

Preparation of Interesterified Plastic Fats from Fats and Oils Free of *Trans* Fatty Acid

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Interesterified plastic fats were produced with *trans*-free substrates of fully hydrogenated soybean oil, extra virgin olive oil, and palm stearin in a weight ratio of 10:20:70, 10:40:50, and 10:50:40, respectively, by lipase catalysis. The major fatty acids of the products were palmitic (32.2–47.4%), stearic (12.0–12.4%), and oleic acid (33.6–49.5%). After storage at 5 °C (refrigerator temperature) or 24 °C (room temperature) for 16 h, the physical properties were evaluated for solid fat content, texture, melting, and crystallization behavior, viscoelastic properties, crystal polymorphism, and crystal microstructure. The interesterified fats contained desirable crystal polymorphs (β' form) as determined by X-ray diffraction spectroscopy. They exhibited a wide plastic range of solid fat content of 52–58% at 10 °C and 15% at 40 °C. The physical properties were influenced by the ratio of palm stearin and olive oil. Harder and more brittle texture, crystallization and melting at higher temperature, higher solid fat contents, and more elastic (G') or viscous (G'') characteristics were observed in the produced fats containing a higher content of palm stearin and lower content of olive oil. The produced fats stored at 5 °C consisted mostly of β' form crystal together with a small content of β form, while those at 24 °C had only β' form. The produced fat with a higher amount of palm stearin appeared to have more β' form crystal and small size crystal clusters. Thus, the physical properties of the produced plastic fats may be desirable for use in a bakery product.

KEYWORDS: Crystal microstructure; interesterification; lipase; olive oil; palm stearin; polymorphism; solid fat content; *trans* fat

INTRODUCTION

Bakery fats (i.e., shortening, margarine, or cooking fats) contain various plastic and melting ranges, and these fats are mostly prepared from vegetable oils by the hydrogenation process. However, hydrogenation may lead to the formation of *trans* fatty acids (TFA), up to 50%, depending on the degree of hydrogenation (1, 2). By comparison to other fatty acids, high intake of TFA is unfavorably associated with coronary heart disease (CHD) (3–6); hence, the concern about the adverse effect of TFA on health limits their application in food. To replace the conventional hydrogenation process, an interesterification process can be one of the alternative technologies (7). The interesterification reaction via enzyme or chemical catalysis can

be used to modify the solid-to-liquid ratio of vegetable oils and fats, and produce various types of plastic fats containing no TFA.

An enzyme-catalyzed interesterification induces a rearrangement of fatty acids within and between triacylglycerols (TAGs) resulting in new altered TAG molecules, and can lead to the modification of original oils and fats while providing desired physical and chemical properties in the restructured fats. In many studies, the interesterification was used for the synthesis of plastic fats in which fully hydrogenated vegetable oil, fractionated oil, or vegetable oil were used as substrates (8–10). Palm stearin, a solid fraction of palm oil, is not used by itself due to its high melting point and β crystal promoting characteristic. However, palm stearin is known to promote the formation of desirable plastic fats when it is interesterified with vegetable oils. Plastic fats should contain proper physical properties including melting and crystallization behavior, solid fat content, crystal structure, and polymorphic form to impart desirable tenderness, texture, mouthfeel, incorporation of air, and extended shelf life (11). In the studies of Ming et al. (12) and Zhang et

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Table 1. Fatty Acid Composition (mol %) of the Produced Interesterified Plastic Fats^a

sample	12:0	14:0	16:0	16:1n-7	18:0	18:1n-9	18:2n-6	18:3n-3	20:0	I.V. ^b
FOP2	0.1 ± 0.0 ^c	1.0 ± 0.0	47.4 ± 0.3	0.2 ± 0.0	12.4 ± 0.0	33.6 ± 0.1	4.7 ± 0.1	0.3 ± 0.0	0.4 ± 0.0	38.0 ± 0.0
FOP4	0.1 ± 0.0	0.7 ± 0.0	37.6 ± 0.3	0.3 ± 0.0	12.1 ± 0.1	43.8 ± 0.1	4.5 ± 0.0	0.5 ± 0.0	0.4 ± 0.0	47.0 ± 0.1
FOP5	0.1 ± 0.0	0.6 ± 0.0	32.2 ± 0.1	0.4 ± 0.0	12.0 ± 0.1	49.5 ± 0.1	4.5 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	51.7 ± 0.0

^a Enzymatically interesterified plastic fats with FHSBO:OO:PS in a weight ratio of 10:20:70 (FOP2), 10:40:50 (FOP4), and 10:50:40 (FOP5). Abbreviations are the following: FHSBO, fully hydrogenated soybean oil; OO, olive oil; PS, palm stearin. ^b Iodine value was calculated from the fatty acid composition. ^c Values are the means of triplicates ± standard deviation from the same batch of interesterified plastic fats.

Table 2. Solid Fat Content (%) of the Produced Interesterified Plastic Fats

sample	temp (°C)			
	10	20	30	40
	Produced Fats ^a			
FOP2	57.6 ± 3.4 ^b A	41.4 ± 5.4 A	25.0 ± 4.0 A	14.7 ± 4.0 A
FOP4	57.3 ± 3.8 A	34.7 ± 3.7 B	16.6 ± 0.8 B	5.0 ± 2.5 B
FOP5	51.6 ± 3.1 A	29.3 ± 2.2 C	12.3 ± 2.1 C	2.4 ± 1.2 C
	Commercial Bakery Fats			
A	56.6 ± 0.7	32.6 ± 0.5	14.8 ± 0.4	3.6 ± 2.6
B	46.9 ± 0.5	27.0 ± 0.8	11.2 ± 0.0	5.1 ± 1.4
C	58.4 ± 3.6	35.4 ± 4.9	14.9 ± 0.5	3.2 ± 0.7

^a Enzymatically interesterified plastic fats with FHSBO:OO:PS in a weight ratio of 10:20:70 (FOP2), 10:40:50 (FOP4), and 10:50:40 (FOP5). ^b Values are the means of triplicates ± standard deviation from the same batch of interesterified plastic fats.

