LWT - Food Science and Technology 46 (2012) 29–35

Contents lists available at SciVerse ScienceDirect

LWT - Food Science and Technology

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

Enrichment of palm olein with long chain polyunsaturated fatty acids by enzymatic acidolysis

S. Nagachinta, C.C. Akoh*

Department of Food Science and Technology, The University of Georgia, Food Science Building, Athens, GA 30602, USA

ARTICLE INFO

Article history:
Received 12 July 2011
Received in revised form
17 October 2011
Accepted 27 October 2011

Keywords:
Structured lipids
Docosahexaenoic acid
Arachidonic acid
Palm olein
Acidolysis

ABSTRACT

Long-chain polyunsaturated fatty acids (LCPUFA) such as docosahexaenoic (DHA) and arachidonic (ARA) acids have great benefits for the development and maintenance of human brain functions. The production of structured lipid (SL) by acidolysis of palm olein with a free fatty acid mixture obtained from DHASCO® and ARASCO®, catalyzed by Novozym 435® in hexane was optimized by response surface methodology (RSM). Three independent factors chosen were substrate mole ratio of total free fatty acids to palm olein (6–18 mol/mol), reaction temperature (55–65 °C), and reaction time (12–24 h). Good quadratic models were obtained for the incorporation of DHA + ARA (response 1) and their incorporation at the *sn*-2 position (response 2) by multiple regression and backward elimination. The models were verified by carrying out acidolysis reaction at several random combinations of the independent factors. The optimal condition generated from the models resulted in 25.25 g/100 g total incorporation of DHA + ARA and 17.20 g/100 g DHA + ARA incorporation at the *sn*-2 position. The fatty acid composition of SL was comparable to that of human milk fat. The SL produced in this study has potential for use in infant formulas as well as in nutraceutical applications for pregnant women.

1. Introduction

Docosahexaenoic acid (DHA, C22:6n-3) and arachidonic acid (ARA, C20:4n-6) are long-chain polyunsaturated fatty acids (LCPUFA) derived from the elongation and desaturation of α-linolenic acid (ALA, C18:3n-3) and γ-linoleic acid (GLA, C18:3n-6), respectively. DHA is present at high levels in the retina, cerebral cortex and sperm, while ARA is present in all biological membrane (Neuringer, Anderson, & Connor, 1988). DHA and ARA constitute the majority of the fatty acids in the brain and their role is to aid in the development and protection of neurological functions (Bourre, 2006; Koletzko et al., 2008; Ryan et al., 2010). These fatty acids are also important in neonatal development of the brain and retina. Studies have shown that children of women receiving prenatal DHA supplements, scored higher on mental processing and visual acuity (Helland, Smith, Saarem, Saugstad, & Drevon, 2003; Williams, Birch, Emmett, Northstone, & Team, 2001). Premature infants fed with DHA and ARA supplemented infant formula, had weight gains comparable to full-term infants on breastfeeding. In contrast, premature infants fed on formula with no DHA or ARA had a lower body weight than full-term infants (Innis et al., 2002). Dietary supplementation of DHA and ARA in adults was shown to improve cognitive dysfunctions due to aging, organic brain lesions and Alzheimer's disease (Kotani et al., 2006). Therefore, the consumption of DHA and ARA benefits people of all ages from infants, mothers of infant and aging adults.

Although conversion of ALA and GLA to LCPUFA products occurs naturally in the human body, studies demonstrated that the conversion of ALA to DHA occurs at a very limiting rate (Burdge & Calder, 2005; Gerster, 1998). Both the fetus and the newborn depend on the maternal supply of DHA and ARA through the placenta and breastfeeding (Boehm et al., 1996; Green & Yavin, 1998). Consequently, an adequate intake of preformed LCPUFA is recommended to maintain optimal tissue function, especially for pregnant and breast-feeding women. Individuals on diets lacking meat and fish, which are primary sources of ARA and DHA, respectively, may suffer from neurological and visual disturbances. Therefore, there is an interest in creating structured lipids (SL) enriched with ARA and DHA for potential uses in the food and nutraceutical industry (Innis, 2004; Osborn & Akoh, 2002).

