LWT - Food Science and Technology 54 (2013) 179–185

Contents lists available at SciVerse ScienceDirect

LWT - Food Science and Technology

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

Production of human fat milk analogue containing α-linolenic acid by solvent-free enzymatic interesterification

Huri Ilyasoglu*

Gumushane University, Department of Nutrition and Dietetics, Baglarbası, 29100 Gumushane, Turkey

ARTICLE INFO

Article history:
Received 7 August 2012
Received in revised form
21 April 2013
Accepted 21 May 2013

Keywords:
Human milk fat analogue
α-Linolenic acid
Enzymatic interesterification
Flaxseed oil
Rosemary extract

ABSTRACT

Human milk fat (HMF) analogue was synthesized by lipase-catalysed interesterification of tripalmitin with extra virgin olive oil, and flaxseed oil in a solvent-free media. Lipozyme® TL IM, immobilized sn-1,3 specific lipase, was used as biocatalysts. Substrate molar ratio, reaction temperature and reaction time were used to model and to optimize the reaction conditions via response surface methodology. Good quadratic models were obtained for the incorporation of palmitic acid and α-linolenic acid. At the optimal conditions generated from the model, the palmitic acid and α-linolenic acid contents of the product were found to be 25.2 g/100 g and 15.9 g/100 g, respectively. The chemical properties (free fatty acid, iodine value, and saponification value), sn-2 positional fatty acid composition, and sterol composition of the final structured lipid (SL) were determined. Furthermore, the oxidative stability of the SL and the SLs with antioxidants (BHT, gallic acid, and rosemary extract) was evaluated. The peroxide and thiobarbituric acid reactive substances (TBARS) values of the SLs showed they had antioxidant activity on the SL. The HMF analogue synthesized in this study may have potential use in infant formulas to provide omega-3 fatty acids (α-linolenic acid).

1. Introduction

Human milk is naturally a unique source of nutrients for newborns, term and pre-term infants. Human milk fat (HMF) provides a major source of energy as well as required nutrients (Yang, Xu, He & Li, 2003). Human milk fat contains oleic acid (27–35 g/100 g), palmitic acid (22–27 g/100 g), and linoleic acid (8–14 g/100 g) as the main constituents. The saturated fatty acid, palmitic acid, is mostly located at the sn-2 position (more than 60 g/100 g) (Xu, 2000). Predominance of the palmitic acid at the sn-2 position of HMF provides the absorption of palmitic acid and reduces the calcium losses in the feces. Palmitic acids esterified at the sn-1,3 positions are hydrolyzed by pancreatic lipase, yielding free palmitic acids which form insoluble calcium soaps in the intestine. The formation of calcium soaps results in the reduction of calcium absorption and loss of dietary energy (Silva et al., 2011).

Developing structured lipids (SLs) with a composition similar to that of HMF has gained much attention. Structured lipids resembling to HMF can be produced by enzymatic interesterification or acidolysis reactions. Numerous studies have been conducted on the production of SLs as HMF analogues. Lard and soybean oil (Nielsen, Yang, Xu & Jacobsen, 2006; Silva et al., 2011; Yang et al., 2003), butterfat, soybean oil, and rapeseed oil (Sorensen, Xu, Zhang, Kristensen & Jacobsen, 2010), tripalmitin and hazelnut oil (Sahin, Akoh & Karaali, 2005; Sahin, Akoh & Karaali, 2006), tripalmitin, vegetable oil blends, and fish oil (Maduko, Park & Akoh, 2008) have been used as substrates. Lard is the only fat resembling to HMF in terms of fatty acid composition. Butterfat includes short-chain fatty acids and minor fatty acids, whose levels are similar to those of HMF. Hazelnut oil is a source of monounsaturated fatty acid (oleic acid), and soybean oil is a source of polyunsaturated fatty acid (linoleic acid). A combination of tripalmitin or lard with vegetable oils has been chosen to obtain a product possessing similar fatty acid composition to HMF. Moreover, fish oil has been chosen to enrich with omega-3 fatty acids. Enzymatic synthesis of SLs can be conducted in a solvent media or solvent-free media. Solvent-free media offers some advantages in terms of economic and safety aspects. No use of solvents reduces the cost of production and avoids the contact of product with low toxic solvents (Esteban et al., 2011).

Flaxseed oil, one of the main sources of omega-3 fatty acids, contains α-linolenic acid (ALn) more than 50 g/100 g (Przybylski, 2005). ALn has been reported to have beneficial effects in health maintenance. ALn is the principal precursors of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) which are essential for

* Tel.: +90 456 233 74 25x1425.
E-mail addresses: huriilyasoglu@yahoo.com, hilyasoglu@gumushane.edu.tr.

0023-6438/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.lwt.2013.05.036

infant growth and development (Teichert & Akoh, 2011). Olive oil is rich in monounsaturated fatty acid as oleic acid, ranging from 55 to 83 g/100 g. Oleic acid, one of the main fatty acid in human milk fat, is known to provide health benefits (Nunes, Pires-Cabral, & Ferreira-Dias, 2011).

