycol Succinate) on the Chromosorb W 60–80 mesh, operated under the following conditions: nitrogen flow, 40 mL/min, hydrogen flow, 40 mL/min, column temperature, 180 °C, injector temperature, 220 °C, detector temperature, 230 °C. The fatty acids were identified using standard fatty acid methyl esters from Sigma Aldrich, USA.

Triglyceride composition Triglyceride (TG) composition was determined by high performance liquid chromatography (HPLC): (Model Simadzu LC-10A, Simadzu Corp., Tokyo, Japan). Column used was C-18 (ODS), length 25 cm $\times$ 4.6 mm id, refractive index detector and oven temperature 40 °C. The mobile phase consisted of 63.5% acetone and 36.5% acetonitrile at 1 mL/min flow rate (Haryati et al. 1999). Peaks were identified comparing their retention time with those of authentic standards and reported as trisaturated (GS₃), monounsaturated (GS₂U), monosaturated (GSU₂) and triunsaturated (GU₃) triglycerides.

Cooling curve Cooling curves of the blends and interesterified fats were carried out by modified Shukoff's flask method (Wilton and Wode 1963). The fat was melted and taken in Shukoff's flask. Thermometer was inserted inside the flask and kept at 0 °C. Readings were recorded from 60 °C at

Table 1 Effect of interesterification on melting endotherms of different fat blends and interesterified fats determined by DSC

Sample	Peak ₁ (°C)/ ΔH (J/g)	Peak ₂ (°C)/ ΔH (J/g)
Sal:PSt 50:50/B	22.5 (19–27)/8.5	34.1(29–37)/30.4
Sal:PSt 50:50/8 h*	21.0 (20–25)/11.8	42.5(33–45)/20
Sal:PSt 60:40/B	-	34.1/53.4
Sal:PSt 60:40/6 h*	34 (34–39)/14	11.6 (4–20)/9.5; 26 (24–28)/2
Sal:PSt 70:30/B	-	32.95 (28–35)/54.2
Sal:PSt 70:30/8 h*	26.5 (25–28)/25.55	36.6 (30–40)/7
Mg:PSt 70:30/B	11.2, 22.8/9.5	34.7/29.5
Mg:PSt 70:30/8 h*	13.2 (8.5–16)/20	40.25 (34–43)/13.8

J/g: Joule/gram; PSt: Palmstearin; Mg: Mango fat; B: Blend; *after interesterification; Onset and end set temperatures of each peak are given in parenthesis.

Fig. 3 Cooling curves of: **a** 1, 3=Sal: PSt 60/40, 70/30 blends; 2, 4=Sal:PSt 60/40, 70/30 interesterified; 5, 6=Mango/PSt 70/30 blend and interesterified samples respectively. **b** 1,2=Cooling curves of mango

fat: PSt 60/40, 70/30 blends and 3, 4=after interesterification respectively; PSt: Palmstearin

1 min intervals. This shows the solidification or crystallization characteristics of fats.

Slip-melting point Slip-melting point of the samples was carried out by open capillary method according to AOCS method Cc 4–25, (AOCS 2002).

Differential scanning calorimetry (DSC) A Mettler (Zurich, Switzerland) differential scanning calorimeter (DSC-30) was used to determine melting characteristics of the samples. The heat flow of the instrument was calibrated using indium. The PT-100 sensor calibrated using indium, zinc and lead. To ensure the homogeneity and to destroy all crystal nuclei, the samples were heated to 60 °C, about 15 mg of molten sample was accurately weighed into standard aluminum crucible and cover crimped in place. An empty aluminum crucible with pierced lid was used as reference. For melting characteristics, the samples were stabilized according to IUPAC (1987) method, by keeping at 0 °C for 90 min, 26 °C for 40 h and 0 °C for 90 min prior to introduction into DSC cell. Although, this tempering method applies to fats with extended stabilization like cocoa butter, it ensures complete transformation to the stable form and the results are comparable. Thermograms were recorded by heating at the rate of 2.0 °C/min from –10 to 60 °C. The peak temperatures, heat of fusion values (ΔH) and percentage of liquid at various temperatures were

recorded directly using TC-10A data processor and STARE program. The solid fat content (SFC) was calculated from the percentage of liquid and melting profiles were drawn by plotting SFC vs temperature. The curve and SFC at various temperatures indicate the plasticity of the fat/fraction and suitability for a specific end use.

