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Lipase-catalyzed synthesis and characterization of flaxseed oil-based structured lipids

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ABSTRACT

The biosynthesis of structured lipids (SLs) was carried out by the interesterification of flaxseed oil (FO) and tricaprylin (TC) in an organic solvent medium (OSM), using selected commercial lipases, including Amano DF, Novozym 435, Lipozyme TL-IM and Lipozyme RM-IM. The fatty acyl chains of the synthesized triacylglycerols (TAGs) were identified by atmospheric pressure chemical ionization/mass spectrometric (APCI/MS) analysis, while the fatty acid positional distribution of the MLM- and MML-SLs (M-medium and L-long chain fatty acids) was determined by silver-ion high-performance liquid chromatographic (Ag^+/HPLC) analysis. The effects of reaction temperature (T_r, 30–50 °C), enzyme concentration (E_c, 0.5–4%, w/v), initial water activity (a_w, 0.05–0.43) and reaction time (R_t, 0–72 h) on the efficiency of the enzymes, were studied. The bioconversion yield (%) of the synthesized MLM- and MML-SLs was monitored under the established reaction parameters for each lipase. The maximum yield of MLM-SLs was obtained in the order, of Novozym 435 > Lipozyme TL-IM > Lipozyme RM-IM > Amano DF. Moreover, considering the ratio of the MLM- to MML-SLs produced by each enzyme, Novozym 435 and Lipozyme TL-IM were selected as the most effective enzymes for interesterification of FO and TC.

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1. Introduction

The functional and physical properties, metabolic fate and putative health benefits of various fats and oils are different because of the component fatty acid (FA) residues and their position on the glycerol backbone of triacylglycerols (TAGs) (Akoh, Lee, & Fomuso, 1998; Goderis et al., 1987). Specialty lipids referring to fats and oils with special functional or nutritional properties, include a wide range of products amongst which structured lipids (SLs) are a main class (Xu & Akoh, 2002). Structured lipids, which imply the change in the composition and positional distribution of fatty acids in the triacylglycerols, could be obtained either chemically or enzymatically (Osborn & Akoh, 2002). Through enzymatic transesterification, it is possible to incorporate the desired acyl groups onto specific positions of glycerol; on the other hand, the chemical process is lacking the specificity and may hence lead to the formation of side products, with a subsequent decrease in the yield (Lee & Akoh, 1998a).

Among various types of SLs, MLM-type structured SLs (MLM-SLs), the medium chain FAs (MCFAs) and the essential long chain FAs (LCFAs), esterified respectively, at the *sn*-1,3 and *sn*-2 positions of the glycerol backbone, have been the subject of great interest. Pancreatic lipase is a *sn*-1,3 regioselective enzyme and hydrolyzes the TAGs into *sn*-2 monoacylglycerols (2-MAGs) and free FAs, where the liberated MCFAs are readily absorbed by the portal route and rapidly oxidized in the liver providing energy (Odle, 1997); the 2-MAGs of LCFAs are efficiently absorbed through the lymphatic system (Bugaut, 1987). Hence, MLM-SLs could be used for a

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balanced diet for patients and infants with special requirements (Lee & Akoh, 1998b).

Lipase-catalyzed acidolysis, in which the MCFAs and vegetable oils are used as the sources of acyl donors and essential LCFAs, respectively, is one of the most common approaches for the synthesis of MLM-SLs (Hamam & Shahidi, 2008; Kim & Akoh, 2005; Nunes, Pires-Cabral, & Ferreira-Dias, 2011). However, lipase-catalyzed interesterification seems to be a more proper route than acidolysis with fewer complications in the recovery of SLs (Osorio, Ferreira-Dias, Gusmão, & Da Fonseca, 2001). Although acidolysis reactions, used for the synthesis of MLM-SLs, have been reported extensively in the literature, there is little information on the lipase-catalyzed interesterification between TAGs or ethyl esters of short or medium chain FAs and heterogeneous TAGs, such as flaxseed oil (FO) (Irimescu, Hata, Iwasaki, & Yamane, 2001; Shin, Akoh, & Lee, 2010; Yang, Fruekilde, & Xu, 2003). With the growing recognition of the importance of dietary ω-3 FAs, FO with its high content of linolenic acid has been increasingly used to produce healthy oils (Chen, Ma, Liang, Peng, & Zuo, 2011; Gunstone & Harwood, 2007).

The considerable resolving power of reversed-phase high-performance liquid chromatography (RP-HPLC), is used as an appropriate technique for the separation of TAGs. The main difficulty, when analyzing complex mixtures, is the separation of TAGs with the same equivalent carbon numbers (ECN) value, where the positional isomers cannot be determined (Andrikopoulos, 2002; Buchgraber, Ulberth, Emons, & Anklam, 2004). Silver-ion chromatography is commonly used for the separation of TAG isomers, where the elution order is dependent on the increasing degree of FA unsaturation (Andrikopoulos, 2002).

