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Physiochemical characteristics, fatty acid profile and tocopherol composition of the oil from *Camellia oleifera* Abel cultivated in Henan, China

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SUMMARY: As a unique woody oil tree, *Camellia oleifera* Abel originates from southern China and is now being cultivated in central China, such as the southern Henan province. The aim of this work is to determine some physiochemical parameters, fatty acid profile and tocopherol composition of the *Camellia oleifera* seed oils harvested in southern Henan in the 2016 season. The lipid contents of the kernel are in the range of 28.39-56.94% on dry matter. The acid value (AV), saponification value (SV), iodine value (IV), and refractive index of the oils are in the ranges: 0.22-5.17 mg KOH/g, 178.90-196.91 mg KOH/g, 82.0-87.1 g I_2/100g, and 1.4667-1.4720 (n_D^{25}), respectively. The main fatty acids are: palmitic 7.07-9.52%, palmitoleic 0.09-0.22%, stearic 1.45-2.94%, oleic 77.02-84.33%, linoleic 5.19-11.23%, linolenic 0.53-0.70% and arachidic 0.22-0.70%. The sn-2 fatty acid composition is: 84.36-91.33% oleic, 7.14-14.22% linoleic, and 0.89-1.96% others. The tocopherol content is in the range of 39.55-75.94 mg/100g oil, including mainly α-tocopherol (33.98-67.82 mg/100g) and γ-tocopherol (5.57-8.27 mg/100g) when the oils are extracted with hexane; and 14.91-44.36 mg/100g, including mainly α-tocopherol (9.42-37.08 mg/100g) and γ-tocopherol (5.50-7.33 mg/100g) when the oils are extracted by ethyl ether. The physiochemical properties *C. oleifera* seed oils from southern Henan are similar to those from other districts of China.

KEYWORDS: C. oleifera *oil; Fatty acid profile; Physiochemical properties; Tocopherols*

RESUMEN: ***Características fisicoquímicas, perfil de ácidos grasos y composición de tocoferoles de aceites de* Camellia oleifera *Abel cultivadas en Henan, China***. *Camellia oleifera* Abel es una planta leñosa única, proveniente del sur de China que ahora es cultivada también en el centro del país, en la provincia de Henan. El objetivo de este trabajo es determinar las propiedades fisicoquímicas, el perfil de ácidos grasos y la composición de tocoferoles de aceites de semillas de *Camellia oleifera* de semillas recogidas en el sur de esta provincia en la temporada 2016. El contenido de lípidos del grano está en el rango de 28.39-56.94% sobre materia seca. La acidez, índice de saponificación, índice de yodo e índice de refracción se encuentran en los rangos: 0.22-5.17 mg KOH/g, 178.90-196.91 mg KOH/g, 82.0-87.1 g I_2/100g y 1.4667-1.4720 (n_D^{25}), respectivamente. La composición en ácidos grasos fue: 7.07-9.52% palmítico, 0.09-0.22% palmitoleico, 1.45-2.94% esteárico, 77.02-84.33% oleico, 5.19-11.23% linoleico, 0.53-0.70% linolénico y 0.22-0.70% araquídico. La composición en ácido graso en sn-2: 84,36-91,33% de ácido oleico, 7,14-14,22% de linoleico y 0,89-1,96% de otros. El contenido de tocoferoles está en el rango: 39.55-75.94 mg/100 g, y se componen principalmente de α-tocoferol (33,98-67,82 mg/100 g) y γ-tocoferol (5,57-8,27 mg/100 g) cuando los aceites se extraen con hexano y 14,91-44,36 mg/100 g, formados por α-tocoferol (9,42-37,08 mg/100 g) y γ-tocoferol (5,50-7,33 mg/100 g) en aceites extraídos con éter etílico. Las propiedades fisicoquímicas de los aceites de semillas de C. oleifera del sur de Henan son similares a los de otros distritos de China.

PALABRAS CLAVE: *Aceite de* C. oleifera*; Perfil de ácidos grasos; Propiedades fisioquímicas; Tocoferoles*

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1. INTRODUCTION

The seeds of the camellia (*Camellia oleifera* Abel, Theaceae) have been utilized in China for more than 1000 years (Shanan and Ying, 1982). *Camellia oleifera* oil is a popular cooking oil in south China, with a unique flavor and health benefits, especially in the Huan and Jiangxi provinces, where more than 50% of the vegetable cooking oil is from *C. oleifera* seeds (Tang *et al.*, 1993). Because some characteristics of the *C. oleifera* oil are distinctly similar to those of olive oil, it is also called oriental olive oil (Tang *et al.*, 1993). Numerous studies have shown that *C. oleifera* oil have many physiological functions (Akihisa *et al.*, 1997; Cheng *et al.*, 2014; Lee and Yen, 2006; Li *et al.*, 2011; Miura *et al.*, 2007; Sahari and Amooi, 2013; Tu *et al.*, 2017), which improve the economic value and broaden the utilization of *C. oleifera* oil. The *C. oleifera* is cultivated mainly in Hunan, Jiangxi, Guangxi, followed by Guizhou, Fujian, Guangdong, Zhejiang, Chongqing, Hubei, Sichuan and Yunnan in China (He and He, 2002). Recently, *C. oleifera* Abel has been cultivated widely in southern Henan of China (Yao *et al.*, 2011; Wang *et al.*, 2008).

