J Am Oil Chem Soc (2008) 85:1–11
DOI 10.1007/s11746-007-1155-0

ORIGINAL PAPER

Physical Properties of *trans*-Free Bakery Shortening Produced by Lipase-Catalyzed Interesterification

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Received: 3 May 2007 / Revised: 18 July 2007 / Accepted: 4 October 2007 / Published online: 6 November 2007

Abstract Lipase-catalyzed interesterified solid fat was produced with fully hydrogenated soybean oil (FHSBO), and rapeseed oil (RSO) and palm stearin (PS) in a weight ratio of 15:20:65, 15:40:45 and 15:50:35. The interesterified fats contained palmitic (27.8–44.6%), stearic (15.6–16.2%), oleic (27.5–36.5%) and linoleic acids (8.0–13.5%). After interesterification of the blends, the physical properties of the products changed and showed lower melting points and solid fat contents, different melting and crystallization behaviors as well as the formation of more stable crystals. The produced interesterified fats (FHSBO:RSO:PS 15:20:65, 15:40:45 and 15:50:35 blends) contained desirable crystal polymorphism (β' form) as determined by X-ray diffraction spectroscopy, a long plastic range with solid fat content of 51–63% at 10 °C to 4–12% at 40 °C, and melting points of 39 (15:50:35), 42 (15:50:45) and 45 °C (15:20:65). However, a reduction in tocopherols (α and γ) content and a reduced oxidative stability were observed in the interesterified fats. The physical properties of the interesterifed fats were influenced by the amount of PS, resulting in more hardness and higher solid fat contents for 15:20:65 than 15:40:45 and 15:50:35 blends. The present study suggested that the produced interesterified fats containing *trans*-free fatty acids could be used as alternatives to hydrogenated types of bakery shortenings.

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Keywords Crystallization · Interesterification · Lipase · Melting point · Palm stearin · Polymorphism · Rapeseed oil · Solid fat content

Introduction

Shortening is a solid fat product that is commonly used in the baking industry. Its plasticity allows air incorporation during the creaming stage of making a cake batter or cookie dough formation [1]. The small air bubbles are locked into the shortening and remain in the final batter. The air bubbles expand as the batter is baked, serving as nuclei for leavening gases, and gives a fine and smooth texture to the bakery product. The high melting characteristic of shortening can withstand elevated baking temperatures and hold the shape of the batter longer.

With regard to melting characteristics, consistency and crystal structure, various types of shortening have been produced to impart desirable texture and mouthfeel to different bakery products (i.e., cakes, icing, doughnuts or roll-in). Shortening is normally produced by a partial hydrogenation process, in which vegetable oils (i.e., soybean oil, cottonseed oil or rapeseed oil) are chemically transformed into a solid state. However, the hydrogenation is accompanied with the formation (up to 50%) of *trans* fatty acids (TFA) [2, 3]. TFA is associated with increased risk of developing cardiovascular disease, and it has more adverse effects than saturated fatty acids or *cis* form of unsaturated fatty acids [4–7]. In the US, intake of TFA has been estimated at approximately 2.6% of total energy (5.3 g/day), and the American Heart Association (AHA) recommended TFA intake of <1% of energy [8]. Thus, efforts for reducing the consumption of TFA has led to the prohibition of foods containing >2% TFA as a percentage

of total fats in the Danish market [9], and mandatory TFA labeling in packaged foods in the US [10].

Lipase-catalyzed interesterification is one of the alternative technologies for replacing conventional hydrogenation process to reduce or eliminate TFA [11]. Because a molecular rearrangement of fatty acids on the glycerol backbone takes place during interesterification with lipase catalysis, it has been used in many studies for producing modified solid fats with desired physical and chemical properties [12, 13]. Palm stearin is known to promote the formation of desirable solid fat having β' crystal when it was interesterified with vegetable oils [14]. Recent studies showed that the combinations of palm stearin and sunflower oil (40:60 and 50:50) or palm stearin and coconut oil (70:30) were suitable for table margarine or shortening preparation [13, 14].

In bakery foods, shortening provides a number of desirable physical properties including tenderness and texture, mouthfeel, incorporation of air, heat transfer and extended shelf life [15]. In the present study, lipase-catalyzed interesterification of fully hydrogenated soybean oil (FHSBO), rapeseed oil (RSO) and palm stearin (PS) at different blend weight ratios (15:20:65, 15:40:45 and 15:50:35) was performed to replace the conventional hydrogenation process for producing bakery shortening containing TFA. To determine suitable properties in bakery products, the physical properties of the produced interesterified solid fats were evaluated for hardness, solid fat content, melting and crystallization behavior, polymorphic forms as well as crystal morphology.

Materials and Methods

Materials

Fully hydrogenated soybean oil (FHSBO), rapeseed oil (RSO), and palm stearin (PS) were supplied by the C.J. Co. (Seoul, Korea). Lipozyme RM IM, a *sn*-1,3 selective lipase from *Rhizomucor miehei*, was purchased from Novozymes, Inc. (Franklinton, NC). Heptadecanoic acid methyl ester and tocopherols (α and γ) were purchased from Sigma-Aldrich Chemical Co. (St Louis, MO).

Lipase-Catalyzed Interesterification

Blends of FHSBO, RSO, and PS in weight ratios of 15:50:35, 15:40:45, and 15:20:65 were prepared for interesterification. The melted blend was reacted with Lipozyme RM IM (10 wt% of total substrates) in a 1-L stirred-tank batch-type reactor (11-cm internal diameter and 15-cm height) for 24 h at 65 °C. A semicircular-shaped stirring blade was placed at a distance of 1 cm from the bottom of the reactor, and the mixing speed was set at 300 rpm. Lipase was removed by vacuum filtration after the reaction, and then the reaction product was purified with KDL-4 short path distillation system (UIC Inc., Joliet, IL) in which the temperatures of the evaporator and condenser were set at 185 and 20 °C, respectively, and vacuum pressure was maintained below 1 mm Hg. After the distillation, the free fatty acid (FFA) content was measured following AOCS Method, Ca 5a-40 [16].

