Food Chemistry 119 (2010) 1332–1338

Contents lists available at ScienceDirect

Food Chemistry

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

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

Prakash Adhikari [a], Xue-Mei Zhu [a], Anupama Gautam [a], Jung-Ah Shin [a], Jiang-Ning Hu [a], Jeung-Hee Lee [a], Casimir C. Akoh [b], Ki-Teak Lee [a,*]

[a] Department of Food Science and Technology, Chungnam National University, Daejeon 305-764, South Korea
[b] Department of Food Science and Technology, The University of Georgia, Food Science Building, Athens, GA 30602, USA

ARTICLE INFO

Article history:
Received 6 March 2009
Received in revised form 24 June 2009
Accepted 3 September 2009

Keywords:
Pine nut oil
Palm stearin
Interesterification
DSC
Phytosterol
Margarine stock

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.

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)

* Corresponding author. Tel.: +82 042 821 6729; fax: +82 042 822 6729.
E-mail address: ktlee@cnu.ac.kr (K.-T. Lee).

0308-8146/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2009.09.009

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

Table 1
Fatty acid composition (area%), tocopherol and phytosterol (mg/100 g) of pine nut oil (PN) palm stearin (PS), physical blends and the interesterified products.

Fatty acids[f]	PN	PS	Physical blend (PN:PS)		Interesterified product (PN:PS)	
			40:60	30:70	40:60	30:70
14:0	ND[g]	1.2 ± 0.0	0.8 ± 0.0	0.8 ± 0.00	0.8 ± 0.0	0.9 ± 0.0
16:0	4.8 ± 0.1	55.0 ± 0.0	36.7 ± 0.1	40.3 ± 0.13	35.1 ± 1.1	40.4 ± 0.2
18:0	2.3 ± 0.1	7.4 ± 0.1	5.0 ± 0.0	5.9 ± 0.0	4.8 ± 0.9	5.9 ± 0.1
Δ9–18:1	26.7 ± 0.1	29.0 ± 0.2	28.5 ± 0.1	28.9 ± 0.0	29.5 ± 0.3	29.5 ± 0.2
Δ5, 9–18:2	2.2 ± 0.0	ND	ND	ND	ND	ND
Δ9, 12–18:2	46.3 ± 0.1	7.2 ± 0.2	23.5 ± 0.0	19.7 ± 0.1	23.9 ± 0.0	19.1 ± 0.0
Δ5, 9, 12–18:3[h]	14.1 ± 0.1	ND	5.5 ± 0.0	4.4 ± 0.0	5.9 ± 0.0	4.2 ± 0.0
Δ9, 12, 15–18:3	0.2 ± 0.0	ND	ND	ND	ND	ND
20:0	0.4 ± 0.1	ND	ND	ND	ND	ND
Δ11–20:1	1.2 ± 0.0	ND	ND	ND	ND	ND
Δ11, 14–20:2	0.7 ± 0.1	ND	ND	ND	ND	ND
Δ5, 11, 14–20:3	1.1 ± 0.0	ND	ND	ND	ND	ND
ΣUFA	92.5 ± 0.1	36.2 ± 0.2	57.5 ± 0.1	53.0 ± 0.1	59.3 ± 0.2	52.8 ± 0.2
ΣSFA	7.5 ± 0.1	63.3 ± 0.2	42.5 ± 0.1	47.0 ± 0.1	40.7 ± 0.3	47.2 ± 0.2
α-Tocopherol	14.0 ± 1.4[a]	3.1 ± 0.3[c]	7.6 ± 0.6[b]	6.2 ± 0.1[b]	1.1 ± 0.1[d]	1.3 ± 0.0[d]
γ-Tocopherol	8.9 ± 1.5[a]	0.5 ± 0.1[c]	3.7 ± 0.4[b]	2.7 ± 0.1[b]	0.4 ± 0.1[c]	0.3 ± 0.1[c]
Total	23.0 ± 3.0[a]	3.6 ± 0.5[c]	11.3 ± 0.9[b]	8.9 ± 0.1[b]	1.4 ± 0.2[c]	1.5 ± 0.1[c]
Campesterol	17.1 ± 1.6[a]	6.0 ± 0.4[d]	11.6 ± 0.1[b]	9.6 ± 0.9[b,c]	9.0 ± 1.2[b,c]	8.0 ± 2.4[c,d]
Stigmasterol	ND	2.3 ± 0.3[a]	1.5 ± 0.3[a]	1.9 ± 0.4[a]	1.5 ± 0.2[a]	1.8 ± 0.6[a]
β-Sitosterol	125 ± 2.2[a]	17.9 ± 1.4[e]	63.9 ± 1.6[b]	49.5 ± 3.3[c]	52.7 ± 2.0[c]	39.9 ± 5.8[d]
Total	142 ± 0.6[a]	26.1 ± 1.2[e]	77.0 ± 1.3[b]	61.0 ± 3.8[c]	63.2 ± 0.9[c]	49.6 ± 8.8[d]
SMP (°C)	–	51.0 ± 0.3	45.1 ± 0.8	48.8 ± 0.3	34.8 ± 0.4	39.5 ± 0.0

