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Characteristics and Feasibility of *Trans*-Free Plastic Fats through Lipozyme TL IM-Catalyzed Interesterification of Palm Stearin and *Akebia trifoliata* Variety *Australis* Seed Oil

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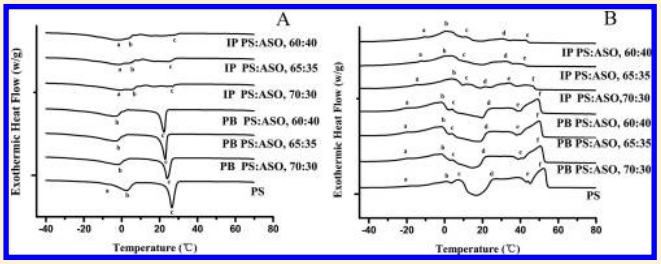
ABSTRACT: *Akebia trifoliata* var. *australis* seed oil (ASO) was used as an edible oil in China. However, in-depth research studies on ASO have yet to be conducted for production of plastic fats in food industry. In this work, an immobilized lipase from *Thermomyces lanuginosus* (TL IM) was employed to catalyze palm stearin (PS) with different ratios of ASO in a laboratory-scale operation at 60 °C. The physical properties [e.g., fatty acid profile, slip melting point (SMP), solid fat content (SFC), polymorphic form, and microstructure] of physical blends (PBs) were analyzed and compared with those of the interesterified products (IPs). Results showed that SMPs of IPs (33.20−37.60 °C) decreased compared with those of PBs (48.03−49.30 °C). Meanwhile, IPs showed a good SFC range from 16.11% to 28.29% at 25 °C with mostly β' polymorphic forms determined by X-ray diffraction analysis. It should be mentioned that no *trans* fatty acids (TFAs) were detected in any products, suggesting much more health-benefits of IPs. Texture tests showed that PBs (3318.19 ± 86.67 g) were markedly harder than IPs (557.02 ± 12.75 g). Conclusively, our study demonstrated that ASO can be utilized to produce *trans*-free plastic fats with good qualities through lipase-catalyzed interesterification.



KEYWORDS: *plastic fat, trans-free fatty acids, interesterification, Akebia trifoliata var. australis seed oil*

INTRODUCTION

The search for new sources of edible oils has gained attention because of increasing demands for edible oils in China. *Akebia trifoliata* (Thunb.) Koidz. var. *australis* (Diels) Rehd., a woody climbing vine, is widely found in Asia, especially in Korea, Japan, and China.[1] The stem of this plant is traditionally used to activate blood circulation and inhibit inflammation in Japan and China. The dried young leaves of this plant can be used as a tea alternative, and the fruits are used to make sweeteners, juice, and fruit vinegar.[2,3] *A. trifoliata* var. *australis* seed oil (ASO) was used as an edible oil in southern China during the period of shortage of edible oil. These seeds are composed 39% of oil, but they are regarded as a waste material and discarded directly in herb planting regions nowadays. In a previous study, we determined protein from the seeds and pectin from the peels of *A. trifoliata* var. *australis*.[4,5] Studies on the application of ASO have not been reported so far.

Some fats and oils have limited applications in food products when used in their original state.[6] Modification such as interesterification (catalyzed by chemicals or enzymes) is necessary to change the physiochemical characteristics of such oils.[7] Interesterification effectively alters the fatty acid type or their positions in triacylglycerol (TAG) molecules and consequently changes the melting and crystallization properties of fats or oils.[6,8] Interesterification catalyzed by enzymes is widely used to produce modified lipids possessing nutritional fatty acids or desirable physical properties; this technique requires mild reaction conditions and simple post-treatment and produces lipids of higher nutritional quality.[9] Although with a bright application prospective, enzymatic interesterification for production of modified lipids still face many technique bottlenecks such as how to minimize the acyl migration and stabilize enzyme activities et al. Lipase from *Thermomyces lanuginosus* (TLL) is a basophilic and noticeably thermostable enzyme. Also, TLL is a *sn*-1,3-specific lipase, which are commercial available both in soluble and immobilized form. It has been reported that immobilization of enzymes on a support can enhance the performances of an enzyme in a synthetic process with many advantages.[10] For instance, the enzyme can be fully dispersed on the support surface after immobilization, which will prevent aggregation or other inactivation phenomena; on the other hand, multipoint covalent immobilization may produce a more rigid structure,

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less sensitive to conformational changes; thus, enzyme activity under drastic conditions may become higher than that of the free enzyme.[10,11] With these improvements, the immobilized form of *Thermomyces lanuginosus* (TL IM) was more popular in modification of fats and oils. Palm stearin (PS) is an excellent alternative for the production of plastic fats with addition of other vegetable oils because of its solid fat content (SFC) and ability to promote β' crystallization. [12]

The main objective of this paper is modification ASO and PS catalyzed by TL IM to produce desirable low-*trans* plastic fats as an alternative for plastic fats. After interesterification, the properties of produced fats [fatty acid profile, slip melting point (SMP), solid fat content (SFC), polymorphic form, and microstructure et al.] were characterized by gas chromatography (GC), differential scanning calorimetry (DSC), X-ray diffractometry (XRD), and confocal laser scanning microscopy. Also, the *sn*-2 positional fatty acid compositions were determined to check the occurrence of acyl migration side reaction.