Fatty Acid Composition

The fatty acid composition of samples was determined after converting fatty acids into corresponding fatty acid methyl esters (FAME). After methylation the fatty acid composition was determined with Hewlett-Packard 6890 gas chromatography (GC) equipped with an auto injector and a flame-ionization detector (Agilent Technologies, Little Falls, DE) using a fused-silica capillary column (SP^{TM}-2560, 100 m × 0.25 mm i.d., 0.25-μm film thickness, Supelco, Bellefonte, PA). The temperatures of the injector and detector were set at 250 and 260 °C, respectively. The oven was heated to 150 °C and held for 5 min. Then the temperature was increased to 220 °C at the rate of 4 °C/min, and held for 30 min. FAMEs were identified by comparison with relative retention times of standard mixtures (GLC-461, Nu-Chek Prep, Inc., Elysian, MN). Methyl ester of heptadecanoic acid was used as an internal standard. Fatty acid composition at the *sn*-2 position was determined by the Grignard reaction with ethyl magnesium bromide described by Lee et al. [17]. Thin-layer chromatography (TLC) plates were pre-impregnated with 0.4% boric acid. After spotting the sample on TLC plate, it was developed in diethyl ether/hexane/acetic acid (50:50:1, *v*/*v*/*v*), and sprayed with 0.2% 2,7-dichlorofluorescein in methanol. A band corresponding to *sn*-2 monoacylglycerol was isolated and methylated for GC analysis as described above. Triplicate analyses were performed.

Melting Point

The capillary melting point was determined by AOCS Official Method Cc 1-25 [16]. Each sample (about 10 mm high from the bottom) in an open capillary tube was tempered in a refrigerator (4 °C) for 16 h. The sample tube was heated in a water bath at the rate of 0.5 °C/min until the solid fat in the tube was completely melted. The temperature at which the solid fat in the tube became clear was measured, and the average of three measurements was reported as the melting point.

Tocopherols

High performance liquid chromatography (HPLC) (Younglin, Anayang, Korea) was used for tocopherol analysis. A UV detector was set at 295 nm, and the column was a Chromsep Cartridge, LiChrosorb Diol (5 μm, 3 mm × 100 mm, Chromapack, Rartian, NJ). The mobile phase was a mixture of hexane fortified with 0.1% acetic acid, and the flow rate was 1 mL/min. Standards of α and γ-tocopherol was used for quantification.

Oxidative Stability Index (OSI)

Oxidative Stability Instrument (Omnion, Rockland, MA) was used to evaluate oxidative stability of RSO, PS and the interesterified solid fats following AOCS OSI Method, Cd 12b-92 [16]. The sample (5 g) was put in glass tubes, and conductivity sensor was placed into deionized water (50 mL) in polycarbonate tube. A stream of purified air (2.5 mL/s) was passed through the sample that was held in a thermostated bath set at 110 °C. The effluent air swept volatile organic acids from the samples, and increased the conductivity of deionized water as oxidation proceeded. The oxidative stability index (OSI, expressed in hours) was defined as the point of maximum change of oxidation rate, and the determination was carried out in duplicate.

Texture

Hardness and adhesiveness of samples were evaluated by texture profile analysis (TPA) using a TA-XT2 texture analyzer (Stable Micro Systems Ltd, London, UK). Melted samples (25 g) were placed in 100 mL-beaker, and tempered at 5 or 24 °C for 18 h. A 45° cone probe attached to a 25 kg compression load cell was penetrated twice into the sample at 1.0 mm/s to a depth of 7.5 mm. Hardness was reported as the maximum peak force (g) during the first compression cycle, and adhesiveness was defined as the negative force area (g s) for the first compression. Triplicate measurements were obtained.

Solid Fat Content

A MARAN-20 pulsed nuclear magnetic resonance (NMR) spectrometer (Resonance Instrument Ltd, Oxon, UK) was used to determine solid fat content (SFC) of samples by AOCS Official Method Cd 16-81 [16]. The sample was filled in the NMR tube and melted at 80 °C for 30 min. The samples were tempered at 0 °C for 60 min, and then placed in the water bath at the desired measuring temperature (10, 20, 30 and 40 °C) for 30 min. Olive oil was used as the reference oil. Data were reported as averages of two measurements.

Differential Scanning Calorimetry

The crystallization and melting profiles of samples were determined with a differential scanning calorimetry (DSC) 2010 differential scanning calorimeter (TA Instruments Inc., New Castle, DE). An empty aluminum pan was used as a reference, and samples (5–8 mg) were weighed to ±0.1 mg. The samples were heated to 80 °C and held for 10 min. Then, a crystallization profile was obtained by cooling to −60 °C at a rate of 10 °C/min. After holding for 10 min at this temperature, the melting profile was obtained by heating to 80 °C at 5 °C/min. All samples were analyzed in triplicate. The thermograms were generated and analyzed with Universal Analysis 2000 (TA Instruments Inc., New Castle, DE).

