Fatty acid composition and volatiles of *Camellia oleifera* oil by GC and SPME/GC-MS

**Jiao Jiao YUAN** 1,2, **Jun Ling TU** 2, **Frank G. F. QIN** 2 *, **Bing LI** 1 *

1School of Food Science and Engineering, South China University of Technology, Guangzhou, Guangdong 510640, China
2School of Chemical Engineering and Energy Technology, Dongguan University of Technology, Dongguan, Guangdong 523808, China

Email: yuanjj@dgut.edu.cn

**Abstract.** *C. oleifera* oil is used extensively for cooking oil in our southern China, because of its high nutrition and pharmacological activity. The physico-chemical property of *C. oleifera* oil were analyzed. The results showed that the oil content of *C. oleifera* breeds were 42.28% and 35.84% by extraction method, respectively, from Yunnan and Zhejiang province. The main FA composition of *C. oleifera* oil was similar from two breeds by GC, which was composed of palmitic, stearic, oleic and linoleic acids. In addition, aldehydes, alkenes and acids consisted of volatiles composition pattern of the two *C*. oils by solid-phase-micro-extraction gas chromatography - mass spectrometer (SPME/GC-MS), and aldehydes content was the highest.

1. Introduction

*Camellia oleifera* Abel. (*C. oleifera*), mainly distributed in southern China, such as Jiangxi, Yunnan, Zhejiang and Guangxi provinces, etc., is an important woody oil crop [1]. *C. oleifera* oil, produced from the *C. oleifera* seeds by cold press, solvent extraction, or aqueous enzymatic treatment, is used extensively for cooking oil. The oil is similar to olive oil, having a high percent of oleic acid (75–80%) [2] and high value for human health. The oil, has high content of oleic acid, plays important roles in reducing cholesterol and triglycerides in the blood [3], and is traditionally applied as a health caring medicine to prevent coronary heart disease, hypertension, cerebrovascular disease, arteriosclerosis, stomachache as well as burning injury in China [4]. Therefore, the oil content and fatty acid composition of *C. oleifera* seeds are the most important factors to evaluate the quality traits and productivity of *C. oleifera* oil.

Smell is the synergisitic effect of all volatile components. For a long time, there is no better way to control the quality of synergistic effects, except artificial sensory detection. However, artificial sensory detection and control are always abstract and subjective judgments. Zhong et al. [5] studied the sensory evaluation of *C. oleifera* oil by different extraction processes, which was the best in the taste of the squeeze method, but did not study the volatile compounds of *C. oleifera* oil. Hai et al. [6] used the electronic nose to successfully distinguish among *C. oleifera* oil, corn oil and sesame oil. The fragrance of *C. oleifera* oil has its own unique smell and composition pattern, and therefore it is valuable to study the volatile composition of *C. oleifera* oil.

Solid-phase microextraction (SPME) is proposed and developed rapidly in the early 1990 s, and is sensitive, convenient and solvent-free, relatively independent of the instrument design, easy automation.
Moreover, it is suitable for pretreatment technology of the gas and liquid samples of new samples [7]. SPME technology is to adsorb and enrich the target material in the sample, based on the fused silica fiber as fixed phase (polyacrylate (PA), polydimethylsiloxane (PDMS), carbowax/divinylbenzene (CW/DVB) and carboxen/polydimethylsyloxane(CAR/PDMS) et al) [8]. At present, SPME has been widely used in food [9, 10], environment [11, 12], and natural products fields [13].

The purpose of this paper is to, on the basis of previous research, study the oil content and fatty acid composition of C. oleifera oil from Yunnan and Zhejiang provinces, and analyze volatile compounds by SPME/GC-MS, summarize its characteristic components to form inherent aroma mode of C. oleifera oil.

2. Materials and methods

2.1 Materials

C. oleifera seeds were collected from Yunnan and Zhejiang provinces in October 2016. All samples were peeled for experiments. Standard methyl ethers of oleic, linoleic, palmitic, stearic acids were purchased from Aladdin Chemicals, China. Other chemical reagents of analytical grade were obtained from by Guangzhou Chemical Reagent Co., Ltd., China.

2.2 Extraction of C. oleifera oil by Soxhlet method

C. oleifera seeds were powdered by a laboratory plant grinder. Approximately 10 g of ground sample were weighed and recorded as \( w_0 (g) \), then transferred to a Soxhlet extractor filled with 180 mL petroleum ether (60–90°C), and extracted at 88°C for 6 h. Finally, the solvent was evaporated under vacuum. The residual was dried at 60 °C in vacuum to a constant weight of \( w_1 (g) \). The oil content was calculated and expressed according to the formula: \( w = w_1/w_0 \times 100\% \).

