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Production and characterization of structured lipids with antiobesity potential and as a source of essential fatty acids

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ABSTRACT

The objective of this work was to produce structured lipids (SLs) from olive oil (O), soybean oil (S), and fully hydrogenated crambe oil – FHCO (C) mixtures by enzymatic interesterification, comparing Lipozyme TL IM and *Rhizopus* sp. performances as catalysts, and to evaluate their physical and chemical modifications. Among other blends (OC 90:10 w/w and SC 90:10 w/w), OSC (45:45:10, w/w), presented the most nutritionally interesting amounts of mono- and polyunsaturated fatty acids, as well as behenic acid. Interesterification caused an increase in crystallization time and a decrease in the solid fat content in all blends. The fatty acid redistribution in the TAGs caused a change in thermal behavior, leading to a decrease in the onset and end temperatures during crystallization, which indicates that new TAGs were formed. Regarding regiospecificity, Lipozyme TL IM lipase acted randomly, while *Rhizopus* sp. lipase was specific for the *sn*-1,3 position. Therefore, it was possible to synthesize SLs presenting different physical and chemical properties, compared to the original OSC blend, containing behenic acid at the *sn*-1,3 position and unsaturated fatty acids at the *sn*-2 position, by enzymatic interesterification catalyzed either by Lipozyme TL IM or by *Rhizopus* sp. lipases.

1. Introduction

Structured lipids (SLs) are defined as restructured or modified triacylglycerols (TAGs) obtained by means of chemical or enzymatic interesterification from triacylglycerols containing short, medium, and/or long chain fatty acids, vegetable or animal fats, or genetic engineering. They are synthesized for the purpose of obtaining nutraceutical or functional lipids, which may provide specific health benefits, improve or modify the physical, chemical, and rheological characteristics of oils and fats, and alter or increase the nutritional properties of foods (D'Agostini & Gioielli, 2002; Osborn & Akoh, 2002). The change in the triacylglycerol classes caused by the interesterification reaction can be verified by means of physical and chemical analyses, due to the fact that structured lipids may present different triacylglycerol composition, solid fat content, crystallization behavior, thermal properties and consistency when compared to the original lipid system (Ribeiro, Basso, Grimaldi, Gioielli, Cardoso and Gonçalves, 2009).

Structured low-calorie lipids have been widely used in absorption studies and in clinical nutrition to combat obesity. Previous studies report their production by interesterifying vegetable oils and behenic acid blends (Arishima, Tachibana, Kojima, Takamatsu, & Imaizumi, 2009; Kojima et al., 2013; Kojima et al., 2010). Pancreatic lipase is a digestive enzyme that catalyzes the hydrolysis of triacylglycerols into fatty acids and monoacylglycerols in the intestine. Behenic acid inhibits the performance of pancreatic lipase and consequently prevents the hydrolysis of triacylglycerols and their absorption, promoting the reduction of calories (Arishima et al., 2009). This inhibition can be explained by non-formation of the enzyme-substrate complex (Kojima et al., 2013) or by the low diffusion of behenic acid in the oil phase, which would block the lipase interface (Kanjilal, Prasad, Kaimal, Ghafoorunissa, & Rao, 1999), thus resulting in the prevention of hydrolysis and excretion of fatty acids by feces, providing an antiobesity effect.

Behenic acid can be obtained by the hydrogenation of crambe oil (*Crambe abyssinica Hochst*), which consists of up to 57% erucic acid (C22:1), and can be applied in a diversity of industrialized products (Pitol, 2010). However, it cannot be used for human consumption due

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to its high degree of toxicity, possibly leading to heart lesions and disturbance of other physiological processes. However, when completely hydrogenated, the double bond of erucic acid in its composition is hydrolyzed, leading to its conversion to behenic acid (C22: 0), which can be ingested without nutritional restrictions (No, Zhao, Kim, Choi, & Kim, 2013).

Another important function of structured lipids is to supply essential fatty acids such as linoleic (18:2n-6) and linolenic acids (18:3n-3) (Cao et al., 2013; Rodrigues-Ract, Cotting, Poltronieri, Silva, & Gioielli, 2010; Silva et al., 2011), which are found in vegetable oils, such as soybean oil. These fatty acids are essential for growth and development throughout the human life cycle, as well as promotion of improvements in health (Stein, 1999; Wen Chang, Ten Wu, Na Chen, & Chao Huang, 2004). Similarly, oleic acid (18: 1n-9) (Nunes, Pires-Cabral, Guillén, Valero, & Ferreira-Dias, 2012; Vistisen, Mu, & Høy, 2006), largely found in olive oil, although not essential, plays a very importante role in reducing the risks of metabolic syndrome (Sales-Campos, Souza, Peghini, Silva, & Cardoso, 2013).

Osborn and Akoh (2002) incorporated unsaturated long-chain fatty acids to the *sn*-2 position of triacylglycerols, aiming to increase their bioavailability, as pancreatic lipase exerts less activity on these fatty acids when they are esterified to *sn*-1 and *sn*-3 positions. For this reason, the location of behenic acid at the *sn*-1,3 positions can favor low lipid absorption, without compromising the fatty acids located at *sn*-2. Based on this perspective, the synthesis of this type of SL requires modifications at specific positions in the glycerol backbone, which can be obtained with enzymatic interesterification. Lipases are more promising when specific positional modification of triacylglycerols is aimed, besides generating fewer residues in the production of SLs. In addition, the enzyme can be reused repeatedly, and minor lipids, as well as other compounds with bioactive function, are preserved due to milder reaction conditions when compared to chemical interesterification (Iwasaki & Yamane, 2000).