Polymorphism by X-Ray Diffraction Spectroscopy

Melted samples were placed on rectangular plastic molds, and tempered at 24 °C for 18 h. Polymorphic forms of the samples were determined by a PW 1729 X-ray-generator (Eindhoven, The Netherlands) with a Cu X-ray tube, operating at 35 kV and 20 mA. The short spacings were observed in the 2θ range of 12°–30°, and the scan rate was set at 2.0°/min. The α form shows a single strong spacing at ca. 4.15 Å, while β' form exhibits two strong spacings at ca. 3.8 and 4.2 Å or three spacing at ca. 3.71, 3.97 and 4.27. The β form usually shows a very strong spacing at ca. 4.60 Å, and also can be recognized at the spacing which does not satisfy the criteria for α or β'. In addition, a number of sub-forms were classified and designated as sub-α, sub-β, γ, and pseudo-β' form. The form not satisfying the criteria for α or β' is called sub-β that shows a strong spacing at 4.74 Å or three medium spacings at ca. 3.6, 3.9 and 4.5 Å [18].

Crystal Morphology by Polarized Light Microscopy

The morphology of crystallized samples was observed using polarized light microscopy (Leica Microsystem Inc., Allendale, NJ) with an Axiocam digital camera attached (Zeiss Inc., Göttingen, Germany). The melted sample (10 μL) was put on a preheated glass slide, and a preheated cover slip was placed over the sample. Then the prepared slide was cooled, and kept at 24 °C for 18 h. The digital images of samples were acquired using ImageJ 1.36b

software that is available in the public domain of the National Institutes of Health [19]. The fractal dimension (D) was determined using a particle counting algorithm in which the number of particles was counted within box sizes of varying length, and the slope of the linear regression on a log–log plot, corresponds with D [1].

Statistical Analysis

Statistical Analysis System software (SAS, Cary, NC) was used to perform statistical computations [20]. Analysis of variance (ANOVA) with Duncan's multiple range test was performed to determine significance of difference at $P < 0.05$.

Results and Discussion

Fatty Acid Composition

The fatty acid composition (mol%) of FHSBO, RSO, PS and the produced interesterified solid fats are presented in Table 1. RSO contained high content of unsaturated fatty acids (ΣUSFA, 91.8%) in which the major fatty acids were oleic (C18:1, 56.9%) and linoleic acid (C18:2, 24.7%). FHSBO and PS contained high amounts of saturated fatty acids (ΣSFA) at 99.8 and 69.9%, respectively. Major fatty acids in FHSBO and PS were stearic (C18:0, 83.7% in FHSBO) and palmitic acid (C16:0, 63.2% in PS), respectively, and such fatty acid composition resulted in relatively high melting points (Table 1). After interesterification by lipase catalysis, the fatty acid composition and melting point of the products were changed based on the different blending ratios of FHSBO:RSO:PS. In most interesterified solid fats, the major fatty acids were palmitic (27.8–44.6%), stearic (15.6–16.2%), oleic (27.5–36.5%) and linoleic acid (8.0–13.5%). As the weight ratio of RSO in the blend increased, the content of ΣSFA (mainly due to palmitic acid) decreased while ΣUSFA (mainly due to oleic and linoleic acid) increased. Therefore, the melting points of interesterified fats decreased with an increased ratio of RSO, representing 45.5, 42.3, and 39.3 °C in FHSBO:RSO:PS 15:20:65, 15:40:45, and 15:50:35 blends, respectively. *Sn*-2 positional fatty acid composition (mol%) is given in Table 2. FHSBO and PS consisted of 97.4 and 73.4% ΣSFA, respectively, while RSO consisted of 89.6% ΣUSFA in which oleic (53.0%) and linoleic acid (27.7%) were the main fatty acids. For the interesterified solid fats, higher saturation was observed at *sn*-2 position, and ΣSFA ranged from 66.2% (15:50:35) to 82.4% (15:20:65) due to higher amount of palmitic (39.5–58.3%) and stearic acid (21.9–24.7%). Oleic acid was the major unsaturated fatty acid at the *sn*-2 position, representing 14.3, 22.8, and 24.9% in FHSBO:RSO:PS 15:20:65, 15:40:45, and 15:50:35 blends, respectively. As compared with the physical blend of substrates (FHSBO, RSO and PS), a significant extent of changes in *sn*-2 position was observed in the interesterified fats. The *sn*-1,3 specific lipase used in our study hydrolyzes TAG, and leads to formation of free fatty acids and *sn*-1,2 (2,3)-DAG that is very unstable. Therefore, acyl migration from *sn*-1,3 to *sn*-2 position or remigration from *sn*-2 to *sn*-1,3 position takes place, and acyl re-esterification forming TAG also occurs during the reaction [21]. This acyl migration can explain the change of fatty acid composition at *sn*-2 position after interesterification, and the migration is undesirable side reaction, however, it could not be avoided.

Tocopherols and Oxidative Stability

Table 3 shows that the major tocopherols in RSO were α-tocopherol (118.5 mg/100 g) and γ-tocopherol (233.7 mg), whereas γ-tocopherol was predominant in FHSBO. PS contained only a small amount of α-tocopherol. After interesterification, the reaction products contained high amounts of free fatty acids (1.4% for FHSBO:RSO:PS 15:50:35, 3.1% for 15:40:45, and 10.6% for 15:20:65 blends), but were reduced to below 0.4% after short-path distillation (Table 3). The distillation reduced the free fatty acid content, but also decreased the amount of tocopherols in the purified produced solid fats. Compared with the noninteresterified blends, the amount of α-tocopherol was reduced by 63 and 80% in the interesterified fat blends of 15:50:35 and 15:40:45, respectively, while γ-tocopherol was decreased by 17 and 32%, respectively. Besides, no tocopherol was detected in 15:20:65 blend. Such reduction in tocopherols has been reported during modified lipid production with lipase [22–25], in which the loss was associated with the manufacturing process that includes the reaction and purification steps. High content of free fatty acids may contribute to the loss of tocopherols during purification step (i.e., deacidification or distillation) [26]. Haman and Shahidi [27] reported that tocopherols were esterified with the fatty acids and could be removed during short-path distillation. According to the oxidative stability study of the interesterified fats, the OSI induction times (h) were lower in FHSBO:RSO:PS 15:20:65 (0.5 h) than in 15:40:45 (1.6 h), and 15:50:35 (1.2 h) blends. Tocopherol is one of the natural antioxidants which can retard or prevent lipid oxidation, thus the lower presence of tocopherol resulted in the decrease of the induction times of the interesterifed solid fats (Table 3). The fatty acid composition can also affect the oxidative stability since saturated fats are more stable to lipid oxidation. Further, the OSI of 10:20:65 blend was the lowest among the interesterified blends; thus, the reduction of