Palm olein, a low-melting point fraction of palm oil, has been widely used in edible-oil blends for food applications. It is liquid in warm climates and blends well with other vegetable oils. The fatty acid composition of palm olein (palmitic acid 40, oleic acid 43 and linoleic acid 11 g/100 g) makes it an excellent source of palmitic and oleic acids to be added into infant formula to mimic the fatty acid

* Corresponding author. Tel.: +1 706 542 1067; fax: +1 706 542 1050.
E-mail address: cakoh@uga.edu (C.C. Akoh).

0023-6438/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.lwt.2011.10.027

composition of human milk. Although the fatty acid composition of vegetable oils used in infant formula are matched to human milk fat, the fat absorption in formula-fed infants is still lower (Hanna, Navarrete, & Hsu, 1970; Lien, 1994). This lower absorption is due to the differences in stereospecific structure of the triacylglycerols (TAG) of vegetable oils and human milk fat. Most of the palmitic acid in human milk (>60 g/100 g of the palmitic acid) is located at the *sn*-2 position, whereas in vegetable oils, it is predominantly located at the outer positions (Innis, Dyer, Quinlan, & Diersen-Schade, 1995; López-López et al., 2001; Tomarelli, Meyer, Weaber, & Bernhart, 1968). Pancreatic lipase specifically hydrolyzes the *sn*-1, 3 positions of TAG to produce free fatty acids from these positions and 2-monoacylglycerol. The 2-monoacylglycerol (2-MAG), is a well-absorbed form of most fatty acids since it readily forms mixed micelles with bile acids and cannot form insoluble soaps with divalent cations (Jandacek, Whiteside, Holcombe, Volpenhein, & Taulbee, 1987; Lien, 1994). Absorption of eicosapentaenoic acid (EPA, C20:5n-3) and DHA was higher when they were predominantly in the *sn*-2 position than when they were randomly distributed between the three positions (Christensen, Hoy, Becker, & Redgrave, 1995). The levels of DHA and ARA at the *sn*-2 position of human milk fat are 0.4–0.7, and 0.3–0.7 g/100 g of the total fatty acids in the *sn*-2 position, respectively. Total amount of DHA and ARA in human milk fat are 0.2–0.5, and 0.4–0.6 g/100 g of the total fatty acids, respectively. However, levels of these fatty acids vary depending on the diets of the mothers (Straarup, Lauritzen, Faerk, Hoy, & Michaelsen, 2006; Yuhas, Pramuk, & Lien, 2006).

Previously, enrichments of LCPUFA including ARA, DHA and EPA into TAG structure of borage oil, evening primrose oil and tripalmitin have been studied (Sahin, Akoh, & Karaali, 2006; Senanayake & Shahidi, 1999; Shimada et al., 2000). Stereospecific analysis of SL using pancreatic lipase is often used to provide useful information about digestion in the intestine. The aim of this study was to enrich the TAG structure of palm olein with DHA and ARA derived from single cell oil TAG (DHASCO® and ARASCO®), by an acidolysis reaction. Response surface methodology (RSM) was used to model and optimize the reaction conditions to generate high incorporation of DHA and ARA with high contents at the *sn*-2 position.

2. Materials and methods

2.1. Materials

Palm olein (San Trans25) was kindly donated by IOI- Loders Croklaan (Channahon, IL). DHA-containing single cell oil (DHASCO®) and ARA-containing single cell oil (ARASCO®) from alga *Crypthecodinium cohnii* and fungus *Mortierella alpina*, respectively, were generously provided by Martek Bioscience Corp. (Columbia, MD). Novozym® 435 (a nonspecific lipase) was purchased from Novo Nordisk A/S (Bagsvaeard, Denmark). The lipid standards Supelco 37 component FAME mix, C15:0 pentadecanoic acid (>98% purity), triolein, and 2-oleoylglycerol were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO). Other solvents and chemicals were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO), J.T. Baker Chemical Co. (Phillipsburg, NJ), or Fisher Scientific (Norcross, GA).

