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ORIGINAL PAPER

Valorization of Beef Tallow by Lipase-Catalyzed Interesterification with High Oleic Sunflower Oil

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Abstract Although beef tallow (BT) has been considered a hard low-*trans* fat convenient to be used in several bakery applications, it has some undesirable characteristics like fatty acid composition, crystallization behavior, graininess formation and poor plastic range. This work studied the modification of BT by blending at different percentages with high oleic sunflower oil (HOSFO) followed by the enzyme-catalyzed interesterification of the blends. The reduction in the solid fat content achieved by the simple blending was enhanced by the interesterification process, as a result of the increase in the concentration of the diunsaturated monosaturated type triacylglycerols. Interesterification strongly impacted too on the crystallization behavior of the blends, since products showed more homogeneous and regular crystals than the starting mixture. Results show that lipase catalyzed interesterification of BT with HOSFO offers a useful tool for the design of fats with adjustable physicochemical properties, improved with respect to that of the starting fats.

Keywords Beef tallow · High oleic sunflower oil · Lipozyme TL IM · Melting profile · Crystal morphology

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Introduction

Beef tallow (BT) is an important animal fat widely used in the food industry due to its advantageous properties—such as high thermal and oxidative stability, ideal plasticity at room temperature, and typical aroma after baking [1, 2]. However, in certain applications, BT is known to exhibit undesirable physicochemical properties and crystallization behavior. It has a poor plastic range and a high percentage of solids at room temperature, making it inadequate to be used for certain foods. It contains a relatively high level of saturated triacylglycerols that do not melt in the mouth, producing an unpleasant sandy sensation. Additionally, if temperature fluctuates over a large range during handling, storage or transportation, BT based fats could suffer the growth of granular crystals with diameters up to 2–3 mm or above, a phenomenon also termed graininess formation, which is one major drawback that impairs the consistency and plasticity of BT based shortenings and margarines [2].

Jin and coworkers [1] found that the phase behavior of higher melting point TAG (triacylglycerols), such as POP, POS and SOS, is closely related to the grainy crystals, mainly of the β form, in edible beef tallow shortenings. The agglomerating of these high melting point TAG, together with forming of β-form crystals, was observed to occur in BT-based shortening with fluctuation of temperature, both in consequence causing the formation of granular crystals, phenomenon similar to the well known "fat bloom" occurring in cocoa-butter chocolate [1].

The modification of BT composition in order to obtain a fat with a wider range of thermal and melting properties can be performed via different well known methods, involving dry fractionation or the blending with oils from different origin, but these methods have several limitations related with the preservation of the structure of the TAG.

Additionally, products may not often result with the desired physicochemical [3] or nutritional properties [4], because the characteristics of the individual fats will be, to some extent, retained in the final blend.

Thus, the rearranging of the fatty acids in the triacylglycerol molecules by the interesterification process appeared as a more versatile tool that has been widely used to provide plastic fats with a proper quality [5, 6].

Although chemically catalyzed interesterification has been traditionally used by industry and well known industrial procedures and equipment are readily available, lipase catalysis has become an interesting alternative, since reactions are more specific, it requires milder reaction conditions and less waste can be produced.

MacKenzie and Stevenson [8] performed the directed interesterification of tallow using lipase catalysis, resulting in oleins containing significantly higher levels of unsaturated fatty acids than obtained by the conventional fractionation without lipase.

Foglia and coworkers [9] studied the lipase-catalyzed interesterification of BT with high oleic sunflower oil (HOSFO) or soybean oil (both at ratios BT/oil of 1:1), by means of either an immobilized 1,3-selective lipase or acyl-specific lipase, in order to provide interesterified blends of glycerides with a wider range of thermal and melting properties. This work demonstrated that the enzyme-catalyzed interesterification of fats and oils is a viable alternative to physical blending or chemical interesterification.

HOSFO has been gained attention due to its high content of n-9 monounsaturated fatty acids (C18:1 >85%), which confers to this oil a high oxidative stability and beneficial properties for human health. To date, however, there are very few records on the use of this oil as raw material for the softening of hard fats via interesterification.

The objective of this study was to analyze in detail the changes produced by the enzymatic interesterification of blends BT/HOSFO at different proportions, as an alternative for the valorization of the original BT. The effect of the percentage of HOSFO added to BT on TAG composition, fusion thermograms, and crystal morphology were evaluated.

Experimental Procedures

Materials

Premier jus beef tallow was generously provided by Frigorífico Tacuarembó S.A., Tacuarembó, Uruguay. Refined high oleic sunflower oil (COUSA S.A., Montevideo, Uruguay) was acquired in a local market.

Lipase form porcine pancreas was supplied by Sigma-Aldrich (PPL Type II, activity equal to 11.6 U/mg, in μmol of fatty acids hydrolyzed per minute per mg lipase). Lipozyme TL IM (from *Thermomyces lanuginosus*) was generously provided by Novozymes, Denmark.

Organic solvents, analytical standards and reagents used in the derivatization step required for some of the lipid analysis performed were supplied by Dexin S.R.L, Montevideo, Uruguay (representative of Sigma-Aldrich Company).

Interesterification Reactions

Blends with different proportions of BT and HOSFO were prepared (containing 10, 20, 30, 40 and 50 wt.% of HOSFO). Blends were heated at 60 °C and stirred for 15 min for total homogenization. 7 g of each blend were transferred to screw cap tubes and 0.7 g of Lipozyme TL IM (previously dried under a vacuum at 60 °C for 30 min) were added. The tubes were vented with N_2 and placed inside an orbital shaker at 60 °C, 200 rpm, for 24 h. After the incubation period the enzyme was separated from products by centrifugation (3,000 rpm, 15 min) and the lipid fraction destined to the analysis methods described below. This procedure was performed in duplicate and the products destined to independent analysis. Alternatively, the interesterification was performed under identical conditions on pure beef tallow (0% HOSFO).

