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The effects of enzymatic interesterification on the physical-chemical properties of blends of lard and soybean oil

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ABSTRACT

The main goal of the present research effort was to evaluate the physical-chemical properties of blends of lard and soybean oil following enzymatic interesterification catalyzed by an immobilized lipase from *Thermomyces lanuginosa* (Lipozyme™ TL IM). Lipase-catalyzed interesterification produced new triacylglycerols that changed the physical-chemical properties of the fat blends under study. Solid fat content (31.3 vs 31.5 g/100 g), consistency (104.7 vs 167.6 kPa), crystallized area (0.6 vs 11.8) and softening point (31.8 vs 32.2 °C) of lard increased after interesterification, and this was mostly due to the increase of SSS (saturated) + SSU (disaturated-monounsaturated) triacylglycerols. These contents (SSU + SSS) increased in lard after interesterification from 42.9 to 46.7 g/100 g. The interesterified blends exhibited lower values for the physical properties when compared with their counterparts before enzymatic interesterification. The interesterification of blends of lard with soybean oil increased the amounts of UUU (triunsaturated) and SSS triacylglycerols and reduced the amounts of UUS (diunsaturated-monosaturated) triacylglycerols. The interesterified blends of lard and soybean oil demonstrated physical properties and chemical composition similar to human milk fat and they could be used for the production of a human milk fat substitute.

1. Introduction

Structured lipids (SLs) are often referred to as a new generation of fats that can be considered as nutraceuticals: foods or parts of foods that provide medical or health benefits beyond basic nutrition, including the potential for prevention and/or treatment of certain diseases (Akoh, 2002). SLs are produced by chemical or enzymatic modification of triacylglycerols (TAGs) (Willis & Marangoni, 1999). In the past few years, enzymatic modification of TAGs has gained preference over chemical modification (Alim et al., 2008; Fomuso & Akoh, 2002; Haman & Shahidi, 2005; Lee et al., 2008; Xu, Fomuso, & Akoh, 2000).

The use of lipases for the modification of fats and oils has many benefits when compared to chemical processes. Lipases are well known for their efficacy under mild reaction conditions (pH, T and P), leading to reduced costs and energy consumption. The use of lipases in a natural reaction system can also reduce environmental pollution by reducing production of side products, since these reactions mimic natural pathways. The availability of lipases from a wide range of biological sources offers a tremendous potential for a post-production modification in industrial application (Yamane, 1987). However, the most important property of lipases that has led to their overwhelming interest remains their specificity. This property has been shown to be a versatile tool for the preparation of a wide variety of novel TAGs (Ghazali, Hamidah, & Che Man, 1995; Marangoni & Rousseau, 1995; Willis, Lencki, & Marangoni, 1998).

Typical applications of SLs include (but are not limited to) margarines, modified fish oil products, cocoa butter, human milk fat replacer, and many other lipid products (Haman & Shahidi, 2005; Jensen, 2001; Nielsen, Yang, Xu, & Jacobsen, 2006).

Human milk contains ~3–5 g/100 g total lipids, with more than 90 g/100 g of milk fat being constituted by TAGs, in which fatty acids represent ~88 g/100 g of the total lipids (Chang, Abraham, &

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John, 1990). TAGs are a major source of energy in both breast milk and infant formulæ, providing half of the dietary energy (Kennedy et al., 1999).

Human milk fat (HMF) contains mostly long-chain fatty acids. The major saturated fatty acid (SFA) in the human milk fat TAGs is palmitic acid (16:0), which represents about a fourth of the fatty acids present in breast milk. HMF contains ~70 g/100 g of its palmitic acid esterified at the *sn*-2 position of TAGs. Stearic (~5–7 g/100 g), oleic (~30–35 g/100 g), and linoleic acids (~7–14 g/100 g) are generally esterified at the *sn*-1,3 positions of TAGs (Nielsen et al., 2006; Silva et al., 2007). This feature gives a unique structure to HMF, and lard is the only animal fat that has a similar structure (Chang et al., 1990). 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 (Filler, Mattson, & Fomon, 1969; Thomson, Keelan, Garg, & Clandinin, 1989). 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, and resulting in reduced absorption of both calcium and fat (Lien, 1994; Lien, Boyle, Yuhas, Tomarelli, & Quinlan, 1997; Lucas et al., 1997). Due to the formation of such calcium soaps, stool hardness, constipation and, in some cases, bowel obstructions may occur. Thus, presence of palmitic acid acyl residues at the *sn*-2 position of HMF increases absorption of 16:0 in the infant and reduces calcium losses in the faeces (Chappel, Clandinin, Kearny-Volpe, Reichman, & Swyer, 1986). Therefore, such unique TAG molecules with palmitic acid esterified at the *sn*-2 position has a significant function in the development of infants.