2.3 FA analysis of the extracted C. oleifera oil by GC

As FA of C. oleifera oil presented in the form of fatty acid triglycerides in general, it must be transformed to be methyl esters of fatty acids means of sodium hydroxide. According to Chinese Standard GB/T 22223–2008, 0.2 mL of the extracted C. oleifera oil were put in 10 mL tube. Two mL of 0.5 mol/L sodium hydroxide–methanol was added into the tube, shook, and then placed at 60 °C in water-bath for 30 min, 5 mL n-hexane were added. The supernatant was taken for injection to a gas chromatography spectrometer (HP6890 series, Agilent Technologies Inc.), equipped with a Hp-5 capillary column (30 m×0.25 mm×0.25μm). The injector and detector temperature were set at 280°C. The oven temperature was programmed from 100 °C to 270°C with a speed of 5°C/min and a final hold of 5 min. The signals from the detector were integrated as normalized percentages from the calibration curve by the HP software, and the main four individual fatty acid (oleic, linoleic, palmitic, stearic acid) were expressed as % of the total fatty acids. The unsaturated acids were considered as the sum of the oleic acid and linoleic acid.

2.4 Analysis of volatiles by SPME/GC-MS

1.00 g C. oleifera oil sample was taken into a 20 mL sealing cover bottle with magneton, and was preheated at 100 °C for 10 min. The solid-phase-micro-extraction (SPME) device was inserted into empty bottles through the lid, and fiber needle (CAR/PDMS) was put on the sample surface about 1 cm. Fiber needle was extracted for 60 min of equilibrium time, then SPME was inserted into gas injection port quickly, and was desorbed under 250°C for 5 min in the splitless mode. The fiber needle was withdrawn in the device and SPME was pulled out of injection port. Then gas chromatography - mass spectrometry instrument was testing.

GC-MS was performed using a HP-5MS column (30 m × 0.25 mm × 0.25 μm). The column was held at 45°C for 2 min followed by a ramped temperature increase to 250°C for 5 min at a rate of 4°C/min. Carrier gas was helium and the injector’s and MSDs’ transfer line temperature were held at 280°C. No split ratio was used and the MS was operated in EI mode (70 eV) at a source temperature of 230°C. The eluted products were identified and analyzed using Nist02 library.
3. Results and discussion

3.1 Oil content of C. oleifera seeds
Oil content of C. oleifera seeds were obtained by Soxhlet method, and the two seeds had different results. The oil content of Yunnan province was 42.28%, and the Zhejiang province was 35.84%. Therefore, C. oleifera breeds of high oil content from Yunnan province will be chosen as good cultivars to breed and cultivate in a large scale, at the meantime, they will play a significant role in providing materials for the processing of C. oleifera oil in industrial scale.

3.2 FA composition of C. oleifera oil
FA composition of C. oleifera oils were analysed by traditional GC method (seen in Fig. 1), and FA content of C. oleifera oils was showed in Table 1. We can see the main FA composition of C. oleifera oil is similar from two breeds, which is composed of palmitic, stearic, oleic and linoleic acids. However, the relative content of individual FA has great difference among them (Table 1). The result may be related to different breeds. Oleic acid ranges from 73.40% to 81.82%, linoleic acid from 6.53% to 13.11%, palmitic acid from 8.78% to 11.68%, and stearic acid from 1.81% to 2.87%. Moreover, unsaturated FA content of Yunnan was higher than that of Zhejiang. In contrast to reported studies [14, 15], the total unsaturated FA of C. oleifera oil was much higher in this study.

![Fig. 1 GC chromatogram of fatty acid methyl esters](image)

Table 1 Individual fatty acid content from different breeds of C. oil (%)

| Breeds    | Oleic acid | Linoleic acid | Palmitic acid | Stearic acid | Unsatrated FA |
|-----------|------------|---------------|---------------|--------------|---------------|
| Yunnan    | 81.82      | 6.53          | 8.78          | 2.87         | 88.05         |
| Zhejiang  | 73.40      | 13.11         | 11.68         | 1.81         | 86.51         |

3.3 Volatiles by SPME/GC-MS
The SPME/GC-MS total ion chromatograms (TIC) of two C. oils were seen in Fig. 2. Compared to the Nist02 mass spectrometry standard library, products were identified combined with mass spectrometry analysis of each substance. Furthermore, their GC relative contents were calculated according to the area normalization method.