Table 3. Texture Properties of the Produced Interesterified Plastic Fats^a

sample	5 °C			24 °C		
	hardness (g)	adhesiveness (g s)	cohesiveness (no unit)	hardness (g)	adhesiveness (g s)	cohesiveness (no unit)
FOP2	21494 ± 2025 ^{b,c} A	179 ± 21 B	0.21 ± 0.01 A	6406 ± 548 A	258 ± 54 A	0.16 ± 0.03 A
FOP4	14221 ± 1090 B	400 ± 148 A	0.18 ± 0.02 B	995 ± 46 B	110 ± 12 B	0.11 ± 0.00 B
FOP5	12704 ± 359 B	543 ± 85 A	0.17 ± 0.01 B	241 ± 6 C	19 ± 1 C	0.13 ± 0.02 AB

^a Enzymatically interesterified plastic fats with FHSBO:OO:PS in a weight ratio of 10:20:70 (FOP2), 10:40:50 (FOP4), and 10:50:40 (FOP5). ^b Values are the means of triplicates ± standard deviation from the same batch of interesterified plastic fats. ^c Values with different letters in the same column are significantly different ($P < 0.05$).

Table 4. Color Values of the Produced Interesterified Plastic Fats^a

sample	L value (lightness)	a value (redness/greeness)	b value (yellowness/blueness)
FOP2	63.97 ± 0.58 ^{b,c} A	-4.93 ± 0.30 A	12.99 ± 0.72 B
FOP4	65.65 ± 0.60 A	-4.15 ± 0.20 A	13.75 ± 0.66 B
FOP5	50.48 ± 0.74 B	-10.43 ± 0.30 B	35.33 ± 0.87 A

^a Enzymatically interesterified plastic fats with FHSBO:OO:PS in a weight ratio of 10:20:70 (FOP2), 10:40:50 (FOP4), and 10:50:40 (FOP5). ^b Values are the means of triplicates ± standard deviation from the same batch of interesterified plastic fats. ^c Values with different letters in the same column are significantly different ($P < 0.05$).

al. (10), the interesterified plastic fats of palm stearin and sunflower oil or coconut oil catalyzed by lipases from *Pseudomonas* sp. or *Rhizomucor miehei* were suitable for table margarine or shortening preparation.

Olive oil is the best source of monounsaturated fatty acids (i.e., oleic acid) which have a beneficial effect on the risk factors for CHD including plasma lipids and lipoproteins (13). Extra virgin olive oil contains natural antioxidants such as carotenoids, tocopherols, and phenolic compounds which may provide an effective defense system against free radical attack. In the present study, plastic fats containing no TFA were prepared from fats and oils free of TFA. To impart plasticity and desirable physical properties, extra virgin olive oil was blended with fully hydrogenated soybean oil and palm stearin at different weight ratios, and interesterified via lipase catalysis in a batch-type reactor. The physical properties of produced interesterified plastic fats were studied to evaluate their possible use in the bakery industry.

MATERIALS AND METHODS

Materials. Extra virgin olive oil (OO), palm stearin (PS), and fully hydrogenated soybean oil (FHSBO) were obtained from C.J. Co. (Seoul, Korea). FHSBO contained 0.89% TFA and the major fatty acids were 16:0 (palmitic acid, 15.1%) and 18:0 (stearic acid, 83.7%). OO contained 16:0 (11.9%), 18:0 (3.4%), 18:1n-9 (oleic acid, 76.6%), and 18:2n-6 (linoleic acid, 6.2%), while PS was composed of 16:0 (63.2%), 18:0 (4.8%), 18:1n-9 (24.8%), and 18:2n-6 (5.1%). Lipozyme RM IM, a *sn*-1,3-selective lipase from *Rhizomucor miehei*, was purchased from Novozymes, Inc. (Franklinton, NC). Trielaidin and triolein with purity of 99%, as standards, were obtained from Nu-Chek-Prep, Inc. (Elysian, MN).

Production of Interesterified Plastic Fat. FHSBO, OO, and PS in a weight ratio of 10:20:70, 10:40:50, and 10:50:40 were blended to prepare the interesterified plastic fats FOP2, FOP4, and FOP5, respectively. The melted physical blend of FHSBO, OO, and PS 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. The mixing speed was set at 300 rpm. After reaction, two reaction products from different batch reactions were combined, and the lipase was removed by vacuum filtration. The reaction product was then purified by a KDL-4 short path distillation system (UIC Inc., Joliet, IL). Vacuum pressure was maintained below 1 mmHg, and the temperatures of evaporator and condenser were set at 185 and 20 °C, respectively.

Trans Fat Analysis. Bruker Tensor 27 Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR) operated by OPUS software (Bruker Optics, Inc., Billerica, MA) was employed for the determination of *trans* fat content in samples, using AOCS Official Method Cd 14d-99 (14). The fat standards of trielaidin (*trans* fat)/triolein blend at 0.5%, 1%, 5%, 10%, 20%, and 40% were prepared and used to generate a calibration curve. Melted standards and samples (each 10 μL) were placed on top of the heated surface of ATR crystal at 65 °C, in which

