Bioresource Technology 101 (2010) 7456–7461

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Production of medium-chain triacylglycerols from corn oil: Optimization by response surface methodology

Tarık Öztürk [a], Guldem Ustun [b], H. Ayse Aksoy [b,*]

[a] Istanbul Technical University, Institute of Science and Technology, Program of Molecular Biology Genetics and Biotechnology, Maslak TR-34469, Istanbul, Turkey
[b] Istanbul Technical University, Faculty of Chemical and Metallurgical Engineering, Chemical Engineering Department, Maslak TR-34469, Istanbul, Turkey

ARTICLE INFO

Article history:
Received 13 July 2009
Received in revised form 11 March 2010
Accepted 28 April 2010
Available online 23 May 2010

Keywords:
Acidolysis
Caprylic acid
Corn oil
Lipozyme TL IM
Response surface methodology

ABSTRACT

Structured lipids (SLs) having long-chain fatty acids at *sn*-2 and medium-chain caprylic acid (CA, 8:0) at their *sn*-1,3-positions from corn oil (CO) were obtained and optimized by response surface methodology (RSM) with a three-level, three-factor face-centered cube design. Compositions of triacylglycerol species (TAGs) in SLs were also investigated by reverse-phase high performance liquid chromatography.

Lipozyme TL IM from *Thermomyces lanuginosa* was used for the acidolysis of CO with CA in *n*-hexane. The effects of substrate molar ratio, enzyme amount, and reaction time on CA incorporation into CO were optimized. The optimum conditions were 13.2% (wt.) enzyme, 3.9:1 caprylic acid/corn oil molar ratio, and 3.1 h reaction time. At optimum conditions, 21.5 ± 0.8 mol.% caprylic acid containing SLs was obtained. This product was characterized by 50% of triacylglycerol species with equivalent carbon number (ECN) C30, C32, C36, and C38, and 50% of triacylglycerol species with ECN C42, C44, and C46.

1. Introduction

Structured lipids (SLs) are modified triacylglycerols (TAGs) to alter the fatty acids' composition or their location in the glycerol backbone (Xu, 2000; Akoh, 2002). SLs are produced by incorporation of selected fatty acids into oils by chemical and enzymatic processes. Recently, the use of lipases to catalyze the fatty acid incorporation into oil for improving the nutritional, therapeutic, and industrial value of oils is being actively investigated by many researchers.

Medium-chain TAGs have been used to meet the nutritional needs of patients with special dietary requirements (Low et al., 2007). Because they undergo nearly complete hydrolysis, they are absorbed as free fatty acids and transported to the liver for oxidation and thereby provide fast energy (Mu and Porsgaard, 2005). First promising studies on benefits of medium-chain (M) and long-chain (L) fatty acids containing SLs were investigated by Jandacek et al. using 2-linoleoyl-1,3-dioctanoyl glycerol and 2-oleoyl-1,3-dioctanoyl glycerol (Jandacek et al., 1987). It was concluded that these triacylglycerols hydrolyzed more rapidly than TAGs comprising all long-chain fatty acids in digestive tract. Further studies demonstrated that not only the fatty acid composition but also their positions in SLs were important and MLM-type structured lipids were a good source of essential fatty acids for patients suffering from fat absorption or pancreatic problems. Therefore, designing SLs with selected fatty acids at specific locations in the TAGs for medical applications has attracted much attention (Akoh, 2002; Hamam and Shahidi, 2008; Lai et al., 2005; Lee and Akoh, 1998).

