

Extraction and Characterization of Seed Oil from Naturally Grown Chinese Tallow Trees

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Abstract Seeds were collected from locally and naturally grown Chinese tallow trees (CTT) and characterized for general physical and chemical properties and fatty acid composition of the lipids. The effects of four different solvents (petroleum ether, hexane, diethyl ether, and 95 % ethanol) and two extraction methods (supercritical carbon dioxide (SC-CO₂) and conventional Soxhlet) on the properties of the CTT seed oil, including Chinese vegetable tallow (CVT) and stillingia oil (SO), were also investigated. In general, the yields of CVT and SO did not vary based on solvent for Soxhlet extraction and solvent-free SC-CO₂ extraction, except that the yield of CVT from SC-CO₂ extraction was substantially lower. Nevertheless, the CTT seed oil, extracted by SC-CO₂ displayed better quality than those extracted by Soxhlet extraction in terms of color, residual precipitation, and acid value of the oils. The pretreatment of CTT seed by 3 % aqueous sodium bicarbonate solution likely promoted the hydrolysis of

triglyceride and caused the high acid value in the CVT samples. The iodine value at around 180 indicated that the SO is a highly unsaturated drying oil. Palmitic (76 %) and oleic (23 %) are two dominant fatty acids in CVT while linolenic (43 %), linoleic (31 %), and oleic (13 %) are the dominant fatty acids in SO.

Keywords Fatty acid compositions · Lipid yield · Oilseed crop · Soxhlet extraction · Stillingia oil · Supercritical carbon dioxide extraction

Introduction

Bio-based materials have received increasing global attention because of increasing environmental and economic concerns regarding fossil fuels. Vegetable oil is an alternative raw material to prepare bio-based materials. The Chinese tallow tree (*Sapium sebiferum*, CTT) is native to China and is one of the most promising oilseed crops [1, 2]. This fast-growing tree has more than 100,000 metric tons annual production of seed oil in China [3]. Seeds of CTT are the size of a pea, and an outer coating of vegetable tallow and fiber cover a hard, brittle shell which contains a small embryo and abundant endosperm. The endosperm is composed of a high protein meal and a drying oil [4]. The CTT seed contains approximately 40–70 % fatty acids and yields two different types of fats: a nontoxic “Chinese vegetable tallow (CVT),” which is in the external tallow coating, and mildly toxic kernel oil in the endosperm which is commonly called “stillingia oil (SO).” Interest in CTT seed oil has been historically focused on CVT for the manufacture of soap, candles, cocoa butter equivalent, and dairy creamer [5, 6]. SO is used to produce textiles, varnish, native paint, lighting, and heating oils [1]. More

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recently, SO has also been used as a feedstock for biodiesel production because of its non-edible nature and high yield of fatty acids [3].

Chinese tallow trees were introduced into North America as an economic plant in the late 1700s [7] and have been considered an invasive species throughout the southeastern United States since the mid 1900s [8]. A major pathway for the invasiveness of CTT is via seed dispersed by birds [3]. Removing and using the CTT seed as a feedstock for bio-material production could be an effective way to control its dispersion and therefore would significantly decrease the species invasiveness [9, 10]. In addition, CTT seed oil is not edible by humans. Utilizing CTT seed oil would avoid competition with food sectors.

A number of studies on extraction of vegetable oil using the Soxhlet extraction method have been published, however, only a few on CTT seed oil were found [7]. Soxhlet extraction is a standard technique and the main reference to which other extraction methods are compared [11, 12]. Although Soxhlet extraction is a well-established technique, it is also time consuming and the solvent residue in the extracting products could be harmful to human health as well as the environment [12]. In addition, degradation and decomposition of thermo-sensitive compounds could occur since relatively high temperature is required for Soxhlet extraction [12].

In recent years, supercritical carbon dioxide (SC-CO₂) extraction has attracted increasing attention as an alternative to conventional methods for lipid extraction because SC-CO₂ can overcome most disadvantages of conventional Soxhlet extraction. Although SC-CO₂ extraction is not a cost-effective process for typical oil extraction, it is an almost pollution-free method and has many advantages, such as performing faster with more efficiency at relatively low temperature and providing the extracts with no residual organic solvents, which makes it an ideal solvent for natural products [12].

