LWT - Food Science and Technology 76 (2017) 156–163

Contents lists available at ScienceDirect

LWT - Food Science and Technology

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

Interesterified *trans*-free fats rich in *sn*-2 nervonic acid prepared using *Acer truncatum* oil, palm stearin and palm kernel oil, and their physicochemical properties

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ARTICLE INFO

Article history:
Received 7 April 2016
Received in revised form
23 October 2016
Accepted 26 October 2016
Available online 27 October 2016

Keywords:
Acer truncatum oil
Nervonic acid
trans-free margarine
Interesterification

ABSTRACT

This study obtained *trans*-free fats containing nervonic acid, a fatty acid with beneficial effects for mental health. Four fats containing 1.28–2.41% (w/w) nervonic acid were prepared by chemical or enzymatic interesterification of palm stearin (PS), *Acer truncatum* oil (ATO) and palm kernel oil (PKO). The chemical procedure was catalyzed using MeONa, whereas the enzymatic procedure was catalyzed with a 1,3 −specific lipase. As a result, the interesterified fats showed a similar fatty acid profile to their corresponding physical blends measured by gas chromatography, but contained more nervonic acids at the *sn*-2 position, which was more conducive to metabolism and absorption. The content of tripalmitoylglycerol (PPP) and 1,3-dipalmitoyl-2-oleoyl glycerol (POP) or 1,2-dipalmitoyl-3-oleoyl glycerol (PPO) in the interesterified fats decreased significantly, resulting in improved plasticities. All interesterified fats showed a satisfactory content of tocopherols and oxidative stability. A combination of differential scanning calorimetry, X-ray diffraction spectroscopy and polarized light microscopy results suggested that the interesterified fats formed spherulitic needle-like crystals in the β′ form, while the physical blends formed dendritic crystals in the β form. These data indicated that the *trans*-free interesterified fats rich in nervonic acid may have a great potential in margarine applications.

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1. Introduction

Nervonic acid (*cis*-tetracos-15-enoic acid; 24:1 Δ15) is a functional fatty acid widely distributed in the sphingolipid fractions of nervous system tissues in vertebrates (Merrill et al., 1997; Poulos, 1995). Many mental disorders including schizophrenia (Assies et al., 2001), psychosis (Evans et al., 2003), and attention deficit disorder (Chen, Hsu, Hsu, Hwang, & Yang, 2004) are related to reduced nervonic acid levels in blood or brain tissues. Moreover, demyelination (Sargent, Coupland, & Wilson, 1994) and Zellweger syndrome (Tanaka, Shimizu, Ohtsuka, Yamashiro, & Oshida, 2007) are negatively associated with nervonic acid levels. Thus, inclusion of oils rich in nervonic acid in the diet is essential for reducing the risk of these diseases.

To date, only a few plant seed oils have been reported to contain nervonic acid (Taylor et al., 2010), including *Lunaria annua* (Money plant), *Borago officinalis* (Borage), *Cannabis sativa* (Hemp), *Acer truncatum* (Purpleblow maple), *Tropaeolum speciosum* (Flame flower) and *Cardamine graeca* (Bittercress). Most of these plants are not suitable for commercial production due to their limited availability, with the exception of *Acer truncatum* (*A. truncatum*). *A. truncatum* is a common plant in China. Our recent study using supercritical carbon dioxide extraction demonstrated that *A. truncatum* seed oil (ATO) contains about 92% unsaturated fatty acids, including 6.2% nervonic acid.

In general, fats with a wide plastic range are used as bakery shortenings, margarines and spreads. Hydrogenation, interesterification and fractionation are the primary processing technologies for modifying fats to achieve the plasticity (Nor Aini & Miskandar, 2007; Yazdi & Alemzadeh, 2011). However, partially hydrogenated fats contain a large amount of *trans* fatty acids, raising safety concerns such as a higher risk of coronary heart disease (CHD)

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http://dx.doi.org/10.1016/j.lwt.2016.10.054
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(Willett et al., 1993). The U.S. Food and Drug Administration (FDA) has recently removed partially hydrogenated oils from the generally regarded as safe (GRAS) list because of their *trans*-fat contents. On the other hand, it is difficult to obtain fats with satisfactory plasticity through fractionation because of limitations that preclude its commercial use. As the demand for *trans*-free plastic fats grows, interesterification which is a physical-chemical or enzymatic process that obtains *trans*-free fats, may represent a preferable method to hydrogenation and fractionation. Many fats produced by interesterification have been explored as margarine fats for bakery applications (Adhikari & Hu, 2012; Adhikari et al., 2010; Ruan et al., 2014).

In the production of margarine, medium-chain triacylglycerols (MCT) containing fatty acids with 6–12 carbon atoms have been commonly used. The hydrolysis and digestion of MCTs are often faster *in vivo* compared with long-chain triacylglycerols (LCTs). Furthermore, MCTs can be hydrolyzed more thoroughly and may be capable of reducing body fat (Norizzah, Chong, Cheow, & Zaliha, 2004; Ruan et al., 2014; Willett et al., 1993). Thus, coconut (CNO), palm (PO), and palm kernel (PKO) oils which consist of MCTs have been typically used to produce functional margarine (Norizzah et al., 2004; Ruan et al., 2014).

