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## Characterization of Medium-Chain Triacylglycerol (MCT)-Enriched Seed Oil from *Cinnamomum camphora* (Lauraceae) and Its Oxidative Stability

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**ABSTRACT**: Medium-chain triacylglycerol (MCT)-enriched oil was extracted by supercritical fluid extraction of carbon dioxide (SFE-CO<sub>2</sub>) from *Cinnamomum camphora* seeds. The SFE-CO<sub>2</sub> process was optimized using the Box–Behnken design (BBD). The maximum oil yield (42.82%) was obtained under the optimal SFE-CO<sub>2</sub> conditions: extraction pressure, 21.16 MPa; extraction temperature, 45.67 °C; and extraction time, 2.38 h. Subsequently, the physicochemical characteristics, fatty acid composition, triacylglycerol (TAG) composition, tocopherol content, and DSC profile as well as oxidative stabilities of *C. camphora* seed oil (CCSO) were studied. Results showed that CCSO contained two major medium-chain fatty acids, capric acid (53.27%) and lauric acid (39.93%). The predominant TAG species in CCSO was LaCC/CLaC (ECN 32, 79.29%). Meanwhile, it can be found that CCSO had much higher oxidative stabilities than coconut oil due to the higher content of tocopherols in CCSO ( $\alpha$ -tocopherol, 8.67  $\pm$  0.51 mg/100 g;  $\gamma$ -tocopherol, 22.6  $\pm$  1.02 mg/100 g;  $\delta$ -tocopherol, 8.38  $\pm$  0.47 mg/100 g). Conclusively, CCSO with such a high level of MCTs and high oxidative stabilities could be potentially applied in special food for specific persons such as weak patients and overweight persons because oils enriched in MCTs can be rapidly absorbed into body to provide energy without fat accumulation.

**KEYWORDS:** *Cinnamomum camphora* seed oil, physicochemical characteristics, fatty acid composition, TAG composition, DSC profile, oxidative stability

#### INTRODUCTION

*Cinnamomum camphora* (Lauraceae), commonly known as camphor tree, is an evergreen tree and widely distributed in southeastern China. Many cities plant *C. camphora* along main roadsides, parks, and schools. As prescribed in traditional Chinese medicine, *C. camphora* is used as a folk medicine for treating inflammation-related diseases such as rheumatism, sprains, bronchitis, and muscle pains.<sup>1</sup> Also, its wood, bark, and leaves can be used to extract essential oil, which has important antifungal activities as well as repellent and insecticidal activities.<sup>2,3</sup> Many compounds such as alkaloids, essential oil (mainly cinnamaldehyde, terpenoid, sesquiterpene and phenylpropanoid), and type II ribosome-inactivating proteins (cinnamomin and camphorin) are reported as the main constituents in *C. camphora*.<sup>4–6</sup>

Recently, researchers have reported that the oil extracted from *C. camphora* seeds had a unique fatty acid profile.<sup>7,8</sup> Up to 90% of total fatty acids were capric acid C10:0 and lauric acid C12:0, both of which belong to medium-chain fatty acids (MCFAs). MCFAs refer to a mixture of fatty acids that generally consist of 6-12 carbons. It is well-known that medium-chain triacylglycerol (MCT) oils such as coconut oil and palm kernel oil can be used for the dietary treatment of malabsorption syndrome due to their metabolic properties.<sup>9</sup> MCTs that consist of three MCFAs on the skeleton of glycerol can be rapidly hydrolyzed, and the resulting MCFAs are directly absorbed to the liver via the portal vein and used as energy sources without using the carnitine transport system for mitochondrial entry.<sup>10</sup> Meanwhile, MCT oil also demonstrates good properties to produce biodiesel,<sup>11</sup> two-stroke engine lubricant.<sup>12</sup> Such a high amount of MCTs in *C. camphora* 

seed oil (CCSO) could potentially be applied in the nutritional, pharmaceutical, and nonfood industries. However, to our knowledge, most *C. camphora* seeds naturally grow and perish year by year and are never considered for effective application in the food and nonfood industries. In addition, there is no information on the physicochemical properties, TAG composition, DSC profile, and oxidative stabilities of CCSO.

