Synthesis of structured lipids containing behenic acid from fully hydrogenated *Crambe abyssinica* oil by enzymatic interesterification

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Abstract Low-calorie structured lipids (SLs) rich in behenic and oleic acids were produced by enzymatic (EI) and chemical interesterification (CI) of high oleic sunflower oil and fully hydrogenated *Crambe abyssinica* oil in proportions of 60:40, 50:50, 40:60 and 30:70 (w/w), respectively. The immobilized lipase from *Thermomyces lanuginosus* (Lipozyme TL IM) was used for 3 h at 70 °C at a dosage of 7% (w/w) free of organic solvents. The original blend (BE) and the reaction products were evaluated for their fatty acid (FA) and triacylglycerols (TAG) compositions, solid fat contents, thermal analysis, regiospecific distribution of the FA, microstructure and polymorphism. The tendency was towards a reduction in the C₅₂, C₅₄, C₆₀, C₆₂ and C₆₄ TAG contents and an increase in the C₅₆, C₅₈ e C₆₆ TAG contents, therefore the solid fat content was reduced. There was lower acyl migration at the *sn*-2 position of the TAGs in EI as compared to CI in all the blends ratios. Needle-like crystals were predominant in the IE while large symmetrical spherulites were observed in the BE. Reduction in the mean crystal diameter was observed in all the blends, in addition to modifications of the crystal morphology. X-ray diffraction analysis showed a predominance of the β' form crystals in both the IE and CI. The SLs were produced for application in the food industry as bakery/confectionery

fats in the proportions of 60:40/50:50 and as additive in the crystallization of lipids in the proportions of 40:60/30:70.

Keywords Structured lipids · Enzymatic interesterification · Lipase · *Crambe abyssinica* oil · High oleic sunflower oil · Regiospecificity

Introduction

Crambe abyssinica (Hochst) is an oil-rich plant belonging to the cruciferous family. It is a native of the Mediterranean region, but the species has shown good adaptation to other climatic conditions (Souza et al. 2009). This species has been cultivated in Brazil due to industrial interest in extracting the seed oil (26–38%), and due to its high protein content and great potential for cultivation in the off-season, in the Midwest region, which expresses characteristics favorable to crop rotation with direct planting after soybean harvest (Panno and Prior 2009). Its oil can be distinguished from others by its high erucic acid content (50–60%), along chain fatty acid. This acid contains 22 carbon atoms and only one double bond, and has special industrial uses (Laghetli et al. 1995). Fully hydrogenated *Crambe* oil is a white solid that has potential value as a component of wax compositions. The hydrogenation process converts the erucic acid (C22:1) into behenic acid (C22:0). Behenic acid can be used as a functional component of low-calorie fats and dietary fats containing behenic acid are expected to reduce visceral fat deposition (Kojima et al. 2010). Furthermore, fats containing behenic acid may be used as crystallization additives in lipids systems (Mori 1989). The high oleic sunflower oil has about 89% oleic acid in its composition compared to the traditional oil which contains less than 50%. This increased

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amount of oleic acid provides high thermo-oxidative stability (Labalette et al. 2012; Alberio et al. 2016) and such oil would be highly valuable in foods (e.g. fried products, bakery produce and margarines). In order to match the food industry's demands for different fats and oils research has been carried out to modify vegetable oils to produce novel products that improve functionality, absorption, metabolism and nutrition (Akoh 1995). Vegetable oils are generally rich in unsaturated fatty acids (UFA) at the *sn*-2 position of the TAG and saturated fatty acids (SFA) in the *sn*-1 and *sn*-3 (*sn*-1,3) positions of the TAG. It has been postulated that saturated fatty acids at the *sn*-2 position might affect the hepatic lipid metabolism differently than the same fatty acids at the *sn*-1 or *sn*-3 positions (Mu and Porsgaard 2005). Enzymatic interesterification of oils and hardfats has been studied over recent decades, using specific enzymes immobilized in batch and continuous processes and related to the reaction conditions (i.e. temperature, enzyme concentration, time, solvent addition, agitation velocity) in order to incorporate a desired fatty acid into the TAG structure, aiming to lower acyl migration at the *sn*-2 position and reduce the production of intermediate products such as diacylglycerols (DAG) and monoacylglycerols (MAG) (Ifeduba et al. 2016; Speranza et al. 2015; Pande et al. 2013). These fats can be applied in bakery products, shortening, margarine, and also used as crystallization additives (Zhao et al. 2014; Meng et al. 2013). The use of additives in continuous fat systems has been investigated by various researchers, as also the influence on the physical properties of the fats, reducing costs and improving final product quality (Basso et al. 2010; Smith et al., 2011; Oliveira et al. 2015). Polymorphism and molecular interactions are specific properties of fat crystallization. High similarity of the acyl chain characteristics of these additives with the fats, such as length and number of double bonds, allows for their integration into the crystal matrix and/or adhesion onto a crystal growth site (Sato 2001; Smith et al. 2011). Ribeiro et al. (2013a) investigated five hardfats as modifiers of the process of cocoa butter (CB) crystallization process. The best result was found with fully hydrogenated Crambe oil, with a stabilizing effect on CB polymorphism. Thus, the objective of this study was to evaluate the physicochemical properties of the SLs obtained from the enzymatic interesterification (EI) catalyzed by a *sn*-1,3 specific lipase in binary blends of HOSO and FHCO in different weight ratios (60:40, 50:50, 40:70, 30:70) using 7% (w/w) Lipozyme TL IM at 70 °C for 3 h. Chemical interesterification (CI) was carried out as a control. The following physicochemical properties of the SLs were analyzed: fatty acid methyl ester (FAME) and TAG compositions, solids fat contents (SFC), differential scanning calorimetry (DSC),