Fatty Acid Composition

Samples were treated with BF_3/MeOH according to AOCS Ce 1b-89 [10] in order to convert the triacylglycerols to the corresponding methyl esters. The esters were analyzed by capillary gas chromatography, using a GC Shimadzu GC-2014, equipped with FID and a capillary column SP 2330 (25 m × 0.5 mm × 0.25 μm). The temperature program started at 160 °C, followed by a heating step (4 °C/min) to 230 °C, and remained at 230 °C for 10 min. Nitrogen at 40 kPa at column head was used as carrier gas, with a split ratio of 1:80. Procedure was performed in duplicate and average values reported.

Triacylglycerol Composition

Samples were dissolved in acetone (5 mg/mL) and directly analyzed using an HPLC Shimadzu Prominence 20A (Shimadzu, Corporation, Kyoto, Japan), equipped with an evaporative light scattering detector Shimadzu ELSD-LTII, two columns Supelcosil TM C18 (25 cm × 4.6 mm × 5 μm). The analysis started delivering a flow rate of 1 mL/min of a solvent mixture comprised by acetone/acetonitrile (1:1), with an increasing linear gradient of chloroform to 20% at 60 min, this solvent composition remained for 20 min and finally it was returned to the

starting composition at 85 min. Peaks were identified using pure TAG standards and considering the order of elution according the corresponding equivalent carbon number (ECN). Two replicate analyses were performed and average values were reported.

Analysis of Fatty Acids in the *sn*-2 Position

The nature of fatty acids in the *sn*-2 position of triacylglycerols was determined according to the AOCS method no Ch 3–91 [10].

DSC Analysis

Thermal profiles and solid fat content (SFC) curves were determined by differential scanning calorimetry (DSC), using a calorimeter TA Q20 (TA Instruments), equipped with a Refrigerated Cooling System RCS90, according to the AOCS method no. Cj 1–94 [10]. The peak areas, the partial areas and the percentage of SFC were determined from the melting profiles using the software TA Universal Analysis 2000 (version 3.9A). Calibration of the DSC equipment was performed using a standard of metallic Indium Two replicate analyses were performed and average values reported.

Polarized Light Microscopy

Samples were melted at 70 °C and a drop transferred onto a glass slide with the aid of a capillary tube. The drop was covered with a pre-heated cover slip before transfer to an incubator at 25 °C and maintaining at this temperature for a period of 20 h. Duplicate slides were prepared for each sample. After this period, the slides were transferred to a plate at a temperature of 25 °C (Mettler Toledo, FP82 Microscope Hot Stage). With the aid of a polarized light microscope (Olympus, model BX 50) connected to a digital video camera (Media Cybernetics), images were taken from three different visual fields at a magnification of 40×, and then a single image selected for the analysis [11]. The Image-Pro Plus software, Version 4.5.1.22 (Media Cybernetics) was used to take the images and carry out the quantitative analysis of the results.

Results and Discussion

Fatty Acid Composition

Table 1 shows the fatty acid composition of BT and HOSFO, and that corresponding to their blends at different proportions. The gradual reduction in the content of saturated fatty acid (SFA) and the corresponding rising in the content of monounsaturated fatty acids (MUFA) as the percentage of HOSFO increased can be appreciated.

TAG Composition

Table 2 shows the TAG composition of the raw materials, their blends in different proportions and the products obtained by interesterification of each blend catalyzed with Lipozyme TL IM for 24 h. According to Table 2,

Table 1 Fatty acid composition of the starting raw materials and their blends in different percentages (for simplicity, only major fatty acids are shown)

FAME	BT	HOSFO	HOSFO (wt.%)				
	% (SD)	% (SD)	10 % (SD)	20 % (SD)	30 % (SD)	40 % (SD)	50 % (SD)
14:0	2.8 (0.14)	nd	2.5 (0.07)	2.2 (0.21)	2.0 (0.07)	1.7 (0.01)	1.4 (0.07)
16:0	25.7 (0.25)	3.5 (0.07)	23.5 (0.14)	21.3 (0.07)	19.1 (0.14)	16.8 (0.14)	14.6 (0.07)
16:1	2.8 (0.21)	nd	2.5 (0.21)	2.2 (0.01)	2.0 (0.01)	1.7 (0.14)	1.4 (0.02)
17:0	1.4 (0.07)	nd	1.3 (0.07)	1.1 (0.07)	1.0 (0.07)	0.8 (0.00)	0.7 (0.40)
17:1	0.5 (0.07)	nd	0.5 (0.71)	0.4 (0.00)	0.4 (0.07)	0.3 (0.07)	0.3 (0.03)
18:0	26.7 (0.07)	2.5 (0.07)	24.3 (0.14)	21.8 (0.21)	19.4 (0.21)	17.0 (0.21)	14.6 (0.07)
18:1	37.9 (0.20)	88.2 (0.49)	42.9 (0.28)	48.0 (0.14)	53.0 (0.31)	58.0 (0.14)	63.1 (0.17)
18:2	0.8 (0.07)	4.6 (0.01)	1.2 (0.07)	1.6 (0.07)	1.9 (0.07)	2.3 (0.07)	2.7 (0.07)
18:3	0.7 (0.00)	nd	0.6 (0.07)	0.6 (0.07)	0.5 (0.07)	0.4 (0.07)	0.3 (0.01)
Fatty acid type							
SFA	56.6 (0.53)	6.0 (0.14)	51.6 (0.42)	46.4 (0.57)	41.5 (0.49)	36.3 (0.35)	31.3 (0.61)
MUFA	41.2 (0.48)	88.2 (0.49)	45.9 (1.20)	50.6 (0.14)	55.4 (0.28)	60.0 (0.35)	64.8 (0.12)
PUFA	1.5 (0.07)	4.6 (0.01)	1.8 (0.21)	2.2 (0.14)	2.4 (0.14)	2.7 (0.14)	3.0 (0.07)

SD standard deviation, *SFA* saturated fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids, *nd* non-detectable

Table 2 Triacylglycerol composition of the starting fatty materials, their blends (B) in different percentages and the interesterification products (P)