Seed oil was conventionally obtained by mechanical pressing or organic solvent extraction in the oil industries. Mechanical pressing is quite a simple method; however, in most cases, the yield is lower.<sup>13</sup> Although solvent extraction is effective, with almost complete recovery of the oil, the organic solvent is dangerous to handle and the residue in oil is harmful to human health.<sup>14</sup> Considering the drawbacks of mechanical pressing and organic solvent extraction, supercritical fluid extraction (SFE) with supercritical carbon dioxide (SC-CO<sub>2</sub>) to extract oil was recently developed as an excellent alternative due to its unique advantages of nontoxicity, nonflammability, low cost, and lack of residue or pollution in the extract.<sup>15,16</sup> The oil extracted by SC-CO<sub>2</sub> is of outstanding quality, and the yield is comparable to that obtained by organic solvent extraction.<sup>17</sup>

In the present study, the effects of the main operating parameters (extraction pressure, temperature, and extraction time) for supercritical carbon dioxide extraction of CCSO from *C. camphora* seeds were investigated using the Box–Behnken design (BBD). The

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 Table 1. Experimental Program and Results for the Oil Yield

 Obtained from the Box-Behnken Design

	$X_1$	X <sub>2</sub>	$X_3$	
run	(pressure, MPa)	(temperature, °C)	(time, h)	oil yield (%)
1	-1(10)	-1 (35)	0(2)	39.33
2	-1(10)	1 (55)	0(2)	39.64
3	1 (30)	-1(35)	0(2)	39.92
4	1 (30)	1 (55)	0(2)	40.54
5	0 (20)	-1(35)	-1(1)	39.95
6	0 (20)	-1(35)	1(3)	41.21
7	0 (20)	1 (55)	-1(1)	40.62
8	0 (20)	1 (55)	1(3)	41.45
9	-1(10)	0 (45)	-1(1)	39.29
10	1 (30)	0 (45)	-1(1)	41.16
11	-1(10)	0 (45)	1 (3)	40.96
12	1 (30)	0 (45)	1 (3)	41.48
13	0 (20)	0 (45)	0(2)	42.89
14	0 (20)	0 (45)	0(2)	42.73
15	0 (20)	0 (45)	0(2)	43.01

physicochemical characteristics, fatty acid composition, TAG composition, and tocopherol content as well as DSC profile of CCSO were then studied. The oxidative stabilities of CCSO were determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay and  $\beta$ -carotene bleaching test.

#### MATERIALS AND METHODS

**Materials and Chemicals.** *C. camphora* seeds were collected from Nanchang University campus in Jiangxi province, China. Samples were vacuum-dried to a constant weight and then ground into pieces by a blender. Carbon dioxide (99.9%) was purchased from Wanli Gas Corp. (Nanchang, China). Coconut oil was purchased from a local market. All reagents were of analytical reagent grade.

**Supercritical Fluid Extraction.** All SC-CO<sub>2</sub> extraction trials were carried out in a Nantong supercritical fluid extraction system (1 L sample capacity) (model HA120-50-01, Jiangsu, China). The schematic flow diagram was described in detail in a previous study.<sup>18</sup> Briefly, samples of 50.00 g of *C. camphora* seed powder were weighed accurately and placed into the stainless steel extraction vessel. The CO<sub>2</sub> flow rate was maintained at about 45 L/h during extraction. When the desired pressure, temperature, and flow rate were reached, the extraction was started. After extraction, the extraction vessel was depressurized and oil was collected from the separation vessel. The extraction yield was expressed as the ratio of seed oil to the amount of dry seed powder placed in the extraction vessel.

