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Structuration of lipid bases zero-trans and palm oil-free for food applications

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ABSTRACT

This work evaluated structured lipids (SLs) through chemical and enzymatic interesterification (CSLs and ESLs). Blends of soybean oil and peanut oil 1:1 wt% were used, with gradual addition of fully hydrogenated crambe to obtain a final behenic acid concentration of 6, 12, 18, and 24 %. Chemical catalysis used sodium methoxide (0.4 wt%) at 100 °C for 30 min, while enzymatic catalysis used Lipozyme TL IM (5 wt%) at 60 °C for 6 h. Major fatty acids identified were C16:0, C18:0, and C22:0. It was observed that with gradual increase of hard fat, the CSLs showed high concentrations of reaction intermediates, indicating further a steric hindrance, unlike ESLs. Increased hard fat also altered crystallization profile and triacylglycerols composition and ESLs showed lower solid fat, unlike CSLs. Both methods effectively produced SLs as an alternative to trans and palm fats, view to potential future applications in food products.

1. Introduction

Lipids are essential in the human diet, primarily serving as an energy source and playing crucial physiological, metabolic, and nutritional roles (Zuin et al., 2022). However, in their natural form, they do not exhibit the necessary plastic fat properties for application in foods, in terms of consistency and oxidative stability, due to their specific composition of fatty acids (FAs) and triacylglycerols (TAGs) (Zhang et al., 2021). Moreover due of the growing concern over the consumption of trans fatty acids (TFAs), and their adverse health effects, coupled with the prohibition of their use and with the proposition to restrict and/or limit the use of saturated fats, the food industry had to adopt alternative technologies, thus the lipid interesterification or lipid synthesis, also known as structured lipids (SLs), emerged as a viable and promising method for the production of dietary lipids without trans isomer content (Viriato et al., 2018; Xie et al., 2015).

SLs are restructured TAGs obtained through chemical and/or enzymatic interesterification or transesterification, or even through genetic engineering methods, aiming to alter and rearrange the position, composition, and/or distribution of FAs on the glycerol molecule based on their unsaturation levels, chain length, and positional distribution (Guo et al., 2020; Moreira et al., 2020). In this context, interesterification has proven to be the primary alternative for preparing plastic fats, providing desirable modifications in the physicochemical, nutraceutical, and bioactive properties of oils and fats for their application in food products (Abed et al., 2017; Ribeiro et al., 2009).

A variety of oil/fat sources can be used in the synthesis of SLs, with palm oil and its fractions being commonly and widely used raw materials in the development of new lipid bases. These can be fully hydrogenated and/or combined with various sources of vegetable oils, producing a lipid base rich in palmitic and oleic acids that is low-cost and accessible in the interesterification process (Mills et al., 2017; Ribeiro et al., 2017). However, its use has faced discrimination due to growing concerns about environmental claim and social implications associated with expanding cultivation areas and still during its fractionation and refining process, it can generate excessive concentrations of undesirable reaction intermediates and contaminants like 3-monochloropropane-1,2-diol (3-MCPD) esters and glycidyl esters which can

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be associated with toxicological effects (carcinogenic and/or genotoxic) (Beekman et al., 2019; Mills et al., 2017; Sim et al., 2020). Thus, the importance of using new fat sources as lipid bases with accessible and economically viable functionality equivalent to partially hydrogenated and palm fats is highlighted (He et al., 2018; Mota et al., 2020).

Fully hydrogenated crambe hard fat (FHCH) is a hard fat with potential of crystallization additive in lipid bases, however, its use in food products has not been widely reported due to its association with erucic acid (C22:1), present in crude crambe oil (*Crambe abyssinica* H.). Despite that, it's worth noting that the total hydrogenation process converts C22:1 into behenic acid (C22:0) (Guedes et al., 2014; No et al., 2013), which exhibits potential anti-obesogenic properties, this is because it partially reduces gastrointestinal lipid absorption through the inhibition of pancreatic lipase (da Silva et al., 2019; Moreira et al., 2020), preventing the complete hydrolysis and absorption of TAGs and resulting in caloric reduction (Kanjilal et al., 2013). C22:0 can also be naturally found in peanut oil, animal milk fats, native oilseeds, and marine oils (Ahmadi et al., 2008; Albuquerque da Silva et al., 2023).

Peanut oil stands out for its nutritional qualities, characterized by high levels of monounsaturated FAs (MUFA), with oleic acid as the majority component (C18:1 ≈ 70 %). It also contains approximately 3.4 to 4 % of C22:0 (Akhtar et al., 2014; Janila et al., 2016; Zaaboul et al., 2018). According to the United States Department of Agriculture (USDA, 2024), Brazil is the second-largest peanut producer in South America, with the state of São Paulo being the largest producer nationally, where production is carried out through scheduled rotation with sugarcane, primarily oriented towards export. Additionally, peanut oil ranks sixth in global vegetable oil production, with approximately 6.25–6.33 million tons between 2022 and 2023 (USDA, 2024). Soybean oil also stands out due to its low economic cost, nutritional qualities, readily available supply, and the required functionality for application in lipid products like SLs (Guedes et al., 2014).

In previous studies conducted by our research group, as documented in the literature by da Silva et al. (2019), Moreira et al. (2020), Moreira, Ract, Ribeiro, & Macedo, 2017; Moreira, Santos, Gambero, & Macedo, 2017 e Zuin et al. (2022), SLs, composed of olive oil, soybean oil, and FHCH, were successfully obtained and characterized. The *in vivo* results were satisfactory, indicating anti-obesogenic potential through the inhibition of pancreatic lipase. Additionally, the SLs exhibited physicochemical characteristics of plastic fats suitable for application in food products. A limiting factor in the research was the high cost of olive oil for the synthesis of these SLs.

In this context, the objective of this study was to produce and characterize *trans*-fat-free and palm oil-free SLs using blends of accessible and low-cost oils rich in MUFA and polyunsaturated (PUFA) and long-chain fatty acids, primarily C22:0, through chemical and enzymatic interesterification. The SLs were composed of blends of soybean oil and high oleic peanut oil (1:1 wt%), with the gradual addition of FHCH, aiming to increase the C22:0 content to final concentrations of 6, 12, 18, and 24 %. The interesterification products were characterized as for physicochemical properties and thermal behavior in relation to the increase in SFC, with a view to potential future applications as lipid bases in various types of food products. Additionally, the study aimed to add value to peanut and crambe production chains at Brazil through the use of their oils in interesterification synthesis.

2. Materials and methods

2.1. Raw materials

The materials used were: soybean oil (SO) (Liza, Cargill Incorporated, Brazil); high oleic peanut oil (HOPO) (Sementes Esperança Comércio, Importação e Exportação Ltd., Brazil); and fully hydrogenated crambe hard fat (FHCH) (Chemyunion Ltd., Brazil), as a source of C22:0. For the enzymatic interesterification reaction, immobilized lipase Lipozyme TL IM, Novozymes® was used. Sodium methoxide anhydrous powder (Sigma-Aldrich®, Brazil) was used in the chemical interesterification process. All other reagents were of analytic grade.

2.2. Blends preparation

The simple blends (SBs), before interesterification reaction, were prepared mixing SO and HOPO (1:1 wt%). FHCH was gradually added to these blends to assess the adjustment of increasing C22:0 content. The study initiated with a minimum of 6 % of the C22:0, based on previous research by the research group on SLs (Moreira et al., 2020; Moreira, Ract, Ribeiro, & Macedo, 2017; Moreira, Santos, Gambero, & Macedo, 2017; Zuin et al., 2022), and reached a maximum of 24 % of the C22:0. At a C22:0 ratio of 6 %, SB was named SB6 and the FHCH:SO:HOPO ratio was 10:45:45 (wt. %). Likewise, SBs with greater C22:0 ratio were named SB12, SB18 and SB24, with their FHCH:SO:HOPO ratio of 20:40:40, 32:34:34 and 46:27:27 (wt. %), respectively. SBs were prepared after complete melting of the pure raw materials at 100 °C for 10 min to destroy the crystallinity memory under continuous stirring to ensure complete homogenization (Ribeiro et al., 2009). Subsequently, SBs were used in the interesterification reaction.

2.3. Chemical interesterification

The reactions were carried out in a 500 mL Buchner flask, where 200 mL of the SBs was subjected to a batch system under vacuum and agitation at 500 rpm, at 100 °C, for 30 min (Grimaldi, Gonçalves & Ando, 2005) with 0.4 wt% sodium methoxide content used as a catalyst. The amount of catalyst used for the interesterification reaction was determined as a function of each blend's free FAs and peroxide contents by American Oil Chemists' Society Ca 5a-40 and Cd 8b-90 method (AOCS - American Oil Chemists' Society, 2009), and calculated according to Desmet Ballestra, (2014). The reaction was finalized by adding distilled water and 5 wt% citric acid solution. After this step, interesterified samples were carefully washed with heated distilled water (80 °C) to remove soaps and subsequently dried under vacuum at 110 °C for 30 min (Ribeiro et al., 2009).

2.4. Enzymatic interesterification

The reactions were carried out according to the methodology of (Moreira et al., 2020), with adaptations. For this purpose, a 500 mL Buchner flask with 200 mL of the SB was employed in a vacuum system under agitation at 350 rpm, at 60 °C for 6 h. A 5 wt% addition of immobilized lipase Lipozyme TL IM, previously conditioned by deaeration and drying as per the manufacturer's instructions, was utilized in the batch system.