acyl migration, polarized light microscopy and X-ray diffraction. These SLs were projected to have broad applications in foods such as bakery/confectionery fats and as crystallization additives in lipids.

Materials and methods

Materials

The ready-to-eat deodorized high oleic sunflower oil (HOSO) was provided by the company Dow AgroSciences—distributed by the company Quantiq Ltda (São Paulo, SP Brazil). The fully hydrogenated Crambe oil (FHCO), ready for industrial use, was provided by the company Cargill Agrícola S/A (Itumbiara, GO, Brazil). The immobilized enzyme Lipozyme TL IM (*Thermomyces lanuginosus* lipase, *sn*-1, 3 specific, specific activity of 329 IUN/g; IUN = interesterification unit) was donated by the company Novozymes do Brasil Ltda (Araucária—Brazil), and the chemical catalyzer, 99% powdered anhydrous sodium methoxide was from Sigma-Aldrich (St. Louis, MO, USA).

Methods

Preparation of the blends (BE) and conditioning of the enzyme

Two samples containing 100 g of the HOSO and FHCO blends were prepared, melted in each of the following respective proportions: 60:40, 50:50, 40:60 and 30:70 (w/w). The blends were maintained at 80 °C for 30 min with a magnetic stirrer to destroy the crystalline memory, and then stored in glass vials under refrigeration (−5 °C). On a laboratory scale the *sn*-1, 3 specific lipase (Lipozyme TL IM) was conditioned to remove air and moisture. The Lipozyme TL IM had a moisture content of 5% (w/w), and the manufacturer (Novozymes) recommended reducing this content before carrying out the experiment in order to decrease the free fatty acids (FFA) and mono (MAG) and diacylglycerols (DAG) formed by hydrolysis of the fat. An aliquot of 100 g of the binary blend was placed in a 250 mL round bottomed flask and heated in a mineral oil bath at 70–75 °C. When the temperature of the mixture reached 70 °C, 7% (w/w) of the enzyme was added and the blend was stirred at 300 rpm using magnetic stirring under a nitrogen atmosphere for 30 min. The elimination of air was evidenced by the absence of bubbles in the granules. The stirring was turned off, the enzyme decanted and the top oil phase removed with a pipette. The conditioned enzyme was used immediately in the EI.

Enzymatic interesterification (EI)

One hundred gram of each blend produced in the proportions of 60:40, 50:50, 40:60 and 30:70 (w/w) were added to a round-bottomed flask containing 7% of conditioned enzyme, and heated in a mineral oil bath at 70–75 °C with magnetic shaking at 300 rpm under nitrogen atmosphere. The total reaction time was 3 h and the samples were collected for analysis in screw-topped test tubes, maintaining the shaking. Pure nitrogen was passed through the mixture, closing the tube and submitting it to 10 min heating in a water bath at 95 °C to inactivate the enzyme. The FFA content was determined as oleic acid (% in weight) according to AOCS method Ca 5a-40 (AOCS 2009). To remove the FFA (1.5–2.5%), the sample was filtered using a twin-walled separating funnel in a heated water bath to avoid solidification of the mixture. Five drops of a phenolphthalein solution were then added followed by a pre-determined amount of 0.2 M NaOH. The interesterified mixture was then washed with hot water (50–60 °C) until disappearance of the pink color and then filtered through filter paper containing anhydrous sodium sulfate to remove the moisture and biocatalyzer particles. The products were dried under vacuum at 110 °C for 30 min and then stored under refrigeration (–5 °C) in glass vials.

