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Enzymatic Production of Zero-Trans Plastic Fat Rich in α-Linolenic Acid and Medium-Chain Fatty Acids from Highly Hydrogenated Soybean Oil, *Cinnamomum camphora* Seed Oil, and Perilla Oil by Lipozyme TL IM

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ABSTRACT: In the present study, zero-trans α-linolenic acid (ALA) and medium-chain fatty acids (MCFA)-enriched plastic fats were synthesized through enzymatic interesterification reactions from highly hydrogenated soybean oil (HSO), *Cinnamomum camphora* seed oil (CCSO), and perilla oil (PO). The reactions were performed by incubating the blending mixtures of HSO, CCSO, and PO at different weight ratios (60:40:100, 70:30:100, 80:20:100) using 10% (total weight of substrate) of Lipozyme TL IM at 65 °C for 8 h. After reaction, the physical properties (fatty acids profile, TAG composition, solid fat content, slip melting point, contents of tocopherol, polymorphic forms, and microstructures) of the interesterified products and their physical blends were determined, respectively. Results showed that the fatty acid compositions of the interesterified products and physical blends had no significant changes, while the content of MCFA in both interesterified products and physical blends increased to 8.58−18.72%. Several new types of TAG species were observed in interesterified products (SSL/SLS, PLO/LLS, and OLLn/LnLO/LOLn). It should be mentioned that no trans fatty acids (TFA) were detected in all products. As the temperature increased, the solid fat content (SFC) of interesterified products was obviously lower than that of physical blends. The SFCs of interesterified products (60:40:100, 70:30:100, and 80:20:100, HSO:CCSO:PO) at 25 °C were 6.5%, 14.6%, and 16.5%, respectively, whereas the counterparts of physical blends were 32.5%, 38.5%, and 43.5%, respectively. Meanwhile, interesterified products showed more β′ polymorphs than physical blends, in which β′ polymorph is a favorite form for production of margarine and shortening. Such zero-trans ALA and MCFA-enriched fats may have desirable physical and nutritional properties for shortenings and margarines.

KEYWORDS: *Cinnamomum camphora seed oil, interesterification, α-linolenic acid, medium-chain fatty acids, highly hydrogenated soybean oil, plastic fat*

INTRODUCTION

Plastic fat, such as margarine and shortening, is commercially produced by partial hydrogenation of vegetable oils, which increases both the melting point and the stability of the oil.[1,2] However, during the partial hydrogenation process, the formation of trans fatty acids is inevitable.[3] It has been reported that trans fatty acids raised low-density lipoprotein (LDL) cholesterol and lowered high-density lipoprotein (HDL) cholesterol.[4] As a result, the intake of trans fatty acids (TFA) may increase the risk of coronary heart disease (CHD).[5,6] Recently, interesterification of edible fats and oils has been considered as an alternative method for the production of low-trans or trans-free margarines and shortenings with satisfactory melting properties and appropriate crystallization behavior, desirable texture, and mouth-feel.[7−9] Interesterification is generally carried out using a blend composed of several oils, one oil combined with desired fatty acid esters, or just one oil having different fatty acids. It involves an exchange of fatty acid residues between the precursor acylglycerols, accompanied by a concomitant change in the physicochemical properties of lipids.[10,11] As compared to chemical interesterification, enzymatic interesterification has certain advantages such as milder reaction conditions and regiospecificity, which may do less harm to the flavor of the product.[12,13]

Cinnamomum camphora (lauraceae), known as an evergreen tree, is largely distributed in the south area of the Yangtze River in China. In a previous study, we have reported that the oil from *Cinnamomum camphora* (lauraceae) seeds has a unique fatty acid profile, which mainly contains medium-chain fatty acids (MCFA) (capric acid, C10:0, 53.27%; lauric acid, C12:0, 39.93%).[14] As compared to long-chain triacylglycerols (LCT), the digestion, absorption, and metabolism of medium-chain triacylglycerols (MCT) show great differences. MCT that consist of three MCFA on the skeleton of glycerol can be rapidly hydrolyzed in the gastrointestinal tract, and the resulting MCFA are transported in the portal blood directly to the liver without incorporating into chylomicrometers and passing through the lymphatic system.[15] Thus, MCFA with 6−12 carbon chain length provide a rapid source of energy. Furthermore, studies have confirmed that MCFA could offer