2.5. Fatty acids composition

The FAs composition was determined by gas chromatography (GC) with a capillary column-CGC Agilent 6850 Series GC System (Santa Clara, CA, USA), following the method of Hartman & Lago, (1973). According to the Ce 1f-96 method (AOCS - American Oil Chemists' Society, 2009), the FAs methyl esters were separated in a DB-23 Agilent capillary column (50 % cyanopropyl methylpolysiloxane; length, 60 m; internal diameter, 0.25 mm; film thickness, 0.25 μm). The operating conditions were as follows: oven temperature: 110 °C for 5 min, 110–215 °C (5 °C/min), 215 °C for 24 min; detector temperature, 280 °C; injector temperature, 250 °C; carrier gas, helium; split ratio, 1:50; injected volume: 1.0 μL. The FAs were identified by comparing the peak retention times with those of the respective FAs standards. The quantitative composition was calculated by area normalization and expressed as the area percentage.

2.6. Atherogenicity and thrombogenicity indexes

Nutritional quality was assessed using the atherogenicity indices (AI) equation (1) and thrombogenicity (TI) equation (2), calculated by FAs composition (Ulbricht & Southgate, 1991). Additionally, the desired fatty acids (DFA) were calculated, where DFA = MUFA + PUFA + C18:0, and hypercholesterolemic saturated fatty acids (HSFA), where HSFA = C12:0 + C14:0 + C16:0 (Barłowska et al., 2018). When a FA was not detected, it was considered zero in the equation.

$$AI = \frac{C12:0 + (4 \times C14:0) + C16:0}{\Sigma\ MUFA + \Sigma\ FA\omega6 + \Sigma\ FA\omega3} \quad (1)$$

$$TI = \frac{C14:0 + C16:0 + C18:0}{(0.5 \times \Sigma\ MUFA) + (0.5 \times \Sigma\ FA\omega6) + (3 \times \Sigma\ FA\omega3) + \left(\frac{\Sigma\ FA\omega3}{\Sigma\ FA\omega6}\right)} \quad (2)$$

2.7. sn-2 regiospecific distribution of fatty acids

Regiospecific distribution of FAs in the SLs was determined after regiospecific hydrolysis of FAs at the *sn*-1,3 position using *Candida Antarctica* lipase, highly selective for *sn*-1,3, following the Ch 3a-19 method AOCS - American Oil Chemists' Society, 2019 with adaptations. In this process, 0.5 g of the SLs sample and 5 mL of 99.5 % ethanol with 0.22 g of Novozymes® lipase 435 (*Candida Antarctica*) were added to glass bottles with screw caps and subjected to agitation at 180 rpm at 30 °C for 3 h. The resulting blend was then filtered in a 25 mL beaker using filter paper to remove the lipase from the lipid base and ethanol blend. The filtrate was kept in an air-circulating oven at 80 °C for 24 h to allow for ethanol evaporation. For the thin-layer chromatography (TLC) was used Ch 3–91 method AOCS - American Oil Chemists' Society, 2022a, with adaptations. The TLC plates were subsequently used to isolate the lipid classes resulting from partial hydrolysis. For this, 1 mL of diethyl ether was added to the lipid blend and 25 μL were applied in the silica gel TLC plate (Merck, Germany). Commercial standards of TAGs, DAGs, monoacylglycerols (MAGs) and free FAs were also applied to the plate. The lipid classes were separated using a mobile phase of n-hexane:diethyl ether:formic acid (70:30:1, v/v/v), pre-conditioned for 10 min, in a glass chamber. A solution of 2′,7′-dichlorofluorescein (2 v/v %) was used to reveal and identify the monoglyceride bands under ultraviolet light. These bands were scraped off with a spatula, and transferred to filter paper (wrapped). This silica (wrapped) was treated according to the Ch 1–91 method AOCS - American Oil Chemists' Society, 2022b to convert monoglycerides into methyl esters and analyzed by GC, as described in section 2.5. The results were expressed based on the composition of FAs at the *sn*-2 position.

2.8. Partial acylglycerol content (HPSEC)

The analysis of acylglycerol compound classes were determined according to Dobarganes, Velasco & Dieffenbacher (2000), using a liquid chromatograph (Perkin Elmer LC-250) coupled with a refractive index detector (Sicon Analytic). Two divinylbenzene (DVB) Jordi gel columns were used with 100 and 500 Å porosity, 5 μm particle size, 30 cm length, and 7.8 mm internal diameter. The mobile phase used was tetrahydrofuran (THF) HPLC grade at a flow rate of 1 mL/min. The classes of compounds were identified by comparing the elution times with TAGs, DAGs, MAGs, and free FAs.

2.9. Triacylglycerol composition (TAGs)

The TAG composition was obtained according to the Ce 5–86 method (AOCS - American Oil Chemists' Society, 2009), using the Agilent 6850 Series GC System (Santa Clara, CA, USA). The capillary column used was the DB-17 HT Agilent Catalog: 122–1811 (50 % phenylmethylpolysiloxane), with dimensions of 15 m length, 0.25 mm internal diameter, and 0.15 μm film thickness. The chromatograph operating conditions were as follows: column flow = 1.0 ml/min; linear velocity = 40 cm/sec; detector temperature = 375 °C; injector temperature = 360 °C; oven temperature: 280–340 °C at 2 °C/min and 340 °C for 40 min; carrier gas: helium; injected volume = 1.0 μL, split injection, ratio 1:100; sample concentration = 10 mg/ml of THF. TAGs identification was performed using a predictive methodology with the PrOleos software according to (Antoniosi Filho et al., 1995), comparing retention times with the possible TAGs with the highest percentage within groups with a specific number of carbon atoms.

2.10. Thermal crystallization

The thermal analysis of the samples was performed using a Differential Scanning Calorimeter (DSC) model Q250 (TA Instruments, Waters, New Castle, USA) according to the Cj 1–94 method AOCS - American Oil Chemists' Society, 2009, with 8 to 12 mg of each lipid sample. The crystallization curve was obtained by stabilizing the temperature at 25 °C, followed by heating to 80 °C for 10 min, with subsequent cooling to −80 °C at a rate of 10 °C/min. The data were processed using the TRIOS software (TA Instruments, Walters™, USA).

2.11. Crystallization kinetics

The blends were subjected to isothermal crystallization at 25 °C in a nuclear magnetic resonance spectrometer (NMR) equipment (Bruker pc120 Minispec, Germany), using high-precision dry baths (0–70 °C) (TCON 2000, Duratech, EUA) and Lauda circulator heater (E200 Ecoline-star edition). The simple and interesterified blends were melted and maintained at 70 °C for 60 min to destroy the crystalline memory (Ribeiro et al., 2009). Data acquisition was automatically performed every minute for 60 min. The induction time of crystallization (t_{SFC}) was also determined by extrapolating back to the onset time of the linear SFC (SFC_{max}) increase based on the curves of SFC as a function of time. The data obtained under isothermal conditions were fitted to the non-linearized Avrami equation (3):

$$\frac{SFC(t)}{SFC_{max}} = 1 - e^{-kt^n} \quad (3)$$

SFC (t) describes the SFC at crystallization time t, maximum SFC (SFC_{max}) is the limiting SFC as the time approaches infinity, k is the Avrami constant, which considers both nucleation and growth rate, and n is the Avrami exponent, which indicates the crystal growth mechanism. Half-time of crystallization ($t_{1/2}$) was calculated according to the following equation (4) (Chai et al., 2018):

$$t_{1/2} = \sqrt[n]{\frac{0.693}{k}} \quad (4)$$

2.12. Solid fat content (SFC)

The SFC was analyzed using NMR Spectrometer (Bruker pc120 Minispec, Germany), in conjunction with dry baths within the temperature range of 0 °C to 70 °C, Tcon 2000 (Duratech, USA). To ensure the crystallization stabilization of the interesterified blends, the Cd 16b-93 method AOCS - American Oil Chemists' Society, 2009 with modifications (Ribeiro et al., 2009) was employed, involving pre-melting of the blends at 60 °C for 5 min, followed by stabilization at 0 °C for 2 h. Subsequently, readings of the samples were taken in series at temperatures of 10, 20, 25, 30, 35, 40, 45, 50, 55, and 60 °C, every 1 h.

2.13. Consistency

The determination of consistency was carried out using the Cc 16–60 method AOCS - American Oil Chemists' Society, 2009 through the penetration test with a 45° acrylic cone in a TA-XT Plus texture analyzer,

Table 1
Fatty acids composition (%) and nutritional quality index of raw materials and simple blends before and after interesterification synthesis.