Chemical interesterification (CI)

The CI reactions were carried out with the same proportions of HOSO and FHSO used in EI. On a laboratory scale, 100 g of each blend were interesterified under magnetic shaking in the presence of 0.4% (w/w) sodium methoxide (99%) at a temperature of 100 °C. The reaction time was 20 min in vacuum, according to the optimization carried out by Grimaldi, et al. (2005). The reaction was then interrupted by adding a 5% citric acid solution and the interesterified samples washed with distilled water (80 °C). To remove the moisture the samples were filtered through filter paper containing anhydrous sodium sulfate, followed by drying under vacuum at 110 °C for 30 min. The samples were stored in glass vials refrigeration (–5 °C).

Fatty acid composition (FA)

The fatty acid methyl esters (FAME) were prepared according to Hartman and Lago (1973) separated using the AOCS Ce 1f-96 method. The chromatographic operating conditions were: Capillary Gas Chromatograph Agilent 6850 Series equipped with a DB-23 Agilent capillary column (50% cyanopropyl-methylpolysiloxane), dimensions: 60 m, 0.25 mm i.d. and 0.25 µm film. The analytical conditions were: oven temperature: 110–215 °C (5 °C/min), 215 °C—24 min; detector temperature: 280 °C;

injector temperature: 250 °C; carrier gas: helium; split ratio 1:50; Injection volume: 1.0 µL. The qualitative composition was determined by comparing the peak retention times with the peaks of the respective FA standards. The analyses were performed in triplicate.

Triacylglycerol composition (TAG)

An Agilent 7890 gas chromatograph equipped with a FID detector and split/splitless injector was used to separate the TAGs in the reaction product. The samples were dissolved in heptane at a concentration of 2 mg/mL and injected into a capillary DB-5-HT column (15 m × 250 µm × 0.1 µm). The oven temperature was initially programmed at 360 °C with heating to 390 °C at 5 °C/min and remaining at this temperature for 14 min. Hydrogen was used as the carrier gas at 40 cm/s and nitrogen as the make-up gas at 40 cm/s. The injector and detector temperatures were maintained at 360 °C and 390 °C, respectively. The injection volume was 1 µL with a 1:30 split ratio. The trisaturated (S_3), triunsaturated (U_3), disaturated–monounsaturated (S_2U) and monosaturated–diunsaturated (SU_2) TAGs were separated according to the number of carbon atoms of the fatty acids and identified by comparison with the retention times of standard tribehenin (99%) and soybean oil which has a known composition. The analyses were performed in triplicate.

Regiospecific distribution

Approximately 150 mg of each sample were liquefied by heating with hot air, and immediately dissolved in oxygen-free deuterated chloroform (500 µL, $CDCl_3$) containing tetramethylsilane (1%, TMS) as the chemical shift reference. The blends were maintained in an ultrasound bath at 60 °C until a homogenous solution was obtained and then transferred to 5 mm NMR tubes. Quantitative ^{13}C NMR spectra were acquired at 30 °C without twisting under the control of an ICON-NMR (Bruker Biospin, Rheinstetten, Germany) with a Bruker Avance-III (500 MHz) spectrometer operating at 125.69 MHz for ^{13}C , equipped with a 5-mm Broadband Observe (BBO) probe. The following acquisition parameters were used ^{13}C excitation pulse 90° for 10 µs, sweep-width of 238 ppm, repetition time of 25 s (acquisition time 10 s + relaxation delay 15 s), number of scans 128 and receiver gain adjusted automatically. The experimental time was 55 min. The unsaturated fatty acids (UFA) (170.88 ppm) and saturated fatty acids (SFA) (170.91 ppm) esterified at the *sn*-2 position on the TAG could be differentiated on the ^{13}C NMR spectrum, and also the UFA (171.29 ppm) and SFA (171.32 ppm) esterified at the *sn*-1,3 position. The assignment of the UFA and SFA was effected based on the inductive effect of the double

Table 1 Fatty acid compositions (%) of the raw materials, high oleic sunflower (HOSO), fully hydrogenated Crambe oil (FHCO), and the binary blends (HOSO:FHCO)