**Experimental Design.** A three-level, three-variable Box—Behnken design (BBD), requiring 15 experiments, was used to optimize the process parameters for supercritical carbon dioxide extraction of CCSO from *C. camphora* seeds. The pressure (*P*), temperature (*T*), and time (*t*) were independent variables studied to optimize the oil yields (*Y*). The BBD design and coded and uncoded independent variables are listed in Table 1. A second-order polynomial regression model was used to express the yield as a function of the independent variables as

$$Y = \alpha_0 + \sum_{i=1}^{3} \alpha_i X_i + \sum_{i=1}^{3} \alpha_{ii} X_i^2 + \sum_{i\neq j=1}^{3} \alpha_{ij} X_i X_j$$

where *Y* represents the response variables,  $\alpha_0$  is a constant, and  $\alpha_i$ ,  $\alpha_{ii}$ , and  $\alpha_{ij}$  are the linear, quadratic, and interactive coefficients, respectively.  $X_i$  and  $X_j$  are the levels of the independent variables. All data were

analyzed by analysis of variance (ANOVA) for response surface quadratic model of the SAS program (SAS, 2000). Response surface and contour plots were developed using the fitted quadratic polynomial equation obtained from holding one of the independent variables at a central value and varying the levels of the other two variables within the experimental range. The test of statistical significance was based on the total error criteria with a confidence level of 95.0%.

Total and sn-2 Positional Fatty Acid Analysis of CCSO. The analysis of fatty acid methyl esters (FAMEs) was performed on an Agilent 6890N GC-FID equipped with a fused silica capillary column  $(100 \text{ m} \times 0.25 \text{ mm} \times 0.2 \,\mu\text{m})$ . The sample  $(1 \,\mu\text{L})$  was injected, and the injector temperature was held at 250 °C. The oven temperature was initially held at 45 °C for 3 min and then increased to 175 °C at a rate of 13 °C/min, held at 175 °C for 27 min, finally increased to 215 °C at 4 °C/ min, and kept at this temperature for 5 min. The detector temperature was set at 250 °C. Hydrogen was used as the carrier gas with a flow rate of 30 mL/min. Identification of FAMEs was achieved by comparing their retention times with those of standard compounds. The composition of the fatty acids was calculated from their peak areas. The sn-2 positional fatty acid of oil was determined using a previous method described by Hita et al.<sup>19</sup> Briefly, 10 mg of seed oil, 1 mL of buffer solution tris-HCl (1 M, pH 7.6), and 1 mg of pancreatic lipase were placed into a centrifuge tube. This mixture was shaken vigorously. Later, 0.25 mL of 0.05% bile salts and 0.1 mL of 2.2% calcium chloride solution were added. After shaking for 1 min, the mixture was incubated for 3 min at 37 °C. Four milliliters of diethyl ether was added to stop the reaction. After centrifugation, the diethyl ether layer was collected with a Pasteur pipet. The sample obtained was analyzed by TLC and GC.

Triacylglycerol (TAG) Composition Analysis by High-Performance Liquid Chromatography (HPLC). The TAG composition of each sample was analyzed by HPLC according to the method of Lee at al.<sup>20</sup> The HPLC system consisted of an Agilent HPLC series 1100 (Agilent Technologies, Little Falls, DE) with a Sedex 75 evaporative light-scattering detector (ELSD, Sedere, Alfortville, France), operated at 40 °C with a nitrogen pressure of 2.2 bar. A Nova-Pak C18 column (150 × 3.9 mm, Waters, Milford, MA) was used for sample analysis. The mobile phase consisted of (A) acetonitrile and (B) isopropanol/hexane (2:1, v/v) at a flow rate of 1 mL/min with the following elution program: 0–44 min, 20% B; 45–50 min, 46% B; 51–58 min, 100% B, and then returned to the initial flow rate.

**Analysis of Tocopherol Content.** The tocopherol content in the oil sample was determined according to a previous report by Gimeno et al. with a slight modification.<sup>21</sup> Briefly, about 1 g of oil sample was weighed into a flask and fully dissolved in 10 mL of hexane; after filtering with a 0.45  $\mu$ m nylon syringe filter, 10  $\mu$ L aliquots of sample were directly injected to Agilent 1100 series HPLC equipment accompanied with a fluorometric detector. The excitation wavelength was set at 295 nm, and the emission wavelength was set at 325 nm. The column was a Hypersil ODS2 (5  $\mu$ m, 4.6 × 150 mm). The mobile phase was methanol/water (98:2, v/v), and the flow rate was 0.8 mL/min. The corresponding standards ( $\alpha$ -tocopherol,  $\gamma$ -tocopherol, and  $\delta$ -tocopherol) for identification and quantification were prepared in hexane solution.