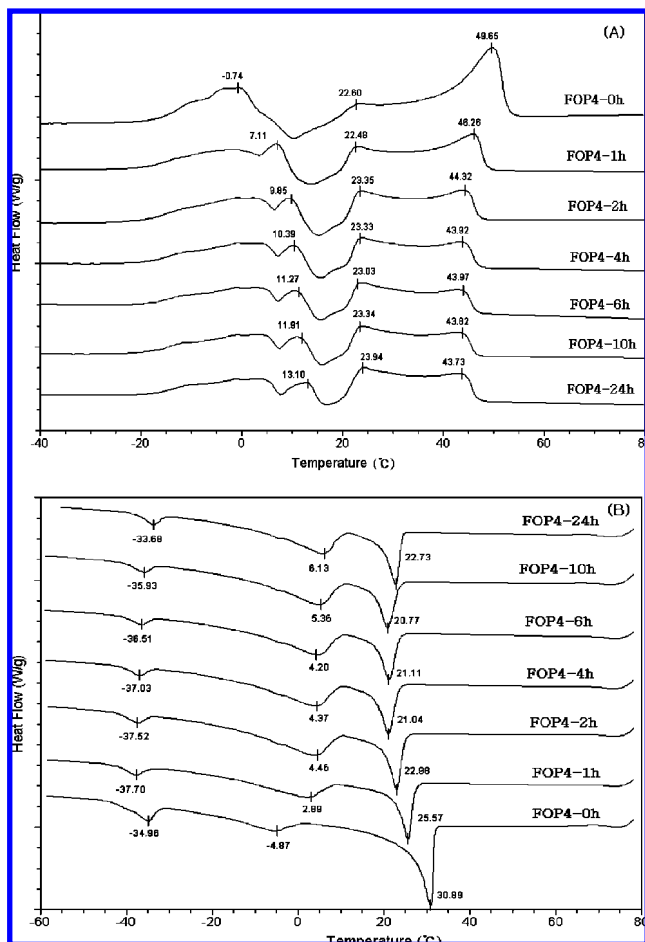


Figure 1. DSC melting (A) and cooling (B) curves of the produced fat FOP4 (FHSBO:OO:PS, 10:40:50) reacted at 0, 1, 2, 4, 6, 10, and 24 h.

the surface was completely covered with the samples. FTIR spectra were measured at 4 cm^{-1} resolution, and the absorption spectra were compared against that of triolein as *trans*-free reference oil. The area under the 966 cm^{-1} band was integrated in the range between 990 and 945 cm^{-1} , and the *trans* fat content as a percentage of total fat was calculated by a linear regression equation obtained from the standard calibration curve: $\text{band area} = 0.1777(\% \text{ trans}) + 0.0037$ (15).

Fatty Acid Composition. After methylation with 2 N KOH in methanol for 30 min, the fatty acid composition of samples was determined with Hewlett-Packard 6890 gas chromatography (GC) equipped with a flame-ionization detector (Agilent Technologies, Little Falls, DE) and a fused-silica capillary column (SP-2560, $100\text{ m} \times 0.25\text{ mm}$ i.d., $0.25\text{ }\mu\text{m}$ film thickness, Supelco, Bellefonte, PA). The temperatures of the injector and detector were set at 250 and $260\text{ }^\circ\text{C}$, respectively. The column was heated to $150\text{ }^\circ\text{C}$, and held for 5 min, then increased to $220\text{ }^\circ\text{C}$ at the rate of 4 deg/min , and held for another 30 min. The methyl ester of heptadecanoic acid was used as an internal standard. Individual fatty acids were identified by comparing retention times with those of the standard mixture (GLC-461, Nu-Chek Prep, Inc., Elysian, MN). Triplicate analyses from the same batch were performed. The iodine value for the interesterified solid fat was determined by calculation from fatty acid composition (14).

Solid Fat Content. Following the AOCS Official Method Cd 16-81 (14), a MARAN-20 pulsed nuclear magnetic resonance (NMR) spectrometer (Resonance Instrument Ltd., Oxon, United Kingdom), was used to determine the solid fat content (SFC) of samples. The sample was placed in the NMR tube and melted at $80\text{ }^\circ\text{C}$ for 30 min. Olive oil was used as the reference oil. The samples were tempered at $0\text{ }^\circ\text{C}$ for 60 min, and then placed in the water bath at temperatures of 10 , 20 , 30 , and $40\text{ }^\circ\text{C}$. The samples were transferred to the measurement port of NMR, and the solid and liquid signals were measured. Duplicate measurements were obtained.

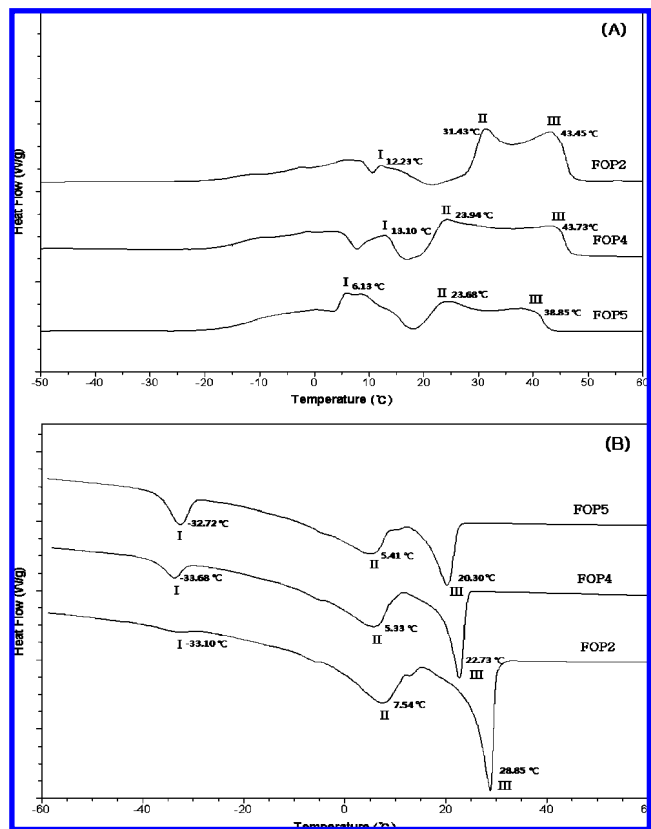


Figure 2. DSC melting (A) and cooling (B) curves of the produced fats (FOP2, FOP4, and FOP5).