Fatty acid (%)	Raw materials (w/w%)		HOSO:FHCO (w/w%)			
	HOSO	FHCO	60:40	50:50	40:60	30:70
C16:0 (P-palmitic)	3.4 ± 0.2 ^d	3.2 ± 0.0 ^a	3.7 ± 0.0 ^{cd}	3.3 ± 0.0 ^{bc}	3.0 ± 0.0 ^b	2.6 ± 0.0 ^a
C18:0 (S-stearic)	3.0 ± 0.2 ^a	31.7 ± 0.3 ^f	14.6 ± 0.2 ^b	17.5 ± 0.2 ^c	20.2 ± 0.2 ^d	23.1 ± 0.2 ^e
C18:1 (O-oleic)	88.0 ± 0.2 ^e	–	51.8 ± 0.6 ^d	42.0 ± 0.4 ^c	34.6 ± 0.4 ^b	25.8 ± 0.0 ^a
C18:2 (L-linoleic)	5.6 ± 0.2 ^e	–	3.6 ± 0.0 ^d	3.2 ± 0.0 ^c	2.4 ± 0.0 ^b	1.7 ± 0.0 ^a
C20:0 (A-arachidic)	–	6.7 ± 0.1 ^e	2.6 ± 0.1 ^a	3.4 ± 0.0 ^b	3.6 ± 0.0 ^c	4.2 ± 0.0 ^d
C22:0 (B-behenic)	–	56.3 ± 0.5 ^e	22.7 ± 0.3 ^a	29.6 ± 0.5 ^b	35.0 ± 0.1 ^c	41.2 ± 0.2 ^d
C24:0 (Lg-lignoceric)	–	2.1 ± 0.0 ^d	1.0 ± 0.0 ^a	1.0 ± 0.0 ^a	1.2 ± 0.0 ^b	1.4 ± 0.0 ^c
Σ Saturated	6.4	100.0	44.6	54.8	63.0	72.5
Σ Unsaturated	93.6	–	55.4	45.2	37.0	27.5

Same letters for the same lines indicate that there are no significant differences between the means evaluated by Tukey's test ($p \leq 0.05$)

bond on the carbonyl group using the method described by Vlahov et al. (2010). The analyses were performed in triplicate.

Solid fat content (SFC)

The solid fat content was determined by nuclear magnetic resonance spectrometry using the Bruker Minispec pc 120 (Silberstreifen, Rheinstetten, Germany) together with Tcon 2000 high precision dry baths (0–70 °C) (Duratech, Carmel, IN, USA). The AOCS Cd 16b-93 method (AOCS 2009) was followed, this being a direct method with readings in series at temperatures of 10, 20, 25, 30, 35, 40, 45, 50, 55, 60 and 65 °C, with tempering for non-stabilized fats. The analyses were performed in triplicate.

Differential scanning calorimetry (DSC)

The crystallization profiles of the samples were determined using a model Q2000 differential scanning calorimeter (DSC) (TA Instruments) according to the AOCS Cj 1-94 method (AOCS 2009), weighing 8–12 mg of sample into aluminum recipients and sealing them. The instrument was calibrated with indium (MP = 156.5 °C, ΔH_f = 28.45 J/g). The samples were heated to 80 °C at 10 °C/min, maintained at this temperature for 10 min, then cooled to –60 °C at 10 °C/min, maintained at this temperature for 30 min, and finally heated to 80 °C at 5 °C/min. The analyses were carried out in duplicate.

Microscopy under polarized light

In order to study the morphology and the crystalline dimensions, the samples were melted in a microwave oven at 70 °C. With the aid of a capillary tube, a drop of sample was placed on a glass slide pre-heated at the controlled

temperature (70 °C) and covered with a coverslip. The slides were prepared in duplicate and maintained for 24 h in an incubator at 25 °C. The morphology of the crystals was evaluated using a polarized light microscope (Olympus BX 51) attached to a digital video camera (Media Cybernetics). The slides were then placed on the support of a heating plate maintained at the same crystallization temperature, and the images captured by the Image Pro-Plus applicative (Media Cybernetics) using polarized light and 40 × amplification. Three visual fields were focused on each slide, and the mean diameter of the crystals selected as the evaluation parameter for the quantitative analysis of the images, calculated using the Image Pro-Plus program, version 7.0 (Media Cybernetics, Bethesda, USA).