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certain physiological benefits such as lowering body fat deposition, reducing lipoprotein secretion, and attenuating postprandial triglyceride response.[16,17] However, it was also observed that MCT with a high amount could increase fasting cholesterol and triglyceride levels. Yet, given in moderate amount in diets with moderate fat supply, MCFA may actually reduce fasting lipid levels as compared to oils rich in mono- or polyunsaturated fatty acids.[18] Recently, MCT oil has been commercially used to produce margarine, which is beneficial for health.[19−21] On the other hand, perilla oil (PO), which is extracted from perilla seeds primarily in Korea and China, has a unique fatty acid composition with a high amount of α-linolenic acid (ALA, C18:3).[22] ALA, an omega-3 polyunsaturated fatty acid, may further convert to other biological metabolites after diet. It was proved to have various biological activities including the retardation and reduction of blood clotting, cancer, cardiovascular disease, inflammatory process, and arthritis.[23−25]

Lipozyme TL IM, which is an immobilized lipase from *Thermomyces lanuginosus*, was supported on granulated silica via ionic adsorption.[10] It has regiospecificity and hydrophilic character. Lipozyme TL IM is less expensive than the commonly applied commercial lipase Lipozyme RM IM (lipase from *Rhizomucor miehei*) and offers an opportunity for the food industry to reduce the process cost and produce high value-added products, such as cocoa butter equivalents, human milk fat substitutes, and other specific-structured lipids.[26] Lipase immobilization usually produces an improvement in enzyme properties, such as lipase activity, specificity, or selectivity.[27] It also increases the mechanical strength, hydrophobic or hydrophilic character, regeneration, and remaining functionality of the immobilized lipase.[28] In addition, enzyme properties in the enzymatic interesterification may be strongly modulated by immobilization via different ways. Thus, lipase immobilization combined with chemical modification or other methods may present some better properties for the process.[27]

The purpose of the present work was to produce zero-trans ALA and MCFA-enriched plastic fat by enzymatic interesterification of highly hydrogenated soybean oil (HSO), CCSO, and perilla oil (PO) by Lipozyme TL IM. The physical properties (fatty acids profile, TAG composition, SFC, SMP, polymorphic forms, and microstructures) of the interesterified products and their physical blends were evaluated to seek a desirable product for margarine and shortening.

MATERIALS AND METHODS

Materials and Chemicals. HSO and PO were purchased in a local oil market (Nanchang, China). CCSO was obtained from *Cinnamomum camphora* seeds by the CO_2 supercritical fluid extraction as described in a previous study.[14] Lipozyme TL IM, a commercial immobilized lipase from a strain of *Thermomyces lanuginosus*, was purchased from Novozymes A/S (Bagsvaerd, Denmark). The specific activity of Lipozyme TL IM was 175 IUN/g, having 0.54 g/mL bulk density and 0.3−1.0 mm particle diameter. Carbon dioxide (99.9%) was obtained from Wanli Gas Corp. (Nanchang, China). #463 of standard fatty acid methyl esters (FAMEs) added with a mixture of four positional conjugated linoleic acid isomers (#UC-59M) were purchased from Nu-Chek Prep Inc. (Elysian, MN). Tocopherol (α, γ, and δ) standards were purchased from Sigma Chemical Co. (St. Louis, MO). All solvents and reagents used in analyses were of chromatographic or analytical grade.

Enzymatic Interesterification. Preliminary experiments were carried out to obtain optimal reaction conditions for production of plastic fat. A mixture (50 g) of HSO, CCSO, and PO at different substrate weight ratios (60:40:100, 70:30:100, and 80:20:100, HSO:CCSO:PO), respectively, was placed in a screw-capped Erlenmeyer flask (250 mL). Lipozyme TL IM (10 wt % of total substrate) was added into the reaction mixture. The interesterification was performed in an orbital-shaking water bath with agitation of 500 rpm at 65 °C for 8 h. After reaction, TL IM lipases were separated from the mixture through a 0.5 μm PTFE syringe membrane filter. To remove free fatty acids in the final products, 50 mL of hexane and 10 drops of phenolphthalein solution were added, followed by titration with 0.5 N KOH in 95% ethanol until a pink color appeared. The reaction mixture was washed several times with warm water until the pink color vanished. The organic layer was passed through an anhydrous sodium sulfate column for removing the moisture, and the solvent was completely evaporated under nitrogen at 40 °C.