Texture. A TA-XT2 texture analyzer (Stable Micro Systems Ltd., London, United Kingdom) was employed to determine hardness, cohesiveness, and adhesiveness with texture profile analysis (TPA). Melted samples (25 g) were placed in 100-mL beaker, and tempered at 5 or $24\text{ }^\circ\text{C}$ for 16 h. A $45\text{ }^\circ\text{C}$ 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. Adhesiveness was defined as the negative force area (g s) for the first compression, and cohesiveness (no unit) was the ratio of the positive force during the second compression to that during the first compression. Triplicate measurements were obtained.

Color Determination. The color of interesterified plastic fat (each 10 g) was measured as Hunter L (\pm , lightness/darkness), a (\pm , redness/greenness), and b (\pm , yellowness/blueness) values with a JC801 colorimeter (Color Techno System Corp. Tokyo, Japan). Samples were measured in triplicate, and the average values were reported.

Crystallization and Melting Behaviors. A Differential Scanning Calorimetry (DSC) 2010 (TA Instruments Inc., New Castle, DE) was used to determine crystallization and melting characteristics of samples. The melted sample was placed into an aluminum pan, and the empty pan was used as a reference. For crystallization behavior, the sample was maintained at $80\text{ }^\circ\text{C}$ for 10 min, and then cooled to $-60\text{ }^\circ\text{C}$ at the rate of 10 deg/min . For melting behavior, the sample was held at $-60\text{ }^\circ\text{C}$ for 10 min, and heated to $80\text{ }^\circ\text{C}$ at the rate of 5 deg/min . All samples were analyzed in triplicate. The melting and crystallization characteristics including thermograms and onset and peak temperatures were recorded and analyzed with a Universal Analysis 2000 (TA Instruments Inc., New Castle, DE).

Rheological Analysis. Viscoelastic measurements were performed on a stress-controlled SR-5000 Dynamic Stress Rheometer (Rheometric Scientific, Piscataway, NJ) fitted with a 40 mm diameter parallel plate geometry with a 0.5 mm gap. Temperature was controlled by a Peltier heating element. Samples were initially subjected to a dynamic stress sweep test ranging from 0.02 to 400 Pa to determine the linear viscoelastic region. A constant stress of 120 Pa was then chosen, and at a constant frequency of 1 Hz , the storage modulus (G') and the loss

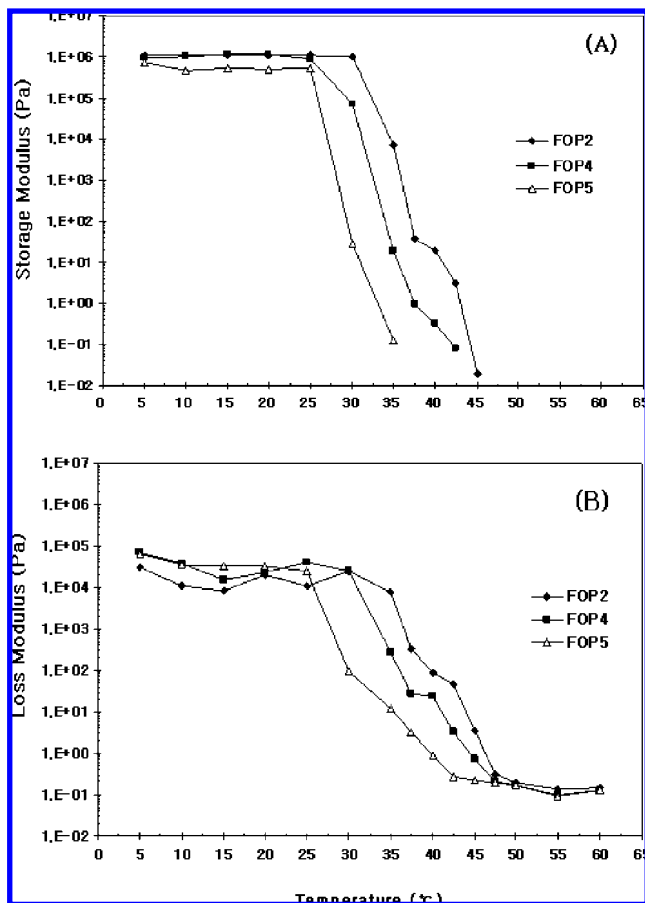


Figure 3. Storage modulus (A) and loss modulus (B) of the produced fats (FOP2, FOP4, and FOP5) as a function of temperature.

modulus (G'') were obtained with a dynamic temperature sweep step test. The sample was cooled to 5 °C (initial temperature) and heated to 35 °C at 5 deg increments. Then the temperature was increased to 50 °C in steps of 2.5 deg, and then to 60 °C (final temperature) in steps of 5 deg. The viscoelastic measurements were taken at each step, and triplicate analyses were performed.

Polymorphism. Melted fat samples were placed on rectangular plastic molds, and tempered at 5 and 24 °C for 16 h. Polymorphic forms of the samples were determined by a ARL Scintag XDS 2000 (Ecublens, Switzerland) with a cobalt X-ray tube, operating at 40 kV and 35 mA. The short spacings were observed in the 2θ range of 18–32°, and the scan rate was set at 2.0 deg/min. The β' form was identified with two strong spacings at ca. 3.8 and 4.2 Å while the β form was identified with a strong spacing at ca. 4.60 Å. Pseudo- β' as subforms were also designated at weak spacing at ca. 4.4 Å (16). The X-ray raw file was managed by Diffraction Management System Software Windows NT (DMSNT, Ecublens, Switzerland), and the Net Intensity file was generated by removing the background, smoothing, and/or correcting the raw data. The relative content (%) of β' and β form was calculated from the intensity (counts per second, CPS) of short spacings, in which the intensity of β' was from 3.8 and 4.2 Å, and that of β was 4.6 Å. Duplicate analysis was carried out.