Acidolysis of vegetable oils such as canola, olive and peanut oils and palm olein and various triacylglycerols (triolein, tripalmitin, triconjugated linoleate, and trieicosapentaenoate) was investigated for the enzymatic synthesis of structured lipids with medium-chain fatty acids (M) located at positions 1 and 3, and long-chain fatty acids at position 2 (MLM) (Famuso and Akoh, 2002; Huang and Akoh, 1996; Lai et al., 2005; Lee and Akoh, 1998; Soumanou et al., 1998; Bektas et al., 2008). Single or multi-step production strategies were employed by these studies. Enzymatic acidolysis reactions were generally catalyzed by *sn*-1,3-specific lipases from *Pseudomonas fluorescens*, *Rhizomucor miehei*, *Rhizopus delemar*, and *Rhizopus javanicus*. The effects of enzyme type and amount, temperature, time, solvent type, substrate molar ratio, and water content on caprylic acid incorporation yield and enzyme specificity were investigated. Enzymatic acidolysis of refined, bleached, and deodorized palm olein with caprylic acid (8:0) was conducted using Lipozyme IM 60 from *R. miehei*, a 1,3-specific lipase (Lai et al., 2005). Caprylic acid content of the produced structured lipids reached to 30.5 mol.% after 24 h of reaction at a substrate mole ratio of 1:5 (palm olein:caprylic acid). Lipozyme IM 60 from *R. miehei* was used as a catalyst for the modification of olive oil with caprylic acid (Famuso and Akoh, 2002). Caprylic acid incorporation was 43 mol.% and fatty acid composition at the *sn*-2 position was kept unchanged.

Lipozyme TL IM is a food grade granulated silica preparation of a microbial 1,3-specific lipase (EC 3.1.1.3) from *Thermomyces*

* Corresponding author. Tel.: +90 212 285 3360; fax: +90 212 285 2925.
E-mail address: aksoyha@itu.edu.tr (H.A. Aksoy).

0960-8524/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biortech.2010.04.096

lanuginosa. *T. lanuginosa* lipase catalyzed the glycerolysis of sunflower oil with similar activity to that of immobilized *R. miehei* lipase (Lipozyme RM IM) (Yang et al., 2003). However, the catalytic activity of *Thermomyces lanuginosa* lipase was much lower than that of Lipozyme RM IM in the acidolysis of sunflower oil and caprylic acid.

The effects of Lipozyme RM IM and Lipozyme TL IM on the incorporation of γ-linolenic acid (GLA) and oleic acid into tripalmitin to produce human milk fat substitutes were investigated and optimized using response surface methodology (RSM) (Sahin et al., 2005). The effect of both enzymes on the GLA and oleic acid incorporation was found to be similar. The optimal conditions for the targeted GLA (10%) and oleic acid (45%) incorporation were 14.8 mol/mol, 55 °C, and 24 h; 14 mol/mol, 55 °C, and 24 h for substrate molar ratio (total FA/tripalmitin), temperature, and reaction time.

Novozyme 435 from *Candida antarctica* was used as a catalyst for the incorporation of polyunsaturated fatty acids (PUFAs) into borage, evening primrose and hazelnut oils and the reaction conditions were optimized using response surface methodology (Can and Özcelik, 2005; Senanayeka and Shahidi, 2002, 2006).

Response surface methodology (RSM) is a popular and an effective optimization technique for investigation of complex processes. RSM consists of a group of mathematical and statistical procedures that can be used to study the relationships between one or more responses and a number of independent variables. RSM is a specially designed regression analysis used to calculate the value of the response, dependent variable, in terms of independent variables (reaction parameters) (Senanayeka and Shahidi, 2002; Meyers and Montgomery, 2002). RSM is mainly used to decrease the number of experiments had to be done to determine statistically acceptable responses at such dependent variable ranges. Responses (results) at certain experimental points are used for a stepwise regression analysis to predict a graphical equation of the response in means of independent variables.

Immobilized *T. lanuginosa* lipase was employed to catalyze the interesterification reaction of corn oil with tristearin in a solvent-free system to produce modified lipids for use in dairy spreads (margarines) (Torres et al., 2002).

Corn oil is rich in essential fatty acid, linoleic acid (50–60%), which aids the body's absorption of vital nutrients and is required for human health. In the literature no record of study on the enzymatic acidolysis of corn oil with caprylic acid has been encountered. Therefore, we have attempted to produce enzymatically MLM-type SLs from corn oil for food and pharmaceutical uses. Since there does not exist any regulations on the content of medium-chain fatty acids in the MCTs in the literature, targeted caprylic acid incorporation was not the main goal of this study. This study was focused in obtaining the MLM-type SLs from corn oil enriched with caprylic acid, and optimized the reaction conditions. The effects of reaction parameters, namely, reaction time, substrates' molar ratio, and enzyme load on caprylic acid incorporation were investigated and optimized by RSM with a three-level, three-factor face-centered cube design.