Fatty acids	Raw materials			Blends				Interesterified samples							
								Enzymatic				Chemical			
	SO	**HOPO**	**FHCH**	**SB6**	**SB12**	**SB18**	**SB24**	**ESL6**	**ESL12**	**ESL18**	**ESL24**	**CSL6**	**CSL12**	**CSL18**	**CSL24**
C14:0	0.13 ±0.1	nd	0.32 ±0.2	0.42 ±0.1	nd	nd	0.11 ±0.1	nd	nd	0.10 ±0.0	nd	nd	nd	nd	nd
C16:0	**14.39 ±1.0**	**6.86 ±0.0**	**4.21 ±0.1**	**8.44 ±0.34**	**8.00 ±0.1**	**7.54 ±1.0**	**7.35 ±1.0**	**8.45 ±0.0**	**9.16 ±1.0**	**7.32 ±0.0**	**7.04 ±0.1**	**8.32 ±0.0**	**8.11 ±0.1**	**7.80 ±0.0**	**6.24 ±2.0**
C16:1	0.11 ±0.0	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
C17:0	0.13 ±0.0	0.12 ±0.8	nd	0.10 ±0.5	nd	nd	nd	nd	nd	nd	nd	0.10 ±0.0	0.10 ±0.0	nd	nd
C17:1	nd	0.11 ±0.0	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
C18:0	5.33 ±1.3	2.96 ±0.0	**36.30 ±0.6**	**5.77 ±0.6**	**8.72 ±0.1**	**12.65 ±2.0**	**16.25 ±0.2**	**6.02 ±0.1**	**8.18 ±0.2**	**10.77 ±2.3**	**16.06 ±0.0**	**5.73 ±0.0**	**8.76 ±0.1**	**13.65 ±1.0**	**16.82 ±0.1**
C18:1	**25.06 ±0.4**	**70.46 ±0.4**	1.16 ±0.5	**47.02 ±0.7**	**41.56 ±0.1**	**34.64 ±1.3**	**30.65 ±0.4**	**46.21 ±0.1**	**42.14 ±0.1**	**34.08 ±0.0**	**30.07 ±0.2**	**47.70 ±0.1**	**43.02 ±0.3**	**36.25 ±2.0**	**30.80 ±0.0**
C18:2t	0.33 ±0.1	nd	nd	0.40 ±0.0	0.14 ±0.1	0.36 ±0.0	0.19 ±0.2	nd	nd	nd	nd	nd	nd	nd	nd
C18:2	**47.87 ±3.2**	**5.45 ±3.9**	0.33 ±0.0	**26.77 ±0.4**	**23.89 ±0.2**	**20.64 ±0.1**	**15.98 ±0.0**	**26.08 ±0.0**	**24.33 ±1.0**	**25.05 ±0.2**	**18.50 ±0.2**	**26.19 ±0.0**	**23.15 ±0.1**	**18.58 ±0.2**	**16.23 ±0.1**
C18:3t	1.00 ±0.3	nd	nd	0.66 ±0.1	0.45 ±0.1	0.34 ±0.5	0.29 ±0.5	nd	nd	nd	nd	nd	nd	nd	nd
C18:3	**5.01 ±0.3**	0.10 ±0.3	nd	2.86 ±0.1	2.24 ±0.1	1.93 ±0.0	1.73 ±0.1	2.93 ±0.0	2.83 ±0.1	1.53 ±0.2	1.51 ±0.5	2.44 ±0.0	2.06 ±0.0	1.59 ±0.1	1.66 ±0.2
C20:0	0.64 ±0.2	1.90 ±0.4	**5.27 ±0.1**	2.09 ±0.2	2.62 ±0.1	2.92 ±0.0	2.53 ±0.1	2.37 ±0.5	1.26 ±0.2	1.76 ±0.2	2.51 ±0.1	2.35 ±0.0	1.58 ±0.3	3.04 ±1.0	2.61 ±0.0
C20:1	0.27 ±0.4	1.92 ±0.3	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
C22:0	1.05 ±0.1	**5.80 ±1.4**	**51.04 ±0.2**	**5.69 ±0.0**	**12.16 ±0.0**	**18.40 ±0.0**	**23.94 ±1.0**	**6.56 ±0.1**	**12.38 ±0.0**	**18.24 ±0.0**	**23.73 ±1.0**	**6.01 ±0.0**	**12.17 ±0.0**	**17.90 ±1.0**	**24.38 ±0.2**
C22:1	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
C24:0	0.36 ±0.0	4.14 ±1.2	2.01 ±0.1	0.97 ±0.0	1.06 ±0.4	1.28 ±0.0	1.20 ±0.0	1.30 ±0.2	nd	0.82 ±2.0	0.92 ±1.0	1.28 ±0.0	1.29 ±0.0	1.48 ±0.5	0.99 ±1.0
∑ MUFA	25.06 ±0.2	**72.38 ±1.7**	1.16 ±0.0	47.02 ±0.1	41.56 ±0.1	34.64 ±0.0	30.65 ±0.4	46.21 ±0.1	42.14 ±0.2	34.08 ±0.0	30.07 ±0.0	47.70 ±0.1	43.02 ±0.2	36.25 ±0.5	30.80 ±0.0
∑ PUFA	**53.88 ±1.5**	5.45 ±0.2	nd	30.29 ±0.2	26.13 ±0.0	22.93 ±0.2	17.71 ±0.0	29.01 ±0.2	27.16 ±0.1	26.58 ±0.7	20.01 ±0.3	28.64 ±0.2	25.21 ±0.1	20.17 ±0.4	17.89 ±0.0
∑Saturated	21.42 ±0.5	21.67 ±0.7	**98.83 ±0.2**	22.95 ±0.2	32.56 ±0.2	**42.78 ±0.5**	**51.26 ±0.0**	24.69 ±0.2	30.98 ±0.3	**38.92 ±0.4**	**50.26 ±0.1**	23.69 ±0.1	31.91 ±0.2	**43.86 ±0.1**	**51.03 ±0.0**
AI	0.19 ±0.1	0.09 ±0.1	3.70 ±0.1	0.13 ±0.1	0.12 ±0.0	0.14 ±0.0	0.16 ±0.4	0.11 ±0.1	0.13 ±0.1	0.13 ±0.1	0.14 ±0.2	0.11 ±0.4	0.12 ±0.1	0.14 ±0.1	0.13 ±0.1
TI	0.38 ±2.1	0.25 ±0.1	**54.41 ±3.5**	0.31 ±0.0	0.42 ±0.1	0.60 ±3.7	0.82 ±3.0	0.32 ±0.4	0.41 ±0.5	0.53 ±0.1	0.80 ±0.2	0.32 ±0.0	0.43 ±0.0	0.67 ±1.2	0.81 ±0.3
DFAI	**84.27 ±2.0**	**80.80 ±0.6**	37.47 ±6.0	**83.09 ±0.2**	76.41 ±2.7	70.22 ±2.0	64.61 ±3.0	**81.23 ±3.4**	77.48 ±3.0	71.43 ±0.1	66.14 ±0.1	**82.07 ±2.0**	76.99 ±2.5	70.07 ±0.1	65.50 ±0.8
HSFI	14.52 ±1.8	6.93 ±0.3	4.54 ±0.9	8.85 ±0.2	8.07 ±0.3	7.62 ±0.6	7.46 ±0.8	8.45 ±1.6	9.16 ±1.0	7.42 ±2.1	7.04 ±0.7	8.39 ±1.5	8.18 ±0.5	7.80 ±0.3	6.24 ±1.4

The values are expressed as mean ± SD (n=3). nd = not detected. Samples coded: SO = soybean oil; HOPO = high oleic peanut oil; FHCH = fully hydrogenated crambe hard fat; SB = simple blend, ESL = enzymatic interesterification, CSL = chemical interesterification. AI = Atherogenicity Index; TI = Thrombogenicity Index; DFAI = Desirable Fatty Acid Index; HSFI = Hypercholesterolemic Saturated Fatty Acid Index. Highlighted values are considered more relevant.

controlled by a computer (Stable Micro Systems®, USA). The samples were preheated (70 °C) for complete crystal melting, placed in 50 mL beakers, and then incubated in refrigerator at 5 °C for 24 h for fat crystallization. Subsequently, they were incubated again for 24 h at each determination temperature (10, 25, and 35 ± 2 °C). The test conditions were as follows: initial distance of 10 mm; speed of 2 mm/s; time of 0.01 s, following the methodology of Stahl et al., (2018). Under these conditions and using the Texture Exponent Extralab program (Stable Micro Systems®, Brazil), the compression force in grams-force (gf) was measured, and penetration data were converted into *Yield Value* and calculated according to equation (5) (Haighton, 1959).

$$C = \frac{K \times W}{p^{1.6}} \quad (5)$$

where C = *yield value*, in gf/cm^2; K = cone angle-dependent factor (4700 for 45°); W = compression force (gf); P = penetration depth at 0.1 mm.

2.14. Statistical data analysis

The determinations were conducted in triplicate for each sample, and the results were expressed as their means ± standard deviation (n = 3). To determine significant differences between the mean values of the results, an analysis of variance (ANOVA) followed by Tukey's test was applied at a level of $p < 0.05$. These statistical analyses were performed using Minitab for Windows version 16.2.2 (Minitab, 2010). For crystallization kinetics analyses, Statistical System version 8.0 (SAS Institute Inc., USA) was employed.

3. Results and discussion

3.1. FAs composition

Table 1 shows the FAs composition (FAC) of the raw materials (SO, HOPO and FHCH) and SBs before and after interesterification process.