TAG	BT	HOSFO	HOSFO (wt.%)										
			0	10		20		30		40		50	
	% (SD)	% (SD)	P % (SD)	B % (SD)	P % (SD)	B % (SD)	P % (SD)	B % (SD)	P % (SD)	B % (SD)	P % (SD)	B % (SD)	P % (SD)
OOL		1.9 (0.3)	0.9 (0.1)	0.2 (0.1)	1.0 (0.2)	0.4 (0.1)	2.3 (0.2)	0.6 (0.1)	2.6 (0.1)	0.8 (0.1)	2.1 (0.1)	1.0 (0.1)	2.8 (0.2)
StStL	4.3 (0.2)	nd	3.9 (0.1)	3.9 (0.1)	4.1 (0.2)	3.4 (0.4)	4.7 (0.2)	3.0 (0.4)	4.3 (0.6)	2.6 (0.4)	3.9 (0.8)	2.2 (0.1)	3.5 (0.3)
POL	3.2 (0.1)	nd	3.1 (0.3)	2.9 (0.2)	2.3 (0.2)	2.6 (0.4)	2.7 (0.5)	2.2 (0.1)	2.0 (0.4)	1.9 (0.1)	1.4 (0.3)	1.6 (0.3)	1.2 (0.1)
OOO	2.5 (0.1)	85.5 (0.4)	4.5 (0.1)	10.8 (0.6)	7.2 (0.4)	19.1 (0.6)	10.7 (0.6)	27.4 (0.6)	16.1 (0.1)	35.7 (0.5)	24.2 (0.6)	44.0 (0.7)	30.9 (1.3)
POO	21.2 (0.6)	5.9 (0.3)	15.5 (0.4)	19.7 (0.7)	18.4 (0.4)	18.1 (0.8)	19.4 (0.5)	16.6 (0.3)	22.1 (0.1)	15.1 (0.3)	23.3 (0.2)	13.6 (1.1)	22.7 (0.5)
PPO	12.1 (0.6)	nd	13.3 (0.5)	10.9 (0.3)	11.4 (0.3)	9.7 (0.5)	9.9 (0.8)	8.5 (0.4)	7.9 (0.1)	7.3 (0.2)	6.0 (0.4)	6.1 (0.2)	4.6 (0.2)
PPP	3.7 (0.1)	nd	1.4 (0.1)	3.3 (0.2)	3.5 (0.2)	3.0 (0.7)	3.2 (0.3)	2.6 (0.4)	2.0 (0.6)	2.2 (0.1)	1.6 (0.3)	1.9 (0.4)	1.2 (0.1)
StOO	9.4 (0.3)	3.6 (0.1)	10.4 (0.4)	8.8 (0.4)	11.4 (0.3)	8.2 (0.5)	15.0 (0.7)	7.7 (0.6)	17.1 (0.1)	7.1 (0.3)	17.8 (0.6)	6.5 (0.3)	17.3 (0.5)
PStO	18.1 (0.8)	nd	18.8 (0.8)	16.3 (0.6)	16.2 (0.6)	14.5 (0.4)	13.7 (0.5)	12.7 (0.2)	11.7 (0.8)	10.9 (0.3)	9.0 (0.2)	9.1 (0.3)	6.4 (0.3)
PPSt	5.0 (0.7)	nd	6.2 (0.1)	4.5 (0.4)	4.5 (0.4)	4.0 (0.7)	3.0 (0.4)	3.5 (0.4)	2.0 (0.7)	3.0 (0.4)	1.2 (0.1)	2.5 (0.4)	0.8 (0.2)
StStO	6.8 (0.2)	nd	6.0 (0.7)	6.1 (0.1)	7.4 (0.4)	5.4 (0.3)	4.8 (0.1)	4.8 (0.6)	4.3 (0.9)	4.1 (0.4)	3.3 (0.2)	3.4 (0.3)	2.4 (0.3)
PStSt	4.4 (0.3)	nd	4.3 (0.2)	4.0 (0.7)	3.5 (0.4)	3.5 (0.4)	1.8 (0.3)	3.1 (0.1)	1.3 (0.1)	2.6 (0.4)	0.9 (0.2)	2.2 (0.1)	0.4 (0.1)
TAG type													
SSS	13.1 (1.1)	nd	11.9 (0.5)	11.8 (1.3)	11.5 (0.9)	10.5 (1.8)	8.0 (0.9)	9.2 (0.8)	5.3 (1.3)	7.9 (0.9)	3.7 (0.6)	6.6 (0.8)	2.4 (0.5)
SSU	41.3 (1.8)	nd	42.0 (2.1)	37.2 (1.1)	39.1 (1.5)	33.0 (1.6)	33.1 (1.6)	28.9 (1.5)	28.2 (2.4)	24.8 (1.3)	22.2 (1.6)	20.7 (0.9)	16.9 (1.1)
SUU	33.8 (1.0)	9.5 (0.4)	29.0 (1.1)	31.4 (1.3)	32.1 (0.9)	28.9 (1.7)	37.1 (1.7)	26.5 (1.1)	41.2 (0.5)	24.1 (0.6)	42.5 (1.1)	21.6 (1.7)	41.2 (1.1)
UUU	2.5 (0.1)	87.4 (0.7)	5.4 (0.3)	11.0 (0.7)	8.2 (0.6)	19.5 (0.7)	13.0 (0.8)	28.0 (0.7)	18.7 (0.1)	36.5 (0.6)	26.3 (0.6)	45.0 (0.8)	33.7 (1.6)

For simplicity only major TAG are shown

SD standard deviation, *O* oleic, *St* stearic, *L* linoleic, *P* palmitic, *SSS* trisaturated, *SSU* disaturated monounsaturated; *SUU* monosaturated diunsaturated, *UUU* triunsaturated, *P* product, *B* blend, *nd* non-detectable

HOSFO is mostly composed by triolein (85.5 wt.%), thus the effect of blending this oil with BT basically produced an increment in the concentration of triolein (OOO) as the percentage of HOSFO in the blend increased, with the corresponding dilutive effect on the rest of the triacylglycerols.