Table 1 Fatty acid composition (mol%) of fully hydrogenated soybean oil (FHSBO), rapeseed oil (RSO), palm stearin (PS), and interesterified fats

Fatty acid	FHSBO	RSO	PS	Interesterified fat blends (FHSBO:RSO:PS)		
				15:20:65	15:40:45	15:50:35
C12:0	0.1 ± 0.0		0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
C14:0	0.5 ± 0.1		1.4 ± 0.0	1.0 ± 0.0	0.7 ± 0.0	0.6 ± 0.0
C16:0	15.1 ± 1.2	5.6 ± 0.0	63.2 ± 0.2	44.6 ± 0.1	33.5 ± 0.1	27.8 ± 0.0
C16:1n-7	0.1 ± 0.0	0.3 ± 0.0	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
C18:0	83.7 ± 1.4	2.1 ± 0.1	4.8 ± 0.0	16.2 ± 0.1	15.7 ± 0.1	15.6 ± 0.1
C18:1n-9	0.1 ± 0.0	56.9 ± 0.0	24.8 ± 0.2	27.5 ± 0.1	33.4 ± 0.1	36.5 ± 0.1
C18:2n-6		24.7 ± 0.1	5.1 ± 0.0	8.0 ± 0.0	11.7 ± 0.0	13.5 ± 0.0
C18:3n-3		8.6 ± 0.0	0.1 ± 0.0	1.9 ± 0.0	4.0 ± 0.0	4.8 ± 0.0
C20:0	0.6 ± 0.1	0.6 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	0.5 ± 0.0	0.5 ± 0.0
C20:1n-9		1.0 ± 0.0		0.2 ± 0.0	0.4 ± 0.0	0.5 ± 0.0
ΣSFA	99.8 ± 0.8	8.3 ± 0.0	69.9 ± 0.1	62.3 ± 0.1	50.5 ± 0.0	44.5 ± 0.0
ΣUSFA	0.2 ± 0.0	91.8 ± 0.1	30.1 ± 0.2	37.7 ± 0.1	49.5 ± 0.0	55.5 ± 0.1
Melting point (°C)	67.5 ± 3.8		53.7 ± 1.4	45.5 ± 0.7	42.3 ± 0.4	39.3 ± 0.4

Table 2 *Sn*-2 positional fatty acid composition (mol%) of fully hydrogenated soybean oil (FHSBO), rapeseed oil (RSO), palm stearin (PS), and interesterified fats

Fatty acid	FHSBO	RSO	PS	Interesterified fat blends (FHSBO:RSO:PS)		
				15:20:65	15:40:45	15:50:35
C12:0	0.1 ± 0.0		0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0
C14:0	0.6 ± 0.0		1.7 ± 0.1	1.4 ± 0.0	1.1 ± 0.1	1.1 ± 0.1
C16:0	11.9 ± 0.8	7.7 ± 0.7	66.1 ± 1.5	58.3 ± 2.1	46.0 ± 2.2	39.5 ± 0.7
C18:0	84.4 ± 0.8	2.7 ± 0.2	5.1 ± 0.0	21.9 ± 1.4	22.3 ± 2.2	24.7 ± 0.3
C18:1n-9	2.6 ± 0.1	53.0 ± 0.9	23.0 ± 0.6	14.3 ± 2.5	22.8 ± 2.5	24.9 ± 0.0
C18:2n-6		27.7 ± 0.6	3.6 ± 0.1	2.9 ± 0.9	5.8 ± 1.6	7.2 ± 0.8
C18:3n-3		7.0 ± 0.3		0.3 ± 0.1	0.9 ± 0.3	1.3 ± 0.1
C20:0	0.6 ± 0.1		0.2 ± 0.0	0.6 ± 0.0	0.6 ± 0.1	0.6 ± 0.1
C20:1n-9		1.8 ± 0.2		0.1 ± 0.1	0.2 ± 0.0	0.4 ± 0.0
ΣSFA	97.4 ± 1.3	10.4 ± 1.0	73.4 ± 1.3	82.4 ± 3.5	70.3 ± 4.4	66.2 ± 1.0
ΣUSFA	2.6 ± 0.1	89.6 ± 1.0	26.6 ± 0.5	17.6 ± 2.8	29.7 ± 3.5	33.8 ± 1.0

tocopherol could be more relevant than the nature of fatty acid composition on oxidative stability.

Solid Fat Content

Solid fat content (SFC) is the percentage of the lipid which is solid at various temperatures, and the values are required to understand the physical properties of solid fat such as shortening. Table 4 shows SFCs of the noninteresterified physical blends, interesterified fat blends and commercial shortenings. The physical blends of FHSBO:RSO:PS 15:20:65, 15:40:45 and 15:50:35 exhibited 54–64% SFC at 10 °C, but were reduced to 14–23% at 40 °C, while the interesterified solid fats had 51–63% SFC at 10 °C, then decreased to 4–12% at 40 °C. Therefore, SFCs of the physical blends were changed after 24 h-interesterification, and the interesterified fats tended to have lower SFC values than the physical blends at all measured temperatures (10, 20, 30 and 40 °C). These results could be because of the rearrangement of fatty acids in triacylglycerols (TAGs) of blends during the interesterification, followed by the formation of altered TAG molecules with lower melting TAG species than those in the physical blends. The