All data are mean values ± standard deviations of duplicate measurements.
[a–e] Values with the same letter in a row are not significantly different ($p > 0.05$). Blend were prepared by blending PN and PS (PN:PS 40:60 and 30:70) in two weight ratios.
[f] All double bonds in the *cis* configuration.
[g] Not detected.
[h] Pinolenic acid (*cis*-5, 9, 12); ΣUFA: total unsaturated fatty acids; ΣSFA: total saturated fatty acid.

pinolenic (Pi, 14.1%). PS contained high amounts (63.3%) of saturated fatty acid (ΣSFA). Palmitic (P) and oleic acids were the most abundant fatty acids in PS (55.0% and 29.0%, respectively), and the interesterified products (35.1–40.4% and 29.5%, respectively). Under our analysis conditions, *trans* fatty acid was not detected. As the weight ratio of PS increased, the content of ΣSFA (mainly palmitic acid) increased. The degree of changes in the physical properties was greatly influenced by interesterification using lipase as biocatalyst.

Sn-2 position is important nutritionally since it is absorbed easily in the body (Quinlan & Moore, 1993). The major unsaturated fatty acids at the *sn*-2 position in PN were linoleic (67.6%) and oleic (29.0%), while PS contained higher levels of palmitic (32.9%) and oleic acids (48.8%) (data not shown). The major fatty acids in the interesterified products were palmitic (38.5–44.2%) and oleic acids (27.3–27.9%), at the *sn*-2 position (data not shown).

Tocopherols are natural antioxidants in vegetable oils which prevent lipid oxidation. The tocopherol content of the PN, PS, physical blends and the interesterified products are presented in Table 1. α-Tocopherol is considered as the most important natural antioxidant. It prevents lipid peroxidation by scavenging radicals in membrane and lipoprotein particles (Esterbauer, Dieber-Rotheneder, Striegl, & Waeg, 1991). Among the α-, β-, γ-, and δ-tocopherols, only α and γ-tocopherols were detected in all analysed samples. PN showed a higher level of total tocopherols (23.0 mg/100 g) than PS (3.6 mg/100 g). Total tocopherols contained in the physical blends (PN:PS 40:60 and 30:70) were 11.3 mg/100 g and 8.9 mg/100 g, respectively. The total tocopherols in the interesterified products (PN:PS 40:60 and 30:70) were 1.4–1.5 mg/100 g. Reduction in tocopherol content of the interesterified product was observed compared to physical blends. Loss of tocopherols generally occurs during purification (i.e., de-acidification) and processing of vegetable oils into solid fats (shortening and margarine). Such reduction of tocopherols was reported previously (Senanayake & Shahidi, 2002).