X-ray Diffraction

The polymorphic habits of the fats before and after inter-esterification (EI and CI) were determined by X-ray diffraction, according to the AOCS Cj 2–95 method (AOCS 2009). The samples were completely melted and crystallized at 25 °C in an incubator during 24 h. The measurements were made in a Philips PW 1710 diffractometer with the Bragg–Bretano (0:20) geometry, and Cu-K α radiation (λ = 1.5418 Å, voltage of 40 kV and current of 30 mA). The measurements were taken in 0.03° steps in 2 θ with an acquisition time of 2 s and scan from 14.5 to 27.5° (2 θ scale). The polymorphic forms were identified as from the short spacings, which are characteristic of the crystals.

Statistical analysis

The results of fatty acid and triacylglycerol compositions, regiospecific distribution and solid fat content were subjected to an analysis of variance (ANOVA) with

STATISTICA Software System Version 8 (StatSoft Inc., Tulsa, OK, USA). Tukey tests were applied for statistical comparisons of means, at a 5% significance level ($p \leq 0.05$).

Results and discussion

In this paper the effect of enzymatic interesterification (EI) on the physicochemical properties of HOSO:FHCO blends in different ratios was evaluated, with a view to understanding the structure of the fat and its functionality. Preliminary tests were carried out with respect to temperature with reaction times of 3, 6 and 9 h, evaluating the thermal behavior (DSC) and acyl migration. A temperature of 70 °C and 3 h of reaction time was defined. Chemical interesterification (CI) was carried out as the control.

Fatty acid composition

The fatty acid compositions of the raw materials and the physical blends in different ratios are shown in Table 1. The major FAs in the FHCO were C22:0 (56.6%), C18:0 (32.0%), C20:0 (6.4%), C16:0 (2.8%), and C24:0 (2.1%). The predominant FA in the HOSO was C18:1 (87.4%), followed by C18:2 (5.8%), C16:0 (3.7%) and C18:0 (3.2%). The higher FA contents were C18:0 (25.6–52.4%) and C22:0 (22.6–40.9%). The FHCO was rich in behenic acid (C22:0) and other studies have also shown high concentrations of behenic acid in FHCO (Ribeiro et al. 2013b; Guedes et al. 2014). The physical blends showed similar total FA compositions.

Triacylglycerol composition (TAG)

Table 2 shows the results obtained for the TAG compositions of the blends, before and after EI and CI. The predominant TAG in the HOSO was triolein (OOO, C₅₄) with 88.3%, followed by proposed TAGs (C₅₂) structure as POO/PSO with 11.4%. With respect to the FHCO, the major TAG was proposed TAGs (C₆₂) structure as SBB with 61.3%, followed by proposed TAGs structure as SAB (C₆₀) with 15.6%. After interesterification an increase in TAGs with carbon number C58 was observed (Fig. 1), with a predominance of proposed TAGs structure as OOB/SOB/SSB when compared to the blends, being higher after EI (32.0–37.5%). There was also a decrease in TAG with carbon number C62 proposed TAG structure as SBB, being greater after EI (7.0–18.2%). Guedes et al. (2014) obtained similar results for the TAG composition after CI. In addition the interesterification resulted in the formation of a new TAG species (BBB, C₆₆) not previously detected in the physical blends. Tynek and Ledóchowska (2005)

Table 2 TAG composition (%) of high oleic sunflower (HOSO) and fully hydrogenated Crambe oil (FHCO), blend (BE) and after chemical (CI) and enzymatic interesterification (EI)