The purpose of this study was to produce human milk fat analogue resembling HMF, and consisting of α-linolenic acids by enzymatic interesterification of tripalmitin with extra virgin olive oil and flaxseed oil in a solvent-free media. Response Surface Methodology (RSM) was used to model and to optimize the reaction conditions. The chemical properties, sn-2 positional fatty acid composition, and sterol composition of the product obtained at the optimal conditions were determined. The effect of different antioxidants on the oxidative stability of the product under accelerated storage condition was also evaluated.

2. Materials and methods

2.1. Materials

Extra virgin olive oil was purchased from a grocery store in Turkey. Flaxseed oil was obtained from the Origo Company (Turkey). Tripalmitin (glycerol tripalmitate, minimum purity 85%), and porcine pancreatic lipase (type 2) were purchased from Sigma Chemical Co. (St Louis, MO). Immobilized sn-1,3 specific lipase, Lipozyme® TL IM was a gift from Novo Nordisk A/S (Bagsvaerd, Denmark/Turkey branch). Organic solvents and TLC plates were obtained from Merck (Darmstadt, Germany). All solvents and reagents used in the study were of chromatographic and/or analytical grade.

2.2. Experimental design

A three-factor and five-level central composite design was used for the response surface methodology studies. The independent variables and experimental design are presented in Table 1. The three factors chosen were substrate molar ratio (Sr, total of olive oil and flaxseed oil (1:1)/tripalmitin), (1–3 mol/mol); temperature (T,°C), (50–60 °C); and time (t, h), (12–24). The ranges of three factors were chosen with respect to the results of prestudy. The ratio of olive oil to flaxseed oil (1:1) was selected to obtain a HMF analogue possessing similar oleic acid and linoleic acid levels to those of HMF. Experiments were run randomly, and duplicate reactions were carried out at all design points.

2.3. Enzymatic interesterification reaction

Reaction mixtures consisted of tripalmitin, extra virgin olive oil and flaxseed oil at different substrate molar ratios were weighed in screw–cap reaction tubes. Lipozyme® TL IM (10 wt% of total substrates) was added to the reaction mixtures. Reactions were carried out in an orbital shaking water bath at 200 rpm. The reaction was stopped by the filtration of the product, and the reaction product was stored at −18 °C until analysis.

2.4. Purification of SLs

The purification of the SLs was performed according to Araujo et al. (2011) with some modifications. The reaction product was dissolved in hexane (10 ml), and 0.8 mol/L alcoholic potassium hydroxide (7.5 ml) was added. After the mixture was agitated, the hydro-alcoholic phase (containing FFAs) and the hexane phase (containing TAGs) decanted. The hydro-alcoholic phase was extracted with twice hexane (2.5 ml). The hexane phases were collected and hexane was evaporated. The overall reaction yield was approximately 78 g/100 g.

2.5. Chemical properties

Free fatty acids, peroxide value, saponification value and iodine value of the substrates and the products were determined in accordance with the AOCS official methods.

2.6. Fatty acid composition

The TLC identification of the free fatty acids and acylgycerols was carried out after the purification process. The purified SLs and standards spotted on silica-gel plates (Merck, Darmstadt, Germany) were developed in chloroform:acetone:methanol (95: 4.5: 0.5). The lipid bands were visualized by spraying the plate with iodine vapour in a chamber. The TLC analysis results showed that the fatty acids effectively separated after the purification step. Therefore, the fatty acid compositions of the SLs were determined by GC.

The determination of fatty acid composition was carried out by gas chromatograph with flame ionization detection (GC-FID). The SLs was dissolved in hexane (10 ml) and 2 mol/L potassium hydroxide in methanol (200 μl) was added. After shaking in a vortex, the upper phase was injected into a Shimadzu GC-2010 Plus gas chromatography equipped with a flame ionization detector, a split/splitless injector and a long capillary column (0.25 mm × 0.20 μm × 60 m, Teknokroma). The oven temperature

Table 1
Central composite design arrangement with levels of factors and palmitic acid and α-linolenic acid incorporation.

Experiment no.	Substrate molar ratio (mol/mol)	Temperature (°C)	Time (h)	Palmitic acid (g/100 g)	α-Linolenic acid (g/100 g)
1	1	50	12	46.8	11.7
2	3	50	12	25.3	17.2
3	1	60	12	38.7	9.9
4	3	60	12	17.0	11.6
5	1	50	24	43.2	11.6
6	3	50	24	26.9	18.4
7	1	60	24	40.3	10.2
8	3	60	24	22.1	15.1
9	0.33	55	18	59.0	5.9
10	3.68	55	18	22.1	17.9
11	2	46.6	18	26.1	12.8
12	2	63.4	18	21.7	10.5
13	2	55	8	21.5	10.5
14	2	55	28	26.2	12.5
15	2	55	18	31.6	15.2
16	2	55	18	30.3	14.2
17	2	55	18	30.2	14.5