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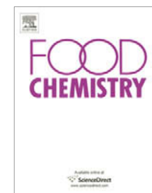

Scaled-up production of zero-*trans* margarine fat using pine nut oil and palm stearin

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ABSTRACT

An interesterified structured lipid was produced with a lipid mixture (600 g) of pine nut oil (PN) and palm stearin (PS) at two weight ratios (PN:PS 40:60 and 30:70) using lipase (Lipozyme TL IM, 30 wt.%) as a catalyst at 65 °C for 24 h. Major fatty acids in the interesterified products were palmitic (35.1–40.4%), oleic (29.5%), and pinolenic acid (*cis*-5, *cis*-9, *cis*-12 18:3; 4.2–5.9%). α-Tocopherol (1.1–1.3 mg/100 g) and γ-tocopherol (0.3–0.4 mg/100 g) were detected in the interesterified products. Total phytosterols (campesterol, stigmasterol, and β-sitosterol) in the interesterified products (PN:PS 40:60 and 30:70) were 63.2 and 49.6 mg/100 g, respectively. Solid fat contents at 25 °C were 23.6% (PN:PS 40:60) and 36.2% (PN:PS 30:70). Mostly β′ crystal form was found in the interesterified products. Zero-*trans* margarine fat stock with desirable properties could be successfully produced from pine nut oil and palm stearin.

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1. Introduction

Margarine and shortening with various melting ranges for bakery products are produced from vegetable oils. Most of them are prepared from partial hydrogenation where *trans* fatty acid (TFA) formation is inevitable. Several studies with conflicting results have been reported on the health effects of *trans* fatty acids; although these studies are still controversial, *trans* fatty acids are associated with coronary heart disease (Lichtenstein, 1993; Mensink & Katan, 1990; Willett & Ascherio, 1994). Similarly, TFA has a negative impact on plasma lipoprotein profile by lowering high-density lipoprotein cholesterol and raising low-density lipoprotein cholesterol (Mensink & Katan, 1990). As a replacement for the partial hydrogenation process, enzymatic interesterification has been shown as an effective way to modify the physical and chemical properties of fat, with desirable functionality and without *trans* fatty acids (Upritchard, Zeelenberg, Huizinga, Verschuren, & Trautwein, 2005). For this purpose, fully hydrogenated oils are interesterified with unsaturated liquid oil. Fats, such as palm stearin and lauric oils, have been used to produce zero-*trans* margarine (Kok, Fehr, Hammond, & White, 1999). Interesterification leads to exchange of fatty acids on the glycerol backbone or to change in the position of fatty acids on the glycerides. It is generally used to customise fat with a range of melting points for different food products and to modify crystallisation.

Conifer nuts contain a very unusual series of C18 polyunsaturated fatty acids (PUFA), in which the first double bond is in the Δ5 position and the next double bond is at the Δ9 or Δ11 position. Pine nut oil is the only commercially available conifer nut oil that is rich in pinolenic acid (PLA) (Imbs, Nevshupova, & Pham, 1998). Pinolenic acid (*cis*-5, *cis*-9, *cis*-12) exerts diverse physiological functions and is used for the prevention or amelioration of various degenerative disorders such as hypercholesterolaemia, thrombosis and hypertension (Sugano, Ikeda, Wakamatsu, & Oka, 1994). It has many biological activities (Deineka & Deineka, 2003), such as reducing blood pressure and attenuation of serum VLDL-TAG and VLDL cholesterol in animals (Asset et al., 1999). Pine nut oil also contains several bioactive and health-promoting substances and they are considered to be an important component of the Mediterranean diet (Hu & Stampfer, 1999).

Palm stearin (PS) is the solid fraction obtained by controlled temperature fractionation, and the liquid fraction is known as palm olein. PS can be used as a source of fully natural hard component in the manufacture of edible fat products, such as margarine and shortening.

The main objective of this study was to produce zero-*trans* margarine fat from PN and PS, with two different weight ratios (PN:PS 40:60 and 30:70). Lipozyme (TL IM) was used as a biocatalyst. The purpose of this interesterification was to obtain fat with suitable melting point and crystallisation behaviour (e.g., more β′ crystal)

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for possible use in margarine. The solid fat contents (SFC), melting and crystallisation behaviour and polymorphic forms were studied to assist with the proper choice of PN and PS amounts required to produce margarine stock with desirable properties.

2. Materials and methods

2.1. Materials

PN and PS were supplied by C.J. Co. (Seoul, Korea). Lipozyme TL IM was purchased from Novozymes A/S (Bagsvaerd, Denmark). The specific activity of Lipozyme TL IM was 175 IU/g, having 0.54 g/ml bulk density and 0.3–1.0 mm particle diameter. Tocopherols (α, γ, and δ) and phytosterols standards were purchased from Sigma Chemical Co. (St. Louis, MO).