EXPERIMENTAL PROCEDURES

Reagents and Materials. PS (SMP, 53.0 °C) was purchased from Fengyi Biotechnology Co., Ltd. (Shanghai, China). ASO were supplied by Jiujiang Regression Biotechnology Development Co., Ltd. (Jiangxi, Jiujiang, China). TL IM from *Thermomyces lanuginosus* was purchased from Novozymes A/S (Bagsvaerd, Denmark) and stored at −18 °C until use according the recommendation. The specific activity of Lipozyme TL IM, which is immobilized on silica gel, was 181 IU/g, having 0.54 g/mL bulk density, and 0.3 to 1.0 mm particle diameter. Standards of fatty acid methyl esters (FAME) (GLC-463) were purchased from Nu-Chek Prep Inc. (Elysian, MN, U.S.A.). All other chemicals used were of either HPLC or analytical reagent grade.

Interesterification. In the preliminary experiment, the interesterification conditions of PS and ASO for producing plastic fats have been screened out. Finally, PS and ASO at ratios of 70:30, 65:35, and 60:40 were successfully achieved to produce plastic fats at 60 °C for 6 h using 10% (wt) of Lipozyme TL IM as a catalyst. The react conditions of Lipozyme TL IM catalyzed interesterification were consistent to our previous reports.[13,14] In order to determine the qualities of produced plastic fats, scale-up experiments were conducted. A total of 50 g of substrate PS and ASO at ratios of 70:30, 65:35, and 60:40 were placed in 250 mL Erlenmeyer flasks with a screw cap. The melted blends were reacted with Lipozyme TL IM (10 wt % of total substrates) in a shaking water bath at 60 °C; the mixing speed was set at 220 rpm. After the reaction, TL IM was separated from the mixtures by filtering. To remove free fatty acids, the same volume of hexane and five drops of phenolphthalein solution were added to the interesterified products (IPs). The mixture was titrated with 0.5 mol/L KOH solution in 95% ethanol until a pink color appeared. IPs were washed with warm water until the pink color disappeared. The upper layer was passed through an anhydrous sodium sulfate column to remove moisture, and the solvent was completely evaporated under nitrogen with moderate heat.

Fatty Acid Composition Analysis. Samples were methylated according to the procedures described by Zhu et al.[15] with slight modification. The fats were placed in a 20 mL tube with a screw cap, saponified by 0.5 mol/L methanolic NaOH, and then methylated by boron trifluoride in methanol (14%). FAMEs were analyzed by GC using an Agilent 6890N gas chromatograph (Santa Clara, CA, U.S.A.) equipped with a flame ionization detector, an autoinjector, and a fused silica capillary column (CP-Sil 88, 100 m × 0.25 mm × 0.2 μm i.d.). The oven was heated to 45 °C and then held at this temperature for 3 min. The temperature was then increased to 175 °C at a rate of 13 °C/min and was held for 27 min. This temperature was finally increased to 215 °C at a rate of 4 °C/min and then held for 35 min. The temperature of the injector and detector were set to 250 and 260 °C, respectively. Nitrogen was used as a carrier gas at a flow rate of 52 mL/min in split mode (50:1). Fatty acid compositions were identified by comparison with the retention times of standard mixtures. Analyses were performed in triplicate.

Fatty Acid Composition at *Sn*-2 Position. *Sn*-2 positional distribution was determined by partial hydrolysis using pancreatic lipase.[15] Ten milligrams of IPs or physical blends (PBs) was dissolved in 10 mL of Tris−HCl buffer (1 mol/L, pH = 7.6), 2.5 mL of 0.05% bile salt, and 1 mL of 2.2% calcium chloride solution; 10 mg of pancreatic lipase was subsequently added to this mixture. The mixture was vortexed for at least 1 min and then placed in a water bath at 37 °C for 3 min; vortexing for 30 s followed. Four milliliters of diethyl ether was added to the mixture to extract the hydrolytic product. The upper layer was dried using a sodium sulfate column. After blowing up by nitrogen gas, applied to a thin-layer chromatography (TLC) plate (Merck KGaA, Darmstsdt, Germany) coated with silica gel G and developed with a solvent mixture comprising diethyl ether/hexane/acetic acid (50:50:1, V/V/V). The plate was air-dried for 30 min and then visualized by spraying with iodine vapor. The band corresponding to the monoacylglycerol was scrapped off, methylated, and analyzed by GC as described above. Analyses were performed in triplicate.

Analysis of TAG by RP-HPLC. The Agilent 1100 HPLC system consisted of quadruples pump with an evaporative light-scattering detector (All-tech 2000ES, U.S.A.) operating at 55 °C and a nitrogen pressure of 1.7 bar. Each sample was dissolved in hexane and 10 μL of the dissolved sample were injected and separated by the Novo-Pak C18 colomn (150 × 3.9 mm, waters, Milford, MA, U.S.A.). Elution solvent consisted of (A) acetonnitrile and B isopropanol:hexane (1:1, v/v) at the flow rate of 1.8 mL/min. [15]

Determination of SMP. The SMP of the mixture was determined according to American Oil Chemists' Society Official Method No. *Cc* 3-25 (2009)[16] using the open tube melting point method. Capillary tubes filled with a 1-cm-high column of fat were stored in a refrigerator at 10 ± 1 °C for 16 h prior to measurement in a beaker of cold water. The water was stirred and heated gradually. The temperature at which the fat in the tube began to rise because of hydrostatic pressure was recorded. This temperature was considered the SMP and determined in triplicate.