The kernel of *C. oleifera* seed is rich in oil/lipid and polysaccharide (Yu *et al.*, 2013). The oil/lipid content of the *C. oleifera* seed and fatty acid composition of its oil are affected by the variety, cultivated soil and surrounding climate (Guo *et al.*, 2013; Yao *et al.*, 2011; Yu *et al.*, 1999). The research is mainly focused on the oil content of *C. oleifera* seeds, the iodine value, saponification value and refractive index and the fatty acid composition of the oils from these seeds cultivated in Hunan, Jiangxi, Guangxi, Zhejiang, Yunnan, Sichuan, Guizhou, Guangdong and Hubei of China have been evaluated (Chen, *et al.*, 2011; Chen *et al.*, 2012; Cheng *et al.*, 2010; Dong *et al.*, 2012; Guo *et al.*, 2008; Guo *et al.*, 2013; Ma *et al.*, 2011; Xi *et al.*, 2006; Yao *et al.*, 2011; Yu *et al.*, 2013; Yuan *et al.*, 2013). Few reports on the minor components (such as Vitamin E and carotene) in *C. oleifera* oil are available. Li *et al.* (2009) analyzed the lipo-soluble vitamin, β-carotene in the seed, oil and shell of *C. oleifera* cultivated in Guizhou (China) by HPLC. The results showed that α- and γ-tocopherol existed in the *C. oleifera* seed, oil and shell, and a very small amount of the β-carotene existed in the crude oil, seed and shell of *C. oleifera*.

However, little information on the physicochemical characterization of the oil obtained from the seeds of *C. oleifera* Abel cultivated in southern Henan of China is available. Yao *et al.* and Wang *et al.* analyzed the lipid content of the *C. oleifera* seeds cultivated in southern Henan of China and the fatty acid composition of the oils from these seeds (Yao *et al.*, 2011; Wang *et al.*, 2008). This investigation was undertaken to determine the physicochemical properties, fatty acid compositions, stereospecific positional distribution of the fatty acids and the tocopherol composition of the oils extracted from *C. oleifera* Abel grown in south Henan of China, and to compare these results with those of the *C. oleifera* oil and the seed oils from other camellia species shown in previous work.

2. MATERIALS AND METHODS

2.1. Materials

Twenty-two samples of *C. oleifera* Abel seeds were collected from South Henan (China), harvested on November 2016. Each sample consisted of about 1.5 kg. These samples were divided into four groups based on the cultivation districts: (1) Guangshan (Gs, n=7; YuGuang variety), (2) Shangcheng (Sc, n=5; YuShang variety), (3) Xinxian (Xx, n=5; YuXin variety), (4) Luoshan (Ls, n=5; YuLuo variety). The detailed information is shown in Figure 1, which describes the places of origin. The standard fatty acid methyl esters (palmitic, stearic, oleic, linoleic, linolenic, and arachidic) and tocopherols (α-, β-, δ-, and γ), pancreatic lipase for the sn-2 position analysis were purchased from Sigma-Aldrich (Shanghai) Trading Co. Ltd (Shanghai, China). Silica gel used for TLC plate preparation was obtained from Qingdao Ocean Chemical Factory (Qingdao, China). The hexane for the fatty acid composition analysis was chromatographic grade, and the other reagents and solvents from local sources were of analytical grade and purified before use.

2.2. Extraction of oil from *C. oleifera* seed

After removal of the hull, the seed was ground into a fine paste using a laboratory grinder. The paste was subjected to Soxhlet extraction with petroleum ether (boiling point, 60-90 °C) or ethyl ester for 8h. Briefly, ground seed (about 50g) was packed in a cellulose thimble, and the open end of the thimble was plugged with cotton to avoid spillage of the seed paste into the miscella. After extraction, a clear miscella was obtained and hence the crude *C. oleifera* oil was obtained after the removal of solvent from the miscella under vacuum.

2.3. Analysis methods

The lipid content of *C. oleifera* seed was analyzed according to the IUPAC method (1.122) (Paquot and Hauntfenne, 1987). The important physicochemical properties of the crude *C. oleifera* oils concerning acid value (AV), saponification value (SV), iodine value (IV), and refractive index were characterized according to the IUPAC Methods 2.201, 2.202, 2.205, and 2.102, respectively (Paquot and Hauntfenne, 1987).