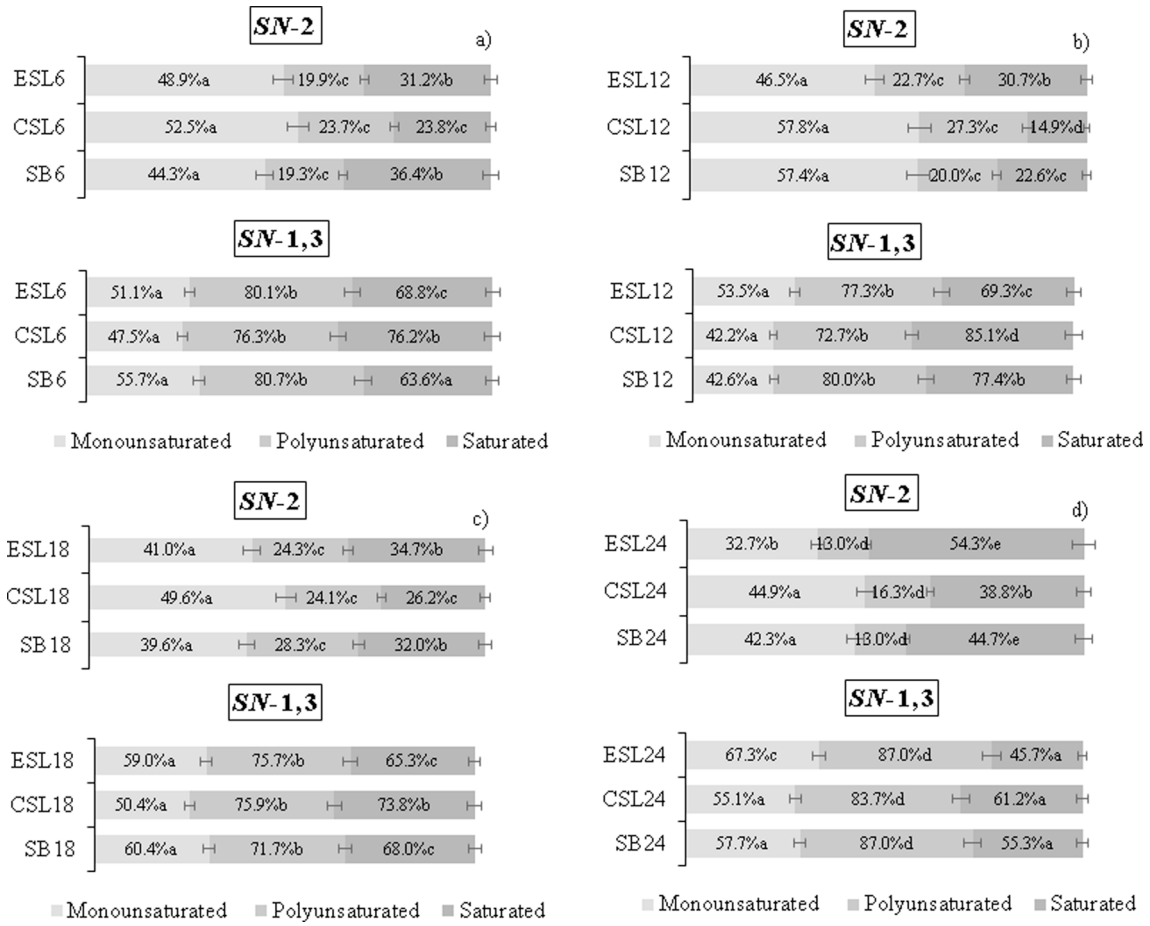

Fig. 1. Regiospecific distribution of fatty acids (wt %) in the *sn*-2 and *sn*-1,3 positions of TAG in the simple blends and interesterified blends according to the behenic acid concentration of 6 (a), 12 (b), 18 (c), and 24 % (d). Samples coded: SB = simple blend, ESL = enzymatic interesterification, CSL = chemical interesterification. Results marked with different letters showed significant differences ($p \leq 0.05$) between blends according to the behenic acid concentration.

For SO, the predominant FAs were C18:2 (47.87 %), C18:1 (25.06 %), C16:0 (14.39 %), and C18:3 (5.01 %). HOPO content of C18:1 (70.46 %), followed by C16:0 (6.86 %), C18:2 (5.45 %), C24:0 (4.14 %), and C18:0 (2.96 %), with concentrations of C22:0 (5.80 %). In FHCH, the major FAs were C22:0 (51.04 %), C18:0 (36.30 %), C20:0 (5.27 %), and C16:0 (4.21 %). The FACs of the raw materials is in line with literature findings for SO (Celus et al., 2018; Onsaard & Onsaard, 2019). Regarding HOPO, it exhibited high oleic characteristics with C18:1 concentration of $\geq$ 70 %, and the levels of C22:0 found were higher than those reported in the literature (≈3.4 to 4 %) (Akhtar et al., 2014; Janila et al., 2016; Zaaboul et al., 2018). As for FAs levels found in FHCH, they differ from studies available in the literature (Guedes et al., 2014; Moreira, Ract, Ribeiro, & Macedo, 2017; Moreira, Santos, Gambero, & Macedo, 2017; Zuin et al., 2022). This variation could be attributed to the variety, crop, cultivation region, edaphoclimatic factors as well as the processing of the FHCH used (Zuin et al., 2022).

Regarding determining raw materials for the SBs, oils were chosen based on their lipid profile and nutritional, technological, and economic benefits. SO and HOPO have high levels of long-chain polyunsaturated FAs (ω-3, ω-6, and ω-9), with HOPO also containing C22:0, offering advantageous functional properties and low production costs (Akhtar et al., 2014; Ribeiro et al., 2009). FHCH was chosen due to its high C22:0 content, a long-chain saturated FA that favors the reduction of lipid gastrointestinal absorption by inhibiting pancreatic lipase (da Silva et al., 2019). Additionally, according to Moreira et al., 2017, it exhibits anti-obesogenic effects by reducing lipolysis rate with partial absorption of TAGs resulting in fecal FA excretion. Technologically, it presents a solid physical characteristic with a high potential for wax compositions that can be used as crystallization additives in lipid bases (Ribeiro et al., 2017). The composition of SBs was determined and adjusted according to minimum concentrations of C22:0 of approximately 6 % found in previous studies available in the literature by da Silva et al. (2019), Moreira et al. (2020), Moreira, Ract, Ribeiro, & Macedo, 2017; Moreira, Santos, Gambero, & Macedo, 2017 e Zuin et al. (2022), which showed positive results in determining anti-obesogenic capacity *in vivo* and technological application in food, with a gradual increase in the percentage of C22:0 up to 24 % (Table 1).

Regarding the FA composition of the samples after enzymatic interesterification, the main unsaturated FAs present in samples ESL6 to ESL24 were C18:1 (46.21 % to 30.07 %) and C18:2 (26.08 % to 18.5 %). The concentrations of saturated FAs increased with the rise in C22:0 content in the samples, with C18:0 varying from 6.02 % in ESL6 to 16.06 % in ESL24 and C16:0 from 7.04 % in ESL24 to 9.16 % in ESL12, with higher concentrations in ESL12. C22:0 was adjusted in the samples, increasing from 6.56 % in ESL6 to 23.73 % in ESL24. The data, especially for C22:0, are consistent with Ribeiro et al., (2017). FACs for chemical interesterification showed higher levels of C18:1 (47.70 % to 30.80 %) and C18:2 (26.19 % to 16.23 %) for unsaturated FAs. For saturated FAs, the highest concentrations were C18:0 (5.73 % to 16.82 %) and C22:0 (6.01 % to 24.38 %). The results obtained by Guedes et al. (2014), are consistent with the CSL12 and CSL24 samples. Observing that the interesterification process neither affected the degree of saturation nor caused isomerization of the FAs double bonds. FAs composition of the starting materials was thus not altered (Table 1).

From a nutritional perspective, the Brazilian Society of Cardiology recommends a lipid diet totaling up to 20 % of saturated FAs, with 55 %

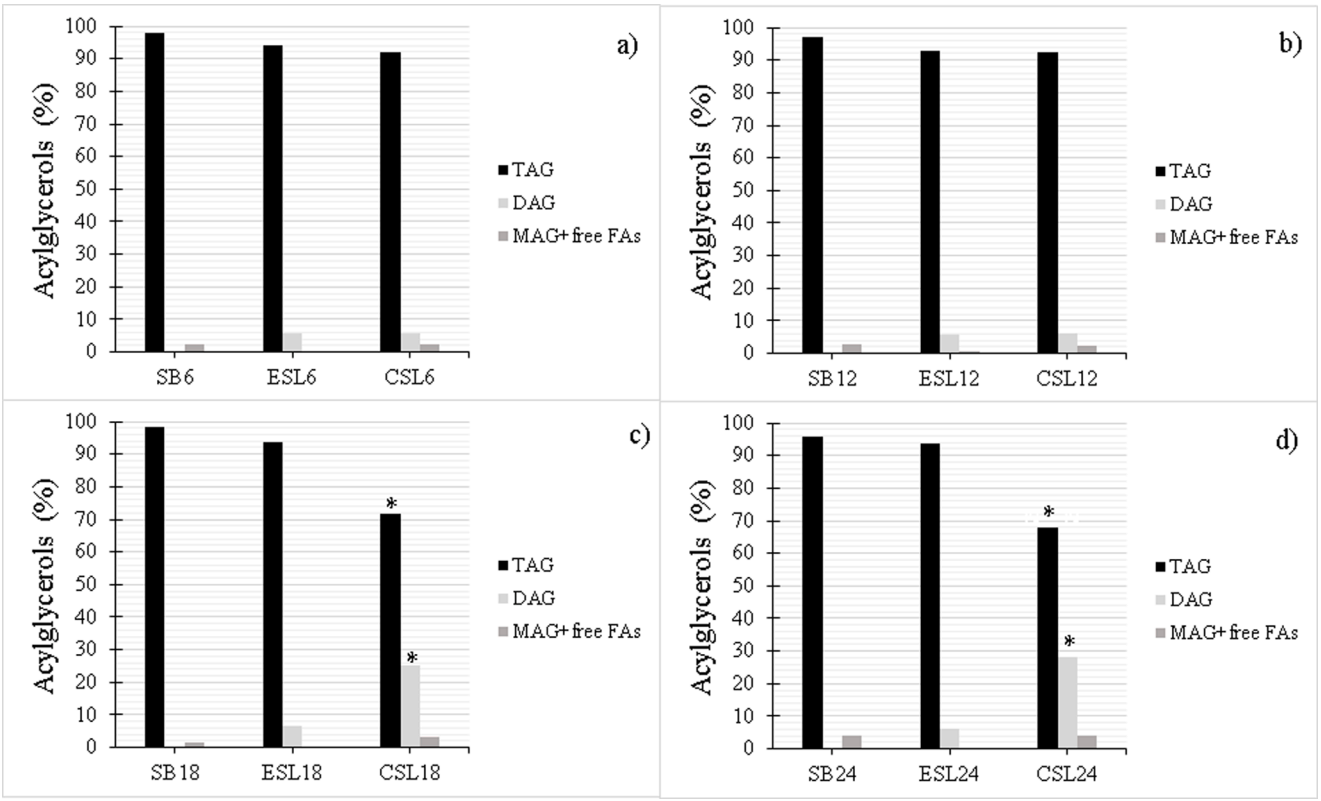

Fig. 2. Classes of acylglycerol compounds (%) in the simple blends and interesterified blends according to the behenic acid concentration of 6 (a), 12 (b), 18 (c), and 24 % (d). Samples coded: SB = simple blend, ESL = enzymatic interesterification, CSL = chemical interesterification. Results marked with * showed significant differences ($p \leq 0.05$) between lipids by chemical and enzymatic interesterification regarding their specific behenic acid composition.