Crystal Microstructure. Samples were melted completely at 80 °C, and each 20 μ L was put on a preheated glass slide. A preheated coverslip was placed over the sample. Then the prepared slide was cooled and stored at 5 and 24 °C for 16 h. The microstructure of crystallized samples was obtained by polarized light microscopy (Leica Microsystems Inc., Allendale, NJ) with an AxioCam digital camera attached (Zess Inc., Göttingen, Germany).

Statistical Analysis. Statistical Analysis System software (SAS, Cary, NC) was used to perform statistical computations (17). 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 the produced interesterified plastic fats is presented in **Table 1**. The major fatty acids were palmitic (16:0, 32.2–47.4%), stearic (18:0, 12.0–12.4%), and oleic acid (18:1n-9, 33.6–49.5%). As expected, an increased weight ratio of OO from 20% to 50% in the substrate blend resulted in a reduced content of palmitic acid and increased content of oleic acid in the plastic fats. Oleic and palmitic acids were the most abundant fatty acid in the FOP5 and FOP2, respectively. The total *trans* fatty acid level in the produced fats was measured by ATR-FTIR, which was developed for rapid quantification without requiring derivatization. This method is based on the measurement of the area of the C–H out-of-plane deformation band at 966 cm^{-1} from a horizontal baseline, which is characteristic of most common *trans* double bonds. No *trans* infrared band was found in the produced plastic fats (FOP2, FOP4, and FOP5), indicating that no *trans* fatty acids were formed. In the commercial bakery shortenings, which were prepared with partially hydrogenated oils, purchased from a local market, the *trans* band was present. The commercial shortenings contained 6.5–15.5% *trans* fatty acids (data not shown, but presented in the Supporting Information section).

Solid Fat Content. The solid fat content (SFC) is the percentage of lipid that is solid at the selected temperatures. Since SFC influences physical properties such as spreadability, hardness, mouthfeel, and stability, the SFC values are required to characterize the properties of plastic fats. The SFC of the fat was measured by NMR procedure. The produced fats FOP2, FOP4, and FOP5 had a 52–58% SFC at 10 °C, then decreased to 2–15% at 40 °C (**Table 2**). The FOP2 had higher SFC values than FOP4 and FOP5 at the selected temperatures, and FOP4 contained more SFC than FOP5. As expected, the addition of PS containing relatively high melting triacylglycerols increased SFC in the produced fats. SFCs of the commercial bakery fats were compared with the produced fats (**Table 2**). The FOP4 or FOP5 showed SFC values that closely matched those of the commercial bakery fats, and the FOP2 contained more SFC at the temperature ranges from 20 to 40 °C.

Bakery fats such as shortening and margarine fats have been modified to provide desirable consistency and quality, and to deliver specific consumer needs in baking, confectionery and cooking applications (11). In the present study, the SFC profile of the produced fats showed a wide plastic range with a gradual slope, which is an important characteristic of fats suitable for use in baking industry.

Texture Profile Analysis. Texture profile analysis (TPA) was performed to evaluate textural properties of the produced plastic fats which were stored at 5 °C (refrigeration temperature) and 24 °C (room temperature) (**Table 3**). The texture was influenced by the storage temperature, and showed a decrease in hardness, adhesiveness, and cohesiveness in most interesterified fats (except for the adhesiveness value in FOP2) when stored at higher temperature (24 °C). For hardness, the produced fats stored at 24 °C were softer than those at 5 °C. Among the produced fats, the FOP2 was the hardest, and FOP5 was the softest due to the different amounts of PS. FOP2 was prepared with 70% PS and FOP5 with 40% PS. This result correlated well with the SFCs because the produced fats having higher hardness values exhibited higher SFCs (**Table 2**). Cohesiveness was measured to evaluate brittleness, and adhesiveness to estimate the stickiness or tackiness of the fats. The cohesiveness values of FOP2 were higher than those of FOP4 and FOP5, indicating that FOP2 was more brittle than the other produced