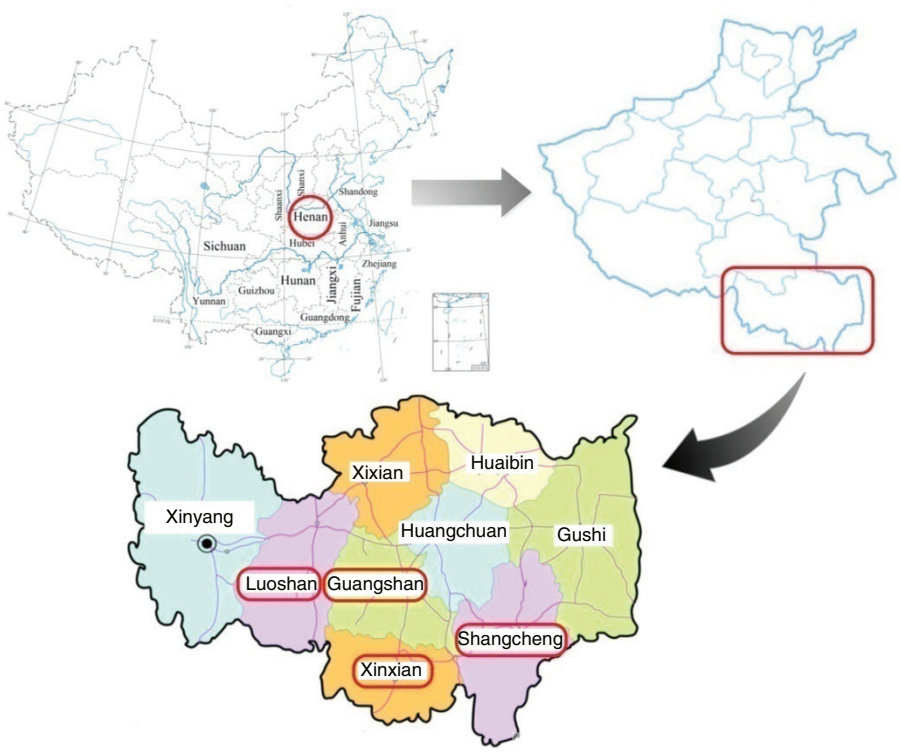

FIGURE 1. Geographical location of the four *Camellia oleifera* Abel cultivation zones in south Henan, China

2.3.1. Fatty acid composition of the crude *C. oleifera* oil

The fatty acid composition of *C. oleifera* oil was analyzed according to the IUPAC method 2.302 (Paquot and Hauntfenne, 1987). The analysis of fatty acid methyl esters was performed on a gas chromatograph (GC) (Agilent 6890N) equipped with a flame ionization detector (FID) and a BPX-70 capillary column (30 m × 0.25 mm, 0.25 μm of film thickness) (SGE Technologies Co. Ltd., Australia). The column, injector, and detector temperatures were set at 180, 230, and 300 °C, respectively. The flow rate of the carrier gas N_2 with a split ratio of 1:20 was set at 70 mL/min. The fatty acids were identified with reference to the retention times of standard fatty acid methyl esters determined under the same conditions.

2.3.2. sn-2 fatty acid distribution of the triacylglycerols in the crude *C. oleifera* oil

The triacylglycerol content was separated from crude *C. oleifera* oil by thin layer chromatography (TLC) using the mixture of hexane:diethyl ether:acetic acid (70:30:1, v/v/v) as developing solvent. The TLC plate was visualized under UV light after spraying with a 0.2% ethanolic solution of 2',7'-dichlorofluorescein sodium salt. Then the triacylglycerol band was collected and extracted with diethyl ether, the obtained triacylglycerol was used for the sn-2 fatty acid composition analysis according to the IUPAC method 2.210 (Paquot and Hauntfenne, 1987). 1,3-Specific pancreatic lipase was employed for the chromatography purified triacylglycerol hydrolyzation. The hydrolyzate of the *C. oleifera* oil oil was separated on TLC using a mixture of n-hexane:diethyl ether:acetic acid (70:30:1, v/v/v) as developing solvent. The TLC bands of the hydrolyzate were visualized under UV light after spraying with a 0.2% ethanolic solution of 2',7'-dichlorofluorescein sodium salt. Then the band containing monoacylglycerol (MAG) was collected and extracted with diethyl ether. The obtained MAG was methylated, and the resulting fatty acid methyl ester was subjected to GC analysis.

2.3.3. Tocopherol content assay of the crude *C. oleifera* oil

The tocopherol (α-, β-, δ-, and γ) contents of the crude *C. oleifera* oil were determined according to our previous report (Liang *et al.*, 2010) with minor modifications. The samples were analyzed by HPLC using a Waters Alliance 2695 system with a silica column (250 × 4.6 mm, 5 μm) (Waters, Singapore) and a Waters 2475 fluorescence detector (Waters, Milford, USA). The column temperature was

maintained at 40 °C. The excitation and emission wavelengths were 298 nm and 325 nm, respectively. The oil samples were dissolved in hexane at 100 mg/mL. A 5 μL volume of the loaded sample was isocratically eluted with *n*-hexane/isopropyl ether (90/10, v/v) at 1.5 mL/min. The absolute contents of tocopherols were determined according to the calibrated standard curves.

2.4. Statistical analysis

All analyses and measurements in this work were performed in at least duplicate. Data were analyzed by one-way analysis of variance (ANOVA) and Tukey's multiple comparison method at $p = 0.05$ using IBM SPSS Statistics for windows version 20.0.

3. RESULTS AND DISCUSSION

3.1. Lipid content of *C. oleifera* seeds

The lipid contents of these 22 seeds in our research were in the range of 28.39-56.94% with an average value of 41.05% on a moisture free basis (Table 1). The lipid contents of the seeds cultivated in Shangcheng were 41.73-56.94% with a mean value of 49.66% (Table 1), and significantly higher than those of the seeds cultivated in GuangShan, Xinxian and Luoshan. The values in our study are comparable to the report (Yao *et al.*, 2011) on the lipid content (46%) of the *C. oleifera* seed from Shangcheng (Henan, China), higher than those (27.21-37.37% with a mean value of 31.31%) of the *C. oleifera* seeds (Xianglin variety cultivated in Henan) as reported by Wang *et al.* (2008). As a comparison, the oil contents of *C. sasanqua* seeds (Gunstone *et al.*, 1994), *C. sinensis* seed (Sahari *et al.*, 2004) and *C. reticulate* Lindl seeds (Li *et al.*, 2013) were 56-70%, 30-33% and 49.4-51.4%, respectively.