X-ray Diffraction Spectroscopy. The polymorphic forms of fat crystals in the blends were determined by Empyrean XRD (PANalytical B.V., Almelo, Netherlands) using Cu Kα radiation (voltage of 40 kV; current of 35 mA; divergence, antiscatter, and receiving slits, fixed at 0.5°, 1°, and 8 mm, respectively). The samples were melted at 70 °C, poured into a rectangular plastic mold, and tempered at 24 °C for 16 h. The short spacing was scanned at 2θ ranging from 16° to 32° at a rate of 0.01°/80 s.[15] The α polymorph was identified by a short spacing of 4.15 Å. β' forms were mainly identified by short spacings of 3.8 and

Table 1. Fatty Acid Composition (Area %) of Palm Stearin (PS), *Akebia trifoliata* Var. *australis* Seed Oil (ASO), the Physical Blends (PBs), and the Interesterified Products (IPs) of PS: ASO 70:30, 65:35, 60:40[a]

			physical blends (PS: ASO)			interesterified products (PS: ASO)		
	PS	ASO	70:30	65:35	60:40	70:30	65:35	60:40
C12:0	0.12 ± 0.01	0.07 ± 0.00	0.11 ± 0.00	0.10 ± 0.01	0.10 ± 0.00	0.11 ± 0.00	0.10 ± 0.00	0.10 ± 0.00
C14:0	1.23 ± 0.01	0.09 ± 0.01	0.89 ± 0.00	0.83 ± 0.01	0.78 ± 0.04	0.89 ± 0.00	0.83 ± 0.03	0.78 ± 0.01
C16:0	59.46 ± 0.01	21.70 ± 0.32	48.15 ± 0.11	46.24 ± 0.34	44.32 ± 0.70	48.12 ± 0.61	46.12 ± 0.29	44.47 ± 1.17
9c C16:1	ND[b]	0.19 ± 0.01	0.06 ± 0.00	0.07 ± 0.00	0.07 ± 0.00	0.06 ± 0.00	0.07 ± 0.01	0.08 ± 0.00
C18:0	5.46 ± 0.17	3.29 ± 0.15	4.80 ± 0.13	4.70 ± 0.13	4.61 ± 0.42	4.81 ± 0.05	4.71 ± 0.04	4.53 ± 0.20
9c C18:1	28.01 ± 0.16	42.40 ± 0.01	32.35 ± 0.07	33.11 ± 0.60	33.78 ± 0.18	32.33 ± 0.94	33.21 ± 0.29	33.78 ± 1.48
11c C18:1	ND	1.30 ± 0.00	0.39 ± 0.01	0.46 ± 0.04	0.52 ± 0.01	0.39 ± 0.06	0.45 ± 0.07	0.52 ± 0.00
9c12c C18:2	5.67 ± 0.02	30.54 ± 0.10	13.14 ± 0.04	14.34 ± 0.06	15.65 ± 0.49	13.18 ± 0.45	14.35 ± 0.14	15.58 ± 0.54
9t C18:1	ND	ND	ND	ND	ND	ND	ND	ND
C18:3	ND	0.43 ± 0.01	0.13 ± 0.00	0.15 ± 0.01	0.17 ± 0.01	0.13 ± 0.01	0.15 ± 0.01	0.17 ± 0.01
ΣMCFA	0.12 ± 0.01	0.07 ± 0.00	0.11 ± 0.00	0.10 ± 0.01	0.10 ± 0.00	0.11 ± 0.00	0.10 ± 0.00	0.10 ± 0.00
ΣSFA	66.28 ± 0.18	25.14 ± 0.10	53.83 ± 0.02	51.77 ± 0.49	49.70 ± 0.33	53.82 ± 1.12	51.67 ± 0.23	49.78 ± 0.95
ΣUSFA	33.68 ± 0.18	74.86 ± 0.10	46.06 ± 0.02	48.12 ± 0.48	50.19 ± 0.33	46.08 ± 1.13	48.23 ± 0.23	50.12 ± 0.95
ΣTFA	ND	ND	ND	ND	ND	ND	ND	ND
SMP(°C)	53.03 ± 0.12		49.30 ± 0.27	48.77 ± 0.06	48.03 ± 0.31	37.60 ± 0.20	34.63 ± 0.71	33.20 ± 0.66

[a]Values are the mean ± standard error. [b]ND = Not detected under this analysis condition.

Table 2. Fatty Acid Composition (Area %) at the *Sn*-2 Position of Palm Stearin (PS), *Akebia trifoliata* Var. *australis* Seed Oil (ASO), the Physical Blends (PBs), and the Interesterified Products (IPs) of PS: ASO 70:30, 65:35, 60:40[a]