Table 2 shows that pure BT underwent a few changes in TAG composition after 24 h of interesterification, which is in agreement with a composition of the original fat close to that corresponding to a random distribution. In order to easily analyze the changes produced by the interesterification process, TAG were grouped by type and the percentage of "Relative Variation" (RV) of the different triacylglycerol type were calculated, defined as:

$$\mathrm{RV} = 100\frac{(\mathrm{TAG_i})_\mathrm{P} - (\mathrm{TAG_i})_\mathrm{B}}{(\mathrm{TAG_i})_\mathrm{B}} \quad (1)$$

where RV is the percentage of relative variation, $(\mathrm{TAG_i})_\mathrm{P}$ is the concentration of TAG type "i" in the product, $(\mathrm{TAG_i})_\mathrm{B}$ is the concentration of TAG type "i" in the original blend.

The most important variation during the interesterification of pure BT was observed in POO concentration, which decreased from 21.2 to 15.5% (Table 2), resulting in a RV of −14% for TAG type SUU (Fig. 1). Although Fig. 1 shows a high RV for UUU type triacylglycerols (RV = +117%), it is a consequence of the increase of OOO, a minor triacylglycerol in BT.

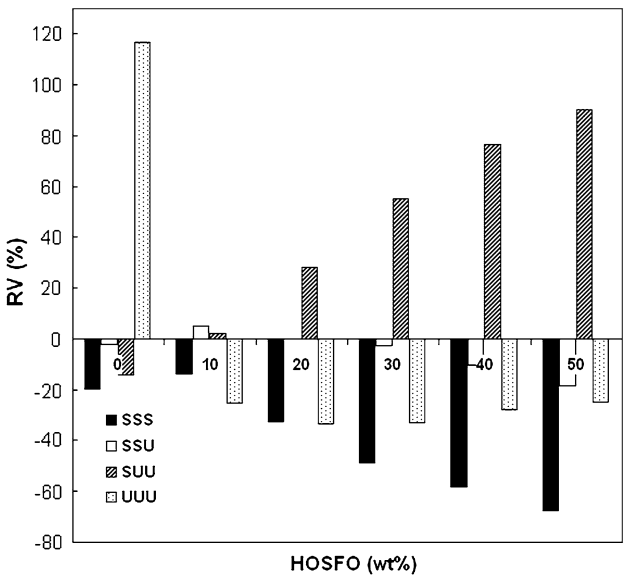

Fig. 1 Percentage of relative variation (RV) of the different triacylglycerol type produced by the enzymatic interesterification of the different blends BT/HOSFO, determined according Eq. 1. (*SSS* trisaturated, *SSU* disaturated monounsaturated, *SUU* monosaturated diunsaturated, *UUU* triunsaturated)

With respect to the effect of the interesterification process on the composition of the different blends, Fig. 1 shows that the concentration of trisaturated triacylglycerols (SSS) diminished as a consequence of the enzymatic reaction. RV values for SSS varied from −14 to −68% as the percentage of HOSFO in the starting blend increased form 10 to 50%, respectively. This gradual diminishing was a consequence of the partial disappearance of all triacylglycerols from this group: PPP, PPSt and PStSt (Table 2).

An opposite trend was shown by the concentration of the diunsaturated monosaturated type triacylglycerols (SUU), which gradually rose as the concentration of HOSFO in the blends was increased. Figure 1 shows a strong effect of increasing the percentage of HOSFO on the concentration of SUU type TAG in the interesterification products: RV raised from 2 to 90% as the percentage of HOSFO in the blend was increased from 10 to 50%.

With respect to the triunsaturated triacylglycerols (UUU, mostly OOO) all the blends showed a similar reduction in their concentration (RV between −25 and −33%), without a unique trend with the percentage of HOSFO.

Concerning one of the main TAG types, SSU, their total concentration raised when the blend with the lowest content of HOSFO was incubated (RV = +5%, corresponding to the blend with 10% of HOSFO), a neutral variation was obtained when the blend with 20% of HOSFO was incubated (RV = 0%), and a gradual decreasing was observed for higher proportions of HOSFO (negative RV values with a decreasing trend, Fig. 1), as expected from the increasing proportion of unsaturated fatty acids provided by HOSFO to the blend.

Fatty Acids in the *sn*-2 Position

The physicochemical properties and nutritional value of oils are determined not only by the fatty acid and triacylglycerol composition but also by the positional distribution of the acyl groups of the triacylglycerols. Our results showed that products did not present any specific distribution of the fatty acids among the three positions of the triacylglycerol molecule (results not shown). Considering that lipase Lipozyme TL-IM has been reported to have *sn*-1,3 positional specificity, the random distribution achieved could be attributed to acyl migration phenomenon, as has been previously observed in the catalysis of interesterification reaction using this enzyme [12].

Thermal Profiles and SFC

Figure 2 shows the heating thermograms scanned at 5 °C/min of the pure raw materials. The thermogram of

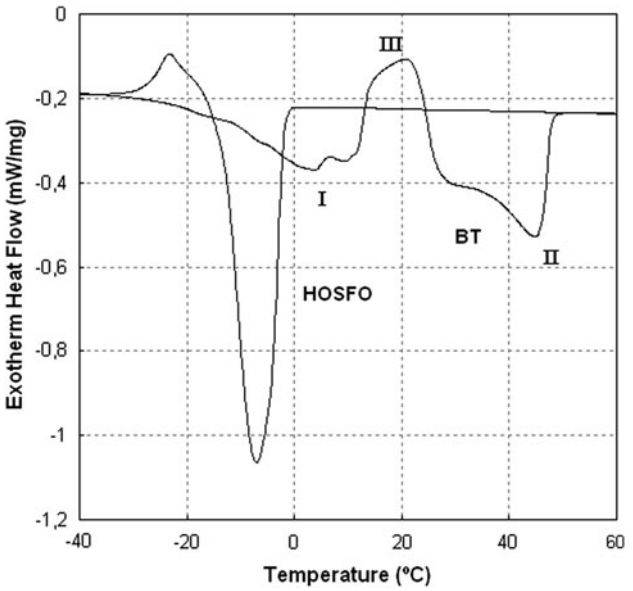

Fig. 2 Heating thermograms of the pure raw materials: beef tallow (BT) and high oleic sunflower oil (HOSFO)