Fatty Acid Composition. The fatty acids of substrates and reaction products were converted into fatty acid methyl esters (FAMEs) according to the procedure described by Zhu and others.[29] The FAMEs were then analyzed by a gas−liquid chromatograph (model 6890N, Agilent Technologies, USA) equipped with an auto injector, a flame ionization detector (FID), and a fused-silica capillary column (CP-Sil 88, 100 m × 0.25 mm × 0.2 μm i.d.). The injector and detector temperatures were maintained at 250 and 260 °C, respectively. The oven was held at 45 °C for 3 min and programmed to 175 °C for 27 min at a rate of 13 °C/min. The temperature was then further increased to 215 °C at a rate of 4 °C/min and held for 35 min. The carrier gas was nitrogen, and the total gas flow rate was 52 mL/min. Fatty acids compositions were identified by comparison with relative retention times of standard mixtures. Duplicate analyses were performed.

Slip Melting Point (SMP) and Solid Fat Content (SFC) Determination. The SMPs of the samples were determined according to AOCS (1990) Official Method Cc.3.25.[30] The SFC was measured by nuclear magnetic resonance (NMR) using Maran SFC (MQC, OXFORD, UK) according to AOCS (1989) Official Method Cd 16b-93.[31] Two replicate analyses were performed for every sample, and the reported value is the average of the two measurements.

High-Performance Liquid Chromatography (HPLC). The composition of the triacylglycerols (TAGs) formed during the enzymatic interesterification was characterized by reversed-phase high-performance liquid chromatograph (HPLC) with a Nova-Pak C18 column (150 × 3.9 mm, Waters, Milford, MA) and an evaporative light-scattering detector (Alltech 2000ES, USA) operating at 55 °C and a gas flow rate of 1.5 L/min. The mobile phases were acetonitrile (solvent A) and 2-propanol/*n*-hexane (1:1, *v*/v) (solvent B). A linear solvent gradient was based on the previous report.[21] The injection volume was 20 μL, and the eluent flow rate was 1.8 mL/min. TAGs were identified by comparing the retention time and equivalent carbon number (ECN). ECN was used to predict the elution order, calculated as ECN = CN − 2DB, where CN is the total carbon except three carbons of glycerol in the TAG molecule number and DB is the total number of double bonds on the fatty acids.

Polymorphism by X-ray Diffraction Spectroscopy. Each melted sample was placed on a rectangular plastic mold and tempered at 24 °C for 24 h. Polymorphic forms of the samples were established by X-ray diffraction (XRD), using a D8-focus (Bruker Int., Germany) with a fine copper X-ray tube, operating at 40 kV and 35 mA.[7] Each analysis was carried out in duplicate.

Analysis of Tocopherols. Quantitative analysis of tocopherol in the samples was performed by HPLC.[14] The Agilent 1100 series HPLC system consisted of HPLC pump (Agilent) accompanied with a fluorimetric detector performing at an excitation wavelength of 295 nm and an emission wavelength of 325 nm. The column was Hypersil ODS2 (5 μm, 4.6 × 150 mm). The mobile phase was methanol/water (98/2, *v*/*v*), and the flow rate was 0.8 mL/min. Standards of α-, γ-, and δ-tocopherol dissolved in hexane were used for identification and quantification.

Statistical Analysis. Data were analyzed by using the Statistical Analysis System software (SAS 2000). Duncan's multiple range test was performed to determine the significance of difference at $P < 0.05$.

Table 1. Fatty Acids Composition (area %) of HSO, CCSO, PO, the Physical Blends, and the Interesterified Products

