J Am Oil Chem Soc (2011) 88:1925–1933
DOI 10.1007/s11746-011-1869-x

ORIGINAL PAPER

Interesterification of Lard and Soybean Oil Blends Catalyzed by Immobilized Lipase in a Continuous Packed Bed Reactor

Roberta Claro da Silva · Fabiana Andreia Schaffer De Martini Soares · Thaís Gonzaga Fernandes · Anna Laura Donadi Castells · Kelly Caroline Guimarães da Silva · Maria Inês Almeida Gonçalves · Chiu Chih Ming · Lireny Aparecida Guaraldo Gonçalves · Luiz Antonio Gioielli

Received: 5 January 2011 / Revised: 1 April 2011 / Accepted: 26 May 2011 / Published online: 14 June 2011

Abstract Structured lipids (SL), formulated by blends of lard and soybean oil in different ratios, were subjected to continuous enzymatic interesterification catalyzed by an immobilized lipase from *Thermomyces lanuginosus* (Lipozyme TL IM) in a continuous packed bed reactor. The original and interesterified blends were examined for fatty acid and triacylglycerol composition, regiospecific distribution, and solid fat content. Blends of lard and soybean oil in the proportions 80:20 and 70:30 (w/w), respectively, demonstrated a fatty acid composition, and proportions of polyunsaturated/saturated fatty acids (PUFA/SFA) and monounsaturated/polyunsaturated fatty acids (MUFA/PUFA), that are appropriate for the formulation of pediatric products. These same blends were suited for this purpose after interesterification because their *sn*-2 positions were occupied by saturated fatty acids (52.5 and 45.4%, respectively), while unsaturated fatty acids predominantly occupied *sn*-1,3 positions, akin to human milk fat. Interesterification caused rearrangement of triacylglycerol species.

R. C. da Silva · F. A. S. D. M. Soares · T. G. Fernandes · A. L. D. Castells · K. C. G. da Silva · L. A. Gioielli (✉)
Department of Biochemical and Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, University of São Paulo, Av. Prof. Lineu Prestes n. 580, B16, São Paulo, SP CEP 05508-900, Brazil
e-mail: lagio@usp.br

M. I. A. Gonçalves
Department of Pharmacy, Faculty of Pharmaceutical Sciences, University of São Paulo, Av. Prof. Lineu Prestes n. 580, B13, São Paulo, SP CEP 05508-900, Brazil

C. C. Ming · L. A. G. Gonçalves
Department of Food Technology, Faculty of Food Engineering, University of Campinas, Caixa Postal 6091, Campinas, SP CEP 13083-970, Brazil

Keywords Human milk fat substitute · Lard · Soybean oil · ^{13}C-NMR analyses · Triacylglycerol composition

Introduction

The interesterification of lipids catalyzed by lipases represents an alternative to chemical interesterification. Eco-friendly processes for modifying fats and oils by utilizing lipases of different microbial origins have been reported by various researchers [1–3]. Lipase-catalyzed interesterification reactions can be performed in a batch-type reactor or in a continuous packed bed reactor [4]. The packed bed reactor is one of the most commonly employed apparatus for solid–fluid contacting in heterogeneous catalysis because it: (1) facilitates contact and subsequent separation; (2) and the continuous removal of inhibitory substances; (3) allows reuse of the enzyme without the need for prior separation; (4) permits the handling of poorly-soluble substrates by using large volumes containing low concentrations of substrate; (5) leads to more consistent product quality and improved enzyme stability due to ease of automation and control [5]; (6) is suitable for long-term and industrial-scale production, in contrast to stirred-tank reactors where enzyme granules are susceptible to breaking down because of mechanical shear stress; and (7) is more cost effective than batch operations [6–8].

Lipids are the major source of energy in human milk or infant formulas [9]. Hence, modification of fats and oils for infant formulas in order to obtain not only the correct fatty acid (FA) composition but also the same positional distribution as in human milk fat (HMF) via interesterification is a focus of investigation. The major saturated fatty acid (SFA) is palmitic acid (16:0), which represents about a

fourth of the fatty acids present in breast milk and is esterified mainly at the *sn*-2 position of triacylglycerols (TAG). Stearic, oleic and linoleic acids are generally esterified at the *sn*-1,3 positions of TAG [10, 11]. This feature lends HMF a unique structure, and lard is the only animal fat that has a similar structure [12]. The palmitic acid residue at the *sn*-2 position is not hydrolyzed by pancreatic lipase and, as 2-monopalmitin, forms a mixed micelle with bile salt which is efficiently absorbed [13]. The palmitic acid residues esterified at the *sn*-1,3 positions are hydrolyzed by pancreatic lipase, producing free palmitic acid residues which form poorly absorbed calcium soaps in the intestinal tract, resulting in reduced absorption of both calcium and fat [14]. Formation of such calcium soaps can lead to stool hardness, constipation and in some cases, bowel obstruction. Thus, the presence of palmitic acid acyl residues at the *sn*-2 position of HMF increases absorption of 16:0 in infants and reduces calcium losses in the feces [15].