**Physiochemical Characteristics of CCSO.** The physiochemical characteristics of oil sample including color, specific gravity, acid value, peroxide value, saponification value, and iodine value were determined in this study. The oil colors at room temperature were noted by visual inspection, and the specific gravity was determined according to the official method of the AOAC. The procedures for determination of chemical indices, such as acid value, peroxide value (AOAC 965.33), saponification number, and iodine value were carried out following AOAC (1990) standard analytical methods. Each oil sample was analyzed in triplicate. A differential scanning calorimeter (DSC) 2010 (TA Instruments Inc., New Castle, DE) was used to obtain the thermograms

 Table 2. Estimates Coefficients of the Second-Order Polynomial Model for Oil Yield

parameter	DF	estimate $(\alpha)$	standard error	<i>t</i> value	p value <sup>a</sup>
intercept	1	42.81	0.12	356.52	< 0.0001
$X_1$	1	0.48	0.06	8.08	0.0013
$X_2$	1	0.23	0.06	3.83	0.0186
$X_3$	1	0.51	0.06	8.49	0.0011
$X_1  imes X_1$	1	-1.52	0.09	-16.00	< 0.0001
$X_2 \times X_1$	1	0.08	0.08	0.91	0.4130
$X_2 \times X_2$	1	-1.43	0.09	-15.10	0.0001
$X_3 \times X_1$	1	-0.34	0.08	-3.97	0.0165
$X_3 \times X_2$	1	-0.11	0.08	-1.27	0.2742
$X_3  imes X_3$	1	-0.57	0.09	-5.99	0.0039
$^a p < 0.01,$ highly significant; 0.01 $ significant; p > 0.05, not significant.$					

of melting and crystallization. The sample was heated to 80 °C and held for 10 min. Thereafter, the temperature was decreased to -60 °C at rate of 10 °C/min. After a 10 min hold at -60 °C, the melting curve was obtained by heating to 60 °C at 5 °C/min. The solid fat content (SFC%) was obtained from melting thermograms by Universal Analysis 2000 (TA Instruments Inc.) according to a previous report by Alim et al.<sup>22</sup> Each DSC thermogram was divided at different temperatures, and the total crystallization energy (J/g) was calculated into percentage (%) at each temperature for SFC (%).

**Determination of Antioxidant Activities.** *DPPH Radical-Scavenging Assay.* The free radical-scavenging activity of CCSO was measured by DPPH assay according to the method of Liu et al.<sup>17</sup> with a slight modification. An aliquot of seed oil (0.1 mL) was mixed with 1.4 mL of ethanol and then added to 1 mL of 0.004% DPPH (Sigma-Aldrich) in ethanol. The mixture was shaken vigorously and left to stand for 70 min in the dark. The absorbance was measured at 517 nm against a blank. Coconut oil, known as MCT-enriched oil, was used as a comparison. The experiment was carried out in triplicate. The capability to scavenge the DPPH radical was calculated using the equation

inhibition percentage  $(I) = 100(A_i - A_i)/A_i$ 

where  $A_j$  and  $A_i$  are the absorbance values of the blank and tested samples, respectively, measured after 70 min.

 $\beta$ -Carotene/Linoleic Acid Bleaching Assay. The oxidative stability of CCSO was evaluated using a  $\beta$ -carotene/linoleic acid system according to the method of Amarowicz et al.<sup>23</sup> with minor modifications. Briefly, 0.2 mL of  $\beta$ -carotene (1 mg/mL) dissolved in chloroform was pipetted into a small round-bottom flask containing 20 mg of linoleic acid (Sigma-Aldrich) and 200 mg of Tween 40 (Sigma-Aldrich). After removal of the chloroform by using a rotary evaporator, 50 mL of distilled water was added to the flask with vigorous shaking to form an emulsion. Aliquots (5 mL) of the prepared emulsion were transferred to a series of tubes containing 0.2 mL of seeds oil of different concentrations, and the absorbance was measured immediately at 470 nm against a blank, consisting of the emulsion without  $\beta$ -carotene. The tubes were placed in a water bath at 50 °C, and the oxidation of the emulsion was monitored spectrophotometrically by measuring absorbance at 470 nm over a period of 120 min. Control samples contained 0.2 mL of water instead of seed oil. The antioxidant capacity of coconut oil was also investigated as a comparison. All determinations were carried out in triplicate. The antioxidant activity (AA) of the sample extract was evaluated in terms of the bleaching of  $\beta$ -carotene using the formula