				physical blend (HSO:CCSO:PO)			interesterified product (HSO:CCSO:PO)		
	HSO	CCSO	PO	60:40:100	70:30:100	80:20:100	60:40:100	70:30:100	80:20:100
C10:0	0.03	56.15	ND	11.74	8.63	5.72	11.34	8.56	5.64
C11:0	ND[a]	0.12	0.05	ND	ND	ND	ND	ND	ND
C12:0	0.02	37.52	ND	6.98	6.33	3.84	6.64	5.97	2.94
C14:0	0.08	1.36	ND	0.30	0.22	0.14	0.29	0.19	0.18
C16:0	11.01	0.46	6.57	6.31	6.55	7.70	6.72	6.60	7.85
9cC16:1	ND	0.14	0.12	0.08	0.04	0.02	0.11	0.07	0.08
C18:0	87.51	0.31	1.90	26.58	31.49	36.54	27.27	31.82	36.48
C18:1	0.35	3.14	14.74	10.83	10.61	9.98	11.91	10.81	10.36
9c12cC18:2	0.02	0.61	15.90	6.20	5.80	5.58	5.92	5.92	5.90
C20:0	0.56	ND	0.13	0.23	0.26	0.32	0.21	0.24	0.33
C18:3	ND	0.19	60.59	30.62	29.92	29.95	29.39	29.58	29.73
9c11cCLA	0.02	ND	ND	0.01	0.01	0.01	ND	ND	ND
C22:0	0.32	ND	ND	0.10	0.11	0.16	0.07	0.09	0.10
C24:0	0.08	ND	ND	0.02	0.03	0.04	0.03	0.04	0.30
ΣSFA	99.61	95.92	8.65	52.26	53.62	54.46	52.67	53.62	53.93
ΣUSFA	0.39	4.08	91.35	47.74	46.38	45.54	47.33	46.38	46.07
ΣMCFA	0.05	93.79	0.05	18.72	14.96	9.56	17.98	14.53	8.58
ΣTFA	ND	ND	ND	ND	ND	ND	ND	ND	ND

[a]Not detected under this analysis condition.

Table 2. Triacylglycerol (TAG) Composition (area %) of HSO, CCSO, PO, the Physical Blends, and the Interesterified Products[a]

					physical blends (HSO:CCSO:PO)			interesterified products (HSO:CCSO:PO)		
ECN[b]	TAG	HSO	CCSO	PO	80:20:100	70:30:100	60:40:100	80:20:100	70:30:100	60:40:100
	peak1	ND[c]	ND	ND	ND	ND	ND	0.63	0.36	1.13
30	CCC	ND	3.41	ND	0.07	0.09	0.10	0.36	1.11	0.75
32	LaCC/CLaC	ND	88.30	ND	1.01	3.16	0.20	1.13	2.04	1.01
34	MCC/LaLaC	ND	8.30	ND	0.11	0.32	6.29	0.58	0.83	0.81
36	LnLnLn	ND	ND	ND	ND	ND	ND	4.32	4.18	3.49
36	LnLnL/LnLLn	ND	ND	33.00	7.70	9.10	9.68	2.50	0.98	3.91
40	LnLL/LnLnO/LnLnP	ND	ND	5.95	6.73	7.79	1.67	1.90	2.02	2.68
42	LLL/POLn	ND	ND	29.93	1.16	1.36	9.80	4.91	7.77	13.58
42	OLLn/LnLO/LOLn	ND	ND	ND	ND	ND	ND	11.86	8.47	7.55
44	LLO/LOL	ND	ND	ND	ND	ND	ND	1.36	2.65	3.70
44	PLL/LPL	ND	ND	6.66	2.25	2.42	1.63	4.66	3.28	4.19
46	LOO/OLO	ND	ND	17.88	0.36	0.47	3.06	1.01	1.80	3.58
46	PLO/LLS	ND	ND	2.55	ND	ND	ND	16.89	9.64	14.10
50	SOO/OSO	ND	ND	1.97	0.39	0.41	0.30	2.42	2.68	5.76
50	SSL/SLS	ND	ND	ND	ND	ND	ND	20.10	35.38	18.74
50	POS	ND	ND	2.05	0.26	0.28	0.53	0.75	4.12	1.80
50	PSP	1.56	ND	ND	0.10	0.12	0.26	3.74	6.10	4.45
52	PSS	43.37	ND	ND	13.23	12.26	10.01	8.12	4.31	6.02
54	SSS	55.07	ND	ND	66.64	62.23	56.47	12.78	2.28	2.73

[a]Abbreviations: Ca, caprylic acid; C, capric acid; La, lauric acid; M, myristic acid; P, palmitic acid; S, stearic acid; O, oleic acid; L, linoleic acid; Ln, linolenic 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]Not detected under this analysis condition.