The main aim of the present study was to produce SL via EIE of lard and soybean oil blends catalyzed by an immobilized commercial *sn*-1,3-specific lipase from *Thermomyces lanuginosus* in a continuous packed bed reactor, and to characterize the chemical properties of the structured lipids thus produced. Lard and soybean oil were chosen due to their desirable nutritional qualities for the development of a fat substitute for human milk fat, and also because they are low cost bio-resources produced on a large scale in Brazil.

Materials and Methods

Materials

Lard and soybean oil were obtained from local commerce (São Paulo, Brazil). Commercial immobilized lipase from *Thermomyces lanuginosus* (Lipozyme TL IM) was kindly supplied by Novozymes Latin America Ltd. (Araucária, Brazil). The enzyme activity of the lipase was 250 IUN/g. All other reagents and solvents were analytical or chromatographic grade.

Betapol was kindly provided by Loders Croklaan, Lipid Nutrition (Wormerveer, Netherlands).

The human milk fat was obtained from samples of human milk donated by the Human Milk Bank of the University of São Paulo.

Methods

Reactant Blend Preparation

Lard and soybean oil were blended at 80:20, 70:30, 50:50, 60:40, 40:60, 30:70 and 20:80 (w/w) proportions, respectively. The blends were prepared after complete melting of the fats at 70–80 °C for 30 min under magnetic stirring. The blends thus obtained were stored at −18 °C until analysis.

Performance of Interesterification Reactions

The EIE was carried out in a continuous tubular glass bioreactor (height 34 cm, internal diameter 2 cm) equipped with an external jacket to maintain constant temperature and fixed bed to support the enzyme (70 g), equipped with a peristaltic pump (VC 360II Ismatec, Switzerland). Soybean oil was initially introduced into the reactor at a flow rate of 1 mL/min to remove air and water from the enzyme until the free fatty acids of the interesterified oil presented a stable value. The residence time was experimentally determined and was defined as the time needed to fill the empty spaces of the reactor packed with immobilized enzyme, using a flow rate of 1 mL/min. The jacketed column was heated by water bath (RE 112, Lauda, Germany) to maintain the bed enzyme at 60 °C (according to the manufacturer's recommendations). After conditioning of the enzyme, the blends were pumped into the reactor at the flow rate of 1 mL/min (residence time of 60 min). Each different interesterified sample was collected (200 mL) after discarding the first 200 mL to avoid cross-contamination with the previous blend, and stored at −18 °C until analysis.

Determination of Fatty Acid Composition

Fatty acids in the triacylglycerols of the interesterified blends were converted into fatty acid methyl esters (FAME). Rapid preparation of methyl esters for gas chromatographic analysis was accomplished by saponifying fats with 0.5 mol equiv./L methanolic potassium hydroxide, followed by refluxing with a solution of ammonium chloride and sulphuric acid in methanol. Rigorous conditions during the saponification and conversion of soaps into methyl esters, and the precipitation of alkali sulfates during the reaction, were avoided, and the degree of esterification was approximately 99.5 g/100 g [16, 17]. Analyses of FAMEs were carried out on a Varian GC gas chromatograph (model 430 GC, Varian Chromatograph Systems, Walnut Creek, CA, USA), equipped with a CP 8412 auto injector. The Galaxie software was used for quantification and identification of peaks. Injections were performed on a 100-m fused silica capillary column (ID = 0.25 mm) coated with 0.2 μm of polyethylene glycol (SP-2560, Supelco, USA) using helium as the carrier gas at an isobaric pressure of 37 psi; linear velocity of 20 cm/s; with make-up gas: helium at 29 mL/min at a split ratio of 1:50; volume injected: 1.0 μL. The injector temperature was set at 250 °C and the detector temperature was set at 280 °C. The

oven temperature was initially held at 140 °C for 5 min, programmed to increase to 240 °C at a rate of 4 °C/min, and then held isothermally for 30 min. Qualitative FA composition of the samples was determined by comparing the retention times of the peaks produced after injecting the methylated samples with those of the respective standards of fatty acids. The quantitative composition was obtained by area normalization and expressed as mass percentage, according to the AOCS Official Method Ce 1-62 [18]. All samples were analyzed in triplicate and the reported values are the average of the three runs.

Triacylglycerol Composition

Analyses of triacylglycerols were carried out on a gas chromatograph (model CGC, Agilent 6850 Series CG System, Santa Clara, USA). A capillary column (50% phenylmethylpolysiloxane, 15 m length × 0.25 mm internal diameter and 0.15 μm film) DB-17HT from Agilent (Santa Clara, CA, USA) was used. Conditions were as follows: split injection, ratio of 1:100; column temperature: 250 °C, programmed up to 350 °C at 5 °C/min; carrier gas: helium, flow rate of 1.0 mL/min; injector temperature: 360 °C; detector temperature: 375 °C; volume injected: 1.0 μL; sample concentration: 20 mg/mL of tetrahydrofuran. Identification of triacylglycerol groups was performed by comparing retention times, according to Antoniosi Filho, Mendes and Lanças [19].

