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Enzymatic incorporation of capric acid into a single cell oil rich in docosahexaenoic acid and docosapentaenoic acid and oxidative stability of the resultant structured lipid

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Abstract

Lipase-assisted acidolysis of a single cell oil rich in docosahexaenoic acid (DHA, C22: 6*n*−3) and docosapentaenoic acid (DPA, C22:5*n*−6), commericallly known as the OMEGA-GOLD oil, with capric acid (CA, C10:0) was carried out. Screening of five commercially available lipases was carried out for oil to CA mole ratio of 1:3 at a temperature of 45 °C, a reaction time of 24 h, 4% (w/w of substrates) PS-30 lipase from *Pseudomonas* sp. and 2% (w/w of substrates and enzyme) water content. Stereospecific analysis indicated that CA was present mainly in the sn-1,3 positions of the triacylglycerol (TAG) molecules while DHA and DPA were mainly esterified to the sn-2 position. Enzymatically modified oil generally had higher conjugated diene (CD) and 2-thiobarbituric acid (TBA) values than its unmodified counterpart. However, the oil subjected to the same reaction steps in the absence of any enzyme, exhibited a significantly ($p < 0.05$) lower oxidative stability. Therefore, removal or alteration of endogenous antioxidants during the process may be primarily responsible for the compromised stability of the modified oil.

Keywords: Acidolysis; Lipase; Structured lipids; Medium-chain fatty acids; Capric acid (C10:0); The OMEGA-GOLD oil; Oxidative stability; Conjugated dienes; Thiobarbituric acid reactive substances; Positional distribution

1. Introduction

Structured lipids (SL) are defined as triacylglycerols (TAG) modified to change the fatty acid composition and/or their location in the glycerol backbone via chemical or enzymatic means. Acidolysis is referred to the exchange of acyl group between an acid (fatty acid) and an ester. Acidolysis is an efficient method for incorporating specific fatty acids into triacylglycerols to achieve a desired functionality. SL are also produced via acidolysis in order to enhance or change the physical and/or chemical properties of TAG.

OMEGA-GOLD oil is a commercial oil derived from microalgae *Schizochytrium* sp. via a fermentation process (Zeller, Barclay, & Abril, 2001). It is a rich source of docosahexaenoic acid (DHA, *n*−3) (41%) and docosapentaenoic acid (DPA, *n*−6) (18%). Medium-chain fatty acids (MCFA) are saturated fatty acids with a carbon chain length ranging from 6 to 12 and are prepared mostly from oils of tropical plants, such as those of coconut and palm. Medium-chain triacylglycerols (MCT) exhibit unique structural and physiological features. MCT rapidly clear from the blood (Babayan, 1987). Structured lipids containing MCFA and long-chain fatty acids (LCFA) have modified absorption rates because MCFA are quickly absorbed and oxidized/metabolized for energy while LCFA are oxidized very slowly. These modified lipids are structurally and metabolically different from simple physical mixtures of MCT and long-chain triacylglycerols (LCT).

In order to examine the oxidative stability of the modified and unmodified OMEGA-GOLD oil, a number of stability tests may be employed. These tests

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include chemical and instrumental techniques (Rossell, 1991; Shahidi & Wanasundara, 1998). The tests detect either the primary or secondary products of lipid oxidation. Conjugated diene (CD) assay is a simple and rapid method for evaluation of the primary products of lipid oxidation (hydroperoxide); it does not require chemical reactions and needs only a small amount of sample in the milligramme range. The 2-thiobarbituric acid (TBA) test, meanwhile, is widely used to examine secondary lipid oxidation products.