RESULTS AND DISCUSSION

Enzymatic Interesterification and Fatty Acid Profile. The interesterification was performed under the optimal reaction conditions, which were obtained by preliminary experiments. Zero-trans plastic fats were produced by enzymatic interesterifications of HSO, CCSO, and PO with different weight ratios of 60:40:100, 70:30:100, and 80:20:100. Rearrangements of fatty acids within and between TAG species resulted in new altered TAG molecules of the restructured fats and oils during enzymatic interesterifications. In the initial stages of the reaction, TAG molecules are hydrolyzed to diacylglycerols, monoacylglycerols, and free fatty acids (FFA). As interesterification progresses, subsequent recombination of the partial acylglycerols and FFA occurs to form new interesterified products with a complex mixture of TAG molecular species.[32] The fatty acid compositions of HSO, CCSO, PO, physical blends, and interesterified products are shown in Table 1. HSO contained a high amount of total saturated fatty acids (SFA, 99.61%), including stearic acid (C18:0; 87.51%) and palmitic acid (C16:0; 11.01%). On the

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contrary, PO contained a high amount of total unsaturated fatty acids (USFA, 91.35%), including α-linolenic acid (ALA, C18:3; 60.59%), linoleic acid (C18:2; 15.90%), and oleic acid (C18:1; 14.74%). The major fatty acids of CCSO were MCFA including capric acid (C10:0, 56.15%) and lauric acid (C12:0, 37.52%). As compared to that of HSO (0.05%), MCFA of physical blends and interesterified products were much higher (9.56−18.72% and 8.58−17.98%, respectively) with no TFA. In addition, the content of ALA in the finally products was increased to approximate 30%. There is no significant difference between the fatty acid compositions of physical blends and interesterified products due to the exchange of fatty acids between and within the TAGs molecules by interesterification catalysis. Therefore, all of the fatty acids are distributed evenly.[33] Tang and others (2012) have previously researched the production of MCT-high plastic fat with no TFA. Because of the unique properties of MCFA and ALA, our trans-free products enriched in MCFA as well as ALA could enhance the nutritional value of the food, which may be potentially useful for the food industries.

Triacylglycerol Analysis. As the TAGs composition of fats and oils is complex, there is no perfect analytical technique to separate all of the TAG species in the fats and oils clearly.[7] The TAG compositions of HSO, CCSO, PO, physical blends, and interesterified products are presented in Table 2. The results showed that significant changes in TAG species occurred after the interesterification reaction (Figure 1). HSO contained

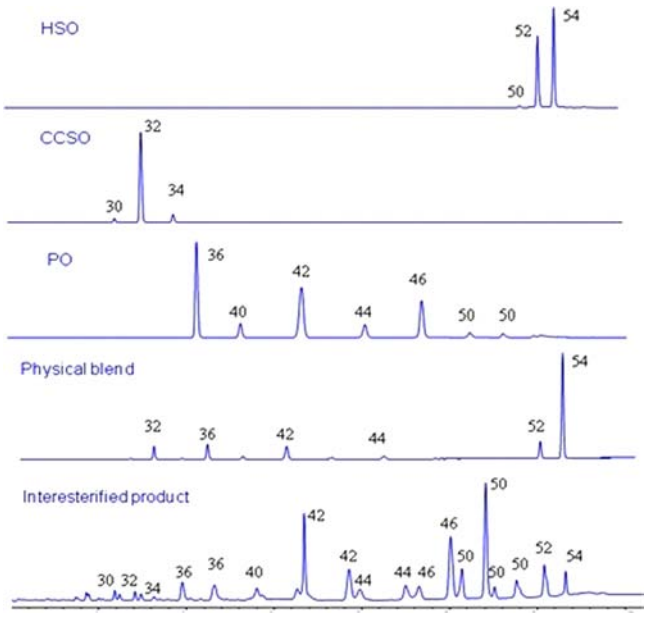

Figure 1. TAG of HSO, CCSO, PO, physical blend (60:40:100, HSO:CCSO:PO), and interesterified product (60:40:100, HSO:CCSO:PO) (peak top number represents ECN of the triaclyglycerol group).