3.2. Some physiochemical properties of *C. oleifera* oil

The acid values (AVs) of the *C. oleifera* oils in our study ranged from 0.22 to 5.17 mg KOH/g with an average value of 1.24±1.17 mg KOH/g (Table 1). The AVs of *C. oleifera* oils from the seeds harvested from Guangshan were 2.01±0.57 mg KOH/g, and similar to those of the oils from the seeds harvested from Xinxian (1.71±0.97 mg KOH/g), however higher than those of the oils from the seeds harvested from Shangcheng (0.33±0.10 mg KOH/g) and Luoshan (0.62±0.13 mg KOH/g) (Table 1).

The saponification values (SVs) of the *C. oleifera* oils in our study ranged from 178.90 to 196.91 mg KOH/mg with an average value of 190.91±4.06 mg KOH/g (Table 1). The SVs of *C. oleifera* oils from the seeds obtained from Guangshan were in the range of 189.69 to 196.91 with an average of 193.44±2.52 mg KOH/g, similar to those of the oils from the seeds harvested from Luoshan (in the range of 191.53 to 194.72 with an average of 192.60±1.28 mg KOH/g), however higher than those of the oils from the seeds harvested from Xinxian (in the range of 183.17 to 192.39 with an average of 188.88±3.85 mg KOH/g) and Shangcheng (in the range of 178.90-192.81 with an average of 187.68±5.33) (Table 1).

The iodine values (IVs) of the *C. oleifera* oils in our study ranged from 82.0 to 87.1 g I_2/100g with an average value of 84.3±1.5 g I_2/100g (Table 1). The IVs of *C. oleifera* oils among the seeds from Guangshang, Shangcheng, Xinxian, and Luoshan

TABLE 1. Fat content of the kernels of *Camellia oleifera* Abel and some characteristics of crude *C. oleifera* oils

Sample		Lipid content* (g/100g)	Acid value (mg KOH/g)	Saponification value (mg KOH/g)	Iodine value (g I_2/100g)	Refraction index (n_D^{25})
Gs $n = 7$	Range	28.39 - 45.38	1.04 - 2.71	189.69 - 196.91	82.0 - 85.5	1.4697 - 1.4713
	Mean	36.65 ± 5.18^{b}	2.01 ± 0.57^{a}	193.44 ± 2.52^{a}	84.6 ± 1.2	1.4706 ± 0.0005
Sc n = 5	Range	41.73 - 56.94	0.22 - 0.44	178.90 - 192.81	82.0 - 85.0	1.4684 - 1.4720
	Mean	49.66 ± 6.57^{a}	0.33 ± 0.10^{c}	187.68 ± 5.33^{c}	83.2 ± 1.4	1.4703 ± 0.0013
Xx n = 5	Range	32.58 - 42.47	0.35 - 5.17	183.17 - 192.39	82.1 - 87.1	1.4667 - 1.4715
	Mean	37.25 ± 3.90^{b}	1.71 ± 0.97^{ab}	188.88 ± 3.75^{bc}	84.6 ± 2.1	1.4701 ± 0.0019
Ls n = 5	Range	33.54 - 45.75	0.48 - 0.83	191.53 - 194.72	84.1 - 85.5	1.4706 - 1.4712
	Mean	42.41 ± 5.11^{b}	0.62 ± 0.13^{bc}	192.60 ± 1.28^{ab}	84.8 ± 0.5	1.4710 ± 0.0002
Total n = 22	Range	28.39 - 56.94	0.22 - 5.17	178.90 - 196.91	82.0 - 87.1	1.4667 - 1.4720
	Mean	41.05 ± 7.19	1.24 ± 1.17	190.91 ± 4.06	84.3 ± 1.5	1.4705 ± 0.0011

*, lipid content of the kernel of *Camellia oleifera* seed, on moisture free basis. Gs, seeds from Guangshang, Henan; Sc, seeds from Shangcheng, Henan; Xx, seeds from Xinxian, Henan; Xx, seeds from Luoshan, Henan. ^{a,b,c}, means within a column with different superscripts differ significantly at $p < 0.05$.

showed no significant differences (Table 1). The refraction index (n^{25}) of *C. oleifera* oils in our study ranged from 1.4667 to 1.4720 with an average of 1.4705±0.0011 (Table 1).

The SVs and IVs of the *C. oleifera* oils in our research are similar to those the *C. oleifera* oils from other province of China. The SV and IV of *C. oleifera Abel.* oil from the seed cultivated in Hunan (China) were 180.5 mg KOH/g and 81.5 g I_2/100g, respectively (Yun *et al.*, 2011). The IV and SV of the *C. oleifera* oil from the seed cultivated in Fujian (China) were 85±2 g I_2/100g and 195±1 mg KOH/g, respectively (Yu *et al.*, 2013).

As a comparison, the refractive index (n^{25}), SV, and IV of *Camellia sasanqua* oil were 1.467-1.469, 193-196 mg KOH/g, and 83-89 g I_2/100g, respectively (Gunstone *et al.*, 1994). The IV and SV of the oils from tea seeds (Lahijan variety of Iranian tea seed, Southern Indian tea seed, and Turkish tea seed) were in the range of 85-91 g I_2/100g and 192-194 mg KOH/g, respectively (Sahari *et al.*, 2004). The refractive index (n^{40}) of *Camellia reticulate f. simpex* seed oil was 1.4711-1.4736 (Huang *et al.*, 2010). The SV and IV of *Camellia sinensis O.Ktze* oil were 189.7 mg KOH/g and 88.5 g I_2/100g, respectively (Yun *et al.*, 2011). The IV, SV and refractive index (n^{40}) of *C. reticulate* Lindl seed oil were 72.1-79.8 g I_2/100g, 174.1-185.0 mg KOH/g and 1.4727, respectively (Li *et al.*, 2013).