pure HOSFO shows one single endotherm peak with a peak temperature (T_p) of −6.4 °C, which should correspond to the melting of triolein. Previous results on the melting properties of single acid triacylglycerols studied by DSC showed that pure triolein could present multiple β'-forms, with the following melting points: β'_3: −12 °C, β'_2: −8 °C and β'_1: −5 °C [13]. According to these results, the main endotherm obtained for HOSFO should correspond to the melting of β' crystals involving probably a mixture of β'_2 and β'_1 forms, with melting points too close to be distinguished. It is interesting to note that the heating thermogram of HOSFO shows an exotherm at the lowest temperatures (Fig. 2). The presence of exotherms in melting thermograms evidences the transformation or rearrangement of one polymorphic form to another one with a higher melting point [14, 15]. Thus, considering that the melting point of α-form crystals of triolein is near −37 °C [13], the exotherm obtained for HOSFO can solely be assigned to the transformation from α to β' form.

The thermogram obtained for pure BT shows two melting zones (endothermic), the first one located at low temperatures and with a T_p^I of 4.5 °C (peak I, Fig. 2) and a second one at higher temperatures, with a T_p^{II} of 45.6 °C (peak II, Fig. 2). According to these temperature ranges the first peak should correspond to the melting of the more unsaturated TAG (SUU + UUU) and the second one to the melting of the more saturated ones (SSS + SSU). An intermediate exotherm peak can also be observed ($T_p^{III} = 21.9$ °C, peak III, Fig. 2) which, as mentioned above, suggests the transformation of one polymorph to another one with a higher melting point. It is well known that the polymorphic behavior of a fat is largely influenced by its fatty acid composition and the positional distribution of these fatty acids on the glycerol backbone, in other words by its triacylglycerol composition. The efficiency of the inter-planar packing of the hydrocarbon chains, which is high in TAG with uniform fatty acid chain lengths, is reduced when different chain length TAG are mixed [15]. Considering that BT can be considered a heterogeneous fat, in terms of the diversity of TAG that comprises, it tends to produce β'-crystals which can persist for long periods [16]. Thus, the occurrence of an intermediate exotherm peak during the melting process can be associated with the transformation or rearrangement from β' to β form, with a higher melting point.

Figure 3a shows the thermograms obtained for the different blends BT/HOSFO which, as expected, have characteristics conferred by both fats. As the percentage of HOSFO in the blend increased the area of peak II diminished, as a result of the "dilution" of the highly saturated triacylglycerols. The same effect can be observed for the exotherm peak III, which gradually decreased with the incorporation of higher percentages of HOSFO. Conversely, the first endothermic peak (I) increased and gradually shifted towards lower melting ranges, closer to that corresponding to the melting ranges of pure HOSFO.

Figure 3b shows the percentage of SFC against temperature, obtained by the relative partial integration of the heating thermograms in Fig. 3a. The effect of the exotherm peak III observed in the heating thermograms is visualized as an "apparent" increase in the SFC. This effect gradually diminished as the percentage of HOSFO in the mixtures increased, accordingly with the diminishing in the area of peak III (Fig. 3a). As expected, the dilution of the hard triacylglycerols from BT caused that the blends with the highest proportions of vegetable oil had the lowest SFC percentages.

Figure 4a shows the heating thermograms scanned at 5 °C/min of the products obtained by the enzymatic interesterification of the different blends BT/HOSFO. It is interesting to note that, as a consequence of the interesterification, the exotherm peak (III) observed in the analysis of pure BT and the blends with HOSFO (Fig. 4a) disappeared in the thermograms of the products. Surprisingly this effect was observed too in the product from the interesterification of pure BT (IBT), even though interesterification slightly modified its TAG composition. Considering that peak III was associated with the transformation of crystals from β' to β form, these results suggest that interesterified fats presented a lower tendency to form β crystals. A preference to form β' crystals, smaller and softer than β, is a valuable property of fats destined to several types of edible products, like spreadable margarines and different type of shortenings.