appreciable amounts of SSS (55.07%) and PSS (43.37%), whereas LaCC/CLaC (88.30%), MCC/LaLaC (8.30%), and CCC (3.41%) were the predominant TAG species in CCSO. PO contained high levels of LnLnL/LnLLn (33.00%), LLL/POLn (29.93%), LOO/OLO (17.88%), PLL/LPL (6.66%), and LnLL/LnLnO/LnLnP (5.95%). The predominant TAG species in physical blends were SSS, PSS, and LnLnL/LnLLn, while other TAG species were also observed. After interesterification, however, SSL/SLS and PLO/LLS were observed as the major TAG species. As compared to physical blends, interesterified products showed an increased amount of several TAG species including LLL/POLn, PSP, PLL/LPL, SOO/OSO, LOO/OLO, and POS, but some TAGs decreased (i.e., SSS, PSS, LnLnL/LnLLn, and LnLL/LnLnO/LnLnP) after the interesterification. Meanwhile, there are several new types of TAG species (i.e., SSL/SLS, PLO/LLS, OLLn/LnLO/LOLn, and LnLnLn) appeared in interesterified products. All of these changes indicated that enzymatic interesterification had taken place. A wide melting range, which is required for bakery products (i.e., margarines and shortenings), can be achieved by using fats containing heterogeneous types of TAGs.[19]

Tocopherol Analysis. The tocopherol contents in the HSO, CCSO, PO, physical blends, and interesterified products are presented in Table 3, respectively. It is well-known that tocopherols, a group of major primary natural antioxidants present in vegetable oils, have a physiological function of scavenging radicals in membrane and lipoprotein particles, and thereby prevent lipid peroxidation.[34] All α-, γ-, δ-tocopherols in HSO, PO, and physical blends were found, while α-tocopherol was below the detection limit in CCSO and interesterified products under our analysis condition. HSO (66.89 mg/100 g) and PO (51.51 mg/100 g) showed a much higher level of total tocopherols than did CCSO (24.18 mg/100 g). Total tocopherols in the physical blends were 51.14−55.59 mg/100 g, while in the interesterified products they were 27.92−36.78 mg/100 g. In most cases, the interesterified products were found to contain a significantly lower level of total tocopherols than the physical blends. The reduction of tocopherols might be due to the oxidation or destruction by interesterification or purification processes. Such reduction was also reported previously.[35,36]

Solid Fat Content (SFC) and Slip Melting Point (SMP). SFC has a great influence on the suitability of fats and oils for a particular application. Generally, the SFC is significantly responsible for many product characteristics in margarines, shortenings, and fat spreads, including their general appearance, ease of packing, spreadability, oil exudation, and organoleptic properties.[37] As shown in Figure 2, SFCs of the samples were different from each other. HSO is a comparatively high melting fat as shown by its SFC value. Even as the temperature increased to 65 °C, SFC was still 67.40%. CCSO had a high SFC at low temperature but melted completely between 15 and 20 °C. This sharp melting profile of CCSO may be due to its high proportions of MCFA. Each SFC of the interesterified products at 30 °C was 3.80% (60:40:100), 8.90% (70:30:100), and 10.80% (80:20:100, HSO:CCSO:PO), which is lower than that of the physical blends, respectively. On the other hand, as compared to other proportion in both physical blends and interesterified products (60:40:100 and 70:30:100, HSO:CCSO:PO), HSO:CCSO:PO (80:20:100) showed higher SFC values at each temperature. It could be explained that more addition of HSO, which is comparatively high melting fat, increased SFC values in the products. When the temperature increased to 40 °C, SFCs of the interesterified products decreased significantly, ranging from 0% to 3.5%. Meanwhile, as compared to the physical blends, the interesterified products showed much lower slip melting points (SMPs) (Table 3). The SMPs of the interesterified products (60:40:100, 70:30:100, and 80:20:100, HSO:CCSO:PO) were 26.52, 37.12, and 39.05 °C, respectively. The changes in the SFCs and SMPs of fats were associated with changes in the TAGs composition during the interesterification.[38] In this study, the physical blends could

Table 3. Contents of Tocopherol (mg/100g) and Slip Melting Points (SMPs) of HSO, CCSO, PO, Physical Blends, and Interesterified Products[a]