Although CTT seed oil has a long history of commercial applications in China and other Asian countries, very few reports on the utilization of CTT seed oil have been conducted in North America perhaps because of its invasive nature. It has been reported that geographic and silvicultural factors could significantly affect the properties of the CTT seed [13]. In addition, extraction conditions (e.g., temperature, time, and type of solvent) have been shown to affect sensory quality, volatile content, and oxidative stability of the extracted oil [14]. The objectives of this study were to compare the effects of different solvents as well as two extraction methods (e.g., conventional Soxhlet and SC-CO₂) on the oil yield and the physical and chemical properties of CTT seed oil from naturally grown CTT seeds harvested in northern Louisiana.

Materials and Methods

Collection of Seed

The CTT seeds were manually harvested from naturally growing trees in Calhoun, Louisiana, USA, in October, 2011. The seeds were air dried for 2–3 days and separated from the husk, branches, and other impurities by hand picking and were put in sealed plastic bags and stored in a freezer at $-20\text{ }^{\circ}\text{C}$ until further use. All chemicals and solvents used in this study were of analytical or HPLC grade and were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Proximate Analysis of CTT Seed

Chinese tallow tree seeds were soaked in hot sodium bicarbonate solution (3 %) for 24 h before separating the tallow coating from the kernel. This treatment rendered the tallow coating soft and tender during separation, which was accomplished by washing. Separated tallow coating and kernels (with shell) were air-dried under room temperature and then stored in a refrigerator until further use. The black shells of the separated kernels were not removed before the grind and oil extraction.

Hundred-grain-weight of the CTT seed was measured by randomly selecting 100 CTT seeds and weighing the seeds by an analytical balance (up to 0.01 g). Three measurements were made and the average weight was reported.

Crude fat/oil content of the CTT seeds was determined by a traditional Soxhlet extraction method with diethyl ether (boiling point $34.6\text{ }^{\circ}\text{C}$) according to a method of the Association of Official Analytical Chemists [15], and expressed as a percentage mass fraction of the initial sample.

Moisture and volatile matter content of the CTT seeds were determined in accordance with ISO method 665 [16]. In brief, approximately 5 g of the CTT seed was ground with a coffee grinder for 1 min and then placed in an oven at $103 \pm 2\text{ }^{\circ}\text{C}$ until reaching a constant mass ($\pm 0.005\text{ g}$). Moisture and volatile matter content were expressed as the mass difference of the CTT seed before and after oven drying as a percentage of the initial mass of the sample. Three measurements were made and the average weight was reported.

Extraction of CVT and SO

In this study, four solvents with different polarities were selected for Soxhlet extraction of the CTT seed; petroleum ether, hexane, diethyl ether, and 95 % ethanol with polarities of 0.01, 0.06, 2.90, and 5.64, respectively. These four solvents are the most commonly used extraction solvents

for seed oil. Approximately 20 g of ground tallow coating or kernel samples were precisely weighed into an extraction thimble (35 × 118 mm) and subjected to Soxhlet extraction for 15 h. The extraction temperatures were 55, 75, 35, and 80 °C for petroleum ether, hexane, diethyl ether, and 95 % ethanol, respectively. After extraction was completed, the solvent was removed under reduced pressure using a rotary evaporator (Laborota 4000 eco/WB/G3, Heidolph, Germany). The lipid obtained was stored in a refrigerator at 4 °C until further analysis.

SC-CO₂ extraction was performed on a laboratory scale SC-CO₂ apparatus (Spe-ed SFE 7070, Applied Separations Inc., Allentown, PA, USA). Approximately 20 g of ground tallow coating or kernel samples were precisely weighed into a 100-mL stainless steel extraction vessel. Extraction experiments were performed at 400 bar for 1.5 h. The CO₂ flow rate was 1.5 L/min, and the chamber temperature was set at 40 °C.

Yield of lipid was calculated using the following equation:

$$\text{Lipid yield (\%)} = \frac{\text{Weight of extracted lipid (g)}}{\text{Weight of sample subjected to extraction (g)}} \times 100\%$$

Physical and Chemical Properties of CVT and SO

Acid, iodine, and saponification values of the CVT and SO samples were determined according to ISO 660 [17], ISO 3961 [18], and ISO 3657 [19], respectively.