This study aimed to obtain new *trans*-free margarine fat containing nervonic acid through chemical interesterification (CIE) and/or eznymatic interesterification (EIE). The 3 oils including *Acer truncatum* oil (ATO), palm stearin (PS) (a high-melting-point fraction of PO) and PKO were selected in this study. ATO was used to supply the nervonic acid, while PKO could provide MCTs. Three different ratios of the oils were employed in the interesterification to obtain optimal margarine properties. EIE was carried out via using a 1,3-specific lipase (Lipozyme RM IM, Novozymes, Bagsvaerd, Denmark), whereas CIE was a randomized process. Physicochemical properties including fatty acid composition (FAC), *sn*-2 position FAC, slip melting point (SMP), solid fat content (SFC), TAG content, melting and crystallization profiles, polymorphisms and crystal morphologies were comprehensively investigated to evaluate the potential application of the prepared interesterificated fats in margarine. In addition, the tocopherol, diacylglycerol (DAG) and monoacylglycerol (MAG) contents as well as oxidative stability were examined.

2. Materials and methods

2.1. Materials

PS and PKO were kindly provided by Kerry Specialty Fats (Shanghai) Co., Ltd. ATO was obtained by solvent extraction from *A. truncatum* seeds followed by refining. Commercial immobilized 1,3-specific lipase from *Rhizomucor miehei*, Lipozyme RM IM, was purchased from Novozymes (Bagsvaerd, Denmark). Sodium methoxide (dry powder) was purchased from Aladdin Reagent (Shanghai) Co., Ltd. The standard mixtures (fatty acid methyl esters MIX, C4-C24) for fatty acids analysis were purchased from SUPELCO (Bellefonte, PA, USA). All other chemicals were analytical grade.

2.2. Chemical interesterification

PS, ATO, and PKO were mixed in 3 weight ratios: 5:4:1, 6:3:1, and 6:2:2, and were labeled as CIE A, CIE B and CIE C, respectively, while the physical mixtures were labeled Blend A, Blend B and Blend C, respectively. Each CIE combination was mixed in a flat-bottomed flask and dried at 105 °C under vacuum for 30 min. After cooling to 80 °C, 0.4% (w/w) sodium methoxide was added as a catalyst. Interesterification was conducted at 105 °C under reduced pressure for 50 min. 10% (w/w) aqueous citric acid was added to inactivate the catalyst, and the mixture was stirred for another 10 min. The reaction solution was washed with hot water until the pH value became 7. Then the oil phase was dried to remove residual water at 105 °C under vacuum for 1 h. The final fats were obtained after purification via bleaching (performed with 1.5% w/w bleaching earth at 105 °C under vacuum for 25 min and filtered at 70 °C) and deodorized at 240 °C for 1.5 h.

2.3. Enzymatic interesterification

PS, ATO and PKO, with a weight ratio of 6:3:1, and labeled as EIE B, were mixed in a flat-bottomed flask and heated to 60 °C before adding Lipozyme RM IM (8% w/w of total substrate weight) as a biocatalyst. The mixture was stirred at 60 °C for 9 h with an agitation speed of 300 rpm. Then the lipase was removed from the reaction mixture using vacuum filtration with Whatman filter paper. The final fat was refined by bleaching and deodorization as that for CIE.

2.4. Gas chromatography (GC) analysis of fatty acids

Fatty acid compositions were determined according to the AOCS official method Ce 1f-96 reported by Adhikari and Hu (2012). After methylation, methyl esters (0.2 μL) were injected into an Agilent 7890 series gas chromatograph (GC) (Agilent Technologies, Little Falls, Delaware, U.S.A) equipped with an auto injector and a flame-ionization detector (Agilent Technologies) with a Varian Cp-Silica 7489 column (Varian CP 100 m × 0.25 mm i.d.; Agilent Technologies Netherlands, Middelburg, Netherlands). The initial oven temperature was 80 °C which was held for 2 min. Then, the oven temperature was increased 10 °C/min to 120 °C. Oven temperature was again increased to 180 °C and held for 2 min, then increased to 206 °C at 2 °C/min, and finally increased to 230 °C at 25 °C/min and held for 5 min. The injector and detector temperatures were set at 250 °C and 280 °C, respectively. Fatty acids compositions were identified by comparison with relative retention times of the standard mixtures. Fatty acid composition at the *sn*-2 position was determined using the pancreatic lipase (Shanghai Maikun Chemical Co. Ltd., Shanghai, China) method (Adhikari & Hu, 2012). Each sample (0.1 g) was placed in a test tube and 0.02 g of pancreatic lipase, 2 mL of Tris buffer (pH = 8), 0.5 mL of sodium taurocholate, and 200 μL of saturated $CaCl_2$ were added. The mixture was stirred at room temperature for 1 min and then at 40 °C for 3 min. This procedure was repeated 3 times. Then, 1 mL of hydrochloric acid and 1 mL of diethyl ether were added to the mixture. Thin-layer chromatographic (TLC) separation was conducted using hexane/diethyl ether/ethyl acetate/formic acid (60:38:2:1, v/v/v/v) as the mobile phase, and the monoacylglycerol band was scraped, methylated, and analyzed by GC.

2.5. Analysis of tocopherol, monoacylglycerol (MAG), diacylglycerol (DAG), and triacylglycerol (TAG)

The tocopherol content was determined according to the AOCS official method Ce 8–89 as described by Follegatti-Romero, Piantino, Grimaldi, and Cabral, (2009). The DAG and MAG contents were analyzed using the AOCS official method Cd 11d-96 by HPLC-ELSD according to Vu et al. (2008). The TAG species of physical blends and interesterified fats was analyzed using GC according to Adhikari & Hu (2012) following an AOCS official method.

2.6. Oxidative stability

The oxidative stability of the samples was determined as the induction time according to the Rancimat method (Rancimat 743,

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