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High performance liquid chromatographic separation of interesterified palm oil with tributyrin

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Abstract

Short-chain triacylglycerol, tributyrin (glyceryl-*sn*-1,2,3-tri-butyric acids), was interesterified with palm oil to produce mixtures of structured triacylglycerol (SL-TAG) species as a low-calorie lipid. Lipozyme RM IM from *Rhizomucor miehei*, known as a 1,3-regioselective immobilized lipase, was used as a catalyst. During 24 h reaction, the reaction mixture was analyzed with Hypersil® BDSCPS high performance liquid chromatography (HPLC) column to determine neutral lipids composition. Also, the compositional changes of TAG, as well as monoacylglycerol (MAG)/diacylglycerol (DAG) as by-products, were determined. After 24 h, 5.8 g/100 g DAG was found in the reaction mixture, while 89.5 g/100 g TAG was observed. The reaction mixture was also analyzed with Nova-Pak® C18 and Chrompack Si60 columns for separation of SL-TAG species. It was found that the normal-phase (NP)-HPLC with Chrompack Si60 column readily separated SL-TAG species composed of short- and long-chain acyl residues. Further, mass spectrometer was employed for identifying the separated SL-TAG molecules. SL-TAG containing two butyryl and one long-chain acyl residue (e.g., PBB and OBB) were most abundant in the interesterified product.

Keywords: APCI mass spectrometry; Evaporated light-scattering detection; High performance liquid chromatography; Interesterification; Palm oil; Tributyrin

1. Introduction

Palm oil is one of the traditional fats is human diet and has been widely used throughout the world. In 2005 global palm oil production was 33,733,000 metric tones, accounting for 24% of the worldwide production of total dietary oil (Basiron, 2007). Recently, compositional modification of palm oil is an important application, in which functional fatty acids (i.e., conjugated fatty acid, medium- or short-chain fatty acid, omega-3 fatty acid) are incorporated into triacylglycerol (TAG) molecules in palm oil.

Structured lipids (SL) are TAG molecules that have intentionally predetermined fatty acyl groups on the glycerol backbone. Through lipase-catalyzed interesterification, for example, such modified fats/oils can be obtained by exchanging acyl groups between ester bondages of lipids, resulting in improved characteristics due to the change in composition and distribution of fatty acids (Akoh, 1995; Akoh & Moussata, 2001; Iwasaki & Yamane, 2000; Lee & Akoh, 1998; Senanayake & Shahidi, 2002; Torres, Munir, Blanco, Otero, & Hill, 2002; Willis, Lencki, & Marangoni, 1998). However, during interesterification between TAG molecules, mono- (MAG) and diacylglycerol (DAG) are produced as by-products along with the newly synthesized SL-TAG.

Use of SL as a low-calorie lipid is of interest. SL with saturated short-chain fatty acids can be intentionally produced because they provide about 3.5–6 kcal/g whereas long-chain fatty acids provide about 9–9.5 kcal/g. SALATRIM™ (*short and long acyltriglyceride molecules*) is one example of low-calorie lipid, produced by interesterification

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of highly hydrogenated vegetable oils with TAGs of short-chain fatty acids (acetic, propionic, and/or butyric acids) (Huang et al., 1994; Smith, Finley, & Leveille, 1994; Softly et al., 1994). Recently, separation of SL on reverse or normal-phase (NP) high-performance liquid chromatography (HPLC) and detection with evaporated light-scattering detector (ELSD) have been extensively studied (Lee, Jones, Lee, Kim, & Foglia, 2003; Ruiz-Gutiérrez & Barron, 1995) because the ELSD is not very sensitive to the change in mobile phase gradient during separation. Otherwise, refractive index detector is recommended for isocratic separation while ultraviolet detector has limitation on the detection of lipid species that lack double bonds. Generally, HPLC is suitable for the separation of TAG molecules while analysis of TAG containing short-chain fatty acids using NP- and reverse-phase (RP)-HPLC has not been widely applied. Mangos, Jones, and Foglia (1999) reported the NP-HPLC separation of TAG molecules containing acetic and long-chain fatty acids in which interesterification of triacetin and hydrogenated soybean oil was conducted.

In this study, an immobilized *Rhizomucor miehei* lipase (Lipozyme RM IM) was used for interesterification of palm oil with tributyrin to produce SL as a low-calorie lipid. During 24 h reaction, compositional changes of TAG as well as MAG/DAG as by-products were studied. The compositional changes of SL-TAG molecular species were examined using RP-HPLC. Finally, the reaction mixture was separated by NP-HPLC with mass spectrometric detection (MSD) to characterize the individual SL-TAG molecular species in SL products.