The aim of the present study, which is a part of an ongoing research work (Bai et al., in press), was to characterize the TAGs, synthesized by the interesterification of FO and tricaprylin (TC) in organic solvent media (OSM), and to investigate the efficiency of selected commercial lipases for the synthesis of MLM- and MML-SLs. The effects of selected parameters, including reaction temperature (T_r), enzyme concentration (E_c), solvent initial water activity (a_w) and reaction time (R_t), on the bioconversion yield (%) were investigated.

2. Materials and methods

2.1. Materials

Flaxseed oil (FO) was a gift from Arista Industries, Inc. (Wilton, CT, USA). Selected commercial immobilized lipases including, Novozym 435 from *Candida antarctica*, Lipozyme RM-IM from *Rhizomucor meihei* and Lipozyme TL-IM from *Thermomyces lanuginosus*, were acquired from Novozymes Nordisk A/S (Bagsværd, Denmark). Lipase DF from *Rhizopus oryzea* was donated by Amano Enzyme (Nagoya, Japan). Porcine pancreatic lipase, bile salts, tricaprylin (purity > 99%) and rod shaped molecular sieves (3 Å) were purchased from Sigma-Chemical Co. (St-Louis, MO, USA). All HPLC grade organic solvents and ACS grade salts, used for pre-equilibration of the reaction medium initial water activity (a_w), were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Gas–liquid chromatography (GLC) standards were purchased from Nu-Check Prep (Elysian, MN, USA).

2.2. Fatty acid composition and positional distribution

2.2.1. Preparation of FAMEs

The fatty acid (FA) profile of FO was obtained by GLC analysis of the methylated FAs. Fatty acid methyl esters (FAMEs) were obtained according to a modification of the method of Rocha-Uribe and Hernandez (2004). Five to ten milligrams of flaxseed oil were solubilized in 0.6 mL of hexane and 60 μL of 2 M sodium methoxide in 20% methanol. The mixture was incubated at 65 °C in a reciprocal shaking water-bath (Model 25, Precision Scientific, Chicago, IL, USA). After 20 min of incubation, 1 mL of a 10% sulphuric acid solution in methanol was added to the mixture, followed by incubation for 30 min in a water-bath at 85 °C. The FAMEs were extracted twice with 4 mL of hexane and recovered for analysis.

2.2.2. GLC analysis of FAMEs

The FAMEs were analyzed by GLC, using an Agilent 6890 Series Chromatograph (Agilent Technologies, Wilmington, DE, USA), equipped with an HP-INNOWax column (30 m × 30 mm I.D. × 0.25 μm film thickness) purchased from Agilent Technologies, with flame ionization detector (FID) and ultra-high purity helium as the carrier gas at a flow rate of 1 mL/min with a split ratio of 1:20. The injector and detector temperatures were set at 150 and 230 °C, respectively. The initial column temperature was 150 °C for 1 min before its increase to 180 °C, at a rate of 10 °C/min, followed by a 1 °C/min increase rate to 220 °C within 40 min and held there for an additional 5 min. The FAMEs were identified by comparing their retention times with those of the standards. Nonadecanoic acid (19:0) was used as the internal standard for quantitative determination of FAs.

2.2.3. sn-2 Positional analysis

Pancreatic hydrolysis was used to determine the positional distribution of FAs in flaxseed oil (Luddy, Barford, Herb, Magidman, & Riemenschneider, 1964). Five milligrams of flaxseed oil were mixed with 2 mL of Tris–HCl buffer (1 M, pH 7.6), 0.5 mL of 0.05% of bile salts, 0.2 mL of 2.2% $CaCl_2$ and 3 mg of pancreatic lipase. The mixture was incubated in a water-bath at 37 °C for 3 min, then vortexed vigorously and extracted 2 times with 3 mL diethyl ether. The components of the mixture were then separated by preparative thin-layer chromatography (TLC), using Silica gel 60 GF plates of 20 × 20 cm with fluorescent indicator (Whatman, Fisher Scientific, Fairfield, NJ, USA), where the extracted sample was diluted in 100 μL chloroform and deposited on the TLC plate. The separation was performed according to the method described by Hita et al. (2007) in a development chamber, containing a solvent mixture of chloroform/acetone/acetic acid (96:8:2, v/v/v). The separated bands were visualized under UV (235 nm) in a fluorescence analysis cabinet (Spectroline, Model CX20, Westbury, NY, USA) and the band corresponding to the 2-MAGs was scraped-off and extracted 3-times with 4 mL of diethyl ether. The suspension of the extracted product was filtered through a fritted glass filter and concentrated by

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