Statistical data analysis For experimental samples, average of triplicate was reported, whereas for commercial samples, error bars for different types are reported. Duncan's multiple range test (DMRT) was applied to differentiate the slip melting points (Table 2) and triglycerides species (Table 3) among the means of different samples at a probability (P) of <0.05 (Duncan 1955).

Table 2 Slip melting point of blends and interesterified fats

Sample	Slip melting point (°C)	
	Blend	Interesterified - 8 h
Sal:PSt (50:50)	36.5 ^c	39.0 ^c
Sal:PSt (60:40)	33.8 ^b	38.0 ^b
Sal:PSt (70:30)	32.2 ^a	34.5 ^a
Mg:PSt (70:30)	34.0 ^b	42.0 ^d

PSt: Palmstearin; Mg: Mango fat; Means in the same column with different superscripts are significantly different ($P < 0.05$; $n = 5$) as per DMRT

Table 3 Triglyceride composition (%) of sal, mango fat blends with PSt before and after interesterification

Sample	GS ₃	GS ₂ U	GU ₂ S	GU ₃
Sal:PSt 50:50/Bl	7.3 ^e	60.0 ^e	29.5 ^e	3.2 ^e
Sal:PSt 50:50/8 h*	12 ^f	48.7 ^b	37.9 ^e	1.4 ^c
Sal:PSt 60:40/Bl	1.7 ^a	65.3 ^f	22.6 ^a	10.4 ^f
Sal:PSt 60:40/8 h*	5.6 ^c	65.1 ^f	26.7 ^b	2.6 ^d
Sal:PSt 70:30/Bl	6.0 ^d	60.5 ^d	32.5 ^d	1.0 ^b
Sal:PSt 70:30/8 h*	4.4 ^b	50.0 ^c	45.0 ^f	0.6 ^a
Mg:PSt 70:30/Bl	7.5 ^e	51.1 ^c	38.7 ^e	2.7 ^d
Mg:PSt 70:30/8 h*	4.6 ^b	43.3 ^a	50.4 ^g	1.7 ^c

PSt: Palmstearin; Mg: Mango fat; Bl: Blend; GS₃: Trisaturated; GS₂U: Di-saturated; GU₂S: Di-unsaturated; GU₃: Tri-unsaturated glycerides; *after interesterification. Means in the same column with different superscripts are significantly different ($P < 0.05$; $n = 5$) by DMRT

Results and discussion

Sal: Palm stearin blends It has been observed that there is a sudden increase in FFA in samples with and without added moisture after 2 h of lipase catalyzed reaction and thereafter increase is not significant. FFA formation without added moisture was less (increased from 0 to 5% in 2 h) compared to that with addition of moisture (increased from 0 to 16% in 2 h). Reaction time and temperature had a linear positive and negative effect, respectively, on the increase of FFA during the Interesterification reaction. Higher FFA contents were observed in samples containing higher amounts of PSt and when longer reaction times and lower temperatures were used (Natália et al. 2009). The increase in temperature from 60 to 70 °C led to an increase in FFA content. Reshma et al. (2008) reported an increase in FFA was observed in the first hour of enzymatic esterification, after that it stabilized. Reactions were carried out without addition of moisture to minimize FFA formation. The blends of sal: palm stearin (PSt) 50:50; 60:40 and 70:30 were selected for enzymatic interesterification to prepare plastic fats suitable for bakery, confectionery and other culinary purpose to replace vanaspati. DSC melting endotherms showed a major peak in the range of 33–34 °C for all native blends (Fig. 1a), which resulted in short melting range (Fig. 2a).