CN	HOSO:FHCO (%w/w)											
	Raw materials			60:40			50:50			40:60		
	HOSO	FHCO		BE	CI	EI	BE	CI	EI	BE	CI	EI
50	0.6 ± 0.1 ^a	ND		ND	0.6 ± 0.1 ^a	ND	ND	ND	ND	ND	ND	ND
52	11.2 ± 0.2 ^f	1.3 ± 0.4 ^a		8.0 ± 0.2 ^e	6.6 ± 0.1 ^d	7.2 ± 0.3 ^d	6.7 ± 0.2 ^d	6.0 ± 0.0 ^c	5.7 ± 0.2 ^c	5.7 ± 0.1 ^c	5.8 ± 0.1 ^c	4.3 ± 0.3 ^b
54	88.2 ± 0.1 ⁱ	2.5 ± 0.3 ^a		53.6 ± 0.0 ^k	41.5 ± 0.8 ⁱ	41.8 ± 0.2 ⁱ	46.3 ± 0.5 ^j	34.8 ± 0.7 ^g	34.1 ± 0.5 ^g	37.2 ± 0.3 ^h	26.8 ± 0.3 ^e	24.8 ± 0.6 ^d
56	ND	8.4 ± 0.8 ^e		3.0 ± 0.3 ^a	8.3 ± 0.2 ^c	7.9 ± 0.1 ^c	4.0 ± 0.1 ^{ab}	8.6 ± 0.2 ^{ef}	8.5 ± 0.3 ^{ef}	4.7 ± 0.1 ^{bc}	6.1 ± 0.1 ^d	9.1 ± 0.1 ^{ef}
58	ND	7.5 ± 0.4 ^c		3.1 ± 0.5 ^a	31.6 ± 0.1 ^d	32.9 ± 0.0 ^d	3.3 ± 0.2 ^a	34.1 ± 0.4 ^e	35.4 ± 0.4 ^{ef}	4.4 ± 0.0 ^b	36.0 ± 0.5 ^f	38.0 ± 0.5 ^f
60	ND	15.9 ± 0.3 ^g		6.0 ± 0.1 ^c	3.4 ± 0.4 ^a	2.9 ± 0.6 ^a	7.4 ± 0.3 ^d	4.6 ± 0.2 ^b	4.6 ± 0.2 ^b	8.9 ± 0.6 ^c	6.9 ± 0.1 ^{cd}	6.2 ± 0.1 ^c
62	ND	61.0 ± 0.1 ⁱ		25.0 ± 0.3 ^g	7.5 ± 0.2 ^a	6.8 ± 0.2 ^a	30.5 ± 0.7 ^f	10.5 ± 0.1 ^b	10.4 ± 0.1 ^b	37.0 ± 0.3 ^e	16.3 ± 0.8 ^c	15.6 ± 0.8 ^c
64	ND	3.4 ± 0.1 ^g		1.3 ± 0.6 ^g	0.5 ± 0.1 ^a	0.5 ± 0.1 ^a	1.8 ± 0.0 ^{de}	0.7 ± 0.2 ^{ab}	0.6 ± 0.4 ^{ab}	2.1 ± 0.1 ^f	1.4 ± 0.2 ^{de}	1.1 ± 0.2 ^{cd}
66	ND	ND		ND	ND	ND	ND	0.7 ± 0.1 ^{ab}	0.7 ± 0.1 ^a	ND	0.7 ± 0.1 ^{ab}	0.9 ± 0.1 ^b

Same letters for the same lines indicate that there are no significant differences between the means evaluated by Tukey's test ($p \leq 0.05$)

CN carbon number, ND no detected

Fig. 1 CGC separation of the triacylglycerol profile of high oleic sunflower oil (HOSO) and fully hydrogenated *Crambe abyssinica* oil (FHCO) of enzymatic interesterification in proportion of 50:50

studied the interesterification of olive oil (as the source of oleic acid) with completely hydrogenated high erucic rapeseed oil (as the source of behenic acid), and the acidolysis of olive oil with behenic acid catalyzed by the enzyme *Lipozyme RM IM*. Interesterification resulted in the formation of new TAGs mainly proposed TAGs structure as BOO, BOP and BOB.

Regiospecific distribution

The regiospecific analysis of TAG by ^{13}C NMR showed the distribution and contents of SFA and UFA in the glycerol backbone. This analysis provided important information about how the stereospecific structure influences the lipid

metabolism and the bioavailability of the FA. The methodology provided good results with minimal sample preparation and a significantly shorter experimental time. The technique is well suited for studying the interesterification of vegetable fats and oils (Guedes et al. 2014; Speranza et al. 2015). Figure 2 shows the percentages of UFA and SFA presents at the *sn*-2 position of the glycerol before and after EI and CI. There was a decrease in UFA at position *sn*-2 in all blends, due to the proportion of HOSO. Acyl migration was lower after EI than after CI, so the results were higher (28.6–58.2%) as compared to the results after CI (25.5–56.2%). The SL obtained after EI proportion of at 40:60 showed more UFA at the *sn*-2 position of the TAG (37.3%) as compared to the others blends. Figure 3 shows the results for UFA and SFA presents at the *sn*-1,

Fig. 2 Distribution of the fatty acids (%) at the *sn*-2 position on the TAG of high oleic sunflower (HOSO) and fully hydrogenated Crambe oil (FHCO) blend (BE), after chemical (CI) and enzymatic interesterification (EI)

Same letters for the same columns indicate that there are no significant differences between the means evaluated by Tukey's test ($p \leq 0.05$)

Fig. 3 Distribution of the fatty acids (%) at the *sn*-1,3 positions on the TAG of of high oleic sunflower (HOSO) and fully hydrogenated Crambe oil (FHCO) blend (BE), after chemical (CI) and enzymatic interesterification (EI)

Same letters for the same columns indicate that there are no significant differences between the means evaluated by Tukey's test ($p \leq 0.05$)

3 position of the TAG, before and after EI and CI. The SFA content increased at the *sn*-1, 3 position in all the blends with increase in the proportion of FHCO. After EI the range for

SFA was 47.3–71.4% and after CI it was 45.9–74.1%. The majority of the FAs determined at the position *sn*-1, 3 were saturated, which is the typical pattern for vegetable oils.