Fatty Acid Composition Analysis

Fatty acid compositions were determined as fatty acid methyl esters by GC/MS. In brief, around 50 mg lipid was dissolved in 5 mL hexane in a centrifuge tube followed by adding 0.5 mL potassium hydroxide methanol solution (2 N). The tube was capped and vigorously agitated for 5 min and was then centrifuged at 4,500 rpm (2,268g) for 10 min. An aliquot of the top hexane solution was transferred into a sample vial and analyzed in a GC/MS instrument (Agilent 5975/5790, Santa Clara, CA, USA) equipped with a DB-WAX capillary column (30 m × 0.25 mm × 0.25 μm, Agilent, Santa Clara, CA, USA). The column temperature was programmed to increase from 150 to 220 °C at 6 °C/min. Helium was used as the carrier gas at a flow rate of 20 mL/min. The temperature of the injector was set at 240 °C. The spectrum was compared with a standard spectrum (NIST).

Statistical Analysis

Statistical analysis of the data was carried out using SAS (version 9.1, SAS Institute, Cary, NC). All extractions and

analysis of samples from each extraction were performed in duplicates, and the results were expressed as mean values with standard deviation. A one way ANOVA was performed by using Tukey adjustment to determine significant difference ($\alpha = 0.05$) among the different treatments.

Results and Discussions

Proximate Analysis of CTT Seed

Hundred-grain-weight is a well established physical parameter of the CTT seed, which is used to assess yield and basic qualities of the seed [20]. The hundred-grain-weight of the CTT seed used in this study is 12.54 g. It is lower than those reported in literatures, which is probably due to the different geographic locations and the way the CTT was grown (i.e., cultivated vs. wild growth) [20].

The results of proximate analysis of the dried CTT seed are presented in Table 1. The CTT seed consisted of approximately 23 % tallow coating, 68 % kernel, and 6 % moisture and volatile matter. The tallow coating content was lower than the average tallow coating contents (32 %) of the CTT seed reported by Potts and Don [21]; however, the kernel content was similar to that previously reported (67 %). This result indicated that the tallow coating of the CTT seed tends to be more affected by cultivation or geographic factors than the sections of the kernel. It has been reported that tallow coating content significantly increased with rising annual accumulated temperature and annual precipitation because of a change in longitude from west to east [13].

The results of crude fat/oil content of CTT seed is also listed in Table 1. The lipid content of the whole CTT seed was around 44 %, while the yield of CVT and SO from the tallow coating and kernel were 81 and 33 %, respectively. The oil content of CTT seed is comparable to castor seed (40–60 %) and jatropha seed (30–50 %), two major sources of non-edible oils for industrial use [22]. Therefore,

Table 1 Proximate analysis of the CTT seed

	Components of the CTT seed (%)	Crude fat/oil content (%)
Whole seed	100.00 ± 0.00	44.15 ± 0.91
Tallow coating	22.81 ± 0.59	80.76 ± 1.02
Kernel ^a	67.64 ± 0.16	32.61 ± 0.45
Moisture and volatile Matter	5.83 ± 0.41	–

Data are presented as mean values ± standard deviations ($n = 2$)

^a With kernel shell

CTT is also a promising oilseed crop for industrial feedstock applications.

Lipid Yield by Soxhlet and SC-CO₂ Extraction

The lipid yields under different extraction conditions are shown in Fig. 1. Both the CVT and SO yields were similar among selected extraction solvents. The results indicate that all four solvents had similar efficiencies at extracting lipids from CTT seed. Nevertheless, besides the major component of lipids in CTT seed, other minor components that have been extracted by different solvent might vary because of the different polarities of the solvents.

The yield of CVT and SO by SC-CO₂ extraction was 46 and 31 %, respectively (Fig. 1). It is interesting to note that the yield of SO by SC-CO₂ extraction was similar to those from Soxhlet extraction; however, the yield of CVT was substantially lower than Soxhlet extraction. The solubility of fatty acids in SC-CO₂ extraction depends on the length of the hydrocarbon chain and the presence of functional groups, as well as the extraction parameter, such as pressure and temperature [23]. The CVT and SO mainly consist of C16 and C18 fatty acids, which are of similar hydrocarbon chain length. The most obvious difference between CVT and SO is that most fatty acids in CVT are saturated while those in SO are unsaturated. Markon [24] has reported that triglycerides rich in saturated fatty acids generally have low solubility in SC-CO₂. It could also be related to the high melting point of CVT compared to SO, which is due to the high content of saturated fatty acids. Our observations during the SC-CO₂ extraction showed that, unlike smooth dripping during the extraction of SO, a small amount of snow flake-shaped CVT was piled around the end of the extraction outlet during the extraction. This semi solid CVT could clog the flow and delay the extraction, which may reduce the lipid yield. Increasing the extraction temperature and/or combining solvent extraction with