Recently, many research studies were done attempting to produce SLs with a composition similar to that of HMF for use in infant milk formulæ or as HMF substitute (Maduko, Akoh, & Park, 2007a, 2007b; Mukherjee & Kiewitt, 1998; Sahin, Akoh, & Karaali, 2005; Silva & Gioielli, 2006; Srivastava, Akoh, Chang, Lee, & Shaw, 2006; Thomson et al., 1989).

The main goal of the research effort entertained herein was to produce SLs via enzymatic interesterification of lard and soybean oil blends catalyzed by a immobilized commercial *sn*-1,3-specific lipase from *Thermomyces lanuginosa*, and to characterize both chemical and physical properties of the structured lipids thus produced. These new lipids could be applied to child nutrition resembling human milk fat.

2. Materials and methods

2.1. Materials

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

2.2. Methods

2.2.1. Reactant blend preparation

Lard and soybean oil were blended in 80:20, 60:40 and 50:50 (w/w) proportions, respectively. These proportions were used in previous studies of our group and showed similar fatty acid composition and physical properties in relation to human milk fat of Brazilian mothers (Silva et al., 2007; Silva & Gioielli, 2006). The blends were prepared after complete melting of the fats at 70–80 °C for 30 min under magnetic stirring, and the mixtures thus obtained were stored at refrigeration conditions (~4 °C).

2.2.2. Performance of interesterification reactions

Before the interesterification, the immobilized lipase was deaerated according to the protocol described by the supplier. Lipase-catalyzed interesterification reactions were carried out in a magnetically stirred cylindrical glass reactor thermostatted at 60 °C. For each reaction, thermal equilibration of the appropriate binary mixture (60 °C) was allowed to proceed for ~10 min; after this time period, a known amount of commercial lipase preparation (5 g/100 g) was added to the mixture and the reaction was allowed to proceed under mild (~750 rpm) magnetic stirring for a timeframe of 6 h (Criado, Hernandéz-Martins, Lopéz-Hernadéz, & Otero, 2007; Díaz Gamboa & Gioielli, 2003; Gioielli et al., 1994; Zhang et al., 2001). The headspace above the reaction mixture was continuously supplied with (anhydrous) nitrogen. By the end of the reaction timeframe, the remaining interesterified blend was filtered using plain filter paper, and then stored at −18 °C until analysis was in order.

2.2.3. Determination of fatty acid composition

Fatty acids in the triacylglycerols of the interesterified mixtures were converted into fatty acid methyl esters (FAMEs). A rapid preparation of methyl esters for gas chromatographic analysis has been accomplished by saponifying fats with 0.5 mol equi/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 sulphates during the reaction were avoided, and the degree of esterification was approximately 99.5 g/100 g (Hartman & Lago, 1973; Rodrigues & Gioielli, 2003; Rodrigues, Torres, Mancini-Filho, & Gioielli, 2007). Analyses of FAMEs were carried out in a Varian GC gas chromatograph (model 3400CX, from Varian Ind. Com. Ltda., Brazil), equipped with a split-injection port, a flame-ionization detector and a software package for system control and data acquisition (model Star Chromatography Workstation version 5.5). Injections were performed in a 30-m fused silica capillary column (ID = 0.25 mm) coated with 0.25 μm of CP-Wax 52CB (Chrompack, Chromtech, MN, USA) using helium as carrier gas at a flow rate of 1.5 mL/min and a split ratio of 1:50. The injector temperature was set at 250 °C and the detector temperature was set at 280 °C. The oven temperature was initially set at 150 °C for 5 min, then programmed to increase to 215 °C at a rate of 3 °C/min. After drawing up air into the filled syringe (sample volume 1 mL) and inserting the needle into the heated injector, samples were injected manually after a dwell-time of ~2 s. Qualitative fatty acid 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 (1997a, 1997b) Official Method Ce 1-62. All samples were analyzed in duplicate and the reported values are the average of the two runs.

2.2.4. Softening point

The softening point of reaction samples was determined using the open tube melting point method, according to the AOCS (1997a, 1997b) Official Method Cc 3-25. These analyses were performed in triplicate.

2.2.5. Consistency

Consistency of samples withdrawn from the reaction mixture was determined via penetration tests using a 45° acrylic cone fitted to a constant speed Texture Analyzer (model TA-XT2, from Stable Micro Systems, Surrey, UK). The consistency was calculated as a "yield value" (KPa), according to the equation proposed by Haighton (1959) (see Eq. (1)).