3.3. Fatty acids composition and positional distribution of *C. oleifera* Oil

The fatty acid composition of the *C. oleifera* oils in our research was 7.07-9.52% palmitic, 0.09-0.22% palmitoleic, 1.45-2.94% stearic, 77.02-84.33% oleic, 5.19-11.23% linoleic 0.53-0.70% linolinic, and 0.22-0.70% arachidic acid (Table 2). The proportion of unsaturated fatty acids (UFAs) and saturated fatty acids (SFAs) were 87.80-90.30% and 9.69-12.19%, respectively. And the ratios of UFAs/SFAs were in the range of 7.20-9.32.

The palmitic contents of the oils from *C. oleifera* seeds obtained from Guangshan and Luoshang were higher than those of the oils from *C. oleifera* seeds cultivated in Shangcheng and Xinxian (Table 2). The oleic contents of the oils from *C. oleifera* seeds obtained from Guangshan, Xinxian and Luoshang were similar, and lower than that of the *C. oleifera* seeds obtained from Shangcheng (Table 2). Linoleic contents of the oils from *C. oleifera* seeds obtained from Guangshan, Xinxian and Luoshang were similar, and higher than that of the *C. oleifera* seeds obtained from Shangcheng (Table 2). The geographic differences among the seeds from different districts could be attributed to the differences in the fatty acid composition of the oils.

The fatty acid composition of the *C. oleifera* oils in our study was comparable to those of the oils

Table 2. Fatty acid composition of *C. oleifera* oils

Fatty acids	Gs, *n* = 7		Sc, *n* = 5		Xx, *n* = 5		Ls, *n* = 5		Total, *n* = 22	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean
C16:0	8.61 - 9.13	8.92 ± 0.18^{a}	7.07 - 9.52	8.24 ± 0.84^{c}	7.84 - 8.89	8.45 ± 0.46^{bc}	8.43 - 9.01	8.83 ± 0.19^{ab}	7.07 - 9.52	8.64 ± 0.54
C16:1	0.14 - 0.19	0.17 ± 0.02^{a}	0.09 - 0.15	0.11 ± 0.02^{b}	0.11 - 0.22	0.16 ± 0.04^{a}	0.11 - 0.14	0.12 ± 0.01^{b}	0.09 - 0.22	0.14 ± 0.03
C18:0	1.90 - 2.14	2.01 ± 0.09^{b}	2.08 - 2.71	2.34 ± 0.24^{a}	1.45 - 2.94	2.16 ± 0.60^{ab}	2.03 - 2.37	2.20 ± 0.12^{ab}	1.45 - 2.94	2.16 ± 0.33
C18:1	77.14 - 79.47	78.14 ± 0.75^{b}	77.38 - 84.33	81.09 ± 2.58^{a}	77.02 - 81.73	79.41 ± 1.76^{b}	78.00 - 78.70	78.41 ± 0.20^{b}	77.02 - 84.33	79.16 ± 1.89
C18:2	8.72 - 10.54	9.76 ± 0.61^{a}	5.19 - 9.91	7.32 ± 1.96^{b}	6.68 - 11.23	8.75 ± 1.85^{a}	8.92 - 9.98	9.49 ± 0.34^{a}	5.19 - 11.23	8.91 ± 1.60
C18:3	0.57 - 0.66	0.61 ± 0.03^{b}	0.53 - 0.70	0.62 ± 0.05^{ab}	0.58 - 0.70	0.65 ± 0.04^{a}	0.60 - 0.69	0.65 ± 0.03^{a}	0.53 - 0.70	0.63 ± 0.04
C20:0	0.30 - 0.48	0.40 ± 0.05^{a}	0.22 - 0.39	0.29 ± 0.05^{b}	0.27 - 0.70	0.42 ± 0.15^{a}	0.23 - 0.42	0.30 ± 0.05^{b}	0.22 - 0.70	0.36 ± 0.10
SFAs	11.08 - 11.53	11.33 ± 0.16^{a}	9.69 - 11.99	10.86 ± 0.75^{b}	10.30 - 12.19	11.03 ± 0.65^{ab}	10.86 - 11.68	11.33 ± 0.26^{a}	9.69 - 12.19	11.15 ± 0.52
UFAs	88.46 - 88.92	88.67 ± 0.16^{b}	88.00 - 90.30	89.14 ± 0.75^{a}	87.80 - 89.70	88.97 ± 0.65^{ab}	88.31 - 89.14	88.67 ± 0.26^{b}	87.80 - 90.30	88.84 ± 0.52
UFAs/SFAs	7.67 - 8.03	7.83 ± 0.12^{b}	7.34 - 9.32	8.25 ± 0.65^{a}	7.20 - 8.71	8.10 ± 0.51^{ab}	7.56 - 8.21	7.83 ± 0.20^{b}	7.20 - 9.32	7.99 ± 0.43

Gs, seeds from Guangshang, Henan; Sc, seeds from Shangcheng, Henan; Xx, seeds from Xinxian, Henan; Ls, seeds from Luoshan, Henan. C16:0, palmitic acid; C16:1, palmitoleic acid; C18:0, stearic acid; C18:1, oleic acid; C18:2, linoleic acid; C18:3, linolenic; C20:0, arachidic. SFA, saturated fatty acid; UFA, unsaturated fatty acid.. ^{a,b,c}, means within a row with different superscripts differ significantly at $p < 0.05$.