2.2. Preparation of free fatty acid (FFA) from DHASCO and ARASCO

Preparation of free fatty acids from single cell oils was carried out according to Senanayake and Shahidi (1999). Twenty-five grams of oil (treated with 5 mg butylated hydroxytoluene) was saponified using a mixture of 5.75 g of KOH, 11 ml of distilled water and 66 ml of aqueous ethanol (95 ml/100 ml) by refluxing for 1 h at 60 °C. To the saponified mixture, 60 ml of distilled water was added, and the unsaponified matter was extracted into hexane (2 × 100 ml) and discarded. The aqueous layer containing saponifiable matter was acidified to pH about 1.0 with 4 mol/l HCl. The liberated fatty acids were extracted into hexane 50 ml. The hexane containing free fatty acids was dried over anhydrous sodium sulfate, and the solvent was removed in a rotary evaporator at 40 °C. Free fatty acids were flushed with nitrogen prior to storage in the freezer at −80 °C.

2.3. Preparation of DHA and ARA concentrate by urea complexation and low temperature solvent crystallization

The separations of DHA and ARA from the hydrolyzed fatty acids mixtures of DHASCO and ARASCO, respectively were performed using urea-fatty acid complexation according to Senanayake and Shahidi (1999) while low temperature solvent crystallization followed a method described by Vali, Sheng, and Ju (2003) with modification. Ten grams of free fatty acids was mixed with 30 g of urea in 150 ml aqueous ethanol (95 ml/100 ml) and heated at 60 °C with stirring. The urea-fatty acid complex was allowed to crystallize at room temperature first and then placed in a freezer at 4 °C for a period of 24 h. The crystals formed were separated from the liquid by suction filtration through Whatman® filter paper grade no. 1. The filtrate was diluted with an equal volume of distilled water and acidified to pH 4–5 with 6 mol/l HCl. An equal volume of hexane was then added and the mixture was stirred thoroughly for 1 h. Hexane layer containing liberated fatty acids was separated from the aqueous layer containing urea using a separatory funnel. The hexane layer was then washed with distilled water to remove any remaining urea and dried over anhydrous sodium sulfate. The solvent was subsequently removed at 40 °C using a rotary evaporator. The method of Vali et al. (2003) for low temperature solvent crystallization was modified as given below. Four gram of free fatty acids was dissolved in 300 ml methanol. The mixture was stirred at 50 °C until all dissolved. The solution was allowed to cool and stored at −80 °C for 48 h. Then, the liquid and solid phases were separated immediately by vacuum filtration. Methanol was removed in a vacuum rotary evaporator at 40 °C. The concentrated fractions obtained from both methods were flushed with nitrogen and stored at −80 °C. The fatty acid composition and average molecular weight of these fractions were determined using a gas chromatographic (GC) procedure as described below.

2.4. Determination of fatty acid profiles

Samples were converted to fatty acid methyl esters (FAME) following AOAC official method 996.01 with modification. Briefly, 100 mg of oil was weighed into a Teflon-lined test tube, and 1 ml of C15:0 in hexane (20 mg/ml) was added as internal standard (Vali et al., 2003) and dried with nitrogen to remove solvent. Two milliliters of 0.5 mol/l NaOH in methanol was added followed by incubation for 5 min at 100 °C to saponify the lipid. To the sample was added 2 ml of boron trifluoride (BF_3) in methanol (14 g/100 ml), vortexed for 1 min and incubated at the same condition to allow methylation. Two milliliters of hexane and 2 ml of NaCl-saturated solution were added and vortexed for 2 min to stop the reaction and to extract the FAME. To separate the organic and aqueous phases, the sample was centrifuged at 1000 rpm (approximately 100× g) for 5 min. The upper layer was recovered into a GC vial and analyzed. Supelco 37 component FAME mix was used as FAME external standard and ran in parallel with the samples.