Phytosterol is known to block the absorption of cholesterol from the diet into the bloodstream. Phytosterols (campesterol, stigmasterol, and β-sitosterol) were detected in all the interesterified products. PS contained lower total phytosterols (26.1 mg/100 g) compared with PN (142 mg/100 g). The total phytosterol concentrations in the interesterified products (PN:PS 40:60 and 30:70) were 63.2 and 49.6 mg/100 g, respectively. When the ratio of PS increased, the amount of phytosterol decreased. The most predominant phytosterol was β-sitosterol (17.9–125 mg/100 g) followed by campesterol (6.0–17.1 mg/100 g) in all analysed samples (Table 1).

3.2. Solid fat contents (SFC)

DSC melting and crystallisation thermograms of PS, physical blends and the interesterified products are presented in Fig. 1. PS showed six (A–F) melting peaks, and a broad melting peak was observed at 48.9 °C which represents highest melting triacylglycerols. The highest melting peaks of the interesterified products (PN:PS 40:60 and 30:70) were observed at 36.7 °C and 40.3 °C, respectively. Physical blends (PN:PS 40:60 and 30:70) showed four similar melting peaks (Fig. 1A). The crystallisation peaks were observed at 1.0 °C and 25.9 °C in PS. Similarly, crystallisation peaks of the interesterified products (PN:PS 40:60 and 30:70) were observed at −0.5 °C and 13.9 °C and −0.1 °C and 17.9 °C, respectively (Fig. 1B). When the PS amount was increased the melting and crystallisation peaks were moved towards a higher temperature. SFC influences physical properties such as hardness, mouthfeel and spreadability and SFC values are needed to characterise the physical properties of fats. The SFC of the interesterified products (PN:PS 40:60 and 30:70) at 25 °C were 23.6% and 36.2%, respectively. SFC of the interesterified products (PN:PS 40:60 and 30:70) at 40 °C were decreased to 0.7% and 7.6%, respectively (data not shown). As expected, the addition of PS increased the SFC in the interesterified products and physical blends. SFC profile with wide plastic