			physical blends (PS: ASO)			interesterified products (PS: ASO)		
fatty acid	PS	ASO	70:30	65:35	60:40	70:30	65:35	60:40
C12:0	0.22 ± 0.01	0.04 ± 0.01	0.16 ± 0.00	0.18 ± 0.00	0.18 ± 0.00	0.21 ± 0.06	0.66 ± 0.00	0.83 ± 0.17
C14:0	1.05 ± 0.01	0.17 ± 0.01	1.13 ± 0.11	1.20 ± 0.28	0.70 ± 0.04	1.41 ± 0.15	1.21 ± 0.01	1.31 ± 0.03
C16:0	34.86 ± 0.81	8.44 ± 0.21	29.96 ± 0.00	28.19 ± 0.36	24.80 ± 0.02	49.80 ± 0.33	47.32 ± 0.46	45.93 ± 1.27
9c C16:1	1.13 ± 0.02	0.15 ± 0.02	1.00 ± 0.00	0.75 ± 0.07	0.65 ± 0.03	0.18 ± 0.01	0.20 ± 0.01	0.20 ± 0.00
C18:0	5.49 ± 0.37	2.34 ± 0.43	4.65 ± 0.21	4.94 ± 0.04	4.09 ± 0.01	9.70 ± 0.23	9.77 ± 0.07	9.91 ± 0.63
9c C18:1	45.26 ± 0.05	52.11 ± 0.06	45.84 ± 0.14	45.21 ± 0.24	48.54 ± 0.12	23.91 ± 0.69	26.69 ± 0.29	27.52 ± 1.57
9c12c C18:2	10.76 ± 0.32	35.63 ± 0.32	16.35 ± 0.31	18.62 ± 0.52	19.88 ± 0.21	12.92 ± 0.32	13.23 ± 0.12	13.70 ± 0.12
C18:3	1.23 ± 0.18	1.14 ± 0.02	0.91 ± 0.06	0.88 ± 0.01	1.16 ± 0.08	1.87 ± 0.05	0.92 ± 0.01	0.56 ± 0.01
ΣMCFA	0.22 ± 0.01	0.04 ± 0.01	0.16 ± 0.00	0.18 ± 0.00	0.18 ± 0.00	0.21 ± 0.06	0.66 ± 0.00	0.83 ± 0.17
ΣSFA	41.61 ± 0.44	10.98 ± 0.22	35.91 ± 0.11	34.53 ± 0.69	29.77 ± 0.05	61.11 ± 0.30	58.96 ± 0.38	57.98 ± 1.70
ΣUSFA	58.39 ± 0.44	89.02 ± 0.22	64.09 ± 0.11	65.47 ± 0.69	70.23 ± 0.05	38.89 ± 0.30	41.04 ± 0.38	42.02 ± 1.70
ΣTFA	ND[b]	ND	ND	ND	ND	ND	ND	ND

[a]Values are the mean ± standard error. [b]ND = Not detected under this analysis condition.

4.2 Å or three minor short spacings at 3.17, 3.97, and 4.27 Å. β forms were observed by a short spacing of 4.6 Å.[17]

Differential Scanning Calorimetry. Melting and crystallization thermograms were determined using DSC (Model No. DSC-204 F1, NETZCH Corporation, Germany) equipped with a thermal analysis data station. Nitrogen (99.99% purity) as the purge gas was used at a flow rate of 20 mL/min. An empty aluminum pan was used as a reference, and the samples were precisely weighed (7 ± 0.5 mg) for DSC analysis. The samples were heated to 80 °C and held at this temperature for 10 min. The temperature was subsequently decreased at a rate of 10 °C/min to −60 °C. After holding for 10 min at −60 °C, the temperature was returned to 80 °C at a rate of 5 °C/min and maintained for 5 min. The solid fat content (SFC, %) was determined from the melting thermograms obtained by the thermal analyzer. For calculating solid fat content SFC, each DSC thermogram was divided into 5 °C increments (0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, and 55 °C), and the total melting energy (J/g) was converted into a percentage to determine the SFC at each temperature increment.[18]

Microstructural Analysis. The samples were melted at 70 °C in a water bath and kept at this temperature for 30 min to destroy crystal memory. About 10 mg of melted fat was placed on a preheated microscope slide and covered with a preheated coverslip with the aid of a capillary tube. The samples were cooled to 25 °C and maintained at this temperature for 20 h. The crystal morphology of the fat samples was studied under isothermal conditions (25 °C).[13] The coverslips were fixed on the stage of a Zeiss inverted microscope and observed under a Carl Zeiss 710 confocal laser scanning microscope (Carl Zeiss Inc., Oberkochen, Germany) connected to a digital video camera. All photomicrographs were obtained with a 40 × 0.75 Zeiss Plan Neofluar objective.

Induction Time of Oxidation. The induction times of produced IPs (PS:ASO 70:30) and IPs with different dosages of α-tocopherol (50, 100, and 200 ppm) were determined using a Metrohm 679 Rancimat (Brinkaman Instruments Co., Des Plains, IL, U.S.A.). For Rancimat analysis, 3.0 g of the sample was placed in a Rancimat reaction vessel kept at a constant temperature of 110 °C. A stream of air was passed through the vessel at a rate of 10 L/h. The vapors released during oxidation together with the air were passed into a flask containing 50 mL of demineralized water and an electrode for measuring conductivity.[19] Analyses were performed in triplicate.

Table 3. Triacylglycerol (TAG) Composition (Area %) of PS, ASO, the Physical Blend (PB) and Interesterified Product (IP) of PS:ASO 70:30[a]

ECN[b]	TAG	PS	ASO	PB	IP
42	LLL	ND[c]	1.17	ND	ND
44	LLO/LOL/LaPO/LaOP/OLaP	ND	10.19	3.32	0.52
44	PLL/LPL	ND	5.52	1.40	0.81
46	LOO/OLO	ND	21.56	5.49	3.90
46	PLO/OPL/POL	1.95	17.69	6.48	13.58
46	PPL/PLP	3.25	2.24	2.78	8.63
48	OOO	0.72	2.53	1.37	3.20
48	POO/OPO	10.78	15.34	13.08	27.88
48	POP/PPO	48.45	16.87	39.33	25.85
48	PPP/PLS/PSL/SPL	28.75	ND	18.27	10.11
50	POS/PSO/OPS	1.28	ND	0.76	0.99
50	SSL/SLS	2.05	4.47	3.38	1.97
50	PPS/PSP	1.67	ND	1.08	0.86
	others	1.10	2.42	1.07	1.68

[a]Abbreviations: La = lauric acid; P = palmitic acid; S = stearic acid; O = oleic acid; L = linoleic acid. [b]Equivalent carbon number (ECN) = CN-2DB, where CN is carbon number of TAG and DB is total number of double bonds in TAG. [c]ND = not detected.