Table 3 Tocopherols and oxidative stability index (OSI) value of FHSBO, RSO, PS and interesterified fats

	FHSBO	RSO	PS	Interesterified fat blends (FHSBO:RSO:PS)		
				15:20:65	15:40:45	15:50:35
Tocopherols (mg per 100 g)						
α-Tocopherol	36.5 ± 0.4	118.5 ± 1.4	33.1 ± 4.1	ND	13.53 ± 1.2	28.1 ± 0.3
				(50.7 ± 2.9)[a]	(67.7 ± 2.3)	(76.3 ± 2.1)
γ-Tocopherol	298.2 ± 14.4	233.7 ± 17.9	ND	ND	93.31 ± 1.4	133.5 ± 4.1
				(91.5 ± 1.4)	(138.2 ± 5.0)	(161.6 ± 7.0)
OSI value (h)[b]	ND	6.0 ± 0.3	11.5 ± 0.3	0.5 ± 0.0	1.6 ± 0.2	1.2 ± 0.2
Free fatty acid (% oleic acid)[c]				0.42[c]	0.28	0.21
				(10.57)[d]	(3.10)	(1.41)

ND not detected

[a] Tocopherol content of blend (before interesterification)

[b] Evaluated at 110 °C with Oxidative Stability Instrument

[c] Free fatty acid content after short path distillation

[d] Free fatty acid content after reaction

Table 4 Solid fat contents (%) of the physical blends, interesterified fats, and commercial shortenings

Sample	Temperature (°C)			
	10	20	30	40
Physical blends (FHSBO:RSO:PS)				
15:20:65	63.7 ± 0.2	44.3 ± 2.6	32.0 ± 2.1	22.9 ± 1.9
15:40:45	54.4 ± 0.2	37.1 ± 1.1	24.3 ± 1.3	17.1 ± 0.8
15:50:35	53.7 ± 3.3	36.8 ± 4.4	21.7 ± 1.8	13.8 ± 1.4
Interesterified fat blends (FHSBO:RSO:PS)				
15:20:65	62.9 ± 2.1	42.0 ± 4.8	26.3 ± 1.7	11.6 ± 0.8
15:40:45	51.1 ± 0.7	29.6 ± 0.6	14.2 ± 1.4	5.9 ± 0.3
15:50:35	50.6 ± 5.7	29.1 ± 2.8	11.0 ± 0.1	3.9 ± 2.6
Commercial shortenings				
Shortening A	47.1 ± 0.8	28.0 ± 0.6	12.9 ± 0.9	4.9 ± 1.3
Shortening B	56.7 ± 0.7	32.6 ± 0.5	14.8 ± 0.4	3.6 ± 2.6
Shortening C	56.3 ± 5.4	30.4 ± 4.2	11.8 ± 0.6	2.9 ± 0.7
Shortening D	58.2 ± 0.4	33.5 ± 1.9	14.6 ± 0.4	4.7 ± 2.7

rearrangement could also alter polymorphic forms of physical blends ($\beta' > \beta$ form), and produced more β' polymorphic forms in interesterified blends ($\beta' >> \beta$) (Table 5). Since the β form has higher melting point than β' form, the altered polymorphic formation might cause lower SFC values in the interesterified blends. In the interesterified fats, the FHSBO:RSO:PS 15:20:65 blend had higher SFCs values than 15:40:45 and 15:50:35 blends at each temperature, and 15:40:45 contained higher SFCs than 15:50:35. The result could be expected since the addition of PS containing relatively high melting fat increased SFCs in the interesterified fats. Compared with commercial shortening products, the interesterified fat FHSBO:RSO:PS 15:40:45 and 15:50:35 blends showed SFCs values that closely matched those of commercial shortenings (Table 4).

The plasticity of shortening helps air incorporation to increase the volume during the creaming stage in cake batter and cookie dough formation. The plasticity or melting range of fats can be determined by SFC. Normally, a long (wide) plastic range is required for a bakery shortening [28]; therefore, the SFC is one of the important characteristics of shortenings used to assess their performance in bakery products. The interesterified fats produced in the present study showed a gradual slope with a wide plastic range from 10 to 40 °C in SFC profiles. Thus, these fats could be suitable for use as bakery fat-like shortening.

Melting and Crystallization Properties

The melting and cooling curves of the blends and interesterified fats were determined by DSC. In the melting thermograms of the physical blends (FHSBO:RSO:PS 15:20:65, 15:40:45 and 15:50:35), peak A gradually disappeared and peak B appeared as the amount of PS in the blends increased. Also peak D at 52 °C, representing high melting TAGs, increased in size (Fig. 1). After interesterification, a change in the shape of the melting curve was observed since peak D in the blends disappeared while peak E and peak F–G appeared in the interesterified fats, indicating that interesterification altered the melting profiles and induced the formation of a softer fat. In the interesterified fat, when the amount of PS increased, peak E became smaller and shifted toward a higher temperature showing melting at 11.4 °C in FHSBO:RSO:PS 15:50:35

Table 5 X-ray diffraction pattern of the physical blends and interesterifed fats

Solid fats	Short spacing (Å)					Polymorphic form
Physical blends (FHSBO:RSO:PS)						
15:20:65	3.84 (s)	4.22 (s)	4.32 (w)	4.51(vw)	4.60 (vw)	$\beta' >> \beta$
15:40:45	3.82 (s)	4.20 (s)	4.32 (m)		4.59 (m)	$\beta' > \beta$
15:50:35	3.78 (m)	4.20 (m)		4.51 (m)		$\beta' > \beta$
Interesterifed fat blends (FHSBO:RSO:PS)						
15:20:65	3.85 (s)	4.20 (s)	4.30 (m)	4.50 (w)		$\beta' >>> \beta$
15:40:45	3.84 (m)	4.19 (s)	4.27 (m)	4.51 (w)		$\beta' >> \beta$
15:50:35	3.85 (w)	4.25 (m)	4.35 (vw)	4.59 (vw)		$\beta' >> \beta$
Substrates						
FHSBO	4.11 (vs)					α
PS	3.87 (s)	4.19 (s)	4.34 (m)	4.59 (m)		$\beta' + \beta$