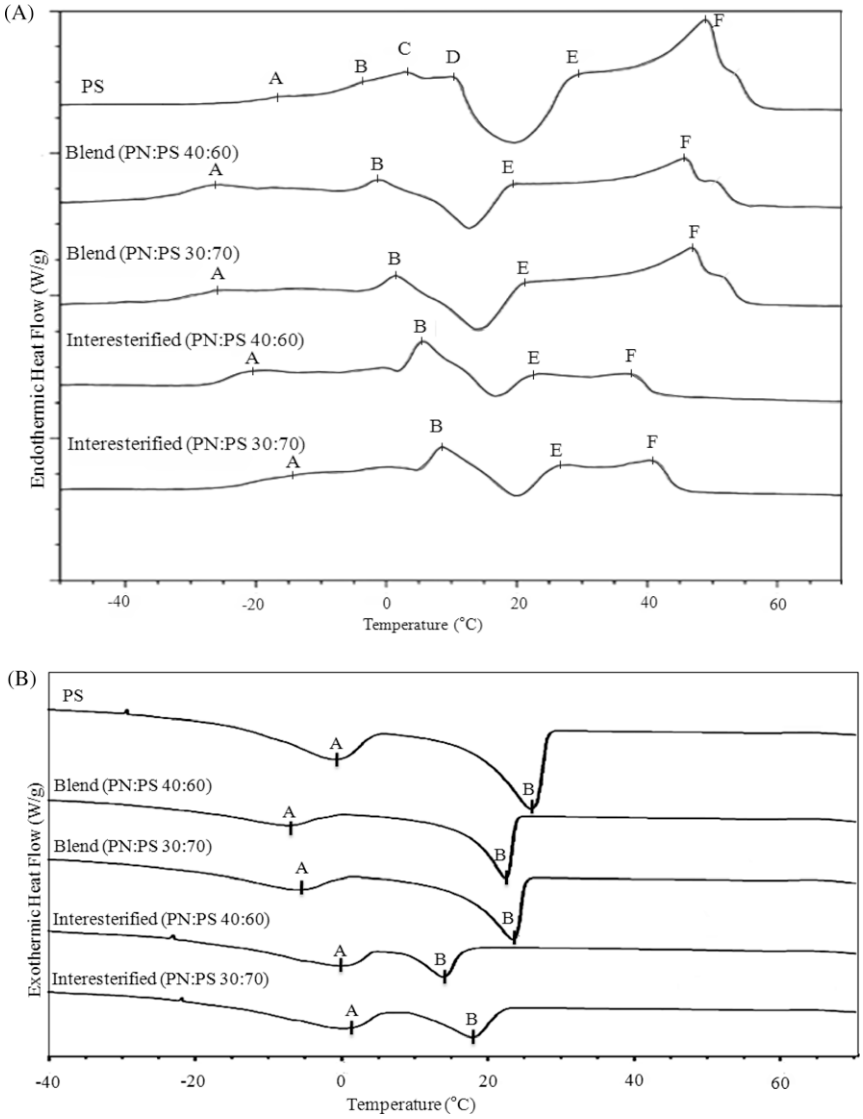

Fig. 1. (A) Melting thermograms of PS, physical blends and the interesterified products (PN:PS 40:60 and 30:70) and (B) crystallisation thermograms of PS, physical blends and the intereserified products (PN:PS 40:60 and 30:70).

range and gradual slope is a major characteristic of fats for use in the baking industry (Lee, Akoh, Himmelsbach, & Lee, 2008).

3.3. Triglyceride (TAG) composition

The TAG composition obtained by RP-HPLC of PN, PS, interesterified product and physical blend are presented in Table 2. The predominant TAGs of PN were LLPi (24.7%), OLL (17.0%), and LOPi (17.0%), whereas PS contained appreciable amounts of POP (43.7%), PPP (25.6%) and POO (15.2%). Physical blend (PN:PS 40:60) also contained high levels of POP (31.7%), PPP (19.0%) and POO (13.1%). In the interesterified products, it is clearly noticeable that most of the reaction occurred within 1 h. After 1 h reaction, there were no major changes among the TAG species until 24 h (Table 2). Some TAGs were increased (i.e., POL, PLO, PLP, PLL and POS) and some TAGs decreased (i.e., LOPi, OLL, POP, PPP) (Table 2) after the interesterification. Chen, Chong, Ghazali, and Lia (2007) reported greater decline in TAG concentration and formation of more FFA during 12 h reaction. In our analysis, after 24 h reaction, small amounts of FFA (0.6% and 0.4%), diacylglycerols (DAG; 0.9% and 1.2%) and monacylglycerols (MAG; 2.2% and 3.5%) were found in the interesterified products (PN:PS 40:60 and 30:70), respectively. The presence of a certain amount of DAG in the interesterified product is advantageous for the production of margarine stocks. Another product of lipase-catalysed interesterification is free fatty acids (FFA). The presence of FFA in the product is a disadvantage because hydrolysis of TAG into FFA leads to low yield of desired product and contribute to deterioration of the product quality, such as rancidity (Zainal & Yusoff, 1999).

3.4. Polymorphism and fat crystal microstructure

The main polymorphs of fat crystals are α, β′, and β polymorphic forms. Each polymorph has different characteristics with α form characterised as unstable with the lowest melting point and short spacing at 4.15 Å; β′ form, metastable, intermediate melting point and two strong short spacings at 3.80 Å and 4.20 Å and three minor short spacings at 4.27 Å, 3.97 Å and 3.71 Å; β form, very stable, highest melting point and short spacing at 4.60 Å (Solís-Fuentes, Hernandez-Medel, & Duran-de-Bazua, 2005). The β' form having small crystal size is a desirable polymorphic form for margarines and shortenings because β crystal composed of very large crystals will increase the hardness and decrease the spreadability (Widlak,

Table 2
Triacylglycerol composition (area%) of pine nut oil (PN) and palm stearin (PS), physical blend and interesterified product.