$$AA = 100(R_{control} - R_{sample})/R_{control}$$

where  $R_{\text{control}}$  = average bleaching rates of  $\beta$ -carotene of the control  $[\ln(A_i/A_i)/120]$  and  $R_{\text{sample}}$  = average bleaching rates of  $\beta$ -carotene of

the sample  $[\ln(A_i/A_j)/120]$ , where  $A_i$  = absorbance at zero time and  $A_j$  = absorbance at 120 min.

### RESULTS AND DISCUSSION

Fitting the Models. One of objectives of the present study was to optimize the operating condition to achieve an efficient extraction of CCSO from *C. camphora* seeds using the BBD. The levels of independent variables (pressure, *P*; temperature, *T*; and time, *t*) were determined on the basis of preliminary experiments. All oil yields from *C. camphora* seeds obtained from 15 experimental runs as well as independent variables are summarized in Table 1. ANOVA for the response surface quadratic model was employed to fit the second-order polynomial equation. From Table 2, regression coefficients were determined by employing the least-squares technique to predict quadratic polynomial models for oil yields. The fitted secondorder polynomial equation is

 $Y = 42.81 + 0.48X_1 + 0.23X_2 + 0.51X_3 - 1.51X_1^2 - 1.43X_2^2$  $- 0.57X_3^2 + 0.08X_1X_2 - 0.34X_1X_3 - 0.11X_2X_3$ 

ANOVA results of the response surface showed a very small p value of total model (p = 0.0006), satisfactory coefficients of determination ( $R^2 = 0.99$ ), and no significant lack of fit (data not shown). All of these values indicated that the generated models adequately explained the data variation and significantly represented the actual relationships between the independent variables.

Response Surface Analysis. The best way of expressing the effect of any independent variable on the oil yield is to generate surface response plots of the model, which was done by varying two variables within the experimental range under investigation and holding the third variable at the central point. Figure 1A is the response surface curve showing the predicted response surface of oil yield as a function of pressure and temperature at the fixed extraction time of 2 h. An increase in oil yield was observed by increasing extraction temperature in an early stage of extraction, most likely due to the increased vapor pressure of the solutes that brings about the elevation in the solubility of oil in SF-CO<sub>2</sub>. The trend was, however, reversed, when the temperature reached a certain value under the range of extraction pressure in this work. The decreased density of CO2 at high temperature led to a reduction in solvent power to dissolve the solute.<sup>16</sup> Pressure showed a positive linear effect on oil yield at low-pressure levels. However, the oil yield decreased with increasing extraction pressure at the range of high pressures. This is probably a reflection of the increased repulsive solute--solvent interaction resulting from the highly compressed CO<sub>2</sub> at high pressure levels.<sup>13</sup> Figure 1B shows the response surface showing the effect of pressure and extraction time on the oil yield at the fixed temperature of 45 °C. Extraction time had a positive linear effect on the oil yield for most of the time. The oil yield increased greatly as extraction time increased from the beginning. After 2.5 h, the oil yield gradually reached a plateau. The interaction between pressure and extraction time showed a significant effect on the yield of oil. At low-pressure levels, extraction time was long when the oil yield reached maximum value. At higher pressure levels, however, extraction time was short when the oil yield reached maximum value. Similar trends could be found in the interaction of temperature and extraction time on the oil yield at the fixed pressure of 20 MPa (Figure 1C).



Figure 1. Response surface plots for oil yields extracted by  $SC-CO_2$ : (A) varying extraction pressure and temperature; (B) varying extraction pressure and extraction time; (C) varying extraction temperature and time.