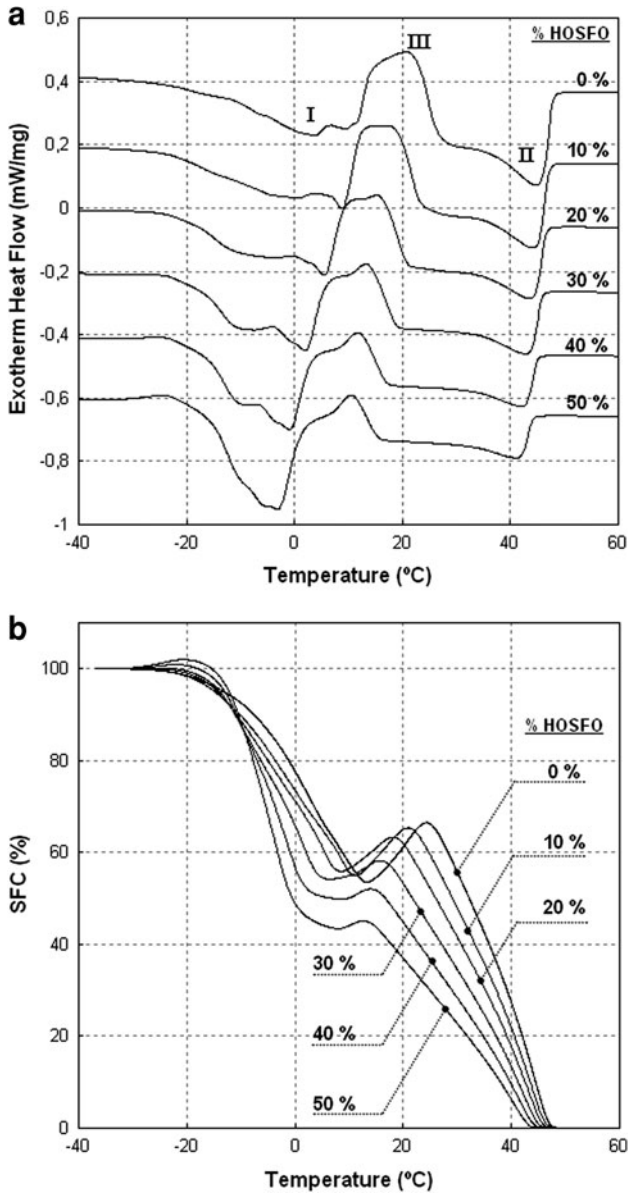

Fig. 3 Heating thermograms of the mixtures BT/HOSFO (**a**), and the corresponding solid fat content (SFC) curves (**b**)

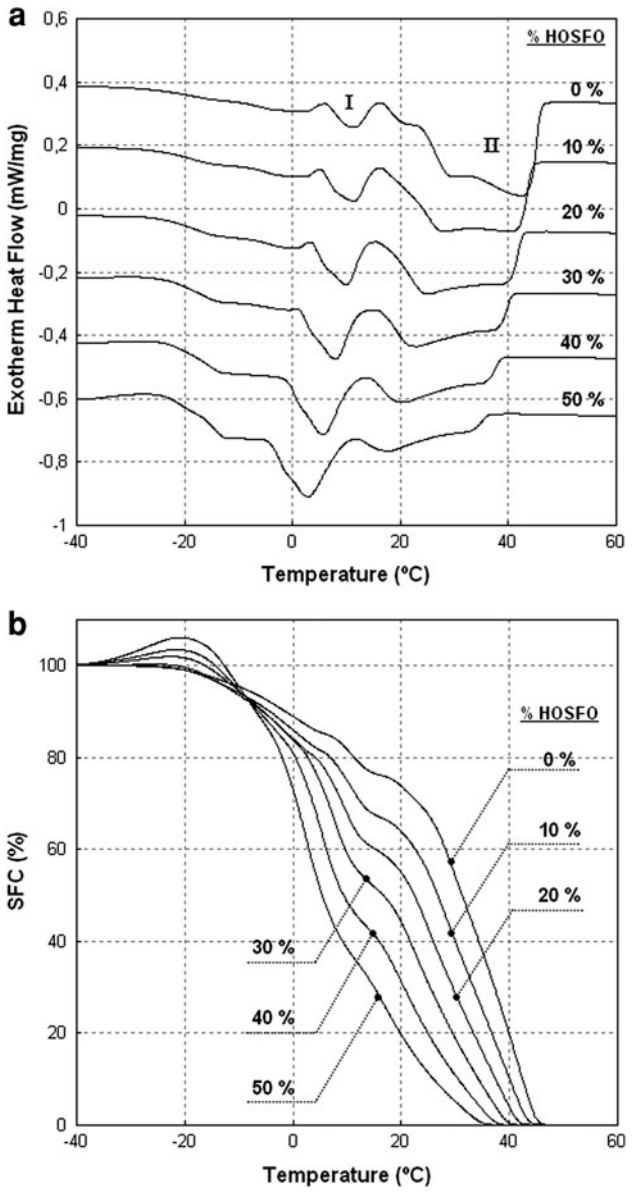

Fig. 4 Heating thermograms of the products obtained from the enzymatic interesterification of the mixtures BT/HOSFO (**a**), and their corresponding solid fat content curves (**b**)

Figure 4a shows that interesterification produced strong changes in the endotherm peak II, peak area diminished with the increase of the percentage of oil and peak temperature (T_P^{II}) shifted from 45.6 °C (pure BT) to temperatures from 20 to 30 °C, depending on the percentage of HOSFO.

Concerning peak I area, it increased with the percentage of oil and, compared with the starting blend, peak became thinner and showed some increase in peak temperature (T_P^{I}). Both changes are in agreement with the diminishing of UUU and the increment of SUU type TAG mentioned before.

Due to the absence of exothermic peaks in the thermograms of the products, the SFC curves show a continuous decreasing trend with temperature (Fig. 4b). It is interesting to analyze the SFC at 37 °C because of its influence on "mouth feel": the $SFC_{BT,37°C}$ is about 40% and that corresponding to $SFC_{IBT,37°C}$ is near 35%, while the SFC at 37 °C corresponding to the blend with 50% is about 13% and diminished to 0% after the interesterification.

Solid fat content is responsible for many important characters of fats, like physical appearance, organoleptic properties, and spreadability. The plasticity or consistency of an edible oil product depends on the amount of solids

and the variation of the solid fat content with temperature, The sharpness of the melting range and other factors like crystal morphology determine the range within which a fat could be considered plastic [15]. According to DeMan [17] a desirable spreadability occurs within a range of roughly 15–35% solids, called the "plastic range of fats".

Figure 5 shows the plastic range, as defined above, for the different blends BT/HOSFO and their interesterification products. The product from the interesterification of pure BT (IBT) had a narrow plastic range of about 5 °C, in a region over room temperature (from 37 to 42 °C). As the percentage of HOSFO increased, the plastic range increased too and shifted to lower temperatures, with the widest plastic range (from 12 to 23 °C) corresponding to the product containing 50% HOSFO. Thus, the increase in HOSFO content produced two effects on the plastic range of the interesterification products: the extension of the plastic range and its shifting to lower temperatures.

According to Fig. 5, the plastic range and hence the spreadability, could be adjusted to product requirements by the convenient selection of the percentage of oil in the blend destined to the interesterification process.

Crystalline Morphology and Microstructure

Figure 6 shows the microscopic images under polarized light at 25 °C of pure beef tallow and two different blends (containing 20 and 50% of HOSFO), before and after interesterification.