Pacheco et al. (2015) studied the acyl migration phenomenon during the lipase-catalyzed interesterification of refined and fully hydrogenated soybean oil. Their research showed that even when using *sn*-1, 3 specific lipases, acyl migration occurred at the *sn*-2 position of the TAG. However, this type of biocatalysts is still preferred as compared to nonspecific ones or chemical catalysts, since they maintain the original profile to a greater extent.

Solid fat content (SFC)

Figure 4 shows the SFC profiles for all the blends before and after EI and CI. After EI and CI at 10 °C all the blends showed higher SFC as compared to the BE. However, at higher temperatures (20–70 °C) the values were lower. This decrease in SFC was due to the interesterification reactions, forming new TAGs from the starting material with both high and low melting points. The SLs obtained after EI and CI were melted completely in the range of 55–65 °C. At 35 °C, the SFC of the EI blends ranged from 10.7 to 46.4%, while the CI blends ranged from 19.6 to 58.2%. The plasticity of the EI blends was higher than that of the CI blends and BE. The formation of new TAGs with

intermediate melting points after interesterification presented a decline in the SFC between 25 and 45 °C, showing more linear melting profiles. Several previous studies reported a decrease in SFC after interesterification due to respective decreases in the trisaturated TAGs and corresponding increases in monounsaturated and diunsaturated TAGs (Lee et al. 2008; Zhao et al. 2014).

Differential scanning calorimetry (DSC)

Figure 5 shows the crystallization profiles of the BE, and of the HOSO:FHCO blends after EI and CI. The FHCO showed one intense crystallization peak at 56.6 °C from saturated TAGs and the HOSO showed one narrow crystallization peak at −36.2 °C for the unsaturated TAGs. The different physical blends (BE) showed crystallization profiles with two peaks in the ranges from −30.7 to −33.2 °C and from 50.4 to 54.8 °C. After interesterification (EI and CI) the crystallization profiles changed, and three peaks diminished, producing other types of intermediary TAGs and plastic fats. After interesterification the second peak, indicating increased concentrations of disaturateds-monounsaturateds and monosaturateds-diunsaturateds TAGs,

Fig. 4 Solid fat content (%) of high oleic sunflower (HOSO) and fully hydrogenated Crambe oil (FHCO) blend (BE), after chemical (CI) and enzymatic interesterification (EI)

Fig. 5 The crystallization profiles of high oleic sunflower (HOSO) and fully hydrogenated Crambe oil (FHCO) blend (BE), after chemical (CI) and enzymatic interesterification (EI). Proportions 60:40, 50:50, 40:60 and 30:70

which affected the intensity of the crystallization profile. The peaks were wider when compared with the individual components (HOSO and FHCO), and after interesterification (EI and CI), the blends (60:40, 50:50 and 40:60) showed three crystallization peaks. The first peak was from -29.5 to -33.2 °C, the second from 29.1 to 32.3 °C and the third from 40.2 to 50.6 °C. EI resulted in lower crystallization peaks than CI, the difference being $2\text{--}3$ °C. After the interesterification process (EI and CI), only the 30:70 (HOSO:FHCO) blend showed two crystallization peaks which were at 32.3 and 50.5 °C after EI and at 34.3 and 50.6 °C after CI. This type of behavior was also observed by Guedes et al. (2014).

Microscopy under polarized light

Polarized light microscopy is a technique used to visualize the microstructural network of fats. The macroscopic rheological properties of networks formed by lipids are essential for the consistency and acceptability of the final plastic fat

products (Narine and Marangoni 1999). Figure 6 shows the crystalline structures of all the BE, CI and EI samples for all the proportions of the HOSO:FHCO blends, formed by rapid cooling at 25 °C/24 h. The microstructure changed considerably after interesterification. The physical blends (BE) showed needle-like crystals with compact nuclei, the needles being long and, distributed radially. After EI and CI, the crystals changed to a more loosely packed needle-like shape. Thus, the results suggested that the amount of FHCO influenced the structure and network of the crystals in the interesterified fats, and that an increasing FHCO content induced the formation of long needle-shaped like crystals with compact nuclei. According to Marangoni and Narine (2002), the average diameter of the fats crystals in food products should be less than 30 μm to avoid a sensation of grittiness in the mouth. The mean crystal diameter of all the HOSO:FHCO blends was between 19.1 and 21.2 μm . After CI it was between 18.2 and 19.4 μm and after EI it was between 16.5 and 18.7 μm , thus EI promoted a decrease in the average crystal diameter as compared to BE.