Jennings and Akoh (1999) successfully incorporated CA (43%) into a fish oil triacylglycerols (containing 40.9% EPA and 33.0% DHA) using immobilized lipase (IM 60) from *Rhizomucor miehei*. Akoh and Moussata (2001) modified fish oil as well as canola oil with caprylic acid using lipozyme IM from *Rhizomucor miehei*. Their results showed that Lipozyme IM incorporated a higher level of caprylic acid (40.1%) into canola oil as than fish oil (29.5%). Shimada et al. (1996) reported that caprylic acid (8:0) was successfully incorporated into tuna oil containing DHA via acidolysis using immobilized lipase from *Rhizomucor delemar*. This specific lipase catalysed the exchange of almost 65% of the fatty acids at the sn-1,3 positions with caprylic acid. Senanayake and Shahidi (2002a, 2002b) used acidolysis to incorporate CA into seal blubber oil. They used immobilized lipase, Lipozyme-IM from *Mucor miehei* and found, incorporation of 25.4% CA into seal blubber oil after 24 h of incubation at 45 °C and a 1% water level. Lipozyme IM catalyzed incorporation of caprylic acid, up to 70%, in the sn-1,3 positions of the modified fish oil (Xu, Balchen, Hoy, & Adier-Nissen, 1998). In another study, Xu (2000) produced SL containing 40% caprylic acid and 35% EPA and DHA with less than 3% caprylic acid at the sn-2 position via acidolysis of menhaden oil with caprylic acid using Lipozyme IM in a solvent-free system.

Iwasaki, Han, Narita, Rosu, and Yamane (1999) reported lipase-assisted acidolysis of a single-cell oil containing docosahexaenoic acid (DHA, *n*−3) (41%) and DPA, *n*−6 (18%) with caprylic acid. Two lipases from *Rhizomucor miehei* and *Pseudomonas* sp. were used as biocatalysts. The end products were modified oils containing caprylic acid in the sn-1,3 positions and DHA and DPA at the sn-2 position of the glycerol backbone. *Pseudomonas* sp. lipase catalysed the exchange of more than 60% of fatty acids in the single-cell oil with caprylic acid, while *Rhizomucor miehei* lipase catalysed incorporation of 23% of caprylic acid into the single-cell oil.

The objectives of this study were to incorporate capric acid, as a rapid source of energy, into the OMEGA-GOLD oil, optimize the reaction conditions for preparation of OMEGA-GOLD-based SL, determine the positional distribution of fatty acids in the enzymatically-modified OMEGA-GOLD oil, and to evaluate the oxidative stability of the resultant SL.

2. Materials and methods

2.1. Materials

Two lipases from *Candida anatrctica* (Novozyme-435) and *Mucor miehei* were acquired from Novo Nordisk (Franklinton, NC). Other lipases, namely *Pseudomonas* sp. (PS-30), *Aspergillus niger* (AP-12), and *Candida rugosa* (AY-30) were obtained from Amano Enzymes (Troy, VA). The OMEGA-GOLD oil was obtained from Monsanto (St. Louis, MO). Standards of fatty acid methyl esters (FAMEs; GLC-461) were purchased from Nu-Check (Elysian, MN). Porcine pancreatic lipase (EC 3.11.3), sodium taurocholate, and silica gel thin-layer chromatographic plates (TLC; 20×20 cm; 60 Å mean pore diameter, 2–25 μm mean particle size, 500 μm thickness, with dichlorofluorescein) were purchased from Sigma Chemical Co. (St. Louis, MO). All solvents used in these experiments were of analytical grade, purchased from Fisher Scientific (Nepean, ON).

2.2. Methods

2.2.1. Acidolysis reactions

In general, the OMEGA-GOLD oil (500 mg) was mixed with CA, at different mole ratios of oil to CA, ranging from 1 to 3, in screw-capped test tubes, and then lipase (2–10% by weight of substrates) and water (1–2.5% by weight of substrates and enzyme) were added in hexane (3.0 ml). The mixture was incubated for different periods (12–48 h) in an orbital shaker at 250 rpm at 25–55 °C. All experiments were reported in the Section 2.2 were triplicated.

2.2.2. Separation of acylglycerols following acidolysis

After a given time period, the reaction was stopped by addition of a mixture of acetone and ethanol (20 ml; 1:1, v/v). In order to neutralize the released and unused free fatty acids, the reaction mixture was titrated with a 0.5 M NaOH solution (using a phenolphthalein indicator) until the colour of the solution turned pink. The acylgylcerols were then extracted into hexane (25 ml). The two layers (aqueous, hexane) were allowed to separate in a separatory funnel, and the lower aqueous layer was discarded. The hexane layer was passed through a bed of anhydrous sodium sulphate to remove any residual water. The hexane was evaporated using a rotary evaporator at 45 °C and the acylglycerol fraction was recovered and a portion of it transferred to special transmethylation vials.

2.2.3. Preparation of fatty acid methyl esters

Fatty acid profiles of products were determined following their conversion to methyl esters. Transmethylation reagent (2.0 ml, freshly prepared 6.0 ml of