However, the solids fat content (SFC) at different temperatures varied depending on the proportions of individual fat/fractions in the blend, which in turn reflected in the melting profiles (Fig. 2a). On interesterification with lipase, melting peaks at lower and higher temperatures appeared (Fig. 1a), the temperatures and enthalpy of peaks differ depending on the proportion of the blend (Table 1). The appearance of other melting peaks both at lower and higher temperatures compared to native blends resulted in increasing the melting or plastic range, which could be clearly seen from melting profiles (Fig. 2b). The samples with higher proportion of high melting PSt (50%) with sal fat showed a melting peak at 42.5 °C, which appeared as a small hump in the sample of sal/PSt with 60:40 blend (Table 1, Fig. 1a). The high melting peak of 50:50 blend is responsible for high solids content at higher temperatures and hence cannot be used in food application as it imparts waxy feeling in the mouth. These results revealed that melting or plastic range of native blends was increased upon interesterification with enzyme, similar to those reported earlier by chemical interesterification (Liu et al. 2010). The samples containing Sal: PSt (60:40, 70:30) after interesterification for 6 or 8 h showed melting profiles similar to those of vanaspati used for bakery or confectionery (Fig. 2b). The solidification curves also showed plastic nature of samples after interesterification unlike native samples, which showed supercooling property and raise in the temperature during crystallization (Fig. 3a, b). The slip melting point of all the blends after interesterification increased (Table 2) as observed with DSC. The increase in plasticity at higher temperature is the result of formation of higher melting glycerides in the form of trisaturated and/or may be due to formation of unsymmetrical mono-unsaturated type triglycerides and also increase in lower melting glycerides (Table 3). Chemical interesterification of canola oil and fully hydrogenated cotton seed oil blends in different proportions resulted in reduction of trisaturated triglycerides and increased in disaturated and monosaturated triglycerides and lowering melting points and SFC (Ribeiro et al. 2009). Chemical interesterification (CIE) caused a more balanced rearrangement of TG species, reduction of trisaturated (GS₃), triunsaturated (GU₃) TG content and increase in monosaturated (GSU₂) TG content (Liu et al. 2010). Investigations on chemical interesterification of rice bran oil, palm

Table 4 Fatty acid composition of blends used for interesterification

Sample	Relative%							
	C _{12:0}	C _{14:0}	C _{16:0}	C _{18:0}	C _{20:0}	C _{18:1}	C _{18:2}	C _{18:3}
Sal:PSt 50:50	0.2 ^b	0.5 ^c	27.0 ^c	21.4 ^a	4.0 ^b	39.5 ^a	7.3 ^c	0.1 ^a
Sal:PSt 60:40	0.2 ^b	0.4 ^b	23.0 ^b	24.0 ^b	5.4 ^d	40.4 ^a	6.4 ^b	0.2 ^b
Sal:PSt 70:30	0.3 ^c	0.3 ^a	19.0 ^a	27.5 ^d	5.1 ^c	42.2 ^b	5.4 ^a	0.2 ^b
Mg:PSt 70:30	0.1 ^a	0.3 ^a	19.5 ^a	26.5 ^c	2.0 ^a	44.0 ^c	7.5 ^c	0.1 ^a

PSt: Palmstearin; Mg: Mango fat. Means in the same column with different superscripts are significantly different ($P < 0.05$; $n = 5$) by DMRT.

oil and palm stearin revealed no change in the overall fatty acid composition on interesterification of the blends owing to the fact that interesterification catalyses exchange of fatty acids between and within the triglyceride molecules, thereby all the fatty acids get distributed evenly (Reshma et al., 2008). However, they also observed that the degree of changes in the physical characteristics was greatly influenced by interesterification, due to altered triglyceride composition and fatty acid composition of the fats and their ratios in the blends.

Noor Lida et al. 2007 reported that after chemical interesterification (randomization), there was a significant increase in trisaturated triglycerides and decrease in other disaturated and diunsaturated glycerides. However, the isomers of monounsaturated triglycerides, which might have been formed were not reported as they could not be separated by HPLC. The fatty acid composition of the samples after interesterification did not show any difference nor contain any *trans* fatty acids (Table 4), whereas the commercial vanaspati consists of about 17.5% *trans* fatty acids and about 50% saturated fatty acids (Reddy and Jeyarani 2001).

Mango: Palm fraction blend As observed with sal: PSt blends, mango: PSt blends also showed similar results. The blend mango/PSt (70:30) showed a major peak at about 35 °C and lower peaks at 11, 23 °C, whereas after interesterification, the higher melting peak shifted towards higher temperature to 40.2 °C (Fig. 1b, Table 1). The native blends showed short melting range (Fig. 2a), whereas after interesterification the melting range increased and is similar to those of commercial hydrogenated fats used for bakery or confectionery (Fig. 2b). The solidification curves (Fig. 3a, b) and slip melting point (Table 2) confirm these results.

Conclusion

The results revealed that lipase catalyzed interesterification extended the plasticity of blends of sal and mango fats with palm stearin and thus were found to be suitable for application in bakery, confectionery and other culinary purposes to replace hydrogenated fats and are nutritionally superior to the latter as they did not contain any *trans* fatty acids.

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