				physical blends (HSO:CCSO:PO)			interesterified products (HSO:CCSO:PO)		
	HSO	CCSO	PO	80:20:100	70:30:100	60:40:100	80:20:100	70:30:100	60:40:100
α-tocopherol	4.72 ± 0.09	ND[b]	2.86 ± 0.01	3.78 ± 0.08	3.37 ± 0.10	3.56 ± 0.16	ND	ND	ND
γ-tocopherol	42.85 ± 1.02	8.70 ± 0.23	45.52 ± 1.19	40.52 ± 0.81	37.13 ± 0.58	38.99 ± 0.52	28.60 ± 0.30	28.04 ± 0.31	22.35 ± 0.29
δ-tocopherol	19.32 ± 0.50	15.48 ± 0.29	3.13 ± 0.07	11.29 ± 0.32	10.65 ± 0.44	11.37 ± 0.21	8.10 ± 0.13	8.74 ± 0.16	5.57 ± 0.02
total	66.89 ± 1.58	24.18 ± 0.47	51.51 ± 1.25	55.59 ± 1.21	51.14 ± 1.09	53.92 ± 0.86	36.70 ± 0.38	36.78 ± 0.45	27.92 ± 0.27
SMP/°C	67.90	16.04		50.51	59.04	55.45	39.05	37.12	26.52

[a]Values presented as means of triplicates ± standard deviation. [b]Not detected under this analysis condition.

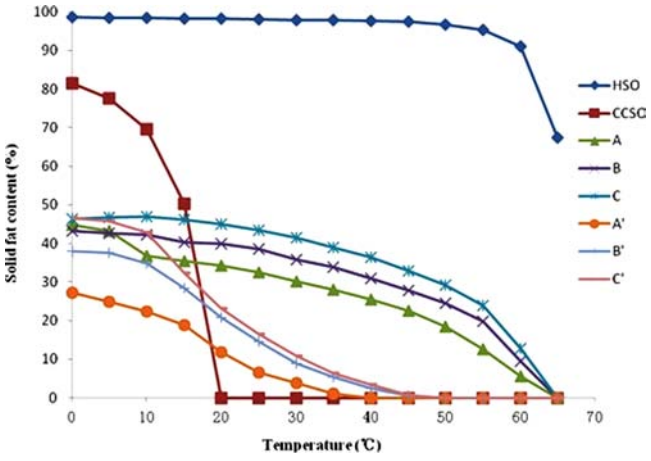

Figure 2. Solid fat content (SFC) of the physical blends (A, 60:40:100; B, 70:30:100; C, 80:20:100) and interesterified products (A′, 60:40:100; B′, 70:30:100; C′, 80:20:100) with different ratios.

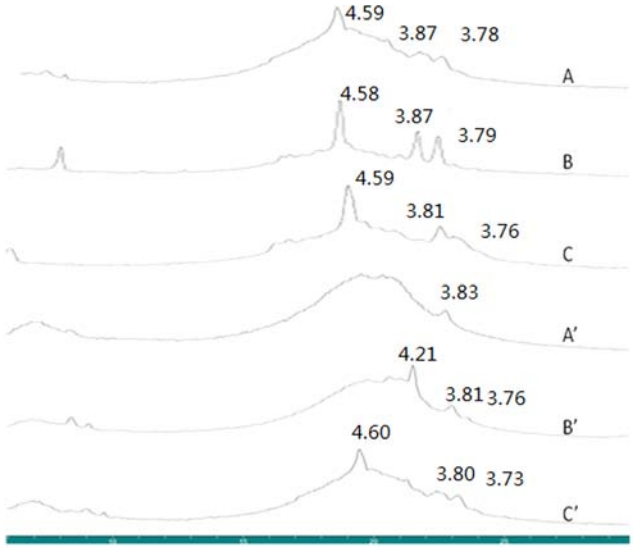

Figure 3. X-ray diffraction spectroscopy of physical blends (A, 80:20:100; B, 70:30:100; C, 60:40:100) and interesterified products (A′, 80:20:100; B′, 70:30:100; C′, 60:40:100) with different ratios.

be suitable for shortening, while interesterified products were more likely to be a source material of margarine. Enough high melting temperature is one of the desirable properties in shortening because shortening should not melt quickly at baking temperature.[39] In addition, higher solid content is desirable for use in cake manufacture as they can retain the air incorporated during baking.[40] The SFC at 35 °C, which significantly influences "mouth feel" or waxy sensations of fats, is important for table margarines.[41] Therefore, these results suggested that the physical blends and interesterified products might be suitable for the formulation of shortenings and margarines, respectively.