2. Material and methods

2.1. Materials

Tributyrin (glyceryl-*sn*-1,2,3-tri-butyric acids), tripalmitin (glyceryl-*sn*-1,2,3-tri-palmitic acids), tristearin (glyceryl-*sn*-1,2,3-tri-stearic acids), triolein (glyceryl-*sn*-1,2,3-tri-oleic acids), and trilinolein (glyceryl-*sn*-1,2,3-tri-linoleic acids) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Palm oil was a gift from CJ Co. (Seoul, Korea). The fatty acids composition of palm oil (g/100 g) are C16:0, 44.7; C18:0, 5.4; C18:1, 39.8, and C18:2, 10.2. All solvents were HPLC grade. Hexane, acetone, acetonitrile and ethylene chloride were obtained from Burdick & Jackson (Muskegon, MI, USA). Methyl-*t*-butyl ether (MTBE) was obtained from J.T. Baker Inc. (Phillipsburg, NJ, USA). Immobilized lipase (Lipozyme RM IM) was provided by Novo Nordisk Biochem North America Inc. (Franklinton, NC, USA).

2.2. Procedures for obtaining interesterified lipids

Tributyrin (0.5 g) was interesterified with an equal weight of palm oil using RM IM lipase (0.1 g, 10 wt% of substrates). Reactions were performed for 24 h at 65 °C. A screw-cap vial placed in a water-jacketed beaker with magnetic stirring was used as a reactor. During 24 h interesterification, reaction mixture was obtained at the designated reaction time (1, 3, 5, 7, 14, and 24 h) and was diluted with 100 mL hexane. Then, aliquot (3 mL) was filtered through a disposable Fluoropore PTFE membrane filter (Sigma Chemical). After evaporating hexane under nitrogen, each sample was re-dissolved in hexane (for normal phase) or acetone (for reverse phase) for HPLC analysis (Lee, Jones, & Foglia, 2002). As standards for HPLC, 0.5 g each of tripalmitin, tristearin, triolein, and trilinolein were, respectively, interesterified with an equal weight of tributyrin under the reaction conditions described above.

2.3. Analysis of interesterified lipids by HPLC

For separation of TAG, DAG, and MAG, a Hypersil® BDSCPS column (4.6 mm × 250 mm, 5 μm, Thermo Electron Corp., UK) was used in the HPLC system, which includes Younglin SP 930D dual pump (Younglin, Anyang, Korea). Sedex 75 ELSD (Afortvill, France) was operated at 40° with nitrogen as a nebulizing gas at a pressure of 2.2 bar. A binary solvent system of hexane and methyl-*t*-butyl ether with each solvent fortified with 0.4 g/100 g acetic acid was used. The flow rate was 1 mL/min. Hexane (100%) was pumped for 5 min, followed by an isocratic period of 10 min, then methyl-*t*-butyl ether (0–80%, v/v) was increased, linearly for 7 min, followed by increase of hexane to 100% for 0.1 min, and an isocratic period of 9.9 min. The area of each peak was integrated by Autochro-2000 software (Younglin).

Non-aqueous RP-HPLC was conducted on the 24-h interesterified reaction mixture with a Nova-Pak® C18 60 Å 4 μm HPLC column, (3.9 mm × 150 mm, Waters Corp., USA). The HPLC system was Younglin SP 930D dual pump (Younglin). Sedex 75 evaporative light-scattering detector (ELSD, Alfortvill, France) was operated at 30 °C with nitrogen as a nebulizing gas at a pressure of 1.5 bar. A binary step gradient of ethylene chloride in acetone was used at a flow rate of 0.8 mL/min using a solvent elution profile: linear gradient from 0% to 15% ethylene chloride (v/v) for 10 min; followed by a linear increase from 15% to 45% ethylene chloride (v/v) over 10 min; hold for 10 min; followed by a linear increase from 45% to 60% ethylene chloride (v/v) over 5 min; and hold for 5 min.

HPLC-mass spectrometry (MSD) was performed with a Hewlett-Packard Model 1050 HPLC coupled with a HP Model 5989A quadrupole mass spectrometer engine interfaced with the Analytica APCI attachment (HP Model 103722) in the positive ion mode. Rheodyne 7125 mannual injector (Cotati, CA, USA) with a10 μL loop was used. The separation was performed with a Chrompack Si60 column (300 × 3.0 mm i.d., 5 μm, Varian Instruments, Walnut Creek, CA, USA) at a solvent flow rate of 0.43 mL/min. A binary solvent system of 15% methyl-*t*-butyl ether and 85% hexane (each solvent was fortified with 0.4% acetic