Fig. 6 Microscopy of high oleic sunflower (HOSO) and fully hydrogenated Crambe oil (FHCO) blends (BE), after chemical (CI) and enzymatic interesterification (EI) at 25 °C/24 h with $\times 40$ amplification

Polymorphism

The type of polymorph(s) present in a fat decidedly affects the macroscopic properties of the crystal and will dictate the melting point of the network (Narine and Marangoni 1999). The polymorphs showed identical

chemical compositions but different crystalline structures and different physical properties. Solid fats present three fundamental polymorphs, designated as α (hexagonal), β' (orthorhombic) and β (triclinic). The thermal stability of these polymorphs follows the order $\beta > \beta' > \alpha$, and β has the highest melting point. The conversion from one to

Table 3 Polymorphic forms of the blends before (BE) and after interesterification (CI and EI), obtained at 25 °C for 24 h

Samples	Short spacings (Å)					Polymorphic form
	4.6	4.2	4.1	3.8	3.7	
FHCO			4.14 (s)			α
60:40 (HOSO:FHCO)						
BE	4.6 (vw)		4.13 (s)		3.75 (w)	$\beta' \gg \beta$
CI	4.6 (vw)	4.23 (m)		3.81 (w)		$\beta' > \beta$
EI	4.6 (w)		4.15 (m)			$\beta' > \beta$
30:70 (HOSO:FHCO)						
BE	4.6 (w)		4.17 (s)		3.78 (s)	$\beta' \gg \beta$
CI	4.6 (vw)		4.13 (s)	3.83 (w)		$\beta' \gg \beta$
EI	4.6 (w)		4.11 (s)			$\beta' \gg \beta$

“>” means a little be more, “>>” much more and “>>>” significantly more. Intensities: v, very; w, weak; m, medium; s, strong

HOSO high oleic sunflower oil, FHCO fully hydrogenated Crambe oil

another form generally occurs in the direction of the more stable forms (Timms 1984). The X-ray diffraction technique is used to identify polymorphism in crystals, by determining the short spacing for characterization. Table 3 shows the X-ray diffraction pattern with the short spacing and polymorphic forms of the 60:40/30:70 (HOSO:FHCO) physical blends (BE) and the fats interesterified (EI and CI) at 25 °C for 24 h. FHCO was crystallized in the α form, showing very strong short spacing at only 4.14 Å. Lopes et al. (2015) found the same polymorphic forms (α) for FHCO. The 30:70 physical blend (HOSO:FHCO) exhibited a mixture of the β' (strong intensity) and β (weak intensity) forms, whereas after interesterification the β' form apparently diminished. The intensity of the 3.83 Å short spacing decreased after CI and disappeared after EI. The 60:40 physical blend (HOSO:FHCO) presented a mixture of the β' (strong intensity but less than the 30:70BE) and β (very weak intensity) forms. After interesterification the β' form also appeared to be diminished. The intensity of the 4.23 Å and 3.81 Å short spacings decreased after CI and 3.81 Å disappeared after EI. The differences in the polymorphic states found after interesterification could be due to the amount of FHCO, indicating that the increase in the amount FHCO is proportional to the increase in the β' crystal form. Ahmadi et al. (2008) studied the EI and CI of the blends of fully hydrogenated canola oil with high oleic sunflower oil and obtained a predominance of the β' crystals form. The small crystals of β' polymorphic are appropriated to the plasticity characteristics desirable in products such as margarines, shortenings and fats for bakery and confectionary products. Conversely, the β polymorphic tends to produce broad granular crystals, resulting in grainy products with low aeration potential, and can be applied as a fat crystallization additive (Sato 2001).

Conclusion

Both the CI and EI processes promoted modifications in the final products when compared to the original blend, such as lower melting points, a more linear profile of the SFC and different thermal behavior. The enzymatic process applied for 3 h of reaction time showed the advantage of selectivity for the production of new TAGs and less acyl migration, as evidenced by ^{13}C RMR. These SLs were projected to have broad applications in foods as bakery/confectionery fats and as crystallization additives for lipids.

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