Table 3.	Comparison of Total and Positional Fatty Acid
Composi	tion of CCSO and Coconut Oil <sup>a</sup>

		composition (%)				
		CCSO			coconut oi	1
fatty acid	total	sn-2	sn-1,3	total	sn-2	sn-1,3
C8:0	0.33	nd	0.49	6.03	0.68	8.71
C10:0	53.27	64.29	47.76	5.69	1.75	7.66
C12:0	39.93	32.01	43.89	50.01	77.43	36.30
C14:0	1.11	0.82	1.25	19.74	14.42	22.40
C16:0	0.56	0.87	0.40	10.25	2.82	13.96
C18:0	0.23	0.00	0.34	3.06	0.91	4.13
C18:1	4.00	1.25	5.37	8.78	1.86	12.24
C18:2	0.91	0.76	0.99	2.48	0.82	3.31
C18:3	0.30	nd	0.49	nd	nd	nd
ΣMCFAs	94.00	97.12	93.39	81.47	94.28	75.07
ΣSFAs	94.79	97.99	93.15	88.74	97.32	84.45
ΣUSFAs	7.91	2.01	6.85	11.26	2.68	15.55

<sup>*a*</sup> All values are mean values of duplicate measurements. ΣMCFAs, total medium chain fatty acids; ΣSFAs, total saturated fatty acids; ΣUSFAs, total unsaturated fatty acids; nd, not detected.

High extraction temperature favored short extraction time to get the maximum oil yield.

The optimum levels of the tested parameters were obtained by solving the second-order polynomial equation. The predicted values were extraction pressure of 21.16 MPa, extraction temperature of 45.67 °C, and extraction time of 2.38 h. The maximum predicted

value of the oil yield was 42.95%. Verification experiments were performed at the optimal conditions and obtained an observed value of oil yield of 42.82%, which was reasonably close to the predicted value of oil yield of 42.95%. This result demonstrated the validation of the RSM model.

Fatty Acid Composition of CCSO. The fatty acid composition of an oil is a critical feature. Nagao et al. have explored the physiological functions and molecular actions of medium-chain fatty acids (MCFAs)/medium-chain triacylglycerols (MCTs) in the development of metabolic syndrome and demonstrated that dietary MCFAs/MCTs suppressed fat deposition through enhanced thermogenesis and fat oxidation in animal and human subjects.9 Table 3 shows the fatty acid composition of CCSO in comparison to coconut oil. Each oil contained >90% total saturated fatty acids (SFAs). The fatty acids of CCSO were predominately contributed by MCFAs including capric acid (53.27%) and lauric acid (39.93%), whereas coconut oil had major fatty acid composition of lauric acid (50.01%), myristic acid (19.74%), and palmitic acid (10.25%). On the other hand, the content of unsaturated fatty acids (USFAs) was low in both oils, accounting for less than 7.91 and 11.26% of the total fatty acids, respectively. The CCSO also contained trace amounts of undecanoic acid and linolenic acid. About 43.89% of lauric acid and 47.76% of capric acid existed at the *sn*-1,3 position of CCSO, whereas the sn-2 position of CCSO was occupied by 64.29% of capric acid. As for coconut oil, 77.43% of lauric acid was at the sn-2 position of coconut oil, and a small amount of caprylic acid and capric acid existed at the sn-2 position of coconut oil.

**TAG Composition of CCSO.** Figure 2 shows the HPLC chromatograms of CCSO and coconut oil. In a reverse-phase HPLC, it was proved that the elution of TAGs is according to their



Figure 2. HPLC chromatograms of coconut oil (A) and CCSO (B).

Table 4. Comparison of Triacylglycerol Composition (Area %) of CCSO and Coconut  ${\rm Oil}^a$ 

peak	$ECN^b$	TAGs	CCSO	coconut oil
1	28	CaCC	1.94	
2	30	CCC	7.81	
3	32	LaCC, CLaC	79.29	14.36
4	34	MCC, LaLaC	10.96	22.63
5	36	LaLaLa, OCC		31.71
6	38	MLaLa, MMC		20.47
7	40	MMLa, PMC, SLaC, PLaLa		2.26
8	42	MMM, SMC, PPC, PMLa, SLaLa		8.56
a				_