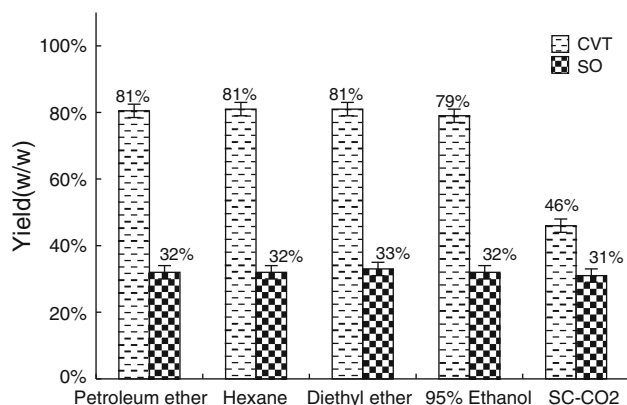


Fig. 1 The lipid yield in CVT and SO with different extraction methods

SC-CO₂ could improve the yield of CVT by SC-CO₂ extraction. Further investigation on this aspect is on going and will be separately reported.

Physical and Chemical Properties of CVT and SO

Figure 2 shows the appearance of CVT from the CTT seed. All CVT samples appear as a white, low density, waxy solid at room temperature regardless of the extraction solvents and methods used. The tallow coating itself is a white fibrous waxy solid that consists mainly of lipids and cellulose [4]. In addition, pretreatment with a 3 % aqueous sodium bicarbonate solution removed most of the water soluble extractives in the tallow coating. Lipids and cellulose were primarily subjected to extraction. Therefore, no significant difference was observed in the appearance of CVTs extracted by different solvents and methods.

On the other hand, SO is a liquid at room temperature and displays different colors with different extraction conditions. The color of SO progressively increased in darkness from pale yellow to light brown with the following method and solvents used in extraction: SC-CO₂, petroleum ether, diethyl ether, hexane, and 95 % ethanol (Fig. 3). As discussed in the previous section, although the yields of SO by different solvents were similar, the composition of extracted oil may be different. Extraction temperature could play an important role in the differences in the resulting SO samples. For instance, the SO extracted by hexane at higher temperature displayed a darker color than that from petroleum ether even though their polarities are similar. It has been reported that the partial thermal degradation of saccharides and protein under a high extraction temperature could increase the color of SO [12].



Fig. 2 CVT extracted from the CTT seed

Different polarities of extraction solvents have also been attributed to the changes in color of SO. Light brown precipitates were observed under room temperature in the SO sample extracted by 95 % ethanol, which is likely because of some polar compounds had dissolved under higher temperature but became less soluble as the temperature decreased. The SO sample extracted by SC-CO₂ had the lightest color, which indicated relative high purity of SO. This result also indicated that SC-CO₂ extraction has the advantages of lower extraction temperature, short extraction duration, and no solvent residue over conventional Soxhlet extraction.

Some physical and chemical properties of CVT and SO, including acid, iodine, and saponification values are listed in Table 2. The acid value is a measure of the free fatty acids (FFA) that are present in lipids and is an important indicator of lipid quality. An increase in the amount of FFA in a sample of lipid indicates the hydrolysis of triglycerides. It is an indicator of inadequate processing and storage conditions (i.e., high temperature and relative humidity, tissue damage). The acid values of CVT and SO were compared and no significant differences ($P \geq 0.05$) were observed between samples from most different extraction conditions except for the acid value of CVT samples

extracted by hexane was significantly higher ($P < 0.05$) than other CVT samples, which could be attributed to the relatively higher extraction temperature with hexane. It is also worth noting that all CVT samples had higher acid value than SO samples. This increase in the acid value of CVT samples could be mainly due to the pretreatment of soaking CTT seeds in hot 3 % aqueous sodium bicarbonate solution. During this pretreatment, hydrolysis could occur under the weak alkaline condition, and therefore have caused the increase in the acid value. On the other hand, the kernel fraction was protected by a shell and has much less influence than the tallow coating during the pretreatment. The CVT extracted by SC-CO₂ had a significantly lower acid value ($P < 0.05$) than those from Soxhlet extraction probably because the CVT samples were not exposed to high extraction temperature and long extraction time during SC-CO₂ extraction.