Texture Profile Analysis. Textural properties (i.e., hardness, adhesiveness, and cohesiveness) of PBs and IPs were determined at 4 and 23 °C using a TA-X2 texture analyzer (Stable Micro Systems, London, U.K.). A 45° conical probe attached to a 5 kg compression load cell was inserted into the sample at 1.0 mm/s to a depth of 10 mm from the sample surface; withdrawal was performed at the same speed. Hardness and adhesiveness were respectively defined as the maximum force (g) and negative force area (g·s) of the first compression. Cohesiveness (unitless) was determined as the ratio of the positive force area during the second compression to that during the first compression. Completely melted samples were placed into 50 mL beakers and tempered at 25 °C for 24 h.[20] Specimens were prepared in triplicate for each analysis.

Statistical Analysis. Statistical analysis was performed using the SAS software package (SAS Institute, Cary, NC, U.S.A.). Duncan's multiple range test was performed to determine significant difference ($P \leq 0.05$).

RESULTS AND DISCUSSION

Total and Positional Fatty Acid Composition and SMP. The *trans* fatty acids (*TFAs*) was not detected in all samples (Table 1). ASO contained palmitic (21.70%), oleic (42.40%), and linoleic (30.54%) acids. The fatty acid profile of ASO was very similar to that of rice bran oil, in which the major fatty acids were palmitic (19.4%), oleic (45.9%), and linoleic (32.1%) acids.[21] The palmitic acid content (21.70%) in ASO was higher than that in other vegetable oils, such as soybean oil, sunflower oil, sesame seed oil, rapeseed oil, and olive oil.[17,22−25] The high palmitic and oleic acid contents of ASO imply its high oxidative stability. The *sn*-2 positional fatty acid compostion in Table 2 showed ASO contained 89.02% unsaturated fatty acids (USFAs), of which oleic acid (52.11%) and linoleic acid (35.63%) were the major fatty acids. These results demonstrated that USFAs in ASO were preferentially located at *sn*-2 position. In the case of PS, more saturated fatty acids (SFAs) were detected where palmitic acid take up to 59.49 and 34.86% of total (Table 1) and *sn*-2 positional fatty acid (Table 2). The high amount of SFAs contributed to higher SMP (53.03 °C) (Table 1).

The total fatty acid compositions PBs and IPs were altered based on the mixing ratios of PS:ASO. With increasing the weight ratio of ASO in PBs and IPs, the ΣUSFA content of PBs and IPs increased while SMP decreased. In the case of positional fatty acids, the obviously different data between PBs and IPs were detected. ΣSFA of IPs (57.98−61.11%) were significantly higher than that of PBs (29.77−35.91%). In some sense, the alteration of positional fatty acid proved the interesterification reaction occurred. Although this data also implied that the interesterification increased the saturation at the *sn*-2 position, which can be attributed to the random replacement of acyl groups in the products via a process called acyl migration. Similar phenomena have been reported previously.[24] Acyl migration is an undesirable side reaction that cannot be avoided during esterification without adopting auxiliary measures. This phenomenon can explain the changes in fatty acid composition at *sn*-2 position after interesterification.

SMP of PBs and IPs are also listed in Table 1. Both in PBs and IPs, reducing ratio of PS caused decrease of SMP. After interesterification, IPs possessed a lower SMP compared to the corresponding PBs. The differences of SMP between PBs and IPs also demonstrated the interesterification occurred. A SMP of 33.2−37.6 °C was ideal for spread fat because it is solid at room temperature and can be melted at body temperature.[6]

TAG Analysis. Table 3 showed the TAG composition of ASO, PS, PB, and IPs of PS:ASO 70:30 with different catalyzed time. The major TAG species in ASO were LLO (10.19%), LOO (21.56%), PLO (17.69), and POO (15.34), whereas POO (10.78%), POP (48.45%), and PPP (28.75%) were the dominant TAG types in PS. Therefore, the TAG species were originated from ASO and PS according to their ratio. After interesterification, however, changes of the TAG species in IP (PS:ASO 70:30) were observed compared to its PB. IP showed a reduced amount on LLO (0.52%), LOO (3.9%), PPP (10.11%), and POP (25.85%), but an increase of PLO (12.54−13.49%) and PLP (7.43−8.27%). The changes of TAG composition suggested that the fatty acid rearranged within and between TAG backbones by Lipozyme TL IM, which may alter physical properties.

SFC Value. SFC is an important determinant of the texture of bakery fats. The SFC is responsible for the various functional characteristics of bakery fat, including organoleptic properties (flavor release and thickness), physical appearance, spread-

ability, and oil exudation.[26] Figure 1 shows the SFC profiles of PS, PBs (PS:ASO 70:30; 65:35; 60:40), and IPs (PS:ASO

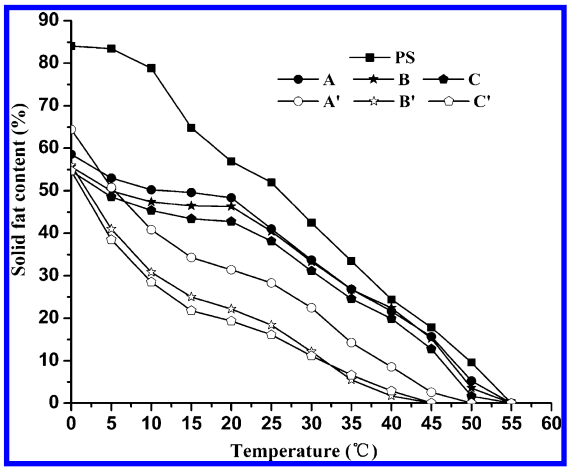

Figure 1. Solid fat content (SFC) of palm stearin (PS), the physical blends (PBs PS:ASO (wt/wt); A 70:30, B 65:35, C 60:40), and the interesterified products (IPs PS:ASO (wt/wt); A′ 70:30, B′ 65:35, C′ 60:40).