MUFA and 25 % PUFA (de Izar et al., 2021). The World Health Organization (WHO) recommends that the total daily lipid should not exceed 30 % of total energy intake with saturated FA below 10 % (WHO, 2020). Excessive lipid intake, especially saturated FAs, can be a health risk, leading to serious lipid metabolic disorders, such as the development of obesity and subsequently exacerbating and/or triggering to non-communicable chronic diseases (NCDs) (Albracht-Schulte et al., 2018; Çakmur, 2020). SLs in this study have saturated FAs values above this recommendation; however, it's important to note that SLs are considered ingredients to be used in food formulations, contributing to a balanced diet without constituting the total lipid intake.

3.2. Atherogenicity and thrombogenicity indexes

Nutritional quality indices, such as AI and TI, are used to understand the different effects of saturated FAs and unsaturated FAs in oils and fats on human health. These indices are obtained through the ratio of saturated and unsaturated FAs (Batista et al., 2017). AI is relevant in the preliminary assessment of atherosclerosis associated with various inflammatory pathways, while TI assesses the potential for clot formation in blood vessels, which can lead to diseases related to platelet aggregation (Khalili Tilami & Kouřimská, 2022). The lowest AI and TI values were obtained for HOPO (AI = 0.09 and TI = 0.25) and SO (AI = 0.19 and TI = 0.38). In contrast, FHCH (AI = 3.70 and TI = 54.41) showed the highest values for both assessed indices, primarily due to its composition in saturated FAs, predominantly C18:0 (36.30 %) (Table 1). The PUFA indices ranged from 65 to 82 %, correlating with the increase in saturated FA in the SLs. However, it is noteworthy that the main saturated FAs for this index is C18:0, which has a hypocholesterolemic effect (Guedes et al., 2014; Kojima et al., 2010). All SLs showed adequate nutritional quality indices, as per Apás et al., (2015). AI and TI values found in the SLs of this study were lower than those reported by Albuquerque da Silva et al. (2023), with concentrations up to 60 % palm stearin.

3.3. Regiospecific distribution of FAs

The regiospecific distribution method allows determining the composition of saturated and unsaturated FAs in the *sn*-1,3 and *sn*-2 positions of TAGs, making it possible to understand how this rearrangement affects lipid metabolism, nutritional effects, and the bioavailability of FAs (Ribeiro et al., 2017). Fig. 1 shows the distribution at *sn*-1,3 and *sn*-2 positions of FAs in SBs before and after interesterification synthesis.

All the SBs showed higher concentrations of saturated FAs in the *sn*-1,3 positions, with the gradual increase of FHCH. According to Kojima et al. (2010), saturated FAs in unmodified vegetable oils and fats are predominant in the *sn*-1,3 positions, maintaining unsaturated FAs in the central position of glycerol. Enzymatic interesterification showed higher concentrations of saturated FAs in the *sn*-2 position compared to the *sn*-2 position in chemical synthesis. However, both syntheses exhibited a balanced distribution of FAs in the *sn*-1,3 and *sn*-2 positions of TAG, with the prevalence of saturated in *sn*-1,3 and the maintenance of unsaturated FAs in *sn*-2. This randomization is a characteristic and expected feature of synthesis with random catalysts. The main saturated FAs found were C16:0, C18:0, and C22:0. C22:0 was found in the *sn*-2 position in samples ESL12 (0.97 %), ESL18 (1.71 %), CSL18 (0.91 %), ESL24 (4.82 %), and CSL24 (2.15 %).

Similar effect was verified by Moreira et al., 2017, who evaluated the regiospecificity of TL IM in SLs formulated by blends of soybean oil, olive oil, and FHCH, in both cases, TL IM positioned C22:0 and unsaturated FAs in all three positions of the TAGs, and the authors further emphasize that there are advantages in incorporating C22:0 in *sn*-2 as it has the ability to partially inhibit pancreatic lipase, preventing the

Table 2
Composition and class of triacylglycerols (TAGs %) of raw materials and simple blends before and after interesterification synthesis.

TAGs	CN	Raw materials			Blends				Interesterified samples							
									Enzymatic				Chemical			
		SO	**HOPO**	**FHCH**	**SB6**	**SB12**	**SB18**	**SB24**	**ESL6**	**ESL12**	**ESL18**	**ESL24**	**CSL6**	**CSL12**	**CSL18**	**CSL24**
POP	50	1.57	1.09	-	1.17	0.85	0.77	-	0.95	1.09	0.72	0.64	0.97	0.88	0.77	-
PStP		-	-	-	**0.14**	-	-	-	-	-	-	-	**0.12**	-	-	-
PStSt		-	-	2.02	-	-	-	**0.58**	-	-	-	**0.75**	-	-	-	**0.73**
POO	52	2.92	**10.88**	-	6.27	4.45	3.54	2.32	5.61	5.19	3.16	2.49	5.83	4.85	3.55	2.16
PLO		**10.77**	1.55	-	**8.00**	5.51	4.21	2.90	6.92	**7.24**	5.06	3.21	**7.04**	5.51	3.65	2.65
PLL		**9.95**	-	-	2.01	1.71	1.25	0.91	2.14	2.52	2.02	1.03	2.13	1.57	0.94	0.81
StStSt	54	-	-	**4.85**	-	-	-	-	-	-	-	-	-	-	-	**0.63**
StOO		1.04	4.66	-	4.18	5.01	5.76	5.29	4.21	4.61	1.30	5.28	4.37	5.46	**6.22**	**5.57**
StLO		3.85	0.67	-	5.34	**6.20**	**6.85**	**6.62**	5.19	6.43	4.74	**6.81**	5.28	**6.20**	**6.39**	**6.82**
OOO		1.81	**36.26**	-	**10.91**	**7.79**	5.46	3.53	**10.98**	**8.26**	**7.58**	3.21	**11.65**	8.89	5.48	3.20
OLO		**10.00**	**7.77**	-	**20.90**	**14.46**	**9.75**	**6.62**	**20.33**	**17.29**	3.03	**6.21**	**21.12**	**15.15**	**8.44**	**5.87**
OLL		**18.47**	0.56	-	**13.34**	**8.95**	5.79	4.14	**12.54**	**12.06**	**11.06**	4.01	**12.76**	**8.61**	4.33	3.60
LLL		**11.37**	-	-	2.37	1.85	1.15	0.86	2.58	2.80	**8.85**	0.86	2.57	1.63	0.74	0.74
PBeSt	56	-	-	5.72	0.17	0.50	1.08	1.52	-	0.31	0.87	1.76	0.18	0.45	1.31	1.70
POBe		-	1.87	-	1.33	3.33	3.07	3.04	1.67	1.69	2.53	3.21	1.46	2.20	3.46	2.93
AOO		-	3.11	-	1.39	1.67	1.33	0.99	1.40	1.15	2.02	0.93	1.46	1.82	1.78	0.93
ALO		-	-	-	1.78	2.07	1.58	1.24	1.73	1.61	0.65	1.20	1.76	2.07	1.15	1.14
ALL		-	-	-	0.57	0.64	-	-	0.53	0.56	3.79	-	0.53	0.59	-	-
StBeSt	58	-	-	-	-	-	2.96	1.74	-	-	3.03	1.87	-	-	**6.05**	2.19
StOBe		-	-	-	0.89	2.62	4.98	**6.95**	1.25	1.50	5.53	**6.81**	1.09	2.48	3.11	**7.54**
StLBe		-	-	-	0.57	2.62	2.96	4.34	0.77	1.05	**8.85**	4.40	0.66	1.41	**7.99**	4.62
BeOO		-	**9.32**	-	3.48	**6.12**	**7.09**	**6.95**	4.91	4.03	3.54	**6.21**	4.37	**6.06**	**8.21**	**6.49**
BeLO		-	1.33	-	4.46	**7.57**	**8.43**	**8.68**	6.06	5.63	2.02	**8.02**	5.28	**6.89**	2.11	**7.96**
BeStSt		-	-	**20.59**	-	-	-	-	-	-	-	-	-	-	-	-
PBeBe		-	-	4.05	-	-	0.66	1.00	-	-	-	1.03	-	-	1.30	0.99
StBeBe	62	-	-	**29.17**	-	-	**1.08**	**2.28**	-	-	**0.76**	**2.20**	-	-	**3.89**	**2.55**
BeOBe		-	0.80	0.81	-	1.80	3.07	4.56	0.73	0.46	2.21	4.01	0.55	1.38	2.00	4.40
BeLBe		-	-	-	-	1.00	1.82	2.85	-	0.66	1.77	2.59	0.29	0.78	0.63	2.70
BeABe	64	-	-	4.05	-	-	-	-	-	-	-	-	-	-	-	-
BeBeBe	66	-	-	**13.78**	-	-	-	**1.00**	-	-	-	**0.86**	-	-	-	**0.99**
Others		29.26	12.36	35.55	11.31	19.55	19.28	21.97	9.51	14.55	21.51	23.03	9.03	15.91	17.17	22.79
$\sum S_3$		-	-	**98.05**	0.43	0.50	3.69	7.02	-	0.31	2.28	9.13	0.42	0.45	5.40	10.52
$\sum S_2U$		7.6	10.75	1.95	9.53	22.05	29.57	**37.17**	9.85	16.33	24.66	**36.62**	11.12	21.32	32.22	**37.01**
$\sum SU_2$		38.15	**41.56**	-	**43.42**	**44.41**	**44.58**	**40.67**	**43.72**	**42.95**	**46.20**	**39.96**	**40.52**	**43.95**	**43.40**	**39.06**
$\sum U_3$		**54.19**	**47.69**	-	**47.19**	33.05	22.15	15.14	**46.43**	**40.41**	26.87	14.29	**48.09**	34.29	18.99	13.41