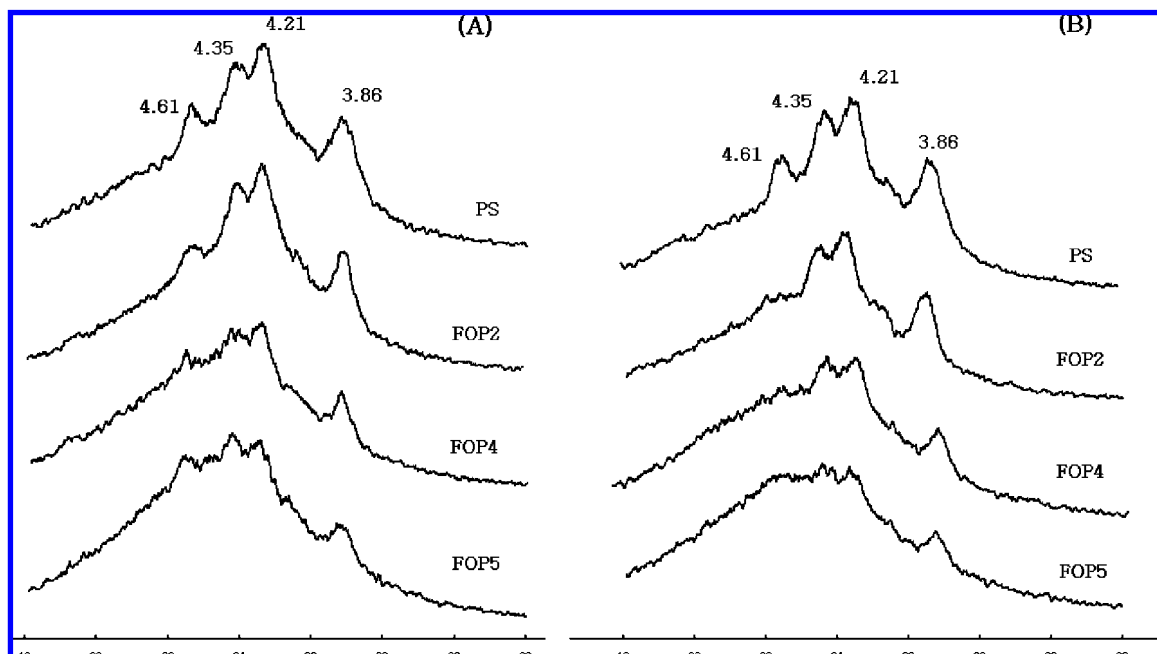


Figure 4. X-ray diffraction spectra of PS and the produced fats (FOP2, FOP4, and FOP5) stored at 5 (A) and 24 °C (B).

Table 5. Content of Polymorphic Forms^a (β and β' Form) in the Produced Interesterified Plastic Fats^b

sample	5 °C					24 °C				
	content (%)		intensity (CPS) ^c			content (%)		intensity (CPS)		
	β'	β	3.8 Å	4.2 Å	4.6 Å	β'	β	3.8 Å	4.2 Å	4.6 Å
FOP2	89 ± 0 ^d	11 ± 0	228 ± 45	379 ± 48	75 ± 8	100 ± 0	0 ± 0	210 ± 18	305 ± 78	nd ^e
FOP4	74 ± 4	26 ± 4	181 ± 23	259 ± 49	150 ± 8	100 ± 0	0 ± 0	142 ± 12	252 ± 2	nd
FOP5	67 ± 1	33 ± 1	112 ± 12	195 ± 19	148 ± 10	100 ± 0	0 ± 0	115 ± 7	133 ± 2	nd

^a Determined by X-ray diffraction: β' , short spacing of 3.8 and 4.2 Å; β , short spacing of 4.6 Å. ^b Enzymatically interesterified plastic fats with FHSBO:OO:PS in a weight ratio of 10:20:70 (FOP2), 10:40:50 (FOP4), and 10:50:40 (FOP5). ^c Counts per second. ^d Values are the means of triplicates ± standard deviation from the same batch of interesterified plastic fats. ^e Not detected.

fats at 5 and 24 °C (Table 3). In addition, FOP2 had a higher adhesiveness value and exhibited more tackiness than FOP4 and FOP5 at 24 °C because some part of the FOP2 was shown to be more likely to adhere to the probe on the upward stroke during the first compression of TPA. However, the pattern was reversed for the fat stored at 5 °C, leading to a lower adhesiveness in FOP2 than in FOP4 and FOP5. This could be because FOP2 containing a high amount of PS was significantly harder, and resulted in a more easily brittle or fractural fat than the other produced fats. No significant texture difference was observed between FOP4 and FOP5 at 5 °C ($P > 0.05$), but storage at 24 °C led to harder and more tacky texture in FOP4 ($P < 0.05$).

Evaluation of Color. Table 4 shows the color differences between the interesterified plastic fats. The lightness of FOP5 was lower than that of the other plastic fats (FOP2 and FOP4). FOP5 showed more greenish and yellowish color than FOP2 and FOP4. The OO contains various amounts of pigments such as chlorophylls and carotenoids, and the chlorophyll and its derivatives are responsible for the greenish color, which has a relationship with a value (18). In the present study, the interesterified fat (FOP5) containing a high amount of olive oil showed a decreased a value and an increased b value compared to the other interesterified fats (FOP4 and FOP2).

Crystallization and Melting Behaviors. The interesterification reaction of the FOP4 (blend FHSBO:OO:PS, 10:40:50) during 24 h was monitored with DSC, and their melting and crystallization behaviors are presented in Figure 1. The melting

thermogram of the noninteresterified FOP4 (0 h) was distinctly different from those of the interesterified fats. After 24 h of reaction, the peak at -0.74 °C of the FOP4 at 0 h was shifted to a higher temperature of 13.10 °C, and the peak at 49.65 °C became smaller and shifted to a lower temperature of 43.73 °C (Figure 1A). The crystallization behavior of noninteresterified FOP4 was also altered during the reaction, and showed a distinct peak at 30.98 °C that became smaller and moved to a lower temperature as the reaction time increased to 1, 2, 4, 6, 10, and 24 h (Figure 1B). Among the substrates, FHSBO had higher melting triacylglycerols, while OO had lower melting triacylglycerols. Before interesterification (at 0 h) each substrate had its own melting and crystallization characteristic, presenting higher and lower melting triacylglycerols together in the physical blend. However, as interesterification by lipase-catalysis was proceeding, rearrangements of fatty acids within or between triacylglycerols occurred and led to the production of new TAG molecules, and in turn, their melting and crystallization behaviors were changed. Figure 2 shows the different melting and crystallization behaviors between the interesterified produced fats (FOP2, FOP4, and FOP5). The FOP2 fat had a more narrow melting range than FOP4 and FOP5. FOP2 contained high-melting triacylglycerols (HMT, peaks II and III) which melted between 22.14 and 49.70 °C, while HMT of both FOP4 and FOP5 started to melt from 18.3 °C, but ended differently at 48.4 and 44.2 °C, respectively. According to the crystallization behavior in the produced fats, peak I gradually disappeared, and peak II became broader and moved toward a higher