Peaks	ECN[a]	TAG	PN	PS	Physical blend (PN:PS 40:60)	Interesterified products (PN:PS 40:60)				
						1 h	2 h	6 h	12 h	24 h
1	40	LLPi	24.7	–	5.6	–	–	–	–	–
2	42	LLL	8.4	–	2.0	–	–	–	–	–
3	42	LOPi	17.0	–	3.8	–	–	–	–	–
4	42	PLPi/LPiP/LPPi	2.1	–	–	–	–	–	–	–
5	44	OLL/LOL	17.0		4.6	2.8	2.7	1.9	2.5	2.7
6	44	PLL/LPL	3.5	–	–	6.1	4.1	4.9	4.1	5.0
7	44	OOPi/OPiO	8.8	–	1.9	–	–	–	–	–
8	46	OLO/LOO	7.2	–	2.9	4.5	4.0	3.3	2.2	4.2
9	46	POL/PLO/OPL	3.6	3.2	5.1	23.0	24.5	26.1	25.1	23.5
10	46	PLP/PPL	–	4.6	3.7	12.2	13.2	14.1	14.6	13.0
11	48	OOO	3.2	–	–	–	–	–	–	–
12	48	POO/OPO	–	15.2	13.1	12.6	14.7	12.1	13.9	11.7
13	48	POP/PPO	–	43.7	31.7	17.8	22.0	21.9	22.3	18.9
14	48	PPP	–	25.6	19.0	3.3	4.4	4.6	4.5	4.6
15	50	POS/PSO	–	2.9	1.5	1.6	2.5	1.6	2.9	2.2
16	50	PPS/PSP	–	2.1	1.1	0.5	1.3	1.5	1.4	1.0
	Others		3.0	2.7	4.0	15.6	6.6	8.0	6.5	13.2

Abbreviations: TAG, triacylglycerol; Pi, Pinolenic; P, palmitic; S, stearic; O, oleic; L, linoleic.
[a] Equivalent carbon number (ECN): CN – 2DB, where CN is a carbon number of TAG and DB is total number of double bonds in TAG.

Hartel, & Narine, 2001). The polymorphic forms of the interesterified products and physical blends are presented in Fig. 2. Interesterified product and physical blend displayed mixture of β′ and β polymorphic forms. Interesterified product showed stronger intensities at 3.86, 4.22 or 4.37 Å than 4.66 Å. In the case of physical blends, strong intensities were observed at 3.86, 4.22 or 4.34 and 4.58 Å. The interesterified product PN:PS 30:70 contained 72.1% β′ polymorphic forms which is higher than for interesterified product PN:PS 40:60 (57.0%). The β′ form (at 3.86 and 4.22 Å) was increased and β form (at 4.66 Å) was decreased, as the amount of PS increased. A similar result was also presented by Lee et al. (2008). Thus, it could be explained that the interesterified product PN:PS 30:70 was harder and more solid than interesterified product PN:PS 40:60 because of the higher solid fat content and more fat crystal (Fig. 3).

Crystal microstructures of the PS, interesterified products and physical blends are presented in Fig. 3. The closely-packed crystals of large rod-like spherulic (round shape crystal) were observed in PS. Physical blend PN:PS 40:60 displayed a similar structure to initial PS whereas physical blend PN:PS 30:70 showed large size