Therefore, the objective of the present research was to synthesize and characterize low calorie structured lipids with improved nutritional properties, in terms of their physical and chemical characteristics, aiming the prevention and treatment of obesity, as demonstrated by Moreira, Santos, Gambero, and Macedo (2017). Soybean and olive oils were used as a source of ω-3 and ω-6 fatty acids, while fully hydrogenated crambe oil represent a source of behenic acid.

2. Material and methods

2.1. Raw materials

Olive oil, soybean oil, and fully hydrogenated crambe oil (FHCO) were used. The extra virgin olive oil, Arbequina variety, was donated by Olivas do Sul (Cachoeira do Sul, Rio Grande do Sul, Brazil). Soybean oil was purchased from a local market in Campinas, São Paulo, Brazil, and the fully hydrogenated crambe oil was donated by Cargill. Immobilized lipase from *Thermomyces lanuginosa* (Lipozyme TL IM) from Novozymes A/S, was kindly donated by the manufacturer and crude lipase from *Rhizopus* sp. was obtained by solid fermentation in the Laboratory of Food Biochemistry of the School of Food Engineering, State University of Campinas, São Paulo, Brazil.

2.2. Obtaining Rhizopus sp. lipase

The crude lipase from *Rhizopus* sp. was obtained according to Macedo, Pastore, and Rodrigues (2004) using the culture medium composed of 40% distilled water and 60% wheat bran (m/m), and incubated at 30 °C for 120 h. The medium was filtered and concentrated with ammonium sulfate until it reached 80% saturation. It was then centrifuged and dialyzed using cellulose membrane (Sigma-Aldrich), against distilled water for 24 h, under refrigeration. Then, the material was frozen and lyophilized, called crude enzyme preparation. The crude lipase activity was determined according to Macedo, Park, and Pastore (1997). A unit of lipase activity (U) was defined as the quantity of enzyme required to release 1 μmol of fatty acid in one minute per milligram of enzyme at 37 °C.

2.3. Characterization of raw materials

Olive, soybean, and fully hydrogenated crambe oils (FHCO) were characterized for their composition in fatty acids (AOCS Ce 1f-96-2009), Iodine and saponification values were obtained according to the methods AOCS Cd 1C-85 (2009) and AOCS Cd 3a-94 (2009), respectively.

2.4. Enzymatic interesterification

Structured lipids (SLs) were produced by interesterification of three blends in different proportions. The first one was prepared with olive oil and FHCO (OC) in the proportion 90:10 (m/m); the second with olive oil, soybean oil, and FHCO (OSC) in the proportion of 45:45:10 (m/m); and the third with soybean oil and FHCO (SC) in the proportion 90:10 (m/m). The maximum amount of FHCO was 10% with the intention to maintain the blends in the liquid state at 60 °C, which corresponds to the maximum temperature of lipase activity.

The interesterification reactions were carried out according to Speranza, Ribeiro, and Macedo (2016), with modifications, in hermetically sealed amber glass bottles with added nitrogen gas to prevent oxidation. The reaction medium was kept under agitation by an orbital-shaking water bath at 180 rpm for 24 h at 60 °C. The samples were melted at 80 °C and then cooled and held at 60 °C for 10 min or until stabilized at this temperature. Subsequently, 5% (w/w) enzyme was added to the substrate or 60 U per gram of lipase total substrate Lipozyme TL IM and or 13 U per gram of *Rhizopus* sp. After the reaction, the enzymes were removed with a 0.45 μm pore polyvinylidene fluoride (PVDF) Millex hydrophilic membrane and stored in a freezer at − 20 °C. The reaction was done on a 100 g substrate scale. The characterization analyzes were done in duplicates.

2.5. Physicochemical characterization of simple mixture and structured lipids

After removal of the lipase by filtration, the structured lipids were purified using ethanol (3:1) and the mixture was maintained in a water bath at 80 °C for 5 min. The alcohol phase was discarded, the procedure was repeated two more times, according to Farmani, Safari, and Hamedi (2006), and the structured lipids were then characterized.

2.5.1. Fatty acid composition

The fatty acid composition analyses were carried out in a gas chromatograph (CGC Agilent 6850 Series GC System), after fatty acid methyl esters (FAMEs) preparation, according to Hartman and Lago (1973). The FAMEs were separated using the AOCS method Ce 1f-96 (2009) using a DB-23 Agilent capillary column (50% Cyanopropyl-methylpolysiloxane), with the dimensions: 60 m length × 0.25 mm internal diameter × 0.25 μm thickness. The operation conditions of the chromatograph were: column flow = 1.0 mL/min; linear velocity = 24 cm/s; detector temperature = 280 °C; injector temperature = 250 °C; oven temperature = 110–215 °C to 5 °C/min, 215 °C for 24 min; carrier gas - helium; injection volume = 1.0 μL; injection split, ratio 1:50. The qualitative composition was determined by comparing the peak retention times with the respective standards for fatty acids.

2.5.2. Partial acylglycerol content (HPSEC)

The classes of acylglycerol compounds were determined according to Dobarganes, Velasco, and Dieffenbacher (2000), using a liquid chromatograph (Perkin Elmer LC-250) with refractive index detector (Sicon Analytic). Two divinylbenzene (DVB) Jordi gel columns were