Samples coded: SO = soybean oil; HOPO = high oleic peanut oil; FHCH = fully hydrogenated crambe hardfat; SB = simple blend, ESL = enzymatic interesterification, CSL = chemical interesterification. S_3 = trisaturated; S_2U = disaturated–monounsaturated; SU_2 = monosaturated–diunsaturated and U_3 = triunsaturated. Highlighted values are considered more relevant.

absorption of MAGs, which, in turn, hinders the absorption of other FAs by not forming chylomicrons. Guedes et al. (2014), in their studies on SLs by chemical interesterification with binary blends of soybean oil and FHCH in the proportion of 20 to 40 % hard fat, found results that support this study. In both studies, the SLs showed a random distribution in the TAG. Ribeiro et al. (2017), obtained SLs through enzymatic and chemical interesterification, using different proportions of high oleic sunflower oil and FHCH. In the SLs composed of a minimum concentration of 40 % hard fat, they found a percentage of 43.8 % saturated FAs at the *sn*-2 after chemical interesterification, which is close to the results found for the CSL24 sample (38.8 %) with 42 % hardfat in this study.

3.4. Partial acylglycerol

The increase in the concentration of reaction intermediates such as MAGs, DAGs, and free FAs is caused by the acyl migration of FAs in TAG, which is a spontaneous thermodynamic process that occurs until dynamic equilibrium is reached. This demonstrates the occurrence of hydrolysis followed by esterification (Kellens & Calliauw, 2013; Ribeiro et al., 2018). To evaluate this process, the analysis of partial acylglycerol content was performed, separating compounds. Fig. 2 shows the distribution of partial acylglycerol content in the SBs before and after interesterification synthesis.

It was observed that the SLs obtained through chemical and enzymatic interesterification processes showed similar TAG levels up to concentrations of 12 % C22:0. However, when the SLs contained higher levels of C22:0, the chemical and enzymatic reactions resulted in different TAG amounts. In the lipids obtained through chemical reaction, a high amount of DAGs (25 % and 28 %) was identified. It is noteworthy that in interesterification reactions, the major fraction of a lipid product is expected to be composed of TAGs (≥90 %) (Rohm et al., 2018; Zuin et al., 2022).

According to Lopes et al. (2016), the randomization in interesterification reactions is related to the catalyst mainly acting on *sn*-1,3 TAGs to produce 1,2- and 2,3-DAGs, leaving the *sn*-2 position practically unchanged. Thus, the FA present in the *sn*-2 of 1,2 and 2,3-DAG can spontaneously migrate, producing more stable 1,3-DAG, which will later form new TAGs with randomly rearranged FAs in positions *sn*-1,3 or *sn*-2 until achieving a balance between all possible combinations. A factor to consider regarding the greater efficiency of enzymatic interesterification may be due to the pre-conditioning of the lipase with an enzymatic conditioning process. According to Moreira et al. (2020), when the lipase undergoes dehydration and deaeration, the aim is to avoid excessive hydrolysis of TAGs, consequently avoiding unnecessary time for the reaction to reach equilibrium, resulting in lower acyl migration, higher efficiency in the interesterification process, better interaction with the active site, and reduced formation of reaction intermediates, especially DAGs. Guedes et al. (2014) found DAGs levels from 4.8 to 6.1 % in SLs with a proportion of 20 to 40 % FHCH. In comparative terms, it is important to note that the concentrations of reaction intermediates such as DAGs found in this study were lower than those reported in studies with palm oil-based SLs with concentrations of 8.16 to 16.25 %

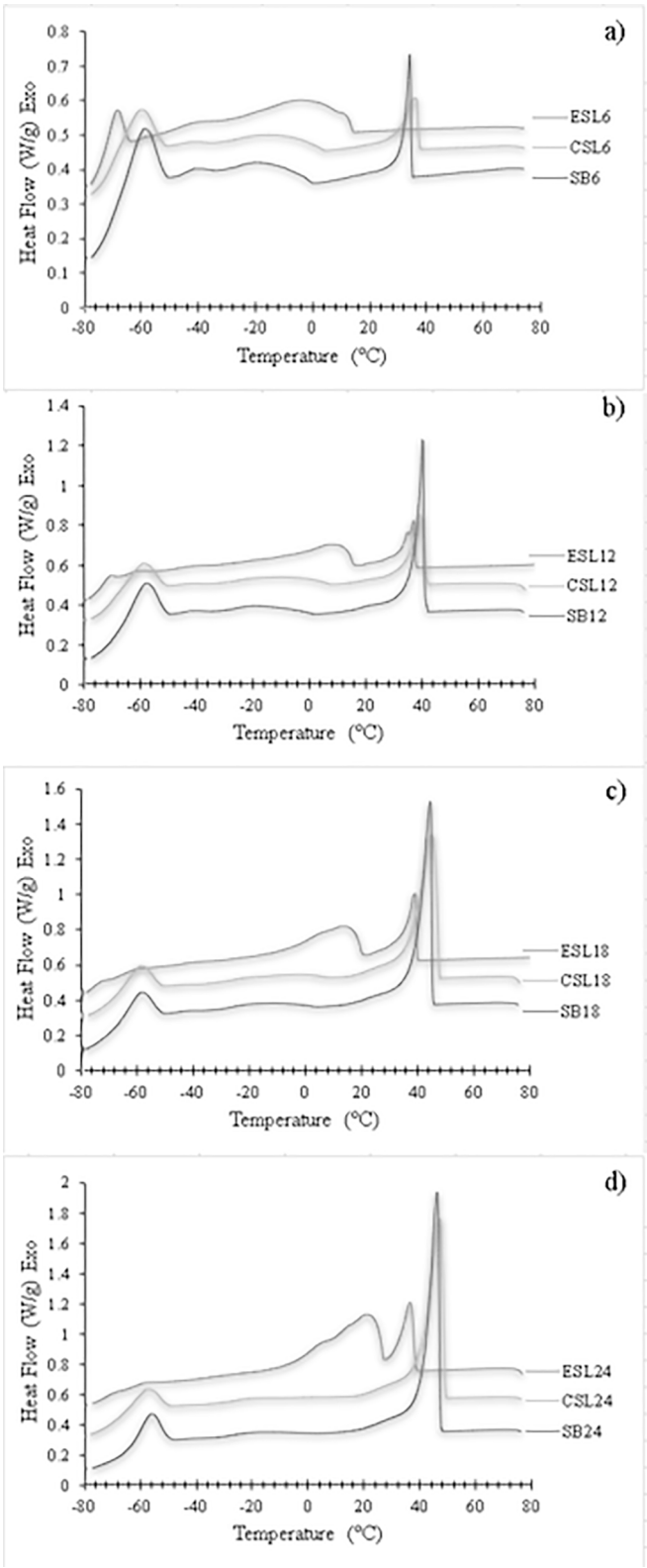

Fig. 3. Crystallization thermograms of simple blends and interesterified blends according to the behenic acid concentration of 6 (a), 12 (b), 18 (c), and 24 % (d). Samples coded: SB = simple blend, ESL = enzymatic interesterification, CSL = chemical interesterification.

DAGs (Zhang et al., 2019).

3.5. TAGs composition

Composition of TAGs is an important parameter for understanding the physical modifications that occur in interesterified oils and fats (Moreira et al., 2020). Table 2 presents the compositions and classes of TAGs in the raw materials, SBs, and interesterified samples, where TAGs are divided into groups based on their carbon number (CN).

TAGs in the SBs are represented by the proportional distribution of the main TAGs in SO, HOPO, and FHCH. The SO presented 46.54 % of TAGs in CN54 and 23.64 % in CN52, with predominant TAGs being OLL, LLL, PLL, PLO, and OLO. In HOPO, CN54 (49.92 %) was the major component followed by CN52 (12.43 %) and CN58 (10.65 %), and the predominant TAGs were OOO, POO, BeOO, and OLO. For FHCH, only trisaturated (S_3) were found, with a prevalence of BeStBe, BeStSt, and BeBeBe TAGs.

TAGs OLO, OLL, and OOO were the main TAGs found in SB6 and its interesterified samples (ESL6 and CSL6), with the formation of TAGs at CN62 during interesterification, such as BeOBe and BeLBe, and ESL6 did not form S_3 TAGs. For ESL12, OLO, OLL, OOO, and PLO were the TAGs with higher percentages, and SB12 and CSL12 presented more similar TAGs with a predominance of OLO, OOO, BeOO, and BeLO. Interesterification resulted in changes mainly in the TAG composition of ESL18, with a reduction in CN60 and CN62, with a predominance of OLO and OLL, unlike SB18 and CSL18 with OLO, BeLO, and BeOO. SB24 and its interesterified samples (ESL18 and CSL18) presented higher amounts of S_3 TAGs compared to other samples, especially at CN52, and with TAGs at CN66 like BeBeBe. Interesterification decreased TAGs with a high melting point and increased those with an intermediate melting point, especially in enzymatic interesterification, and in chemical interesterification, it resulted in the formation of new TAGs like BeBeBe at CN66.

According to Ribeiro et al., 2009, the presence of TAGs such as StLO, StLL, StLSt, and StOSt gives SLs desirable characteristics such as lubricity, aeration, and shine. TAGs like PStSt, even in reduced concentrations, provide structure and moisture barrier, contributing to greater functionality of random FAs. For food applications, it is desirable to have a low concentration of S_3 TAGs such as StStSt, BeBeBe, due to the associated with a disagreeable waxy mouthfeel.