70:30; 65:35; 60:40) as a function of temperature. PS is a comparatively high melting fat that features an SFC of 24.41% even at 40 °C. Residual SFC values of 22.39%, 21.62%, and 19.88% were observed in PBs with PS:ASO ratios of 70:30, 65:35, and 60:40, respectively, at 40 °C. The sharpest decline in SFC appeared at the 25−30 °C range in PBs. In IPs, the fastest decline in SFC occurred at 0−5 °C. This shift is caused by the larger ratio of TAGs in IPs that liquefy at this temperature range. Shifts to lower temperature ranges may also explain the lower SMPs obtained in IPs compared with PBs. However, increasing the proportion of ASO decreased the prominence of the sharp drop in the 5 °C range in IPs. A similar study reported by Ming et al. shows a less pronounced drop in SFC upon decreasing the proportion of sunflower oil in enzymatic interesterified palm stearin−sunflower oil blends.[27]

SFC of IP PS:ASO 70:30 at 0−5 °C was slightly higher than that of PBs, similar to the results obtained by Kim et al. for the enzymatic interesterification of fully hydrogenated canola fat with olive oil.[28] This phenomenon may be attributed to the exchange of fatty acid residues in the two substrates to produce new TAG species after interesterification. The new species synthesized during interesterification could affect SFC profile of product at both high and low temperatures. Overall, IPs showed lower SFC contents than PBs at each temperature interval. For example, at 25 °C, IPs showed 16.11%−28.23% SFC, whereas PBs showed 38.16%−41.06% SFC. An SFC of 15%−35% at this temperature promotes the spreadability of margarine fats.[29] PBs with a higher SFC of 19.88%−21.62% at 40 °C may be used for cake manufacture.[30]

Crystallization and Melting Thermograms. The crystallization and melting properties of PS, PBs, and IPs were evaluated by DSC (Figure 2). The crystallization thermogram of PS (Figure 2A) exhibit two crystallization peaks at 2.4 and 26.4 °C, which correspond to the TAG fractions of high melting temperature and low melting temperature, respectively.[30] Figure 2A also shows the crystallization thermograms of the PS:ASO blends with different weight ratios before and after transesterification with LipozymeTL IM. Two exothermic peaks may be observed in PBs: the first occurred from 22.4 to

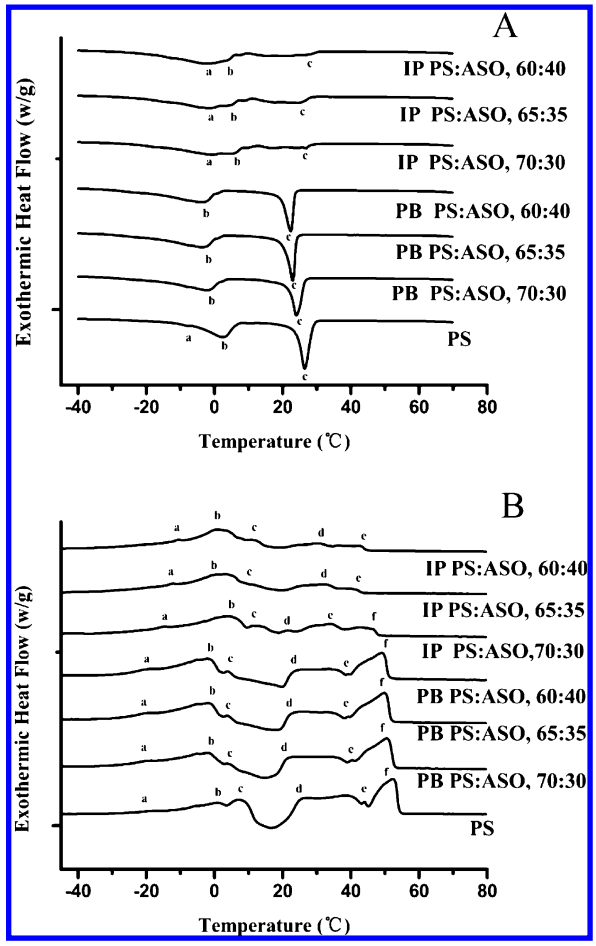

Figure 2. Differential scanning calorimetry (DSC) crystallization (A) and melting (B) thermograms of palm stearin (PS), the physical blends (PBs), and the interesterified products (IPs) of PS:ASO 70:30, 65:35, 60:40.

24 °C and the second occurred from −3.8 °C to −2.2 °C. Increasing the percentage of PS increased the representative peaks of higher-melting TAG (peak b) in terms of heights and areas and shifted them toward higher temperatures. IPs exhibited obviously broader and smaller exothermic peaks than PBs. Peak b was smaller than peak a, which indicated the formation of softer fats.