The iodine value indicates the degree of unsaturation of a lipid. The higher the iodine value, the more unsaturated fatty acid that is present in a lipid and the more reactive is the lipid. It can be seen from Table 2 that there are some fluctuations in iodine values of the CVT and SO samples from different extraction methods. In general, CVT had low iodine values ranging from 22 to 27 g/100 g, which

Fig. 3 SO extracted from the CTT seed



Table 2 Physiochemical properties of the CVT and SO

Lipids	Properties	Soxhlet				SC-CO ₂
		Petroleum ether	Hexane	Diethyl ether	95 % Ethanol	
CVT	Acid value (mg KOH/g)	1.06 ± 0.01 ^a	1.18 ± 0.01 ^b	1.05 ± 0.01 ^a	1.06 ± 0.02 ^a	0.99 ± 0.01 ^c
	Iodine value (g/100 g)	23.7 ± 1.1 ^{a,b}	21.6 ± 1.1 ^b	27.4 ± 0.4 ^{a,b}	27.9 ± 1.2 ^a	26.8 ± 1.3 ^{a,b}
	Saponification Value (mg KOH/g)	177.2 ± 0.6 ^a	175.6 ± 1.8 ^a	174.9 ± 0.9 ^a	174.9 ± 1.1 ^a	177.2 ± 0.6 ^a
	Average molecular weight ^d (g/mol)	949.6 ± 3.2 ^a	958.4 ± 9.6 ^a	962.3 ± 6.0 ^a	962.3 ± 5.1 ^a	950.6 ± 4.6 ^a
SO	Acid value (mg KOH/g)	0.26 ± 0.02 ^a	0.23 ± 0.01 ^a	0.28 ± 0.01 ^a	0.25 ± 0.01 ^a	0.27 ± 0.02 ^a
	Iodine value (g/100 g)	183.8 ± 0.5 ^b	187.1 ± 0.5 ^a	186.4 ± 0.1 ^{a,b}	186.0 ± 0.5 ^{a,b}	185.4 ± 0.7 ^{a,b}
	Saponification value (mg KOH/g)	178.2 ± 1.1 ^b	180.9 ± 0.5 ^{a,b}	179.6 ± 0.9 ^{a,b}	178.1 ± 1.2 ^b	184.3 ± 0.1 ^a
	Average molecular weight (g/mol)	944.3 ± 5.6 ^a	930.3 ± 2.4 ^a	936.9 ± 4.4 ^a	944.9 ± 6.1 ^a	913.4 ± 0.1 ^b

^{a,b,c} Values with same the superscript letter are not significantly different ($P \geq 0.05$)

^d Average molecular weight = $(3 \times 56.1 \times 1000) / \text{saponification value}$

Table 3 Fatty acid compositions and their relative abundance of the CTT seed oil extracted by Soxhlet and SC-CO₂