Rodríguez et al. [7] studied the chemical interesterification of tallow and sunflower oil. As also observed by these authors, the crystal network was heavily influenced by blending and interesterification of beef tallow with a liquid oil, such as high oleic sunflower oil. In this study, the beef tallow crystal network consisted of a dense network of spherulites and small needles, ranging from 2 to 73 μm. This could be attributed to a heterogeneous TAG distribution of BT. The addition of high oleic sunflower oil to tallow resulted in a dilution of the tallow microstructure which varied in concentration but without substantial change in morphology. As a result, the crystallized area and the maximum diameter of the spherulites (59–34 μm) decreased with increasing HOSFO concentration in the blends.

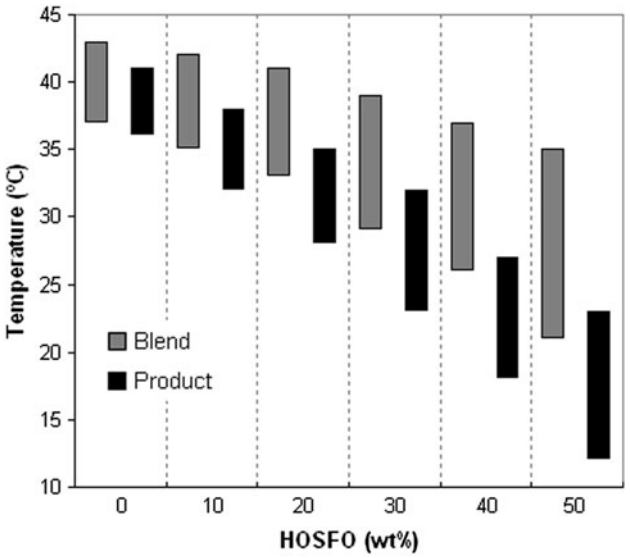

Fig. 5 Temperature ranges corresponding to a solid fat contents (SFC) ranging from 15 to 35% ("plastic range") for the different blends BT/HOSFO and the corresponding interesterification products

The mean crystal diameter of tallow and its blends with HOSFO were close, ranging from 7 to 10 μm, but showed high standard deviation, which is characteristic of crystallized fats when observed using polarized light microscopy [18].

The interesterification caused substantial change on the crystal network morphology of tallow and its blends with HOSFO. It is interesting to note that all the products from interesterification, including pure BT, show more regular and homogeneous crystallization than the corresponding non-interesterified blends. The mean diameters of the interesterified products (7–11 μm) were similar to those of the original blends, but the maximum observed diameters, in the range of 32–33 μm, were lower than those observed in the non-interesterified blends. The crystallized area of the interesterified samples also decreased with increasing HOSFO concentration in the blends. These changes can be responsible for the lower solid fat content of the interesterified blends, as determined by DSC.

According to Rousseau and coworkers [3], the alterations in fat microstructure caused by interesterification result from modifications to morphology and the density of the crystalline network and affect the texture and functionality of the interesterified bases. In addition, the change in solid fat content intrinsic to the process of interesterification influences how the crystalline network is structured. When solid fat content decreases, as observed in this study, changes occur towards greater molecular area and mobility for crystal formation [19]. Thus, according to our results, interesterification of BT with HOSFO tends to avoid sandy mouthfeel caused by large crystals.

Conclusion

The interesterification of BT with HOSFO catalyzed by Lipozyme TL IM under the conditions studied produced randomized fats with lower solid fat content than the starting blend. The softening of the product was the result of the modification of the TAG composition, which led to