^{13}C-NMR Analyses

A proton-decoupled ^{13}C NMR was used to analyze the positional distribution of fatty acids on the triacylglycerol backbone [20–22]. Lipid samples (250 mg) were dissolved in 0.5 mL of deuterated chloroform ($CDCL_3$) in 5-mm NMR tubes, and NMR spectra were recorded on a Bruker Advance DPX spectrometer operating at 300 MHz. The ^{13}C spectra of the lipid samples were acquired with a spectral width of 2,332.090 Hz, pulse of 10.2 μs, and a relaxation delay of 30 s. Determination of ^{13}C was performed at a frequency of 75.8 MHz with a 5 mm multinuclear probe operating at 30 °C, using the method described by Vlahov [20]. The results showed the compositions of saturated fatty acids, oleic acid and linoleic + linolenic acids in *sn*-2 and *sn*-1,3 positions.

Results and Discussion

Fatty Acids Composition

As expected, EIE does not affect the degree of saturation nor cause isomerization [23]. Our results confirmed that

Table 1 Fatty acid composition (g/100 g) of lard, soybean oil and blends, before and after continuous enzymatic interesterification

	Myristic (14:0)	Palmitic (16:0)	Palmitoleic (16:1)	Stearic (18:0)	Oleic (18:1)	Elaidic (18:1t)	Linoleic (18:2)	Linolenic (18:3)	Eicosenoic (20:1)	Eicosadienoic (20:2)	SFA	MUFA	PUFA
Lard (before)	1.5 ± 0.2	24.9 ± 1.1	2.2 ± 0.11	12.2 ± 0.2	39.0 ± 0.7	2.5 ± 0.0	15.7 ± 0.2	0.6 ± 0.0	0.7 ± 0.1	0.6 ± 0.1	38.7	44.5	16.9
Lard (after)	2.0 ± 0.7	26.6 ± 3.8	2.7 ± 0.7	11.1 ± 1.2	37.5 ± 2.7	2.4 ± 0.3	15.9 ± 0.7	0.6 ± 0.0	0.6 ± 0.2	0.5 ± 0.2	39.7	43.3	17.0
80:20 (before)	1.1 ± 0.0	21.7 ± 0.0	1.7 ± 0.0	10.6 ± 0.1	36.2 ± 0.0	2.3 ± 0.0	23.5 ± 0.1	1.7 ± 0.0	0.6 ± 0.0	0.5 ± 0.0	33.4	40.8	25.8
80:20 (after)	1.2 ± 0.1	21.9 ± 0.9	1.8 ± 0.1	10.3 ± 0.2	35.8 ± 0.5	2.3 ± 0.0	23.8 ± 0.3	1.7 ± 0.0	0.6 ± 0.0	0.5 ± 0.0	33.5	40.5	26.0
70:30 (before)	1.3 ± 0.2	22.4 ± 1.6	1.7 ± 0.2	9.2 ± 0.4	33.3 ± 0.8	2.1 ± 0.0	26.9 ± 0.5	2.2 ± 0.0	0.5 ± 0.1	0.4 ± 0.0	32.9	37.7	28.5
70:30 (after)	1.2 ± 0.0	21.4 ± 0.2	1.6 ± 0.0	9.4 ± 0.1	33.7 ± 0.1	2.2 ± 0.0	27.2 ± 0.1	2.3 ± 0.0	0.6 ± 0.0	0.4 ± 0.0	31.9	38.1	29.9
40:60 (before)	0.5 ± 0.0	15.4 ± 0.3	0.7 ± 0.0	6.3 ± 0.2	28.2 ± 0.5	1.8 ± 0.0	42.5 ± 0.6	4.5 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	22.1	30.7	47.0
40:60 (after)	0.6 ± 0.0	16.3 ± 0.0	0.9 ± 0.0	6.6 ± 0.0	28.8 ± 0.0	1.9 ± 0.0	40.1 ± 0.1	4.2 ± 0.0	0.5 ± 0.1	0.0 ± 0.0	23.5	32.1	44.6
30:70 (before)	0.5 ± 0.0	15.1 ± 0.0	0.7 ± 0.0	6.1 ± 0.0	27.9 ± 0.0	1.8 ± 0.0	43.0 ± 0.1	4.6 ± 0.0	0.4 ± 0.1	0.0 ± 0.0	21.6	30.7	47.6
30:70 (after)	0.4 ± 0.0	15.0 ± 0.0	0.7 ± 0.0	6.0 ± 0.01	27.7 ± 0.0	1.7 ± 0.01	43.4 ± 0.0	4.7 ± 0.0	0.4 ± 0.0	0.0 ± 0.0	21.4	30.6	48.0
20:80 (before)	0.4 ± 0.0	14.5 ± 0.5	0.5 ± 0.0	3.1 ± 0.0	25.9 ± 0.2	1.7 ± 0.0	46.3 ± 0.5	5.1 ± 0.1	0.3 ± 0.0	0.0 ± 0.0	19.9	28.4	51.4
20:80 (after)	0.4 ± 0.0	14.2 ± 0.3	0.5 ± 0.0	4.9 ± 0.3	25.5 ± 1.0	1.6 ± 0.1	47.7 ± 1.2	5.1 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	19.6	27.6	52.8
Soybean oil (before)	0.0 ± 0.0	11.4 ± 0.0	0.0 ± 0.0	3.4 ± 0.1	22.8 ± 0.1	1.6 ± 0.1	54.4 ± 0.0	6.3 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	14.9	24.5	60.7
Soybean oil (after)	0.0 ± 0.0	11.6 ± 0.5	0.0 ± 0.0	3.4 ± 0.0	22.9 ± 0.1	1.5 ± 0.0	54.2 ± 0.3	6.3 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	15.1	24.4	60.5