Lipids	Fatty acids (%)	Soxhlet				SC-CO ₂
		Petroleum ether	Hexane	Diethyl ether	95 % Ethanol	
CVT	Myristic (C14:0)	0.10 ± 0.01 ^a	0.09 ± 0.02 ^a	0.10 ± 0.01 ^a	0.10 ± 0.01 ^a	0.11 ± 0.01 ^a
	Palmitic (C16:0)	76.00 ± 0.37 ^a	74.71 ± 0.54 ^a	75.62 ± 0.04 ^a	69.19 ± 0.22 ^b	75.09 ± 0.17 ^a
	Palmitoleic (C16:1)	0.22 ± 0.01 ^a	0.21 ± 0.04 ^a	0.22 ± 0.02 ^a	0.24 ± 0.01 ^a	0.16 ± 0.01 ^a
	Heptadecanoic (C17:0)	n.d.	n.d.	n.d.	0.08 ± 0.02	n.d.
	Stearic (C18:0)	1.30 ± 0.06 ^a	1.44 ± 0.15 ^a	1.21 ± 0.05 ^a	1.23 ± 0.01 ^a	1.12 ± 0.04 ^a
	Oleic (C18:1)	22.04 ± 0.15 ^a	23.40 ± 0.36 ^b	22.68 ± 0.10 ^a	28.07 ± 0.06 ^b	23.35 ± 0.13 ^b
	Linoleic (C18:2)	0.14 ± 0.01 ^a	0.15 ± 0.03 ^a	0.18 ± 0.01 ^a	1.07 ± 0.27 ^b	0.17 ± 0.01 ^a
	SFA ^c	77.40 ± 0.44 ^a	76.24 ± 0.42 ^a	76.93 ± 0.09 ^a	70.61 ± 0.20 ^b	76.32 ± 0.14 ^a
UFA ^d	22.40 ± 0.15 ^a	23.76 ± 0.42 ^b	23.07 ± 0.09 ^a	29.39 ± 0.21 ^b	23.68 ± 0.14 ^b	
SO	8-Hydroxy-5,6-octadienoic (C8:2)	n.d.	0.14 ± 0.03 ^a	0.18 ± 0.01 ^a	n.d.	0.27 ± 0.08 ^b
	2,4-Decaenoic (C10:2)	3.84 ± 0.22 ^a	3.95 ± 0.18 ^a	3.89 ± 0.02 ^a	4.01 ± 0.15 ^a	1.55 ± 0.01 ^b
	Myristic (C14:0)	0.05 ± 0.01 ^a	0.05 ± 0.01 ^a	0.05 ± 0.01 ^a	0.04 ± 0.01 ^b	0.44 ± 0.01 ^b
	Palmitic (C16:0)	6.15 ± 0.15 ^a	6.85 ± 0.10 ^b	6.80 ± 0.17 ^b	6.17 ± 0.16 ^a	6.25 ± 0.05 ^a
	Palmitoleic (C16:1)	0.10 ± 0.01 ^a	0.10 ± 0.07 ^a	0.06 ± 0.01 ^a	0.06 ± 0.01 ^a	0.09 ± 0.01 ^a
	Stearic (C18:0)	2.19 ± 0.14 ^a	2.09 ± 0.06 ^a	2.01 ± 0.07 ^a	2.07 ± 0.03 ^a	2.03 ± 0.01 ^a
	Oleic (C18:1)	12.65 ± 0.03 ^a	12.93 ± 0.27 ^a	12.54 ± 0.11 ^a	12.65 ± 0.22 ^a	12.61 ± 0.02 ^a
	Linoleic (C18:2)	31.44 ± 0.12 ^a	30.73 ± 0.15 ^b	30.48 ± 0.06 ^b	30.74 ± 0.06 ^b	32.20 ± 0.39 ^a
	Linolenic (C18:3)	43.11 ± 0.32 ^a	43.13 ± 0.39 ^a	43.46 ± 0.36 ^a	43.80 ± 0.33 ^b	44.15 ± 0.48 ^b
	Eicosenoic (C20:1)	0.48 ± 0.01 ^a	0.50 ± 0.07 ^a	0.51 ± 0.04 ^a	0.46 ± 0.02 ^a	0.84 ± 0.05 ^b
	SFA	8.39 ± 0.01 ^a	8.99 ± 0.16 ^a	8.86 ± 0.25 ^a	8.28 ± 0.19 ^a	8.30 ± 0.04 ^a
	UFA	91.61 ± 0.01 ^a	91.46 ± 0.80 ^a	91.13 ± 0.24 ^a	91.72 ± 0.19 ^a	91.71 ± 0.03 ^a

n.d. not detected

^{a,b} Values with same superscript letter are not significantly different ($P \geq 0.05$)

^c Saturated fatty acid

^d Unsaturated fatty acid

was close to 20–29 g/100 g reported by Xu and Zhang [25] SO had a much higher iodine value and ranged from 184 to 187 g/100 g, indicating that SO is a drying oil.

The saponification value is the number of milligrams of potassium hydroxide required to neutralize the fatty acids resulting from the complete hydrolysis of 1 g of lipid. In general, the longer the carbon chain of a lipid, the less acid that is liberated per gram of lipid hydrolyzed. It can be used to estimate the average molecular weight (or chain length) of all the fatty acids present [26]. The saponification values of all CTV and SO samples from different extraction methods were similar. They were slightly lower than those values reported by Xu and Zhang [25], which could be due to factors such as geographic location and different harvesting seasons.