<sup>*a*</sup> Abbreviations: TAGs, triacylglycerols; Ca, caprylic acid; C, capric acid; La, lauric acid; M, myristic acid; P, palmitic acid; S, stearic acid; O, oleic acid. <sup>*b*</sup> Equivalent carbon number (ECN): CN-2DB, where CN is a carbon number of TAG and DB is total number of double bonds in TAG.

equivalent carbon number (ECN).<sup>24</sup> TAGs with lower ECN values eluted earlier. On the contrary, TAGs with higher ECN values eluted with longer retention time. Each peak on the chromatograms was well identified according to previous studies<sup>25,26</sup> and their fatty acid composition. As shown in Table 4, the predominant TAG in CCSO was LaCC/CLaC (ECN 32), representing 79.29%. Small amounts of TAG species such as

#### Table 5. Comparison of Tocopherol Content and Physiochemical Characteristics of CCSO and Coconut Oil<sup>a</sup>

	CCSO	coconut oil
tocopherols (mg/100 g)		
α-tocopherol	$8.67\pm0.51$	$0.35\pm0.19$
$\gamma$ -tocopherol	$22.6\pm1.02$	$nd^b$
$\delta$ -tocopherol	$8.38\pm0.47$	nd
physiochemical characteristics		
color	golden yellow	slightly yellow
specific gravity at 25 $^\circ\mathrm{C}$	$0.9217\pm0.01$	$0.9182\pm0.01$
acid value (mg KOH/g oil)	$0.19\pm0.08$	$0.51\pm0.09$
peroxide value (mmol/kg)	$0.25\pm0.14$	$0.71\pm0.18$
saponification value (mg KOH/g)	$278.4\pm3.1$	$261.1\pm1.8$
iodine value (g $I_2/100$ g oil)	$5.91\pm0.22$	$9.23\pm0.67$
solid fat content (SFC% by DSC)		
-15 °C	100	100
−5 °C	98.13	100
5 °C	80.84	100
10 °C	66.18	99.09
20 °C	1.22	63.35
30 °C	0	0.48

 $^a$  Values presented as means of triplicates  $\pm$  standard deviation.  $^b$  Not detected under this analysis condition.



Figure 3. DSC melting thermograms and crystallization thermograms of coconut oil and CCSO.

CaCC (ECN 28, 1.94%), CCC (ECN 30, 7.81%), and MCC/ LaLaC (ECN 34, 10.96%) were also found in CCSO. On the other hand, coconut oil had six major TAG species (LaCC, ECN 34, 14.36%; MCC/LaLaC, ECN 36, 22.63%; LaLaLa/OCC, ECN 38, 31.71%; MMLa/PMC/SLaC/PLaLa, ECN 40, 2.26%; MMM/ SMC/PPC/PMLa/SLaLa, ECN 42, 8.56%). Fatty acid moieties in the TAGs were abbreviated: Ca, caprylic acid; C, capric acid; La, lauric acid; M, myristic acid; P, palmitic acid; S, stearic acid; O, oleic acid. The result showed that the MCTs were predominantly in CCSO. Considering the MCT metabolic properties, CCSO could be potentially applied in special foods for specific persons such as weak patients and overweight persons because oils enriched in MCTs can be rapidly absorbed into the body to provide energy without fat accumulation.<sup>9</sup>

**Physiochemical Characteristics of CCSO.** The physiochemical characteristics of CCSO and coconut oil are given in Table 5. The color of the CCSO was golden yellow, which was much deeper than the color of coconut oil. The specific gravity of CCSO ( $0.9217 \pm 0.01$ ) was similar to that of coconut oil ( $0.9182 \pm 0.01$ ). The acid value of the CCSO ( $0.19 \pm 0.08$  mg KOH/g oil) was lower than that of coconut oil ( $0.51 \pm 0.09$  mg KOH/g oil). CCSO and coconut oil showed very low peroxide values, accounting for  $0.25 \pm 0.14$  and  $0.71 \pm 0.18$  mmol/kg, respectively. Such a low level of the peroxide value demonstrated that it could be stored for a long period without deterioration. The saponification value of CCSO was  $278.4 \pm 3.1$  mg KOH/g and