Figure 2B shows the endothermal profiles of PS and the PS:ASO blends of various ratios before and after interesterification. Five distinct melting peaks (peaks a to e) were observed in PS; peak e (at 52.4 °C) showed the largest and sharpest peak, which indicated that PS contained high-melting temperature TAGs.[23] The endothermic profiles of PBs were similar to that of PS but not to those of IPs. Five melting peaks of PBs ranging from −18.8 to 50.4 °C were observed; here, peak e, which occurred at high temperatures, showed the largest and sharpest peak. After interesterification, endothermic peaks b and c became broader and showed the largest size, whereas peaks d and e became narrower, smaller, and nearly disappeared. All of these changes indicated that most high-melting temperature TAGs are replaced by medium- and low-melting-temperature TAGs through rearrangement of fatty acids between TAGs during interesterification. The melting temperature of bakery fats normally ranges from 39 to 45 °C; IPs have this same melting point range.[22]

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Table 4. Polymorphic Forms of Palm Stearin (PS), the Physical Blends (PBs), and the Interesterified Products (IPs) of PS:ASO 70:30, 65:35, 60:40

fats	short spacing (Å)						polymorphic form
substrates							
PS	3.83 (s[a])	3.90 (s)		4.20 (m[b])		4.60 (m)	$\beta' + \beta$
physical blends (PS: ASO)							
70:30	3.82 (s)	3.90 (m)	4.14 (v[c]w[d])	4.22 (m)		4.61 (vw)	$\beta' \gg \beta + \alpha$
65:35	3.82 (s)	3.90 (m)	4.15 (vw)		4.30 (w)	4.62 (m)	$\beta' > \beta + \alpha$
60:40	3.82 (m)	3.90 (m)	4.14 (vw)	4.22 (m)	4.30 (w)	4.62 (m)	$\beta' > \beta + \alpha$
interesterified products (PS: ASO)							
70:30	3.75 (s)	3.89 (s)			4.30 (m)	4.61 (w)	$\beta' \gg \beta$
65:35	3.74 (m)	3.89 (s)			4.30 (m)	4.61 (w)	$\beta' \gg \beta$
60:40	3.73 (m)	3.89 (m)			4.29 (vw)	4.62 (vw)	$\beta' \gg \beta$

[a]s = strong. [b]m = medium. [c]v = very. [d]w = weak.

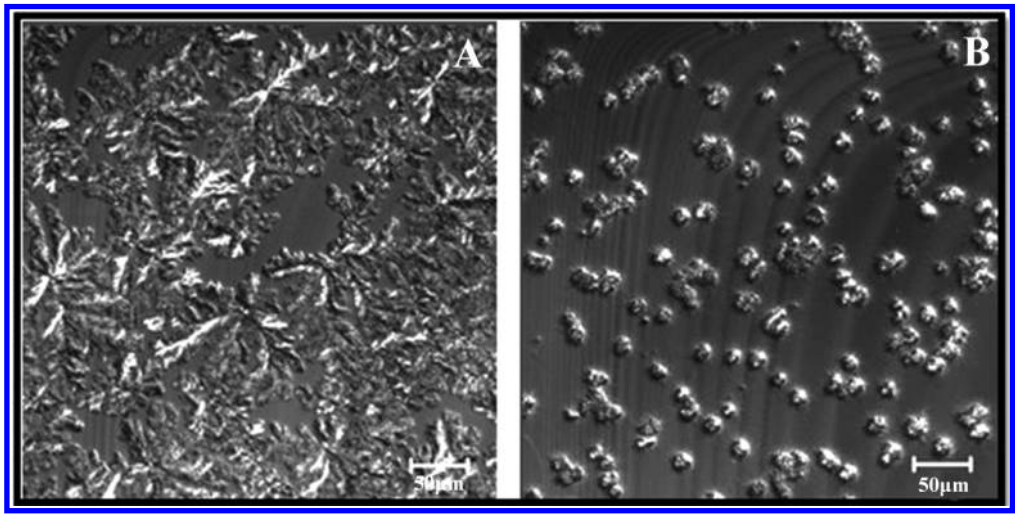

Figure 3. Crystal microstructure of the physical blend (PB, A) and the interesterified product (IP, B) of PS:ASO 70:30.

XRD Spectroscopy. Polymorphic forms of fat crystals are important criteria for determining the functional properties of bakery fats. The major polymorphs of fat crystals include the α (hexagonal), β' (orthorhombic), and β (triclinic) forms. Each polymorphic state has different properties. The α crystal form is characterized as the most unstable form and, therefore, features the lowest melting point. The β' form is a metastable and intermediate-melting polymorph. The β form is the most stable form and consequently has the highest melting point.[32] Table 4 lists the polymorphic forms of PBs and IPs analyzed by XRD.

PS was crystallized by the predominant β' (3.83, 3.90, and 4.20 Å) and β (4.60 Å) forms. PBs showed weak β crystal forms with short a spacing of 4.62 Å, strong β' forms with short spacings of 3.82, 3.90, 4.22, and 4.30 Å, and a very weak α crystal forms with a short spacing of 4.14 Å. IPs showed no α crystal forms, yielding β' forms with strong intensities (3.74, 3.89, and 4.30 Å) and β forms with weak intensity (4.61 Å) instead. We observed that the β' form increased whereas the β form decreased with increasing PS amount, which is attributed to the higher SFC and larger amount of fat crystals in samples with higher PS amounts. Similar results have previously been reported.[24] The β' form is desired for favorable molecular packing of the fatty acid chains of solid fats used to produce margarine or shortening because the small crystal size of this form stabilizes air and imparts better mouthfeel and spreadability; on the other hand, the β form comprises large crystals that give hardness as well as rough and sandy textures.[33]

Microstructural Analysis. The crystal microstructure of PBs (PS: ASO 70:30) and IPs (PS: ASO 70:30) were observed by confocal laser scanning microscopy. Figure 3 shows the distinct crystal morphologies of PBs and IPs. PBs presented very coarse, branched, and leaf-like crystals with crystal sizes of about 50−100 μm in diameter and compact packing. By contrast, IPs (PS: ASO 70:30) formed condensed, uniform, ball-like crystals with diameters of 20−30 μm. These findings agree with the XRD results, which indicated that IPs contain more β' polymorphs and less β polymorphs than PBs. Hartel reported that β' polymorphs have a spherulite-like shapes, whereas β polymorphs feature plate-shaped crystals.[34] Small, compact crystals are ideal for margarine fats and shortenings because these crystals can surround and stabilize air bubbles to impart better textural properties to a food product.