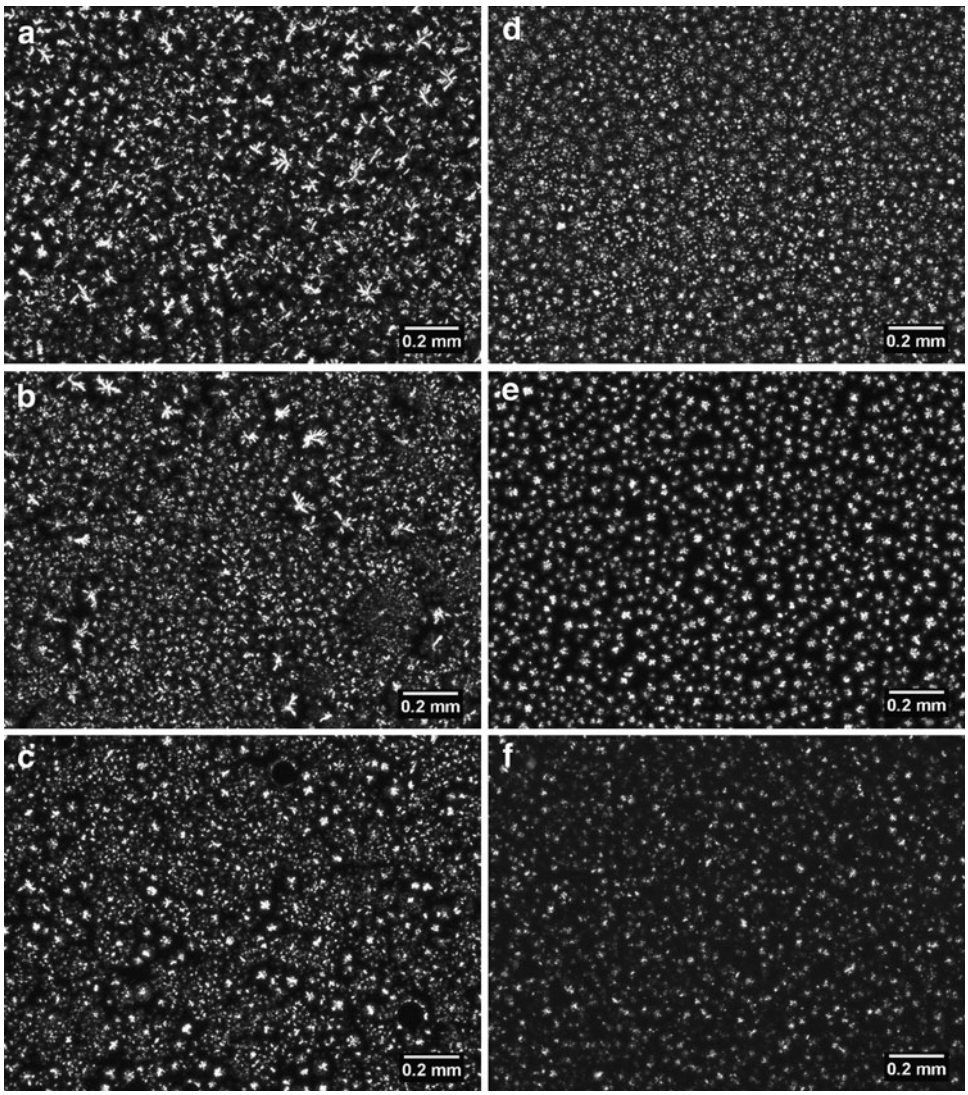

Fig. 6 Microscopic images under polarized light at 25 °C of the blends BT/HOSFO with 0, 20 and 50% HOSFO: (**a**), (**b**) and (**c**), respectively, and the corresponding interesterification products: (**d**), (**e**) and (**f**), respectively. *Bar* 0.2 mm

the increase in the concentration of the diunsaturated monosaturated type triacylglycerols (SUU). The plastic ranges of the blends were highly modified by the interesterification process, conferring the products an improved spreadability at room temperature. Additionally, the products showed smaller and more regular crystals than the corresponding non-interesterified blend, which is beneficial in order to avoid sandy mouthfeel caused by large crystals. Results show that the enzymatic interesterification of BT with HOSFO is an attractive alternative for the design fats with improved physicochemical properties for the food industry.

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References

1. Jin Q, Gao H, Shan L, Liu Y, Wang X (2007) Study on grainy crystals in edible beef tallow shortening. Food Res Int 40:909–914
2. Meng Z, Liu Y, Shan L, Jin Q, Wang X (2010) Reduction of graininess formation in beef tallow-based plastic fats by chemical interesterification of beef tallow and canola oil. J Am Oil Chem Soc 87:1435–1442
3. Rousseau D, Marangoni AG, Jeffrey KR (1998) The influence of chemical interesterification on the physicochemical properties of complex fat systems. 2. Morphology and polymorphism. J Am Oil Chem Soc 75:1833–1839
4. Reena MB, Lokesh BR (2007) Hypolipidemic effect of oils with balanced amounts of fatty acids obtained by blending and interesterification of coconut oil with rice bran oil or sesame oil. J Am Oil Chem Soc 55:10461–10469
5. Norizzah AR, Chong CL, Cheow CS, Zaliha O (2004) Effects of chemical interesterification on physicochemical properties of palm stearin and palm kernel olein blends. Food Chem 86:229–235

6. Chu BS, Ghazali HM, Lai OM, Cheman YB, Yusof S (2002) Physical and chemical properties of a lipase–transesterified palm stearin/palm kernel olein blend and its isopropanol-solid and high melting triacylglycerol fractions. Food Chem 76:155–164
7. Rodríguez A, Castro E, Salinas MC, López R, Miranda M (2001) Interesterification of tallow and sunflower oil. J Am Oil Chem Soc 78:431–436
8. MacKenzie AD, Stevenson DE (2000) Production of high-oleic acid tallow fractions using lipase-catalyzed directed interesterification, using both batch and continuous processing. Enzyme Microb Tech 27:302–311
9. Foglia TA, Petruso K, Feairheller SH (1993) Enzymatic interesterification of tallow–sunflower oil mixtures. J Am Oil Chem Soc 70:281–285
10. AOCS (1997) Official methods and recommended practices of the American Oil Chemists' Society. AOCS Press, Champaign
11. Gamboa OWD, Gioielli LA (2006) Crystallization behaviour of structured lipids produced from palm kernel fat and fish oil. Quim Nova 29:646–653
12. Lee JH, Son JM, Akoh CC, Kim MR, Lee KT (2010) Optimized synthesis of 1,3-dioleoyl-2-palmitoylglycerol-rich triacylglycerol via interesterification catalyzed by a lipase from *Thermomyces lanuginosus*. New Biotechnol 27:38–45
13. Hagemann JW, Tallent WH, Kolb KE (1972) Differential scanning calorimetry of single acid triglycerides: effect of chain length and unsaturation. J Am Oil Chem Soc 49:118–123
14. Garti N, Aronhime JS, Sarig S (1989) The role of chain length and an emulsifier on the polymorphism of mixtures of triglycerides. J Am Oil Chem Soc 66:1085–1089
15. Rao R, Sankar KU, Sambaiah K, Lokesh BR (2001) Differential scanning calorimetric studies on structured lipids from coconut oil triglycerides containing stearic acid. Eur Food Res Techno 212:334–343
16. Foubert I, Dewettinck K, Van de Walk D, Dijkstra AJ, Quinn PJ (2007) Physical properties: structural and physical characteristics. In: Gunstone FD, Harwood JL, Dijkstra AJ (eds) The lipid handbook. CRC Press, Boca Raton, pp 471–534
17. DeMan JM (1992) Fats and oils: chemistry, physics and applications. In: Hui HY (ed) Encyclopedia of food science and technology. Wiley, New York, pp 823–824
18. Ribeiro APB, Grimaldi R, Gioielli LA, Santos AO, Cardoso LP, Gonçalves LAG (2009) Thermal behavior, microstructure, polymorphism and crystallization properties of zero trans fats from soybean oil and fully hydrogenated soybean oil. Food Biophys 4:106–118
19. Himawan C, Starov VM, Stapley AGF (2006) Thermodynamic and kinetic aspects of fat crystallization. Adv Colloid Interface 122:3–33