The melting range of TAGs can be divided into: S_3 (trisaturated) (54 at 65 °C), S_2U (disaturated–monounsaturated) (27 at 42 °C), SU_2 (monosaturated–diunsaturated) (1 at 23 °C), and U_3 (triunsaturated) (−14 at 1 °C) (Hoffmann, 1989). Table 2 also presents the distribution of TAG classes, showing that the gradual increase in FHCH promoted higher concentrations of S_3 and S_2U. Thus, SLs with C22:0 concentrations between 18 to 24 % exhibited low levels of U_3. All samples showed high U_2S content. According to Dollah et al. (2016) and Ribeiro et al., 2009, S_3 and S_2U are primarily responsible for increasing SFC, providing structure to the products. U_2S is related to sensory properties, imparting plasticity, while U_3 TAGs increase lubricity, consequently enhancing the technological functionality of SLs as lipid bases for food applications.

3.6. Thermal behavior

The thermograms of crystallization behavior for the SBs and interesterified samples are presented in Fig. 3. The SBs showed an intense crystallization process, with a high (well-defined) peak between 38 to 48 °C, characteristic of TAGs with a high melting point (S_2U), mainly due to the presence of FHCH (melting point 56.66 °C, S_3 TAGs), this is also related to the presence of a high concentration of long-chain saturated FAs.

The gradual increase of FHCH altered the crystallization behavior of the interesterified samples. The enzymatically interesterified ESL6 did not show the first high peak, with initial crystallization starting at 15.03 °C, indicating that S_3 and S_2U TAGs were redistributed to SU_2 and U_3, unlike what was observed for chemical interesterification in CSL6,

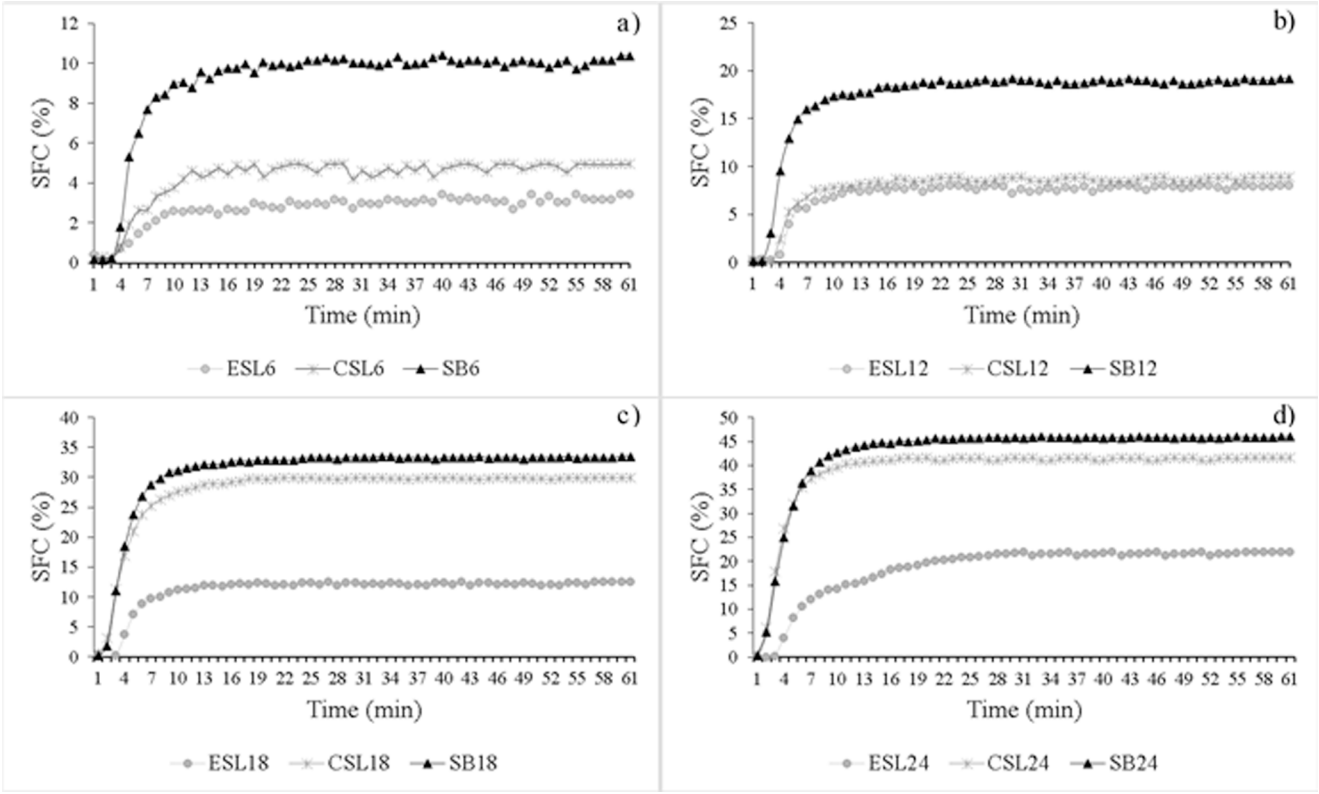

Fig. 4. Crystallization isotherms at 25 °C of simple blends and interesterified blends according to the behenic acid concentration of 6 (a), 12 (b), 18 (c), and 24 % (d). Samples coded: SB = simple blend, ESL = enzymatic interesterification, CSL = chemical interesterification.

Table 3
Induction time (t_{SFC}), maximum solid fat content (SFC_{max}), Avrami constant (k), Avrami exponent (n), half-time of crystallization ($t_{1/2}$), and respective coefficients of determination (R_2) and melting point of isothermal crystallization for the blends before and after interesterification.

Samples	t_{SFC} (min)	SFC_{max} (%)	$t_{1/2}$ (min)	k (10^{-3} min^{-n})	n	R^2	melting point (°C)
SB6	5.00±0.01	10.38±0.24	9.00±0.03	0.005±1.42	2.81±0.03	0.98±0.02	50±0.30
ESL6	7.00±0.02	3.41±0.09	11.00±0.02	0.007±0.92	1.23±0.00	0.96±0.04	20±0.34
CSL6	6.00±0.01	4.97±0.03	13.00±0.04	0.021±1.54	1.86±0.02	0.98±0.02	35±0.47
SB12	4.00±0.02	19.18±0.17	7.00±0.07	0.025±1.00	2.25±0.02	0.99±0.00	60±0.40
ESL12	6.00±0.02	7.98±0.20	8.00±0.01	0.011±1.21	2.11±0.01	0.98±0.01	35±0.42
CSL12	5.00±0.01	8.89±0.14	9.00±0.03	0.017±1.25	2.25±0.01	0.98±0.01	45±0.17
SB18	3.00±0.02	33.49±0.27	9.00±0.03	0.042±1.02	2.04±0.01	0.99±0.00	75±0.56
ESL18	5.00±0.03	12.51±0.05	10.00±0.02	0.017±0.91	2.02±0.01	0.98±0.01	40±0.20
CSL18	3.00±0.02	29.88±0.16	9.00±0.03	0.070±0.87	2.70±0.02	0.99±0.00	60±0.41
SB24	3.00±0.00	45.95±0.58	7.00±0.08	0.058±0.71	1.81±0.01	0.99±0.00	81±0.51
ESL24	5.00±0.02	21.85±0.14	10.00±0.03	0.048±0.93	1.32±0.00	0.99±0.00	50±0.33
CSL24	3.00±0.00	41.66±0.09	7.00±0.08	0.065±0.54	1.90±0.01	0.99±0.00	65±0.46

Samples coded: SB = simple blend, ESL = enzymatic interesterification, CSL = chemical interesterification.

with an initial crystallization temperature at 34.64 °C (S_2U) (Fig. 3a). The other enzymatic SLs (ESL12, ESL18, and ESL24) presented two peaks related to the new formed TAGs, corresponding to the crystallization of FHCH (S_3) with high, medium and low melting point TAGs (S_2U, SU_2, and U_3), thus there was a reduction in the initial and final crystallization temperatures, thus the SLs crystallized at lower temperatures (between 38.19 and 40.87 °C) when compared to their SBs, thermal behaviors similar to those reported by Ribeiro et al. (2017).

The chemically synthesized SLs exhibited a peak related to FHCH (S_3), characterized by a high peak in the temperature range of 4.37 to 47.15 °C, especially in CSLs with higher FHCH content. This could be due to greater saturation or indications of steric hindrance. According to Cahoon & Schmid (2008), the high reactivity stability of saturated systems can hinder the catalyst's access to the active site, resulting in low efficiency in the interesterification process. This would explain the similarity between the crystallization profiles of CSLs and SBs. Such behavior is characteristic of fats with high melting points that are not yet fully stabilized (Humphrey & Narine, 2004; Neves et al., 2020).