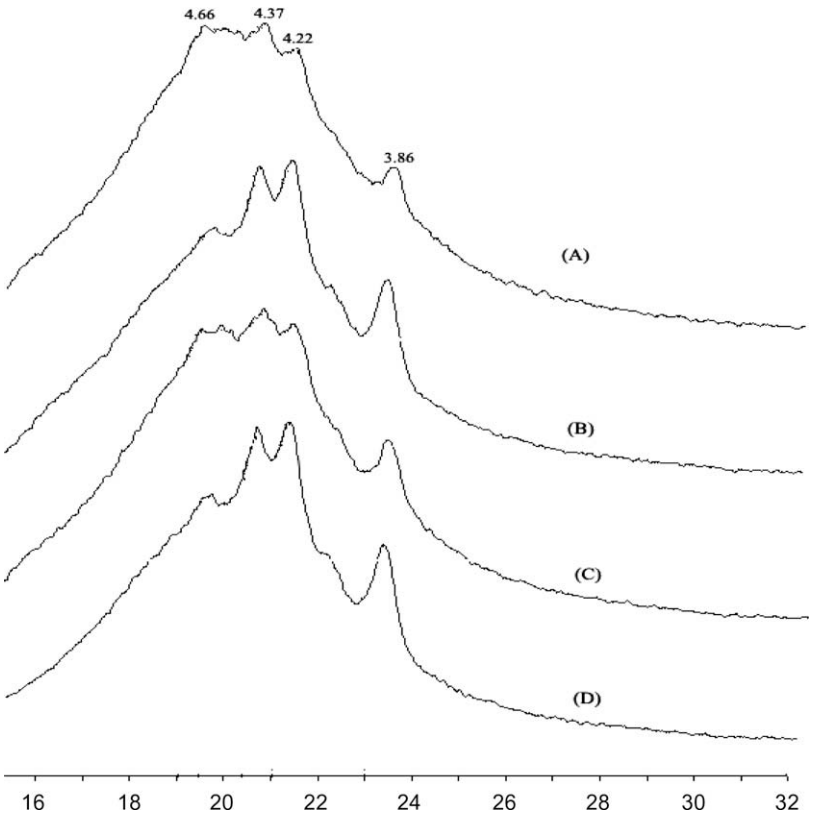

Fig. 2. X-ray diffraction spectroscopy of interesterified product [(A) and physical blend (B) (PN:PS 40:60)], and interesterified product [(C), and physical blend (D) (PN:PS 30:70)].