from the *C. oleifera* seeds cultivated in the other provinces of China reported by previous researchers ($p<0.05$) (Cao *et al.*, 2017; Cheng *et al.*, 2010; Jiang *et al.*, 2010; Xi *et al.*, 2002; Yang, 2010; Yao *et al.*, 2011; Yu *et al.*, 2013; Yuan *et al.*, 2013). The oils from the *C. oleifera* seeds cultivated in Jinhua (Zhejiang, China) consisted of 8.10% palmitic, 0.10% palmitoleic, 1.90% stearic, 80.90% oleic, 8.10% linoleic acid, 0.30% linolenic, 0.60% eicosenoic acid, and UFAs were 90.00% (Cao *et al.*, 2017). The fatty acid composition of the oil from *C. oleifera* seeds cultivated in Hangzhou (Zhejiang, China) was 8.60% palmitic, 0.10% palmitoleic, 1.80% stearic, 78.55% oleic, 9.90% linoleic acid, 0.30% linolenic, 0.70% eicosenoic acid, and UFAs were 89.55% (Cao *et al.*, 2017). The fatty acid composition of the oils from the *C. oleifera* seeds cultivated in Hubei were 6.61-11.01% palmitic, 0.72-2.00% stearic acid, 65.70-84.48% oleic, and 6.14-13.37% linoleic (Cheng *et al.*, 2010). The fatty acid composition of the oils from 35 *C. oleifera* seeds cultivated in Guangxi was 6.68-12.23% palmitic, 1.24-3.21% stearic, 74.49-86.16% oleic, and 1.94-12.97% linoleic acid (Jiang *et al.*, 2010). The oils from *C. oleifera* seeds cultivated in the Jiangxi province consisted of 6.70-1.57% palmitic, 0.49-0.65% stearic, 72.1-90.1% oleic, 2.1-16.6% linoleic acid, and the UFAs were about 90% (Xi *et al.*, 2002). Yang (Yang, 2012) analyzed the fatty acid composition of the oils from 38 *C. oleifera* seeds cultivated in the Jiangxi province in the 2011 season, and the oils consisted of 7.19-9.17% palmitic, 1.50-3.46% stearic, 78.34-82.79% oleic, 6.37-10.05% linoleic acid, and the UFAs were over 88.76%. Yao *et al.* (2011), analyzed the fatty acid composition of the *C. oleifera* oils from several provinces (such as Guangxi, Jiangxi, Hunan, Hubei, Guangdong, Zhengjiang, Anhui, Fujian, and Guizhou) of China. These oils consisted of 7.16-8.87% palmitic, 1.42-2.78% stearic, 76.72-83.18% oleic, 6.59-9.71% linoleic, 0-1.18% linolenic, and 0-0.79% arachidic (Yao *et al.*, 2011). The fatty acid composition of the oil from the *C. oleifera* seeds cultivated in Fujian (China) was 10.20% palmitic, 1.70% stearic, 77.84% oleic, 8.30% linoleic, 0.37% linolenic, 0.59% arachidic (Yu *et al.*, 2013). Yuan *et al.* (2013) evaluated the fatty acid composition of 132 *C. oleifera* oils from the seeds collected from several provinces of China in 2009. The fatty acid composition of these oils was 7.03-13.85% palmitic, 1.35-5.49% stearic, 70.33-86.21% oleic, and 3.25-17.18% linoleic acid.

The fatty acid composition of the *C. oleifera* oils in our study was different from the results of Chen *et al.* (1996) and Ma *et al.* (2011). Chen *et al.* (1996) reported the major fatty acid composition of the oils from the *C. oleifera* seeds cultivated in Hunan province to be 11.32-16.40% palmitic, 63.51-75.08 oleic, and 9.91-19.76% linoleic acid. The study of Ma *et al.* (2011) showed that the fatty acid composition of the oil from *C. oleifera* obtained from Guangxi was 68-77% monounsaturated fatty acids, 7-14% polyunsaturated acids, and 16-18% saturated fatty acids, including 4.4-4.7% nonadecanoic acid (C19:0). The differences in the fatty acid compositions might be attributed to geographic and varietal differences.

The chemical, physical and biological characteristics of lipids are largely dependent on the amount and positional distribution of fatty acids on the glycerol backbone, and therefore, the stereospecific analysis of fatty acids in the triacylglycerol is considered very important when using the lipids for dietary and industrial purposes (Hunter, 2001). The major fatty acids in the sn-2 position of the triacylglycerols in the *C. oleifera* oils in our study were oleic (84.36-91.33% with an average value of 86.74±2.06%), followed by linoleic (7.14-14.22% with an average value of 12.01±2.03%) (Table 3). The palmitic, stearic and arachidic acid contents were lower than 1%, while palmitoleic and linolenic acids were not detected (Table 3). Oleic in the sn-2 position of the oils from the *C. oleifera* seeds obtained from Shangcheng were in the range of 94.65-91.33% with an average of 88.56±2.88%, similar to those of the oils from *C. oleifera* seeds obtained from Xinxian, and higher than those of the oils from C. oleifera seeds obtained from Guangshan and Luoshan. Linoleic in the sn-2 position of the oils from the *C. oleifera* seeds obtained from Luoshan were in the range of 13.27-13.85% with an average of 13.62±0.24%, about the same as those of the oils from the *C. oleifera* seeds obtained from Guangshan and Xinxian, and higher than those of the oils from the *C. oleifera* seeds obtained from Shangcheng. The SFAs content in the sn-2 position of *C. oleifera* oils in our research was in the range of 0.89-1.96% with an average of 1.25±0.25%, and UFA content in the sn-2 position of these oils was in the range of 98.04-99.11% with an average of 98.75±0.26% (Table 3). These differences might be caused by geographic differences.