2.2. Interesterification

Previously small-scale reactions in a shaking water bath were performed to obtain optimal reaction conditions for producing margarine fat in our laboratory. Then, a scaled-up reaction in a batch-type reactor was performed. The interesterified structured lipid (SL) was produced from a lipid mixture (600 g) of pine nut oil (PN) and palm stearin (PS) with two weight ratios (40:60 and 30:70, PN:PS), using lipase (Lipozyme TL IM, 30 wt.%) as a biocatalyst. The blended substrates were reacted in a batch-type reactor for 24 h at 65 °C and the mixing speed was set at 500 rpm, as described by Adhikari et al. (2009). The TAG profile of reacted sample at various times (1, 2, 6, 12, and 24 h) was analysed by HPLC without the removal of free fatty acids (FFA).

2.3. Fatty acid composition

The fatty acid composition of the samples were determined by gas chromatography (GC) after conversion to fatty acid methyl esters (FAMEs) with boron trifluoride in methanol, using the analysis condition described previously (Adhikari et al., 2009).

Positional fatty acid composition of the PN, PS and interesterified product were determined by pancreatic hydrolysis as described previously (Lee & Akoh, 1996).

2.4. Analysis of tocopherols

Tocopherol was determined using HPLC (Lee, Lee, Akoh, Chung, & Kim, 2006). The HPLC system consisted of a Yonglin SP930D dual pump (Yonglin, Anayang, Korea) with a UV detector set at 295 nm. The column was a Chromsep Cartridge, LiChrosorb Diol (5 μm, 3 × 100 mm, Varian, Palo Alto, CA). The mobile phase was a mixture of hexane fortified with 0.1% acetic acid, and the flow rate was 1 ml/min. Standard α-, γ-, and δ-tocopherols were used for the quantification.

2.5. Phytosterol analysis

Each sample was analysed for the quantification of phytosterols as previously described (Lee et al., 2006). Samples were injected onto an M600D (Yonglin) GC equipped with a flame ionisation detector and an Ultra-2 column (5% diphenyl/95% dimethylsiloxane, 30 m × 0.25 mm × 0.25 μm; Agilent, Santa Clara, CA). The initial oven temperature was 220 °C for 0.5 min, increased to 270 °C at a rate of 10 °C/min, and holding for 27 min. Finally, the temperature was increased to 285 °C at 10 °C/min and held for 3.5 min. The injector and detector temperatures were 270 °C and 290 °C, respectively.

2.6. Determination of slip melting point (SMP)

The slip melting points of the samples were determined according to AOCS Official Method Cc. 3.25 (American Oil Chemist's Society, 1990).

2.7. Differential scanning calorimetry (DSC)

A differential scanning calorimeter (DSC) 2010 (TA Instruments Inc., New Castle, DE) was used to obtain the thermograms of melting and crystallisation (Lee & Foglia, 2000). The sample was heated to 80 °C and held for 10 min. Thereafter, the temperature was decreased to −60 °C at 10 °C/min. After holding for 10 min at −60 °C, the melting curve was obtained by heating to 80 °C at 5 °C/min. The solid fat content (SFC, %) was obtained from melting thermograms by Universal Analysis 2000 (TA Instruments Inc.). Each DSC thermogram was divided into different temperatures (10, 15, 20, 25, 30, 35, 40, 45, and 50 °C) and the total crystallisation energy (J/g) was converted into percentage (%) at each temperature for SFC.

2.8. High-performance liquid chromatography

The separation of TAG species from PN and PS was conducted by reversed-phase HPLC as described previously (Adhikari et al., 2009). Twenty microlitres of filtered sample were injected onto Nova-Pak C18 column (150 × 3.9 mm, Waters, Milford, MA). Mobile phase consisted of **(A)** acetonitrile and **(B)** isopropanol/hexane (2:1, v/v) at a flow rate of 1 ml/min with the following profile: 0–44 min, 20% **B**; 45–50 min, 46% **B**; 51–58 min, 100% **B**, and then returned to the initial flow rate.

2.9. Polymorphism by X-ray diffraction spectroscopy

Each melted sample was placed on a rectangular plastic mould, and tempered at 24 °C for 24 h. Polymorphic forms of the samples were determined by X-ray diffraction, using a D/Max-2200 Model Ultima/PC (Rigaku Int. Corp., Tokyo, Japan) with a fine copper X-ray tube, operating at 40 kV and 35 mA (Adhikari et al., 2009).

2.10. Crystal microstructure

The crystal microstructure of the samples was observed using a model confocal laser scanning microscope (Carl Zeiss Inc., Göttingen, Germany). Samples were completely melted and then 10 μl of melted samples were placed on a glass microscope slide. A glass cover slip was placed over the samples to give a homogenous distribution. The samples were cooled at room temperature (25 °C) for 16 h. The microstructure of the crystallised sample was taken at 200 × magnification.

2.11. Statistical analysis

Statistical Analysis System software (SAS Institute, 2000) was used to perform statistical analysis. Duncan's multiple range tests were performed to determine significance of difference at $p < 0.05$.

3. Results and discussion

3.1. Fatty acid composition

The fatty acid compositions of PN, PS, physical blends and the interesterified products are present in Table 1. PN contained a high amount (92.5%) of unsaturated fatty acids (ΣUFA), in which major fatty acids were oleic acid (O, 26.7%), linoleic acid (L, 46.3%) and

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