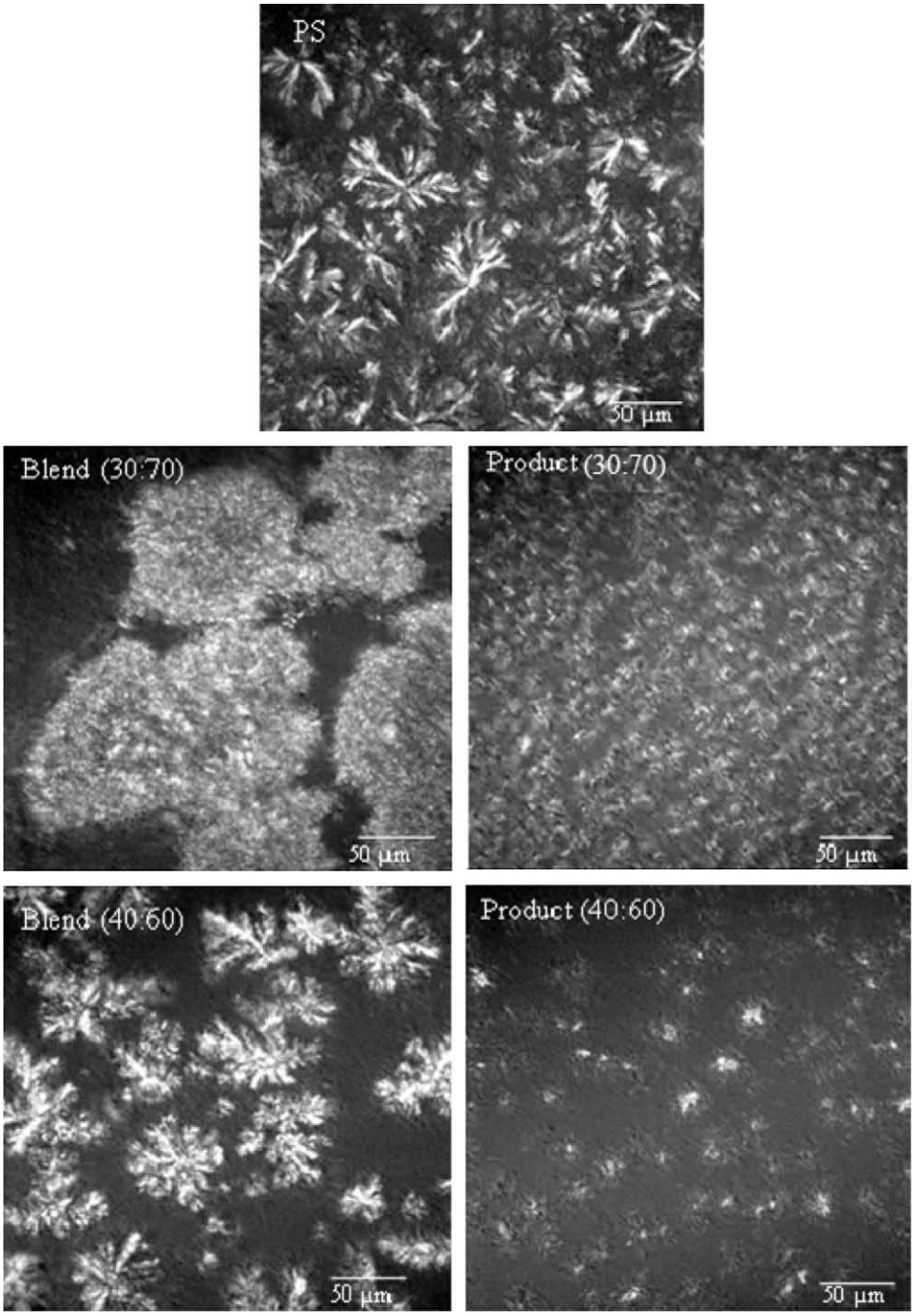

Fig. 3. Crystal microstructure of the PS, physical blends and the interesterified products (PN:PS 40:60 and 30:70).

crystals and different crystals to other analysed samples. The interesterified products displayed different crystal microstructures to the initial PS. Small crystals were observed in the interesterified products compared to physical blends. The crystal of interesterified products displayed spherulite-shaped crystals that tended to be aggregated to form clusters. Compacted small crystals were desirable properties for margarines and shortenings, since these crystals could surround and stabilise air bubbles (Lee et al., 2008).

A zero-*trans* margarine fat stock with desirable physicochemical properties including a wide range of SFCs could be successfully produced from pine nut oil and palm stearin with two different weight ratios (PN:PS 40:60 and 30:70). The interesterified products contained mostly β′ polymorphic form which is a desirable property of margarine fat stock. Our study suggested that the produced margarine without *trans* fatty acids could be used as an alternative to partially hydrogenated fats.

Acknowledgement

This study was supported by Technology Development Program for Agriculture and Forestry, Ministry for Agriculture, Forestry and Fisheries, Republic of Korea.

References

Adhikari, P., Shin, J. A., Lee, J. H., Hu, J. N., Hwang, K. T., & Lee, K. T. (2009). Enzymatic production of *trans* free hard fat stock from fractionated rice bran oil, fully hydrogenated soybean oil and conjugated linoleic acid. *Journal of Food Science, 74*, E87–E96.

American Oil Chemist's Society (1990). *Official and recommended methods of the American Oil Chemist's Society* (15th ed.). Champaign, IL: AOCS Press.

Asset, G., Staels, B., Wolff, R. L., Bauge, E., Madj, Z., Fruchart, J. C., et al. (1999). Effects of *Pinus pinaster* and *Pinus koraiensis* seed oil supplementation on lipoprotein metabolism in the rat. *Lipids, 34*, 39–44.

Chen, C. W., Chong, C. L., Ghazali, H. M., & Lia, O. M. (2007). Interpretation of triacylglycerol profiles of palm oil, palm kernel oil and their binary blends. *Food Chemistry, 100*, 178–191.

Deineka, V. I., & Deineka, L. A. (2003). Triglyceride composition of *Pinus sibirica*. Oil chemistry of natural compounds. *Chemistry of Natural Compounds, 39*, 171–173.

Esterbauer, H., Dieber-Rotheneder, M., Striegl, G., & Waeg, G. (1991). Role of vitamin E in preventing the oxidation of low-density lipoprotein. *American Journal of Clinical Nutrition, 53*, 314–321.

Hu, F. B., & Stampfer, M. J. (1999). Nut consumption and risk of coronary heart disease review of epidemiologic evidence. *Current Atherosclerosis Reports, 3*, 204–209.