that of coconut oil was 261.1  $\pm$  1.8 mg KOH/g. The value is relatively high because both oils mainly contained small-molecule MCTs. The iodine value of the CCSO was  $5.91 \pm 0.22$  g  $I_2/$ 100 g oil, which was lower than that of coconut oil  $(9.23 \pm 0.67 \text{ g})$  $I_2/100$  g oil). As for the solid fat content (SFC), CCSO began to melt at -5 °C, and almost all of the solid fat melted at 20 °C (<1.22% of SFC). On the other hand, coconut oil had a relatively higher SFC than CCSO at the same temperature. The melting and crystallization characteristics of the CCSO profile (Figure 3) showed that CCSO had a major melting peak b with maximum at 16.62 °C accompanied by a distinctive shoulder peak a with a maximum at 3.44 °C. The shoulder peak a represented the melting temperature of unstable crystals of the low-melting TAG species that prematurely melted.<sup>27</sup> Also, a sharp melting peak b' with a maximum at 25.18 °C was found in coconut oil; however, the shoulder peak a' was not obvious compared to CCSO. The crystallization of CCSO and coconut oil occurred with two distinctive peaks. CCSO had peak c with a maximum at -17.65 °C and peak d with a maximum at -13.36 °C. Coconut oil had peak c' with a maximum at -8.35 °C and peak d' with a maximum at 1.20 °C. The crystallization profile is considered to be the temperature at which most of the oil has crystallized. The melting and crystallization temperatures of coconut oil were higher than those of CCSO. The reason could be due to the higher amount of lower melting TAG species in CCSO than in coconut oil. The physiochemical properties suggested that CCSO was comparable to coconut oil.

#### Journal of Agricultural and Food Chemistry



Figure 4. Comparison of oxidative stabilities of coconut oil and CCSO assessed by a DPPH radical-scavenging assay (A) and a  $\beta$ -carotene/linoleic acid bleaching assay (B).

Tocopherol Content and Oxidative Stabilities of CCSO. It is well-known that tocopherols are the natural antioxidants in plant oils that prevent lipid oxidation. As shown in Table 5, CCSO contained much higher amounts of  $\alpha$ -tocopherol (8.67  $\pm$ 0.51 mg/100 g),  $\gamma$ -tocopherol (22.6  $\pm$  1.02 mg/100 g), and  $\delta$ -tocopherol (8.38  $\pm$  0.47 mg/100 g) than coconut oil. Only  $0.35 \pm 0.19 \text{ mg}/100 \text{ g of } \alpha$ -tocopherol and no  $\gamma$ -tocopherol  $\delta$ -tocopherol were found in coconut oil. Such low amounts of tocopherols in coconut oil were also found by Gimeno et al.<sup>21</sup> To further examine the oxidative stabilities of CCSO, the DPPH radical-scavenging assay and  $\beta$ -carotene/linoleic acid bleaching assay were performed. The DPPH radical-scavenging activities of CCSO are presented in Figure 4A compared to coconut oil. It can be observed that CCSO exhibited notable DPPH radicalscavenging activity. The efficacy of CCSO was much higher than that of coconut oil. The values for CCSO ranged from 5.86  $\pm$ 0.37 to 76.27  $\pm$  0.88% as the concentration increased from 0.02 to 0.96 g/mL, whereas coconut oil ranged from 4.24  $\pm$  0.55 to 33.48  $\pm$  0.74%. The lipid peroxidation inhibition activities of CCSO determined by a  $\beta$ -carotene/linoleic acid bleaching test were consistent with the results of DPPH radical-scavenging activities. As shown in Figure 4B, the inhibition ratios of CCSO ranged from 4.15  $\pm$  0.56 to 71.73  $\pm$  2.94% as the concentration increased from 0.02 to 0.96 g/mL, and those of coconut oil ranged from 3.31  $\pm$  0.42 to 31.42  $\pm$  1.09%. These results indicated that the CCSO possessed higher oxidative stabilities

than coconut oil. The reason was probably because CCSO contained much higher amounts of antioxidant compounds (such as tocopherols) than coconut oil.

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#### Notes

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