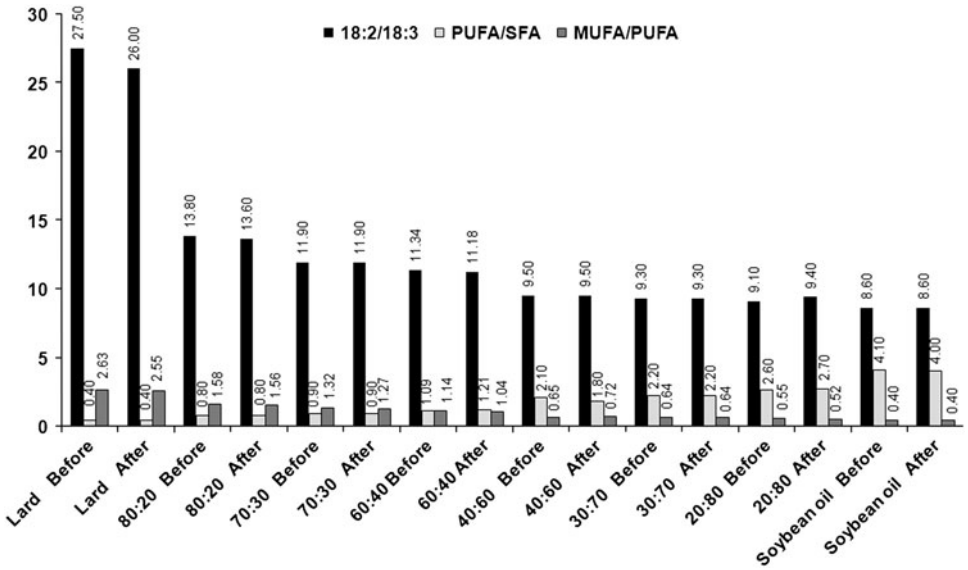

Fig. 1 Ratios of 18:2/18:3, PUFA/SFA and MUFA/PUFA of lard, soybean oil and binary blends, before and after continuous enzymatic interesterification

interesterification did not cause a significant alteration in the fatty acid profile of the initial blends (Table 1). The content of linoleic acid in human milk is dependent on diet and varies according to the feeding habits and geographic region of the population. An adequate intake of essential fatty acids (linoleic and linolenic acids) is crucial for newborn babies. Hence, infant formulas must have a ratio of linoleic/linolenic acids between 5 and 15. In human milk in general, this ratio is ca. 14.4 [10] whereas in human milk from Brazilian mothers, this proportion was found to be 15.2 [11]. In the present study, the ratios of all blends of lard and soybean oil were in the 5–15 range (Fig. 1). Both Food and Agriculture Organization/World Health Organization (FAO/WHO) and the European Union Committee recommend a minimum polyunsaturated/saturated fatty acids ratio (PUFA/SFA) of 1.0 in infant formulas. The PUFA/SFA ratio of lard and soybean oil blends ranged from 0.8 to 2.7. Furthermore, the literature reports values in human milk ranging from 0.23 [10] to 0.49 [11], levels that fall below those recommended. The commercial structured lipid Betapol has a proportion of 0.90 [10]. According to Jensen [9], the amount of oleic acid in breast milk is important to reduce the melting point of the triacylglycerols, thus providing the liquidity required for the formation, transport and metabolism of fat globules in breast milk. Human milk fat provides an MUFA/PUFA ratio of 1.62 [11], while the 80:20 blend had a similar proportion (Fig. 1).