Samples stored at 24 °C for 18 h

S strong, *m* medium, *w* weak, *v* very

blend and 13.9 °C in 15:20:65 blend. In addition, peaks F and G, containing high melting TAGs, also shifted toward a higher temperature and became narrower. This observation suggested that a higher melting solid fat would be formed in the FHSBO:RSO:PS 15:20:65 blend than in the 15:40:45 and 15:50:35 blends. The melting end-points of bakery fats for cakes or doughnuts normally range 39–45 °C, and the blends had this range of melting points after interesterificaiton. The DSC cooling curve shows one distinct crystallization peak A in the physical blends at 33–34 °C (Fig. 2). As expected, peak A became bigger as the amount of PS increased. Such distinct sharp peaks suggested that all crystals in the blends nucleated approximately at the same time [29]. This crystallization behavior of the physical blends was changed by the interesterification resulting in two crystallization peaks (B and C) observed at 6.3 and 18.9 °C in FHSBO:RSO:PS 15:50:35 blend. Furthermore, peak C became distinct and bigger, and shifted toward a higher temperature as the amount of PS increased with crystallization at 22.4 °C in FHSBO:RSO:PS 15:40:45 blend and 29.2 °C in 15:20:65 blend.

Texture

Hardness and adhesiveness of the interesterified fats stored at 5 and 24 °C are presented in Fig. 3. The hardness was affected by the storage temperature showing higher hardness values at 5 °C (refrigeration temperature) than 24 °C (room temperature). At the same temperature of storage, the interesterified fat FHSBO:RSO:PS 15:20:65 blend had significantly higher hardness than the 15:40:45 and 15:50:35 blends ($P < 0.05$), and such an increase could be explained by increased weight ratio of PS in the interesterified fats. Hardness is generally contributed by SFC in which a higher SFC would result in a harder fat (Table 4). In addition, the 15:20:65 blend had a highest adhesiveness value, and exhibited more stickiness than 15:40:45 and 15:50:35 at the storage of 24 °C (Fig. 3). However, the storage at 5 °C lead to reversed pattern of adhesiveness, and showed higher stickiness in 15:40:45 or 15:50:35 than 15:20:65 since the 15:20:65 blend was brittle and fractured at low temperatures due to a large amount of PS.

Polymorphism

Solid fats exhibit polymorphism which results from different patterns of molecular packing on fat crystallization. The different polymorphic states of a substance demonstrate considerably different physical properties [30]. The major polymorphic forms of fats are known as α (hexagonal), β' (orthorhombic) and β (triclinic), The β form is the most stable and has the highest melting point [31]. The α form is the least stable and the lowest melting polymorph, and occurs when the melted fats are crystallized rapidly; but it transforms quickly to a state of the β' form which is intermediate in stability and melting point. A conversion from one to another form generally occurs in the direction of the more stable forms. The polymorphism of solid fat have been investigated with X-ray diffraction spectroscopy in which a short spacing is used to characterize the various polymorphic forms of fats, observed in the 2θ range of 12°–30°.

Table 5 shows the X-ray diffraction pattern with short spacing and polymorphic forms of the physical blends and interesterified fats which were stored at 24 °C for 18 h. FHSBO was crystallized in the α form presenting very strong short spacing at only 4.11 Å while PS had a mixture of β' and β form showing at 4.19, 3.87 and 4.34, and 4.59 Å, respectively. The physical blends exhibited a mixture of β' (strong intensity) and β form (medium or weak intensity), whereas after interesterification the intensity of short spacing representing β form appeared to

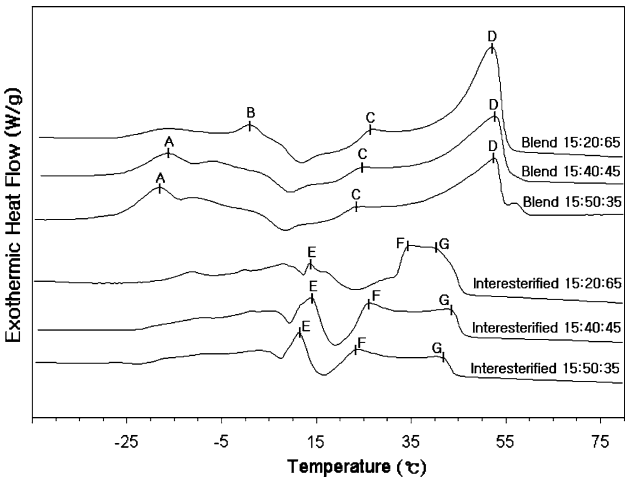

Fig. 1 DSC melting curve of the physical blends (FHSBO:RSO:PS) and interesterified fat blends

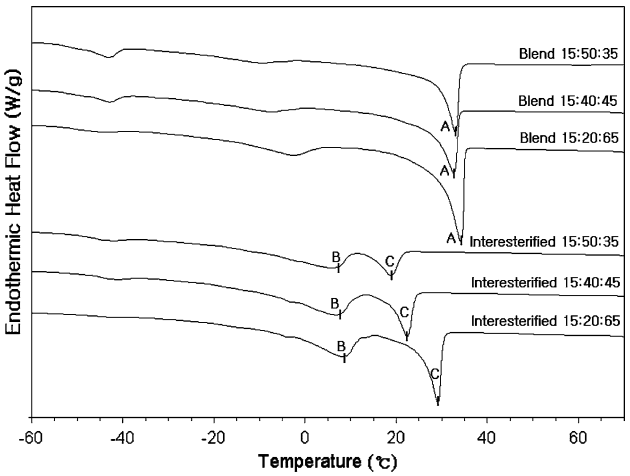

Fig. 2 DSC crystallization curve of the physical blends (FHSBO:RSO:PS) and interesterified fat blends