Fatty Acid Compositions

The fatty acid compositions of the extracted lipids determined by GC/MS are shown in Table 3. Only major fatty acids were selected to examine the changes in lipid

composition as a function of different extraction methods. From the analysis, both CVT and SO contained a great variety of fatty acids. CVT had a high degree of saturated fatty acids (palmitic and stearic acids), comprising more than 76 % of the total fatty acids. *Stillingia* oil had abundance of unsaturated fatty acids (linoleic and linolenic acids), comprising more than 85 % of the total fatty acids. This result is in agreement with the iodine value of the CVT and SO samples discussed in the previous section.

The fatty acids presented in CVT include palmitic, oleic, stearic, linoleic, myristic, palmitoleic, and heptadecanoic acids and their distributions were similar to each other in general. Palmitic and oleic were the two major fatty acids and comprised about 98 % of the total fatty acid content in CVT. The compositions of fatty acids in CVT samples were similar to those reported by Qian and co-workers [27]. The CVT samples extracted by 95 % ethanol had lower saturated fatty acids than others, which was likely because of the high polarity of ethanol. In addition, trace amounts of heptadecanoic acid (~0.10 %), an odd-number straight-chain fatty acid and rare in nature, was also

detected in the samples extracted by 95 % ethanol. The high percentage of oleic acid in ethanol extracted samples was due to the calculation method of the GC/MS. Namely, the highest abundance in the spectrum (i.e., palmitic acid) was set as 100 % and this compound was used as reference compound. The relative abundances of other compounds were calculated as the percentage of the reference compound which was set as 100 % abundance. Therefore, when the actual abundance of the reference compound (palmitic acid) decreased, the relative abundance of other compounds could increase, even if their actual contents might remain the same.

Linolenic, linoleic, and oleic acids were the dominant fatty acids in SO composition. Six major fatty acids (i.e., linolenic, linoleic, oleic, palmitic, stearic, and 2,4-decaienoic acids) comprised about 95 % of the total fatty acid content in SO. The relative abundance of linoleic and oleic acids were higher, while 2,4-decaienoic acid was lower than those reported by Chen [28]. The relative abundance of linolenic acid was similar to those reported [28]. The distributions of fatty acids were similar in all SO samples extracted by different conditions except that the relative abundance of 2,4-decaienoic acid in the SC-CO₂ extracted SO was significantly lower than others. 2,4-Decaienoic acid is a special kind of fatty acid present in SO. It is toxic to humans; however, small doses of SO are used as a purgative and emetic in traditional Chinese medicine because of the presence of 2,4-decaienoic acid. Trace amounts of 8-hydroxy-5,6-octadienoic acid were detected in the SO samples extracted by hexane, diethyl ether, and SC-CO₂. This is another type of fatty acid rarely found in SO. Compared with other vegetable oils for industrial applications, including soybean, sunflower, and safflower oils, SO has a much higher content of linolenic acid, which makes it superior for polymerization and other modifications because of the high content of unsaturated double bonds presented in the fatty acids chain [29].

Conclusion

The objective of the study was to compare the effects of different solvents as well as two extraction methods (e.g., conventional Soxhlet and SC-CO₂) on the oil yield and the physical and chemical properties of CTT seed oil. The hundred-grain-weight of the CTT seed was lower than cultivated CTT seeds previously reported in the literature. The weight percentage of tallow coating of the seed was lower, while the percentage of kernel was similar to those of cultivated species as reported in earlier reports, which indicated that the tallow coating is more susceptible to cultivation and geographic factors. In general, the yields of CVT and SO were similar among four solvents with

different polarities using Soxhlet and solvent-free SC-CO₂ extraction, except that the yield of CVT from SC-CO₂ extraction was substantially lower than the others. Nevertheless, the CTT oil extracted by SC-CO₂ displayed lighter color and lower acid value than those extracted by Soxhlet extraction. On average, CVT had significantly higher acid values than SO regardless of the extraction solvent and method. Among CVT samples extracted at different conditions, CVT samples extracted by hexane had significantly higher acid values than others. The pretreatment of CTT seed by a 3 % aqueous sodium bicarbonate solution and extraction temperature could be the major contributors to the high acid value. The seed of CTT has great potential as an alternative to petroleum feedstock for the manufacture of polymer products, such as polyurethane and epoxy.

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