Crystal Polymorphism and Microstructure. Polymorphic forms are the most important criteria for the functional properties of margarines and shortenings.[42] Three major types of polymorphic forms of fats are known as α (hexagonal), β′ (orthorhombic), and β (triclinic).[43] The α polymorph is the least thermally stable with the lowest melting point. The β′ polymorph, which is metastable with the medium melting point, is responsible for the smooth texture of margarines, whereas the β polymorph, which is the most stable with the highest melting point, is unfavorable in margarine fats due to its association with grainy texture.[44] The polymorphic forms of physical blends and interesterified products were measured by XRD. The physical blends showed strong intensities of short spacing at 4.59 (β) Å and 3.87 and 3.79 Å (β′), whereas stronger intensities were observed at 3.83 and 4.21 Å (β′) than 4.59 Å (β) in the interesterified products. During the interesterification, the intensity of the short spacing β form was reduced, and the β′ form was increased significantly (Figure 3). This is attributed to the rearrangement of fatty acids in the TAGs after the interesterification reaction. The increase of TAGs species obviously led to the decrease of the TAGs molecular symmetry.[21] Such TAGs tend to preferentially crystallize in the β′ forms, which may be suitable for the production of margarines. The crystal microstructures of the physical blend (70:30:100, HSO:CCSO:PO) and interesterified products (70:30:100, HSO:CCSO:PO) were observed by microscopy. As shown in Figure 4, the interesterified product (70:30:100, HSO:CCSO:PO) has crystal morphologies distinctly different from those of the physical blend. The physical blend showed sphere-shaped crystals, whereas small crystal forms were observed in the interesterified products. Such different crystal structures indicated that the interesterified product contained a higher level of β′ form, which was desirable for margarine.

In conclusion, in the present study, the plastic fats were produced from HSO, CCSO, and PO with different weight ratios of 60:40:100, 70:30:100, and 80:20:100 by enzymatic interesterification. The interesterified fats contained 8.58–17.98% MCFA and approximate 30% ALA with no trans fatty acids, which provide many nutritional benefits. On the other hand, desirable physical properties including a wide range of SFCs, SMPs, crystal polymorphs, and microstructures were observed in the produced fats. Results showed that the interesterified products had considerable physical properties for margarine, while physical blends were preferred to be a source material of shortening. However, the expensive cost of enzymes still limits their utilization in the industrial production

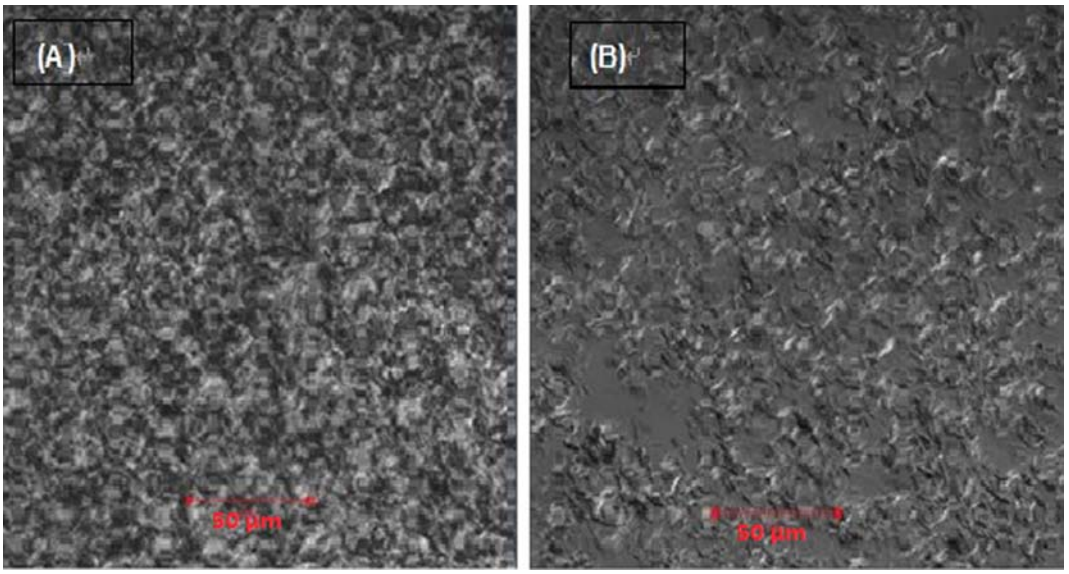

Figure 4. Crystal microstructure of the physical blend (70:30:100, HSO:CCSO:PO) (A) and interesterified product (70:30:100, HSO:CCSO:PO) (B).

of margarine and shortening. How to increase the enzymatic efficiency and reduce the process cost will become the focus of improvement in our research.

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Notes

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