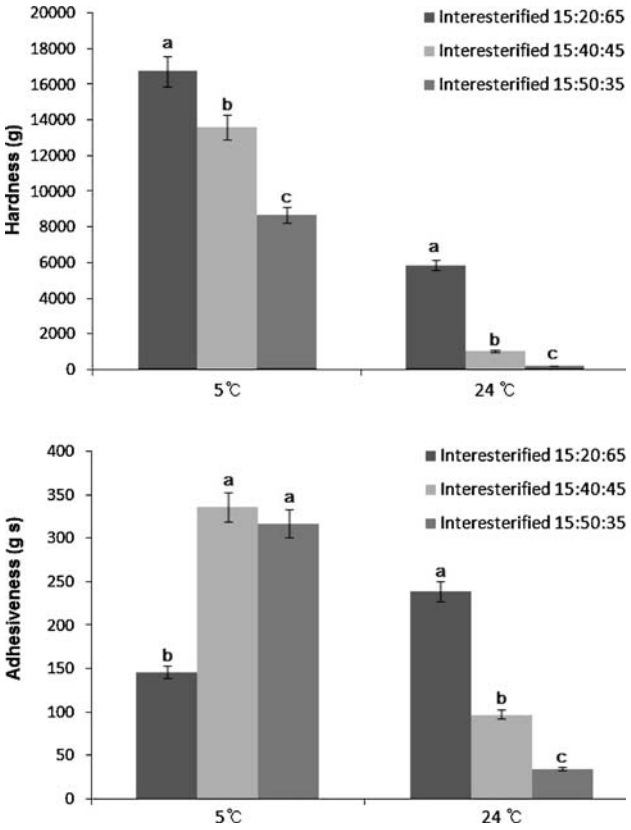

Fig. 3 Hardness and adhesiveness of the interesterified fat blends (FHSBO:RSO:PS) stored at 5 and 24 °C

be diminished, and showed more dominant β' crystal form than the physical blend. That might be due to the rearrangement and randomization of fatty acids in the TAG structure during the interesterification reaction. In the interesterfied fats, FHSBO:RSO:PS 15:20:65 blend was observed to have mostly β' form crystals whereas 15:40:45 and 15:50:35 blends contained β' together with a small amount of sub-β and β forms (4.51 and 4.60 Å). The differences in the polymorphic state of the interesterified fats could be due to the amount of PS in each blend, indicating that the formation of β' crystal tended to increase as the amount of PS increased. PS contained high levels of β promoting TAG (tripalmitin) due to high concentration of palmitic acid (63%) (Table 1). However, when PS was interesterifed with RSO and FHSBO, it induced more diverse structure of TAGs resulting in dominant β' polymorphic form. The form of the crystal is important because it affects the textural and functional properties of shortenings. The β' form is most desirable in baking fats since it has the greatest effect on stabilizing air in the baking dough while the β form is quite unsatisfactory. The interesterified fats produced in this study contained mostly β' form of crystal and could be used as alternatives for bakery fats such as shortening.

Crystal Morphology

The microstructure of solid fats was studied by polarized light microscopy (PLM). Figure 4 shows the distinctly different crystal morphologies of the substrates (FHSBO and PS), physical blends, and interesterified fats. The crystal structure was much different between FHSBO (A) and PS (B). The large rod-like spherulitic crystals were observed in PS whereas long needle-like crystals were densely packed in FHSBO. Such a different structure was also estimated by X-ray diffraction study which showed that FHSBO contained α-form crystals while PS had a mixture of β' and β forms. Both physical blending and interesterification of RSO with FHSBO and PS altered the crystal morphology of individual substrates. The physical