Imbs, A. B., Nevshupova, N. V., & Pham, L. Q. (1998). Triacylglycerol composition of *Pinus koraiensis* seed oil. *Journal of American Oil Chemists' Society, 75*, 865–870.

Kok, L. L., Fehr, W. R., Hammond, E. G., & White, P. J. (1999). Trans free margarine from highly saturated soybean oil. *Journal of American Oil Chemists' Society, 76*, 1175–1181.

Lee, K. T., & Akoh, C. C. (1996). Immobilized lipase-catalyzed production of structured lipids with eicosapentaenoic acid at specific position. *Journal of American Oil Chemists' Society, 73*, 611–615.

Lee, J. H., Akoh, C. C., Himmelsbach, D. S., & Lee, K. T. (2008). Preparation of interesterified plastic fats from fats and oils free of *trans* fatty acid. *Journal of Agricultural and Food Chemistry, 56*, 4039–4046.

Lee, K. T., & Foglia, T. A. (2000). Synthesis, purification, and characterization of structured lipids produced from chicken fat. *Journal of American Oil Chemists' Society, 77*, 1027–1034.

Lee, J. H., Lee, K. T., Akoh, C. C., Chung, K. S., & Kim, R. M. (2006). Antioxidation evaluation and oxidation stability of structured lipids from extravirgin olive oil and conjugated linoleic acid. *Journal of Agricultural and Food Chemistry, 54*, 5416–5421.

Lichtenstein, A. (1993). *Trans* fatty acids, blood lipids, and cardiovascular risk: Where do we stand? *Nutrition Reviews, 51*, 340–343.

Mensink, R. B., & Katan, M. B. (1990). Effect of Dietary *trans* fatty acids on high-density and low-density lipoprotein cholesterol levels in healthy subjects. *The New England Journal of Medicine, 323*, 439–445.

Quinlan, P., & Moore, S. (1993). Modification of triacylglycerols by lipases: Process technology and its application to the production of nutritionally improved fats. *INFORM, 4*, 580–585.

SAS Institute (2000). *SAS Statistics Software, Release 8.2*. Cary: SAS Institute.

Senanayake, S. P. J. N., & Shahidi, F. (2002). Chemical and stability characteristics of structured lipids from borage (*Borago Officinalis* L.) and evening primrose (*Oenothera biennis* L.) oils. *Journal of Food Science, 67*, 2038–3045.

Solís-Fuentes, J. A., Hernandez-Medel, M. D., & Duran-de-Bazua, M. D. (2005). Determination of the predominant polymorphic form of mango (*Mangifere indica*) almond fat by differential scanning calorimetry and X-ray diffraction. *European Journal of Lipid Science and Technology, 107*, 395–401.

Sugano, M., Ikeda, I., Wakamatsu, K., & Oka, T. (1994). Influence of Korean pine (*Pinus koraiensis*)-seed oil containing *cis*-5, *cis*-9, *cis*-12-octadecatrienoic acid on polyunsaturated fatty acid metabolism, eicosanoid production and blood pressure of rats. *Journal of British Nutrition, 72*, 775–783.

Upritchard, J. E., Zeelenberg, M. J., Huizinga, H., Verschuren, P. M., & Trautwein, E. A. (2005). Modern fat technology: What is the potential for heart health? *Proceedings of the Nutrition Society, 64*, 379–386.

Widlak, N., Hartel, R., & Narine, S. (2001). Crystallization and solidification properties of lipids. In J. M. deMan & L. deMan (Eds.), *Polymorphism and texture of fats* (pp. 225–235). Champaign, IL: AOCS Press.

Willett, W. C., & Ascherio, A. (1994). *Trans* fatty acids: Are the effects only marginal? *American Journal of Public Health, 84*, 722–724.

Zainal, Z., & Yusoff, A. M. S. (1999). Enzymatic interesterification of palm stearin and palm kernel olein. *Journal of American Oil Chemists' Society, 76*, 1003–1008.