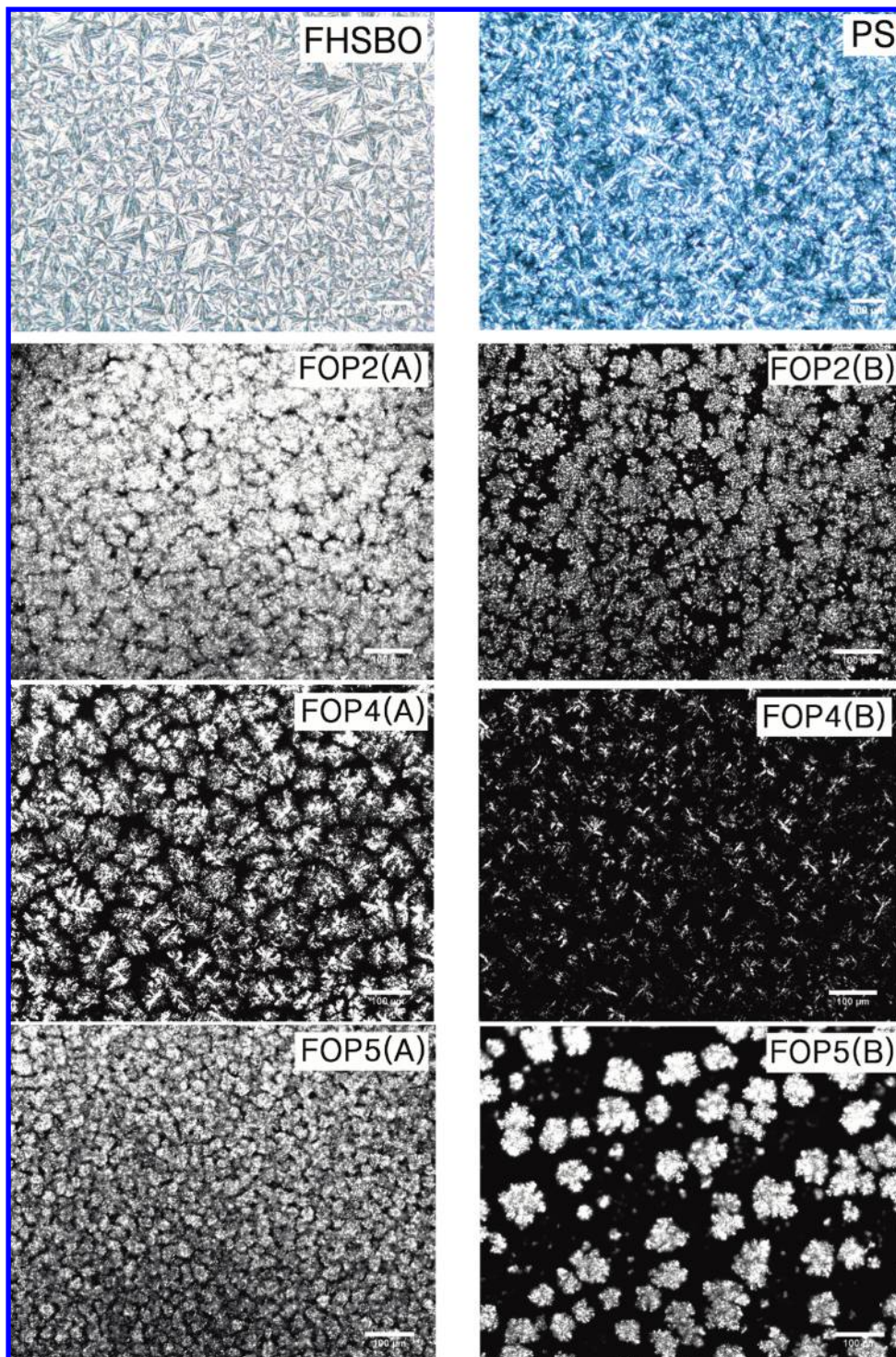


Figure 5. Polarized light micrographs of the produced fats. FHSBO, PS, FOP2(A), FOP4(A), and FOP5(A) were crystallized at 5 °C. FOP2(B), FOP4(B), and FOP5(B) were crystallized at 24 °C. The scale bar represents 100 μm .

temperature due to the increased amount of PS (**Figure 2B**). Furthermore, peak III, representing HMTs, became distinct and bigger, and shifted to a higher temperature, hence FOP2 showed the crystallization of HMT (peak III) starting at 30.34 °C with a maximum peak at 28.85 °C. Therefore, the produced fat prepared with a higher content of PS crystallized and melted at a higher temperature because it contained more HMTs.

Rheological Properties. The viscoelastic properties of the produced plastic fats were evaluated by storage modulus (G') and loss modulus (G''), in which G' relates to elastic or solid behavior of material, while G'' represents viscous or fluid

behavior (19). Each plastic fat contained a different content of solid fats at different temperatures, and consequently the viscoelastic behavior was affected by temperature. In the present study, the G' and G'' values were obtained as a function of temperature from 5 to 60 °C in steps of 2.5 or 5 deg. **Figure 3** shows that the FOP2, FOP4, and FOP5 fats have higher G' values than G'' values up to 25, 30, and 35 °C, respectively. Because G' represents the solid (elastic) behavior of a material, the higher G' values indicated that the produced plastic fats were a viscoelastic solid between these temperature ranges. However, as the temperature kept increasing, G' values decreased,

exhibiting a sharp decline in slope due to the melting of crystalline fats (**Figure 3A**). Each FOP5, FOP4, and FOP2 became a more viscoelastic fluid, and then elastic (or solid) behavior (G' values) disappeared after 35, 42.5, and 45 °C, respectively, showing only viscous fluid property in the produced fats. G'' values remained constant, showing similar viscous behavior up to 25 °C (for FOP5) or 30 °C (for FOP2 and FOP4), and then decreased to 42.5 (for FOP5) or 47.5 °C (for FOP2 and FOP4) and remained steady to the final temperature (60 °C) (**Figure 3B**). A similar pattern of viscoelastic behavior was observed in butter (20) and margarine fats (21, 22).