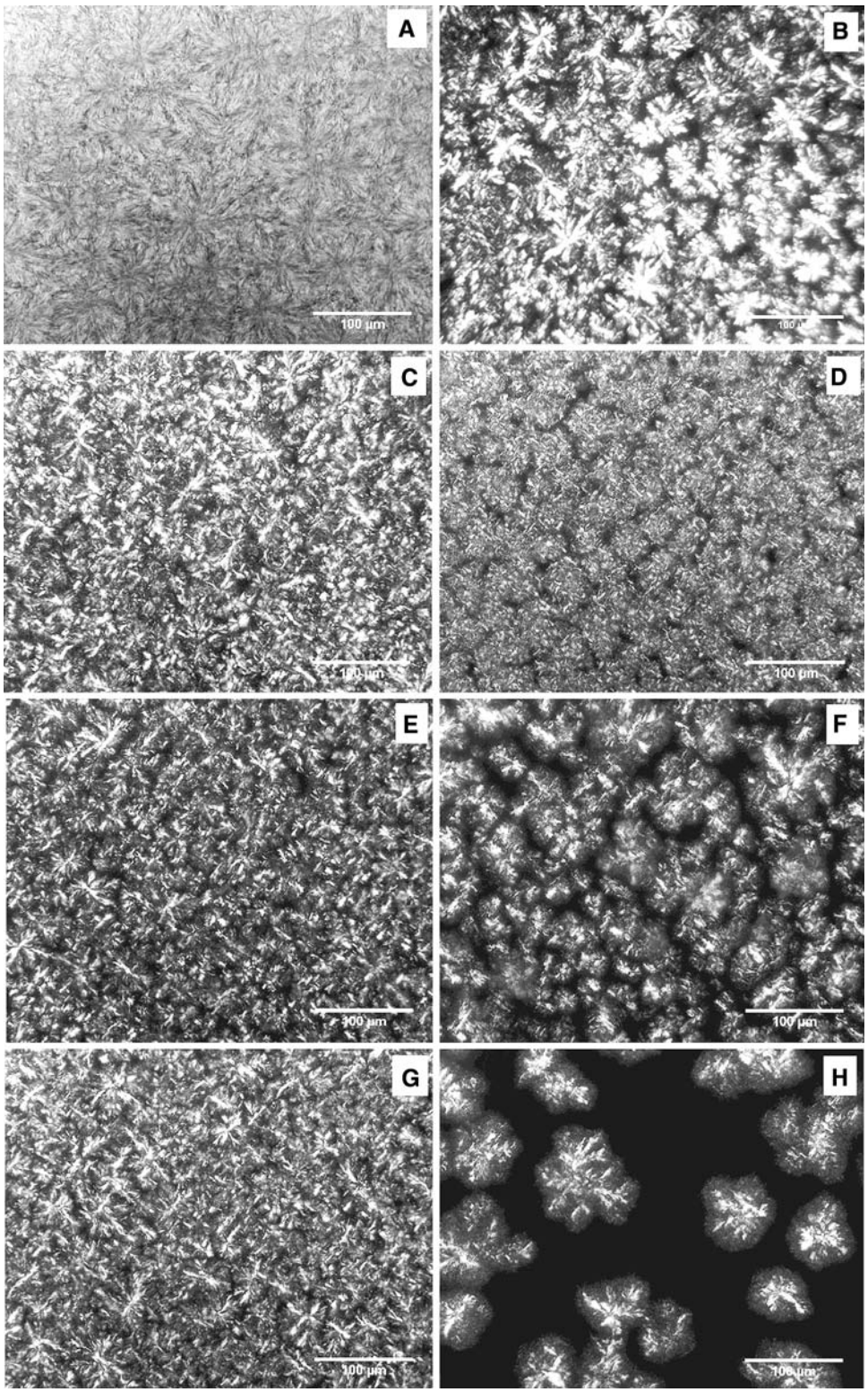

Fig. 4 Crystal morphology of the substrates [FHSBO (**a**) and PS (**b**)], physical blends [FHSBO:RSO:PS 15:20:65 (**c**) 15:40:45 (**e**) 15:50:35 (**g**)] and interesterified fat blends [15:20:65 (**d**) 15:40:45 (**f**) 15:50:35 (**h**)] stored at 24 °C for 18 h

blends (FHSBO:RSO:PS 15:20:65, 15:40:45 and 15:50:35) showed needle-shaped like crystals; whereas the crystals in the interesterified fats displayed discrete morphology when compared to those in the physical blends. In addition, the interesterified fats were composed of fewer crystals than the physical blends. In the physical blends, 15:20:65 blend (C) displayed spherulite-shaped crystals which were more tightly packed with a little space between adjacent crystals

than 15:40:45 (E) or 15:50:35 blends (G). In the interesterified fats, the 15:20:65 blend (D) was composed of more crystals than the 15:40:45 (F) and 15:50:35 (H) blends at 24 °C. The 15:20:65 blend contained smaller and more densely packed spherulite-shaped crystals than the 15:40:45 or 15:50:35 blends. The crystals of 15:20:65 blend tended to be aggregated to form clusters at 24 °C. PS was observed to contain large spherulitic crystals, whereas the addition of PS into the blends and interesterification induced the formation of smaller and densely packed crystals. Therefore, our result suggested that the amount of PS influenced the structure and network of crystals in the interesterified fats, and that the increasing content of PS induced the formation of smaller and more densely packed crystals.

The fractal dimension (D) was determined by a particle counting method and presented in Table 6. The fractal dimension might be useful as a quantitative parameter which may indicate overall complexity or scale-dependency of pattern in crystal network. Normally, crystal networks that arise from more ordered nucleation shows higher fractal dimensions whereas those from more disordered nucleation results in lower fractal dimension [1]. The fractal dimension (D) in the interesterified fats was increased as the weight ratio of PS increased, showing 1.76, 1.62 and 1.45 in FHSBO:RSO:PS 15:20:65, 15:40:45, and 15:50:35 blends, respectively. This suggested that the ratio of PS to the blending altered the spatial distribution of crystals resulting in more ordered crystal network.

Lipase-catalyzed interesterification resulted in the formation of new diverse TAGs and a change in the melting and crystallization properties due to the rearrangement of fatty acids within or between TAGs [12]. The interesterified fat blends (15:20:65, 15:40:45 and 15:50:35) produced from FHSBO, RSO and PS with different weight ratios had altered physical properties compared to the blends (non-interesterified), and showed lower SFCs, and different melting and crystallization behavior, as well as crystal structure. More desirable crystal polymorph (β' form) was found in the interesterified fats. In addition, the physical properties of the interesterifed fats were influenced by the ratio of PS. Higher melting points and SFCs, more desirable crystal structure and polymorphism were observed in the interesterified fat FHSBO:RSO:PS 15:20:65 blend than in the 15:40:45 and 15:50:35 blends when the produced fats were stored at 24 °C. Therefore, the present study shows that the produced interesterified fats containing *trans*-free fatty acids could be used as an alternative to hydrogenated-type shortening.

Table 6 Fractal dimension (D) of the physical blends and interesterified fats

FHSBO:RSO:PS	Physical blends	Interesterified fat blends
15:20:65	1.831	1.762
15:40:45	1.673	1.622
15:50:35	1.632	1.450

Acknowledgments This work was supported by the Korean Research Foundation Grant funded by the Korean Government (MOEHRD), (KRF-2005-214-C00251).

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