Triacylglycerol Composition

In this study, the most abundant TAG in lard were: POO (29.6 g/100 g), POL (15.1 g/100 g), PStO (14.2 g/100 g), PPO (5.6 g/100 g), OOL (5.6 g/100 g) and PPL (5.5 g/100 g). These results for TAG composition of lard are similar to those previously described in the literature [11].

The most abundant TAG in soybean oil were: LLL (21.0 g/100 g), OLL (18.3 g/100 g), PLL (14.0 g/100 g), OOL (12.9 g/100 g), POL (9.5 g/100 g) and LLLn (6.5 g/100 g). Ribeiro et al. [24] described a similar composition of triacylglycerols in soybean oil. The TAG composition of the binary fat blends represents a linear combination of the fats constituting the blends (Table 2).

The triacylglycerols compositions of the samples, before and after interesterification, were divided into classes based on total number of carbons (excluding the carbons of glycerol) and saturation and unsaturation [23, 25]. Based on the number of carbons of triacylglycerols in lard and 80:20, 70:30 and 60:40 blends before interesterification, there was a predominance of C52, while soybean oil and 40:60, 30:70 and 20:80 blends showed a predominance of C54. Continuous EIE promoted an increase in the C48 group for lard and 80:20, 70:30 and 60:40 blends, in the C50 group (except for 40:60 blend) and in the C54 group (except for 40:60 blend). On the other hand, this caused a reduction in the C52 group across all samples, except for the 40:60 blend (Table 2).

For food product formulation, the physical properties of a fat are more easily interpreted when triacylglycerols are designated by their degree of saturation and unsaturation: SSS (trisaturated), SSU (disaturated–monounsaturated), UUS (monosaturated–diunsaturated) and UUU (triunsaturated), instead of by the individual triacylglycerol species [24]. The SSS, SSU, SUU and UUU contents of TAG before and after interesterification are shown in Fig. 2.

The triacylglycerol composition of lard was dominated by the SUU and SSU groups, while soybean oil and the 80:20, 70:30, 60:40, 40:60, 30:70 and 20:80 blends showed

Table 2 Triacylglycerol composition of lard, soybean oil and binary blends before and after enzymatic interesterification

TAG	Lard Before	Lard After	80-20 Before	80-20 After	70-30 Before	70-30 After	60-40 Before	60-40 After	40-60 Before	40-60 After	30-70 Before	30-70 After	20-80 Before	20-80 After	Soybean oil Before	Soybean oil After
PPP	0.2	1.0	0.2	0.9	0.4	0.9	0.2	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
MPO	0.8	1.5	0.6	1.1	0.8	1.0	0.5	0.7	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0.0
PPSt	1.2	1.8	1.0	1.3	0.7	1.2	0.4	0.8	0.1	0.3	0.1	0.5	0.0	0.0	0.0	0.0
PPO	5.6	7.8	4.8	6.4	4.5	5.8	3.7	4.6	1.9	2.1	1.9	2.8	1.3	0.3	1.1	1.3
PPL	5.5	5.2	4.6	5.2	4.0	4.7	3.7	4.5	2.7	3.1	2.9	4.2	2.5	6.2	2.7	2.8
MOL	1.7	1.2	1.2	1.2	0.9	1.2	0.7	0.8	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0
PStSt	1.5	1.1	0.8	0.5	0.8	0.5	0.1	0.5	0.3	0.9	0.0	0.0	0.0	0.0	0.0	0.0
PStO	14.2	10.8	10.9	7.1	9.7	5.5	8.2	4.9	4.8	4.6	4.0	1.6	3.2	1.1	0.5	0.3
POO	29.6	23.4	24.3	18.3	23.1	15.6	19.7	13.1	12.9	12.2	11.6	7.1	9.2	5.7	4.4	3.2
POL	15.1	14.1	14.7	15.0	13.1	15.5	12.8	15.2	12.0	11.6	11.4	13.6	11.4	12.5	9.5	9.7
PLL	3.8	2.6	5.1	5.2	6.3	6.7	7.2	8.5	11.0	10.9	10.7	11.6	12.3	12.0	14.0	12.2
PLLn	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.3	0.0	0.0	3.0	2.7
StStO	1.0	1.7	1.0	1.2	0.7	0.7	0.8	0.9	0.3	2.3	0.0	0.0	0.0	0.0	0.0	0.0
StOO	3.9	5.6	3.3	4.0	2.4	3.0	2.7	2.8	1.6	0.5	1.6	1.3	1.2	1.3	0.7	0.5
OOO	3.6	5.6	4.6	5.3	4.5	5.4	4.3	4.9	3.8	5.2	3.5	2.2	3.2	2.3	2.8	1.5
StOL	4.5	5.6	3.7	6.4	3.4	5.8	4.5	5.5	3.5	3.3	4.0	5.4	3.4	4.2	2.7	2.4
OOL	5.6	8.2	7.7	11.4	8.4	12.6	9.0	13.6	7.6	10.8	11.1	14.9	11.8	14.4	12.9	12.2
OLL	2.4	2.7	6.1	7.4	7.8	9.1	9.5	11.1	17.9	13.4	15.0	18.6	16.8	21.1	18.3	23.3
LLL	0.0	0.0	4.6	2.3	6.7	3.8	9.4	5.5	15.3	14.4	16.3	12.5	18.7	14.8	21.0	21.5
LLln	0.0	0.0	0.0	0.0	0.0	0.0	2.7	0.6	4.3	4.4	5.2	2.9	5.1	4.1	6.5	6.5
LnLnL	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CN																
48	1.1	2.5	0.8	2.0	1.2	1.9	0.7	1.6	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0.0
50	14.0	16.0	11.6	14.1	10.2	12.9	8.5	10.8	5.9	5.5	6.2	7.5	5.0	6.6	3.8	4.1
52	64.1	52.0	55.7	46.0	53.1	44.2	48.0	42.4	41.9	42.4	40.0	34.2	37.8	31.3	31.3	28.1
54	20.9	29.5	31.9	37.9	35.6	41.0	42.8	45.2	52.1	52.1	53.8	57.7	57.2	62.1	64.9	67.8