The compositions of TAG species in MLM-type SLs obtained at critical (optimum) conditions (13.2 wt.% enzyme, 3.9:1 caprylic acid/corn oil molar ratio and 3.1 h reaction time) were also investigated by reverse-phase high performance liquid chromatography (RP-HPLC).

2. Methods

2.1. Materials

A commercially available food grade corn oil "Bizim Yag" was obtained from a local store. It is a product of Besler Gıda and is distributed by Ülker Istanbul, Turkey. The corn oil having fatty acids that consisted of 12% palmitic acid, 2.4% stearic acid, 30.5% oleic acid, and 54.6% linoleic acid was used throughout this investigation.

The caprylic acid was purchased from Sigma–Aldrich (Buchs, Switzerland). Commercially available *sn*-1,3-specific lipase from *T. lanuginosa*, Lipozyme TL IM was supplied by Nova-Nordisk A/S (Copenhagen, Denmark). All other chemicals used in the experiments were of analytical grade and obtained from Merck (Darmstadt, Germany).

2.2. Acidolysis of corn oil and caprylic acid

Acidolysis reactions were conducted in dark-colored, heat-resistant 30 mL reaction flasks. The reaction flasks having substrate mixture (1 g) consisted of corn oil and caprylic acid at different molar ratios, and 5 mL hexane was placed in a temperature-controlled orbital shaker (Edmund Bühler, KS-15, Germany) at 200 rpm and 50 °C. The reactions were started by adding proper amount of enzyme, Lipozyme TL IM, to the reaction mixtures. After a predetermined time the reaction was stopped by 2.5 mL ethanol addition. All reactions were at least duplicated and at least two samples of each run were analyzed.

2.3. Separation of the modified triacylglycerols (SLs) following acidolysis

Hexane (2.5 mL) was added to the reaction samples consisting of modified TAGs (SLs) and free fatty acids (FFAs). TAGs were separated from FFAs according to the method of Senanayeka and Shahidi (2002). FFAs in the reaction samples were neutralized by titration against 0.02 M NaOH using phenolphthalein as pH indicator. After this 50 mL hexane, 25 mL water, and 10 mL saturated NaCl solution were added to the reaction samples for the extraction of TAGs. TAGs containing hexane phase was separated and dried using anhydrous Na_2SO_4. TAGs were recovered by hexane removal using a rotary evaporator.

2.4. Analysis of the acidolysis products

For the determination of fatty acid composition by GC, TAGs were converted into corresponding methyl esters (FAMEs) according to the AOCS Official Method Ce-2-66. The obtained FAMEs were analyzed by a Hewlett-Packard 5890 II gas chromatograph (Hewlett-Packard, Waldron, Germany) equipped with TRB-5ht capillary column (30 m × 0.25 mm × 0.10 μm film thickness of 5% diphenyl and 95% dimethylpolysiloxane; Teknokroma, Barcelona, Spain) and a flame ionization detector. The detector temperature was 280 °C and the injection port temperature was 250 °C. The column temperature was kept constant at 150 °C for 5 min, raised from 150 to 275 °C at a rate of 5 °C/min and kept constant at 275 °C for 10 min. During all analyses the split ratio was 88:1 and nitrogen (1.6 mL/min) was used as carrier gas. The FID detector was supplied with hydrogen and air flow rates at 33 and 460 mL/min, respectively. Individual fatty acids were identified by comparing with the retention times of standards.

The compositions of TAG species of corn oil and SLs separated from acidolysis product obtained at the optimized (critical) conditions (13.2 wt.% enzyme, 3.9:1 caprylic acid/corn oil molar ratio and 3.1 h reaction time) were determined using a reverse-phase high performance liquid chromatography (RP-HPLC) method. RP chromatography is based on the partition of solutes between the mobile phase and the stationary phase (Buchgraber et al., 2004). The Shimadzu HPLC apparatus (Shimadzu, Kyoto, Japan) equipped with a C18 HPLC column (250 mm × 5 μm), Shimadzu SCL-10A VP system controller, SIL-10AD VP auto injector, DGU-14A degasser,