In compliance with the general law for the fatty acid distribution of natural triacylglycerols in vegetable oils, UFAs occupied over 98% of the sn-2 position of the glycerol backbone, in which only a very small amount of thermodynamically unfavorable palmitic, stearic and arachidic acid were detected. As a comparison, the oleic acid in the sn-1, 2, and 3 positions of *C. japonica* L. oil were 66.0%, 93.6% and 94.7%, respectively (Noh and Yoon, 2012). For other high-oleic acid vegetable oils, such as Chufa tuber oil, the major fatty acid composition of the oil was 15.4% palmitic, 65.5% oleic, and 16.2% linoleic, while the major fatty acid composition on its sn-2 position was 75.1% oleic and 23.1% linoleic (Kim *et al.*, 2007). For olive oil, the major fatty acid composition of the oil was 12.1-16.3% palmitic

TABLE 3. sn-2 fatty acid composition of *C. oleifera* oils

Fatty acids	Gs, *n* = 7		Sc, *n* = 5		Xx, *n* = 5		Ls, *n* = 5		Total, *n* = 22	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean
C16:0	0.55 - 0.87	0.66 ± 0.11^{a}	0.42 - 0.80	0.53 ± 0.16^{ab}	0.38 - 0.87	0.54 ± 0.19^{ab}	0.38 - 0.58	0.47 ± 0.08^{b}	0.38 - 0.87	0.56 ± 0.15
C18:0	0.24 - 0.31	0.29 ± 0.03^{a}	0.20 - 0.38	0.31 ± 0.07^{a}	0.26 - 0.35	0.30 ± 0.04^{a}	0.08 - 0.28	0.19 ± 0.09^{b}	0.08 - 0.38	0.27 ± 0.07
C18:1	85.38 - 87.44	86.30 ± 0.63^{b}	84.65 - 91.33	88.56 ± 2.88^{a}	84.36 - 90.52	86.94 ± 2.49^{ab}	85.04 - 85.81	85.33 ± 0.37^{b}	84.36 - 91.33	86.74 ± 2.06
C18:2	11.34 - 12.96	12.31 ± 0.51^{a,b}	7.14 - 14.22	10.24 ± 3.00^{b}	8.51 - 13.68	11.74 ± 2.20^{a,b}	13.27 - 13.85	13.62 ± 0.24^{a}	7.14 - 14.22	12.01 ± 2.03
C20:0	0.37 - 0.47	0.44 ± 0.03^{a,b}	0.28 - 0.46	0.35 ± 0.07^{b}	0.33 - 0.74	0.49 ± 0.17	0.34 - 0.43^{a}	0.39 ± 0.04^{a,b}	0.28 - 0.74	0.42 ± 0.10
SFAs	1.22 - 1.65	1.39 ± 0.14^{a}	1.01 - 1.53	1.20 ± 0.22^{ab}	0.97 - 1.96	1.32 ± 0.39^{ab}	0.89 - 1.20	1.06 ± 0.14^{b}	0.89 - 1.96	1.25 ± 0.25
UFAs	98.34 - 98.78	98.61 ± 0.15^{b}	98.47 - 98.99	98.80 ± 0.22^{ab}	98.04 - 99.03	98.68 ± 0.39^{ab}	98.80 - 99.11	98.94 ± 0.14^{a}	98.04 - 99.11	98.75 ± 0.26

^{a,b,c}, means within a row with different superscripts differ significantly at $p < 0.05$.

and 74.3-77.5% oleic, while the major fatty acid composition on its sn-2 position was 82.2-88.4% oleic (Vichi *et al.*, 2007).

3.4. Tocopherols in crude *C. oleifera* Oil

The total tocopherol contents in the *C. oleifera* oils extracted by petroleum ether were in the range of 39.55-75.94 mg/100g with an average of 58.21±10.54 mg/100g, and the α-tocopherol and γ-tocopherol in these oils were 33.98-67.82 mg/100g with an average of 51.66±10.19 mg/100g and 5.57-8.27 mg/100g with an average of 6.55-8.27 mg/100g with an average of 6.55±0.76 mg/100g, respectively (Table 4). However, the total tocopherol contents in the *C. oleifera* oils extracted by ethyl ether were 14.91-44.36 mg/100g with an average of 30.35±7.77 mg/100g, the *α*-tocopherol and the *γ*-tocopherol contents in these oils were 9.42-37.08 mg/100g with an average of 23.74±7.44 mg/100g and 5.50-7.33 mg/100g with an average of 6.60±0.53 mg/100g, respectively (Table 4). *β*-tocopherol and *δ*-tocopherol were not detected in the *C. oleifera* oils extracted by petroleum ether or ethyl ether, which is consistent with the report of Li *et al.* (2009). The *α*-tocopherol contents in the oils extracted with petroleum ether (33.98-67.82 mg/100g with an average of 51.66±10.19 mg/100g were dramatically higher than those in the oils extracted with ethyl ether (9.42-37.08 mg/100g with an average of 23.74±7.44 mg/100g) (p<0.05) (Table 4). The γ-tocopherol contents in the oils from *C. oleifera* seeds obtained from different locations extracted by the same solvent showed no difference. Moreover, there was no difference between the γ-tocopherol contents in the oils obtained from the same seed extracted with different solvents (petroleum ether and ethyl ether) (Table 4).