3.7. Crystallization kinetics

To assess how interesterification influenced the crystallization process in SLs, crystallization isotherms were determined at 25 °C. Fig. 4 presents hyperbolic curves for SBs and after the interesterification reaction, SLs exhibited sigmoidal curves, indicating that crystallization occurred more slowly, except for CSL18 and CSL24 SLs, with still hyperbolic curves. In Table 3 is possible to observe which interesterification synthesis increased t_{SFC} and reduced SFC_{max}. This is due to the reduction of S_3, which are the inducing TAGs in the formation of crystallization nuclei due to their high melting point and simultaneously resulted in an increase S_2U and SU_2, indicating slower crystallization and altering the formation and structuring of the original crystalline

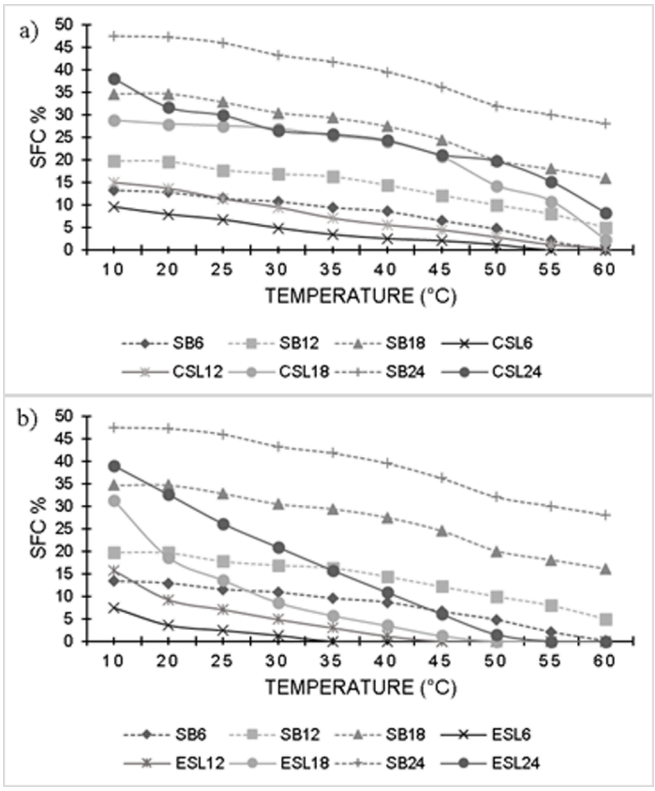

Fig. 5. Solid fat content (%) of simple blends and for chemical and enzymatic interesterification. Samples coded: SB = simple blend, ESL = enzymatic interesterification, CSL = chemical interesterification.

network in the blends. CSL18 and CSL24 SLs exhibited lower t_{SFC} values, similar to their SBs (3 min) and not increased the t1/2 values. According to da Silva et al., (2022) and Ribeiro et al., (2016), this behavior can be associated with high residual levels of minor compounds such as MAGs and DAGs, as well as high concentrations of TAGs formed by long-chain FAs (mainly C22:0), which have a high melting point and lower energy barrier to nucleation. This behavior is consistent with the reduction in k values and the randomization reaction of TAGs.

3.8. SFC

The results indicate that the interesterified samples have different profiles compared to their SBs. Enzymatically interesterified SLs (Fig. 5b) show SFC directly proportional to the gradual increase in saturation, with SFC ranging from 7.33 to 38.95 % at 10 °C, decreasing linearly until complete melting between 35 to 55 °C due to the formation of new TAGs with intermediate melting points. Similar results were found by Guedes et al., (2014) and Ribeiro et al., (2017). ESL6 showed reduced SFC, resulting in a fat with low plasticity not recommended for application in food products that require structure at room temperature. This can be explained by its low total saturated FA content (24.69 %), which should be above 25–30 %, ensuring minimal desirable plastic properties (Petrauskaite et al., 1998). Chemical interesterification showed higher SFC, with a non-linear behavior even with an increase in temperature (Fig. 5a), which may be characteristic of fats with high concentrations of S_3. Similar results were reported by Ribeiro et al., 2009, in chemical interesterifications with concentrations above 20 % of hard fats. The authors discuss the need for a longer tempering time to induce more ordered crystallization.

SFC suitable for each fat depends heavily on the type of application it will be directed to. Products such as spreads and table margarine should not have a high SFC at refrigeration temperatures (ideal SFC 30–35 % at 10 °C) (Arifin et al., 2011). The SLs in this study exhibited a suitable SFC, meeting the requirements for spreadable products, except for the ESL24 and CSL24 samples, which have an SFC of approximately 38 % at 10 °C. SFC between 20 to 22 °C is related to thermal resistance and stability against oil exudation. For food products requiring structure at room temperature (up to 25 °C), the SFC should be above 10 %. Thus, ESL6, CSL6, and ESL12 do not possess these characteristics as they exhibit low SFC (3.51, 7.88, and 9.19 %, respectively). ESL12, ESL18, and CSL12 exhibited suitable plasticity and hardness between 20 and 25 °C for creating products with crystalline structure resistant to deformities, such as baking fats, confectionery, and cookie fillings, which require stability, thermal resistance, and, at the same time, adequate softness (Wassell & Young, 2007; Wirkowska-Wojdyła et al., 2016). The CSL18, CSL24, and ESL24 exhibited significantly elevated SFC between 35 to 40 °C (25.34, 25.75, and 15.73 %), making direct application as a lipid base impractical. It would be advisable to add small proportions (1 to 5 %) of a liquid oil to adjust their physical properties (Ribeiro et al., 2009) or use them as structurants for other fats.

3.9. Consistency

In Table 4, it is possible to observe that the gradual increase of FHCH resulted in an increase in consistency. It was not possible to measure the consistency of the SB6 and ESL6 at 35 °C as they became semi-liquid due to their low melting point. Consistency decreased with the increase in temperature, due to the gradual melting of crystals forming less structured crystalline networks (Ribeiro et al., 2012). This behavior was also observed by Ribeiro et al., 2009.

Consistency of fats can be classified according to the parameters established by (Haighton, 1959). The ESL6, CSL6, ESL12, and CSL12 can be classified as very soft or non-spreadable (yield value < 200 gf/cm^2). The ESL18 and CSL18 showed satisfactory plasticity and spreadability (yield value from 200 to 800 gf/cm^2) at 10 °C and also met the requirements for SFC for application in margarines, spreads, and fillings. Additionally, they demonstrated satisfactory spreadability (yield value between 800 and 1000 gf/cm^2) at 25 °C, attributed to important sensory characteristics such as mouthfeel and low adhesiveness. The CLS24 is

Table 4
Consistency of simple blends and interesterified samples expressed in yield value (gf/cm^2).

T (°C)	Samples											
	SB6	**ESL6**	**CSL6**	**SB12**	**ESL12**	**CSL12**	**SB18**	**ESL18**	**CSL18**	**SB24**	**ESL24**	**CSL24**
10	10.63 ±0.2^{aA}	23.81 ±0.8^{aA}	61.43 ±2.1^{bA}	111.46 ±2.5^{aA}	98.03 ±1.7^{aB}	66.65 ±0.1^{aA}	693.18 ±2.4^{aA}	366.13 ±1.0^{aA}	396.40 ±0.2^{aA}	1840.97 ±0.2^{aA}	1829.32 ±3.0^{aA}	1481.61 ±3.6^{aA}
25	10.37 ±0.1^{aA}	14.56 ±6.0^{aA}	44.27 ±2.0^{bA}	72.89 ±3.0^{aB}	62.56 ±3.1^{aA}	61.12 ±0.5^{aA}	491.39 ±2.0^{aA}	257.17 ±1.6^{aA}	387.37 ±0.5^{aA}	1603.21 ±2.3^{aA}	756.35 ±4.2^{bB}	1126.16 ±3.2^{aA}
35	-	-	33.81 ±1.3^{A}	58.73 ±3.4^{aC}	59.54 ±1.2^{aA}	58.06 ±1.1^{aA}	336.42 ±1.7^{aA}	78.32 ±8.4^{bB}	382.48 ±0.3^{aA}	1579.53 ±1.2^{aA}	200.84 ±5.8^{cC}	890.21 ±4.0^{bB}

The values are expressed as mean ± standard deviation (n=4). Results marked with different letters showed significant differences ($p \leq 0.05$). Lowercase letters represent differences between samples, while uppercase letters indicate differences between sample and temperature. Samples coded: SB = simple blends, ESL = enzymatic interesterification, CSL = chemical interesterification. T = temperature in °C.

classified as very hard at all evaluated temperatures (yield value > 1500 gf/cm^2), and ESL24 is at the limit of spreadability at 10 °C but has satisfactory plasticity and spreadability from 25 °C. However, despite these lipid bases showing greater hardness, they may be suitable for applications in products that require greater thermal stability, such as coatings, fillings, and bouillon cubes. It is also possible to observe that consistency showed significant differences ($p < 0.05$) between samples and temperatures, especially in SLs with higher concentrations of saturated FAs, the variation in temperature may be related to different forms of packaging for formed crystal (Larsson, 1974).

4. Conclusions

Based on the results presented, chemical interesterification showed high concentrations of reaction intermediates, such as MAGs and DAGs, with higher TAG saturation, forming S_3 like BeBeBe, indicating further a steric hindrance. These changes in TAG composition influenced the thermal properties and crystallization kinetics of the CSLs, which showed TAGs with higher melting points compared to the ESLs that formed new TAGs with intermediate melting points and exhibited improved SFC, crystallization, and consistency, resulting in SLs with plastic fat characteristics suitable for various food applications. In contrast, CSLs and ESL24 had higher SFC and harder consistency, limiting their direct use as a lipid base and making them preferable as hard fat or in products requiring greater thermal stability. It should be noted that both catalytic processes were effective in lipid modification. However, in this study, SLs obtained through enzymatic interesterification were preferable as they encompassed plastic fat characteristics suitable for various food applications. Furthermore, we consider enzymatic interesterification to be more advantageous in terms of sustainability. To confirm the technological and nutritional potential of these SLs, our research group has continued ongoing studies on their application in various food categories, aiming for alternatives to trans and palm fats.

CRediT authorship contribution statement

Vanessa Alves: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Guilherme de Figueiredo Furtado:** Writing – review & editing, Supervision, Methodology, Formal analysis, Data curation, Conceptualization. **Valdecir Luccas:** Writing – review & editing, Supervision, Methodology. **Ana Paula Badan Ribeiro:** Methodology. **Juliana Alves Macedo:** Project administration. **Gabriela Alves Macedo:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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