Texture Profile Analysis. Texture profile analysis was performed to evaluate the textural properties (hardness, adhesiveness, and cohesiveness) of PBs and IPs (PS:ASO 70:30) stored at room temperature (25 °C) (Figure 4). Significant changes in all textural attributes except cohesiveness were observed. PBs (3318.19 ± 86.67 g) were markedly harder than IPs (557.02 ± 12.75 g), which agrees with the SFC results, that is, the SFCs of PBs and IPs were 41.06% and 28.29%, respectively, at 25 °C. Hardness is positively related to the amount of solid fat. IPs were softer than PBs, which indicated the higher spreadablility of the former compared with the latter. PBs (PS:ASO 70:30) also showed more significant adhesive-

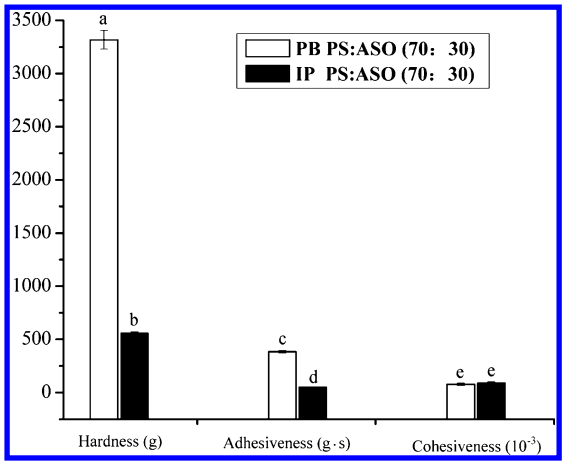

Figure 4. Hardness, adhesiveness, and cohesiveness of the physical blend (PB) and the interesterified product (IP) of PS:ASO 70:30 at 25 °C. Results are expressed as mean ± standard deviation (n = 3).

ness than IPs (PS:ASO 70:30). Adhesiveness reflects the stickiness or tackiness of plastic fats as well as the force required to remove fatty residues in the mouth, which is associated with the waxy taste of some fats. Thus, lower values of adhesiveness are preferred in edible oils.[35] IPs are potentially easier to spread and less persistent because of their lower adhesiveness. IPs possessed higher cohesiveness than PBs at 25 °C but differences between groups were not significant. Cohesiveness is related to the strength of internal bonds that make up the body of a product and describes how crumbly or brittle a food product is. IPs have stronger internal bonds and are therefore more brittle than PBs.

Induction Time of Oxidation. To develop a new lipid product, consideration of the nutritional and physical properties of the products as well as their oxidative stability is important. The oxidative stability of IPs (PS:ASO 70:30) was analyzed by measuring its induction time; longer induction times imply higher oxidative stability. Figure 5 shows that the induction time of IPs with PS:ASO 70:30 was 57 min. This induction time could be prolonged by addition of antioxidants such as α-tocopherol. In this study, the induction times of IPs fortified with 50, 100, and 200 ppm α-tocopherol significantly increased to 75, 91, and 108 min, respectively. α-Tocopherol appears to exert dose-dependent antioxidative effects from 50 to 200 ppm in ASO- and PS-based IPs.

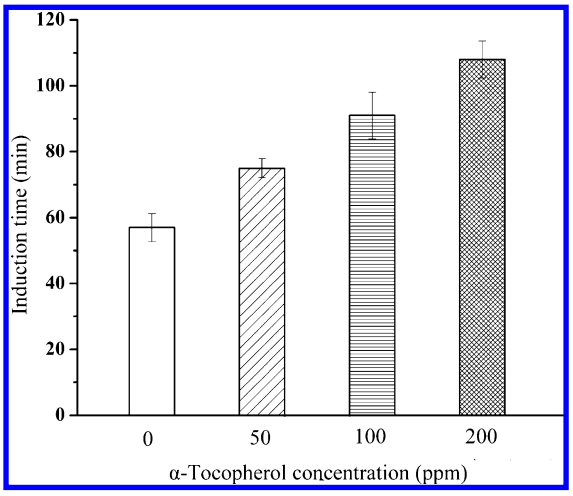

Figure 5. Induction time of the interesterified products (IPs, PS:ASO 70:30) with various antioxidant α-tocopherol concentrations.

In conclusion, the present study demonstrated the characteristics and feasibility of IPs from ASO and PS at different ratios of 70:30, 65:35, and 60:40 as plastic fats. Through analyses of fatty acid composition and physical prosperities, IPs were characterized as low TFAs, relative high content of USFAs (46.08%−50.12%), desirable physical properties (e.g., SFCs, SMPs), suitable crystal forms (β' polymorph), and tender texture; these properties indicated IPs might be used in spreadable margarine, whereas PBs were used as shortening according to their wide SFC range. ASO can be considered as another potential resource for producing plastic fats to meet the increasing demands in the edible oil market. To make a successful application of lipase-catalyzed interesterification to produce plastic fats from ASO, we still need do further research such as how to tackle with the lipase inactivation after several recycling use.

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Notes

The authors declare no competing financial interest.

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