Li *et al.* (2009) reported that the *α*- and *γ*-tocopherols in the *C. oleifera* oil were 1.5-187.7mg/100g and 0.66-1.32mg/100g, respectively. The study by Zhu *et al.*, (2011) showed that α-, γ-, and δ-tocopherols in tea seed (seed of *C. sinensis* O. Ktze) oil were 24.91-55.31mg/100g, 1.68-3.55 mg/100g, and 0.08-1.32 mg/100g, respectively, and β-tocopherol was not detected. In the report of Zhu *et al*. (Zhu *et al.*, 2013), total tocopherol was 29.78-58.63 mg/100g. In tea seed oils (seeds of *C. sinensis* O. Ktze), total tocopherols were 23.2-47.0 mg/100g, with the the major component being α-tocopherol with 22.6-45.0 mg/100g; and β-, γ-, and δ-tocopherol were lower than 2 mg/100g. In the research of Dong *et al.* (Dong *et al.*, 2012), the tocopherol content of tea seed oils (*C. sinensis L.* seed) extracted by press was 24.71 mg/100g with 24.15 mg/100g of α-tocopherol in the oils extracted by the soxhlet method with petroleum ether, the α-tocopherol content was 21.80 mg/100g, and β-, γ- and δ-tocopherols were not detected. In the oils extracted by supercritical CO_2 (15MPa, 35°C), the tocopherol content was 70.11 mg/100g, including

TABLE 4. Tocopherol composition of *C. oleifera* oils (mg/100g oil)

Sample		Tocopherol in oils extracted by petroleum ether					Tocopherol in oils extracted by ethyl ether				
		α	β	γ	δ	Total	α	β	γ	δ	Total
Gs $n = 7$	Range	54.84 - 62.56	-	5.95 - 8.27	-	61.3 - 70.81	17.03 - 33.27	-	5.84 - 7.33	-	22.87 - 40.46
	Mean	59.16 ± 2.68^{a}	-	6.85 ± 0.99^{a}	-	66.01 ± 3.25^{a}	24.99 ± 6.38^{a}	-	6.61 ± 0.57^{a}	-	31.60 ± 6.85^{a}
Sc $n = 5$	Range	36.26 - 61.60	-	6.01 - 6.96	-	42.70 - 67.61	15.30 - 31.90	-	5.75 - 7.13	-	22.21 - 39.03
	Mean	50.76 ± 9.67^{b}	-	6.42 ± 0.34^{a}	-	57.17 ± 9.57^{b}	23.97 ± 6.39^{ab}	-	6.76 ± 0.57^{a}	-	30.72 ± 6.64^{a}
Xx $n = 5$	Range	50.93 - 67.82	-	6.04 - 8.12	-	56.98 - 75.94	20.27 - 37.08	-	6.30 - 7.27	-	27.25 - 44.36
	Mean	56.40 ± 6.84^{ab}	-	6.64 ± 0.88^{a}	-	63.05 ± 7.70^{ab}	29.59 ± 6.60^{a}	-	6.83 ± 0.39^{a}	-	36.43 ± 6.65^{a}
Ls $n = 5$	Range	33.98 - 41.78	-	5.57 - 7.08	-	39.55 - 48.03	9.42 - 20.32	-	5.50 - 6.85	-	14.91 - 26.54
	Mean	37.32 ± 3.12^{c}	-	6.19 ± 0.57^{a}	-	43.51 ± 3.16^{c}	15.92 ± 4.81^{b}	-	6.22 ± 0.49^{a}	-	22.14 ± 4.97^{b}
Total $n = 22$	Range	33.98 - 67.82	-	5.57 - 8.27	-	39.55 – 75.94	9.42 - 37.08	-	5.50 - 7.33	-	14.91 - 44.36
	Mean	51.66 ± 10.19	-	6.55 ± 0.76	-	58.21 ± 10.54	23.74 ± 7.44	-	6.60 ± 0.53	-	30.35 ± 7.77

Gs, seeds from Guangshang, Henan; Sc, seeds from Shangcheng, Henan; Xx, seeds from Xinxian, Henan; Ls, seeds from Luoshan, Henan.
-, not detected. ^{a,b,c}, means within a column followed by the different superscripts represent a significant difference at $p < 0.05$.

68.90 mg/100g α-tocopherol. According to the three methods, supercritical CO_2 extraction might be the best one. Fazel *et al.* (Fazel *et al.*, 2008) evaluated the β-carotene, vitamin E (8 compounds) and polyphenols (8 compounds) in oils obtained from *C. sinensis* seeds, and the results showed that the total content of tocopherols and tocotrienols were 376.0 ± 3.0 mg/kg and 13.4 ± 0.34 mg/kg, respectively, and in vitamin E; the α-tocopherol content (210.0 ± 2.3 mg/kg) was higher than the others.

In conclusion, this work has presented the general properties of the oils from *C. oleifera* seeds cultivated in south Henan of China along with their fatty acid profile. It turns out that physiochemical properties of the oils from the *C. oleifera* seeds cultivated in south Henan of China are similar to those of the oils from the *C. oleifera* seeds cultivated in other districts of China, and could be a good source of natural oil rich in oleic acid and tocopherols.

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