The G' values of FOP5 were lower than those of FOP2 and FOP4 at all measured temperatures. Initially, both FOP4 and FOP5 had similar values, but FOP4 showed lower G' values than FOP2 after 25 °C (**Figure 3A**). These values indicated that the elastic behavior was influenced by SFC and the produced fats showed more elastic (solid) behavior as the amount of PS increased. The G'' values from 5 to 25 °C were similar among the interesterified fats, but at higher temperatures they became highest in FOP2 and lowest in FOP5. The amount of PS also inversely affected the viscous behavior of the produced fats.

Polymorphism. The produced fats were crystallized at 5 and 24 °C, and their polymorphisms were characterized by X-ray diffraction spectra. Liquid molecules in lipid move around at random. Once the lipid is cooled, crystals occur in polymorphs which can be attributed to the different patterns of molecular packing. The three basic polymorphs are known as α (hexagonal), β' (orthorhombic), and β (triclinic). The β form is the most stable and has the highest melting point, while the α form is the least stable and the lowest melting polymorph (23). The β' form is intermediate in stability and melting point. A conversion from one form to another (i.e., β' to β) generally occurs in the direction that favors more stable forms.

The polymorphic forms of the produced fats were determined with the short spacings (**Figure 4**). PS had a mixture of both β' and β polymorphic forms, showing strong intensities at 3.86 and 4.21 Å (for β' form) and 4.61 Å (for β) when stored at either 5 or 24 °C. After interesterification, the produced fat displayed stronger intensities at 3.86, 4.21, or 4.35 Å than 4.61 Å when stored at 5 °C, and consisted of mainly the β' form of the crystal (89.1–67.4%) together with a small content of β form (10.9–32.6%) (**Table 5** and **Figure 4A**). At higher temperature (24 °C), the short spacing (4.61 Å) representing the β form was not found in the produced fats, and only the β' form was observed (**Figure 4B** and **Table 5**). PS contained high levels of β -promoting TAG (i.e., tripalmitin) due to a high concentration of palmitic acid (63%). However, when PS was interesterified with vegetable oils, it induced a more diverse structure of TAGs, and provided the β' form tending TAGs (24). According to the study of Ming et al. (12), the interesterified blends of sunflower oil with palm stearin by lipase-catalysis exhibited the β' polymorphic form and the substrates weight ratios of 60:40 and 50:50 were considered suitable for formulation of a table margarine fat. **Table 5** shows that in the produced fats, the intensity of short spacing for the β' form (at 3.86 and 4.21 Å) increased and that of the β form (at 4.61 Å) decreased as the amount of PS increased. Thus, the content of β' form was higher in FOP2 than in FOP4 and FOP5. **Table 5** shows that the intensities for the overall short spacings obtained from XRD are normally higher in FOP2 than in FOP4 or FOP5. This could be explained by the fact that the FOP2 was more solid than FOP4 or FOP5 due to higher solid fat content at the stored

temperatures; thus, more fat crystals were formed and displayed higher intensities in the FOP2.

The form of crystal is important in bakery fats such as shortening and margarine fats. The β' form is the most desirable because β' crystals give a good aeration to bakery dough, a smooth texture, and good functionality to the bakery product. Narine and Humphrey (25) reported that commercial shortenings (All Vegetable, Blue Crisco, and Golden Crisco) have predominantly the β' polymorphic form. Therefore, the produced fats having mostly β' form crystals in the present study could be used as alternatives for bakery fats.

Crystal Microstructure. The microstructures of the produced plastic fats obtained by polarized light microscopy are shown in **Figure 5**. The crystal morphologies between the substrates and produced fats were distinctly different. FHSBO had densely packed crystals of well-defined Maltese cross shape at 5 °C, and such similar crystal microstructure was previously observed in partially or fully hydrogenated oil by Humphrey et al. (26). The closely packed crystals of large rod-like spherulic were shown in PS. The produced fats (FOP2, FOP4, and FOP5) displayed altered crystal microstructures of initial FHSBO and PS, and exhibited spherulite-shaped crystals which tended to be aggregated to form clusters. The interesterification with a higher amount of PS led to the formation of smaller size crystals in the produced fat, and the FOP2 consisted of more tightly packed small crystals with a little space between adjacent crystals than FOP4 or FOP5. The crystal morphologies of FOP4 were more like Maltese cross-shaped compared to the other produced fats. When crystallized and stored at a higher temperature (24 °C), the produced fats had fewer crystals than those at 5 °C because the fats could hold the liquid oil inside the crystal networks and have a lower content of solid fats. The FOP2 consisted of smaller aggregates of clusters with uniform and small crystals at 24 °C whereas the FOP5 contained larger aggregates of clusters with diverse size. For use in bakery, fats containing the crystal aggregate with compacted small crystal are desired since these crystals could surround and stabilize air bubbles produced during the creaming stage, and allow them to serve as nuclei for leavening gases and to give a fine and smooth texture to bakery products.

In conclusion, the interesterified plastic fats (FOP2, FOP4, and FOP5) were produced from FHSBO, OO, and PS with different weight ratios of 10:20:70, 10:40:50, and 10:50:40, respectively. These interesterified fats contained no *trans* fatty acid, and had 33.6–49.5% oleic acid, which may lower the risk factors for CHD. Desirable physical properties including a wide range of SFCs, crystal polymorphs, and microstructures were observed in the produced fats. In addition, the physical property was influenced by the ratio of PS and/or OO. More desirable crystal microstructure and β' polymorphic form were present in FOP2 than FOP4 or FOP5 when stored either at 5 °C (refrigerator temperature) or at 24 °C (room temperature). Our results suggested that the produced fats containing no *trans* fatty acids could be used as an alternative to partially hydrogenated bakery fats.

Supporting Information Available: Graph giving the *trans* fatty acid levels of commercial shortenings. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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