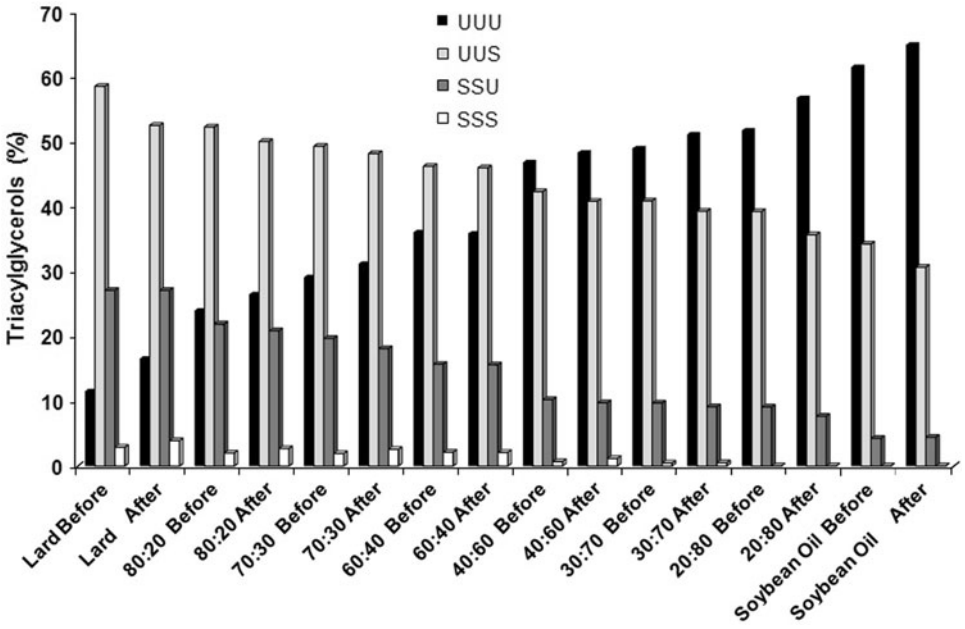

Fig. 2 Distribution of triacylglycerols in lard, soybean oil and binary blends, before and after continuous enzymatic interesterification (*UUU* triunsaturated, *UUS*/*USU* diunsaturated–monosaturated, *SSU*/*SUS* disaturated–monounsaturated, *SSS* trisaturated)

a predominance of UUU and SUU groups. Continuous EIE increased the amount of UUU triacylglycerols (all samples) and SSS (with the exception of soybean oil plus 30:70 and 20:80 blends), while the levels of UUS (all samples) and SSU (with the exception of lard and soybean oil) decreased, indicating that there were exchanges of fatty acids between triacylglycerols. The increase in SSS triacylglycerols of blends of lard and soybean oil after continuous EIE can be explained by the presence of palmitic acid at the *sn-2* position in lard and the presence of saturated fatty acids in *sn*-1,3 positions in soybean oil. Soybean oil (before and after EIE) showed more than 60 g/100 g of triacylglycerols of the UUU group and therefore did not present solid fat content even at refrigeration temperature (5 °C), because the melting point of this group is in the range −13 to 1 °C, remaining liquid at 5 °C.

^{13}C-NMR Analyses

Analysis of the regiospecific distribution of fatty acids in triacylglycerols by NMR is desirable, as the method does not require hydrolysis by pancreatic lipase, with further separation of partial acylglycerols performed by thin layer chromatography and finally, analysis of fatty acids by gas chromatography [26]. However, the technique cannot discriminate between saturated fatty acids, and cannot discriminate between linoleic and linolenic acids, which are assessed together. The NMR analysis technique also allows the determination of fatty acid composition, albeit with the limitations described above for the distinction of saturated fatty acids and linoleic acid/linolenic acid. The results for the fatty acid composition (saturated, 18:1 *cis* + 18:1 *trans* and 18:2 + 18:3) by NMR were similar to those obtained by gas chromatography for all samples of this study. Another point that shows the feasibility of the NMR technique is that the signal of the spectrum corresponding to the *sn*-2 position is always equivalent to around 33.3 g/100 g, while the signal corresponding to *sn*-1,3 positions is always equivalent to around 66.6 g/100 g.

In this study, HMF showed 78.3 g/100 g saturated fatty acids, 12.8 g/100 g of oleic acid and 9.0 g/100 g linoleic acid + linolenic acid in the *sn*-2 position of triacylglycerols (Fig. 3), while values for *sn*-1,3 positions were 46.0 g/100 g saturated fatty acids, 33.1 g/100 g of oleic acid and 20.8 g/100 g linoleic acid + linolenic acid (Fig. 4).

The structured lipid called Betapol showed a different distribution of fatty acids at the *sn*-2 position in relation to HMF, containing 51.4 g/100 g of saturated fatty acids, 28.4 g/100 g of oleic acid and 20.3 g/100 g of linoleic acid + linolenic acid. This lipid contained 50.4 g/100 g of saturated fatty acids, 37.0 g/100 g of oleic acid and 12.6 g/100 g of linoleic acid + linolenic acid at *sn*-1,3 positions.

Lard has a similar regiospecific distribution to that of human milk fat in the *sn*-2 position, with 73.0 g/100 g of saturated fatty acids, 18.4 g/100 g of oleic acid and 8.6 g/100 g linoleic acid + linolenic acid (Fig. 3). However, the distribution at *sn*-1,3 positions differs to that of HMF, with 24.4 g/100 g of saturated fatty acids, 54.1 g/100 g of oleic acid and 21.5 g/100 g linoleic acid + linolenic acid.

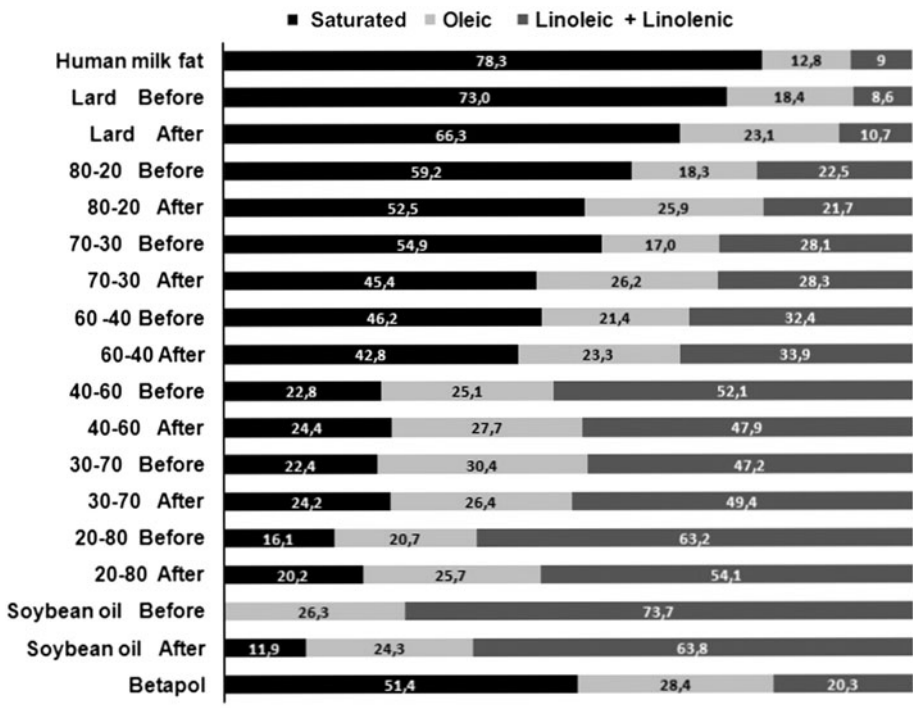

Fig. 3 Distribution of fatty acids in the *sn-2* position of triacylglycerols in human milk fat, Betapol, lard, soybean oil and their blends, before and after continuous enzymatic interesterification

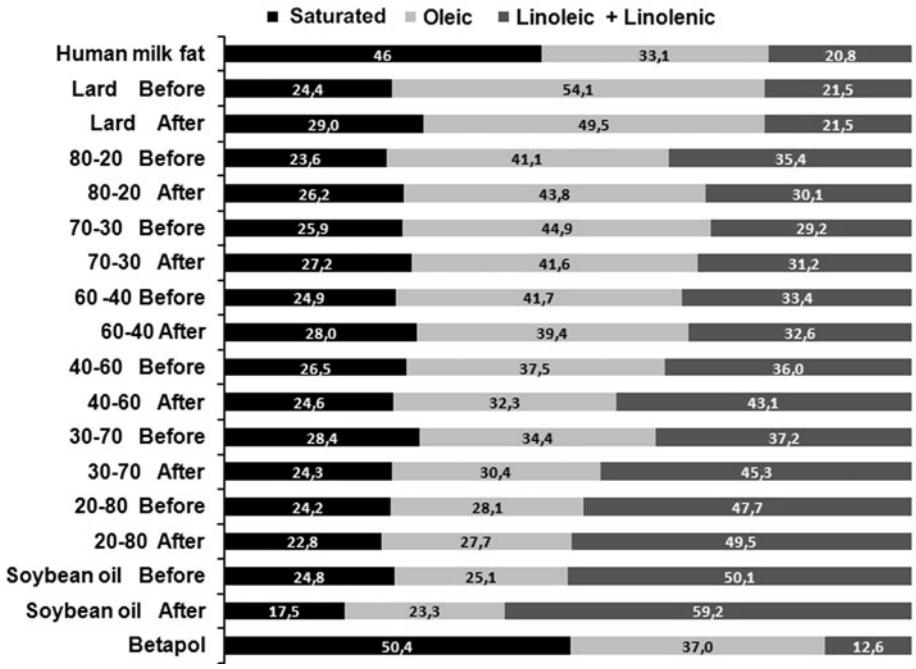

Fig. 4 Distribution of fatty acids in the *sn*-1,3 positions of triacylglycerols in human milk fat, Betapol, lard, soybean oil and their blends, before and after continuous enzymatic interesterification

Therefore, the main differences in these positions involve saturated fatty acids and oleic acid (Fig. 4). A low intensity signal in the *sn*-1,3 positions corresponding to palmitoleic acid (16:1) was also observed, but was not quantified.

Soybean oil is rich in polyunsaturated fatty acids and contains 23.6 g/100 g of oleic acid and 73.7 g/100 g of linoleic acid + linolenic acid at the *sn*-2 position. Therefore, soybean oil is practically free of saturated fatty acids in this position, a typical feature of vegetable oils. However, the literature describes about 3–4 g/100 g of saturated fatty acids, mainly palmitic acid, in the *sn*-2 position when determined by other methods such as enzymatic hydrolysis [26]. In *sn*-1,3 positions, soybean oil showed 24.8 g/100 g of saturated fatty acids, 25.1 g/100 g of oleic acid and 50.1 g/100 g of linoleic acid + linolenic acid, similar values to those found in the literature [26].

According to Quinlan, Lockton, Irwin and Lucas [27], unsaturated fatty acids initially located at the *sn*-2 position should largely remain in this position after using *sn*-1,3 specific lipase, although some degree of acyl migration to the *sn*-1,3 positions can occur [28]. The blends of lard and soybean oil showed lower values of saturated fatty acids at the *sn*-2 position compared to HMF (Fig. 3). However, these values were higher than those found in most infant formulas [29].

In lard and soybean oil blends, the *sn*-1,3 positions are predominantly occupied by unsaturated fatty acids, mainly oleic acid, when there was a predominance of lard, and linoleic + linolenic acids, when there was a predominance of soybean oil. Diets rich in unsaturated fatty acids are beneficial for infant nutrition, because these fatty acids are more easily absorbed than saturated fatty acids in *sn*-1,3 positions [30].

Lard and the 80:20, 70:30 and 60:40 blends showed a decrease in saturated fatty acids at the *sn*-2 position after EIE, while 40:60, 30:70 and 20:80 blends had increased levels of saturated fatty acids. However, the opposite occurred for saturated fatty acids in *sn*-1,3 positions, as the EIE promoted an increase in lard and 80:20, 70:30 and 60:40 blends, and led to a decrease in the saturated fatty acids in 40:60, 30:70 and 20:80 blends.

From a nutritional standpoint, blends containing predominantly lard (80:20 and 70:30) after EIE, proved the most interesting distributions as a HMF substitute, since their *sn*-2 positions are occupied mainly by saturated fatty acids (52.5 g/100 g and 45.4 g/100 g, respectively), while the unsaturated fatty acids predominantly occupy the *sn*-1,3 positions.

Conclusions

The development process presented is an eco-friendly approach for the use of relatively low cost bioresources such as lard and soybean oil, exploiting their intrinsic nutritional and physical properties. Blends of lard with soybean oil in the proportions 80:20 and 70:30, respectively, demonstrated a fatty acid composition and proportions of fatty acids, which are appropriate for the formulation of pediatric products. These same blends were more suited for this purpose after continuous EIE because their *sn*-2 positions were predominantly occupied by saturated fatty acids while unsaturated fatty acids tended to occupy *sn*-1,3 positions, akin to HMF.

Acknowledgments The authors gratefully acknowledge the generous support from the Brazilian research funding agencies, Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP).

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