The volatiles of C. oil of Yunnan Province were identified for 47 compounds, and the result was showed in Table 2. It consisted of 12 aldehydes, 10 acids, 6 ketones, 5 alcohols, 4 alkenes and other 2 compounds, which were 96.99% of the total content. The top 10 content was nonanoic acid (17.77%), octanoic acid (8.78%), oleic acid (7.43%), maltol (6.19%), palmitic acid (5.47%), benzaldehyde (4.44%), (E)-2-decenal (4.36%), 2-undecenal (4.25%), hexanoic acid (3.80%), nonanal (3.76%), and they were 66.25% of the total content.

The volatiles of C. oil of Zhejiang Province were identified for 51 compounds, and the result was showed in Table 3. It consisted of 16 aldehydes, 7 alkenes, 6 acids, 5 esters, 4 alcohols and other 8 compounds, which were 100% of the total content. The top 10 content was nonanal (7.73%), benzaldehyde (7.26%), 2-nonenioic acid (5.02%), palmitic acid (4.77%), 2-undecenal (4.43%), styrene (4.42%), oleic acid (3.99%), (E)-2-decenal (3.93%), phenylacetaldehyde (3.68%) and 2,4-nonadienal (3.35%), and they were 48.58% of the total content.

The volatiles of the two C. oil mainly be aldehydes, alkenes and acids, and the aldehydes content was the highest. The two oils had 23 same compounds, but the content was different, respectively. Long [16] studied the volatiles of C. oil from Jiangxi provinces at 40°C, and he considered the top 10 was nonanal, α-pinene, ethyl acetate, pentanol, benzaldehyde, octanol, α-xylene, nonanol, decanol, octanal. They were 68.40% of the total compounds. It was different from our results, and it might be due to the different experimental condition.

![Figure 2 SPME/GC-MS total ion chromatograms (TIC) of two C. oils](image)

Table 2 Volatiles of Yunnan C. oil

| No. | Retention time/min | Molecular formula | Chemical name | GC content/% |
|-----|-------------------|-------------------|---------------|--------------|
| 1   | 5.22              | C₆H₁²O            | Hexanal       | 0.80         |
| 2   | 8.68              | C₈H₁₆            | Styrene       | 1.54         |
| 3   | 11.02             | C₆H₁₆            | 3-Isopropyl-1-cyclohexene | 0.05      |
| 4   | 11.65             | C₆H₁₂O          | Benzaaldehyde | 4.44        |
| 5   | 12.32             | C₇H₁₆O          | Heptanol      | 0.72         |
| 6   | 13.08             | C₇H₁₈O          | 3,6,9-trioxaundecaidioic acid | 0.75   |
| 7   | 13.33             | C₇H₁₀O          | (E,E)-2,4-Heptadienal | 1.67        |
| 8   | 13.61             | C₈H₁₈O          | Octanal       | 2.00         |
| 9   | 14.86             | C₈H₁₆O₂         | Hexanoic acid | 3.80         |
| 10  | 15.97             | C₈H₁₂O          | (E)-2-Octenal | 1.16         |
| 11  | 16.53             | C₈H₁₄O          | (E,E)-3,5-Octadien-2-one | 1.06 |
| 12  | 16.64             | C₈H₁₈O          | Octanol       | 0.81         |
| 13  | 17.94             | C₈H₁₆O          | Nonanal       | 3.76         |
| No. | Retention time/min | Molecular formula | Chemical name | GC content/% |
|-----|-------------------|-------------------|---------------|--------------|
| 1   | 5.27              | C₅H₁₀O           | Hexanal       | 1.03         |
| 2   | 8.73              | C₆H₁₃             | Styrene       | 4.42         |
| 3   | 11.06             | C₆H₁₁O           | 1-acetyl-1-cyclohexene | 2.46 |
| 4   | 11.57             | C₇H₁₄O           | (E)-2-Heptenal | 0.48         |
| 5   | 11.70             | C₇H₁₄O           | Benzaldehyde  | 7.26         |
| 6   | 13.13             | C₈H₁₇O           | (E,E)-2,4-Nonadienal | 3.35     |
| 7   | 13.64             | C₈H₁₇O           | Octanal       | 2.62         |
| 8   | 13.94             | C₈H₁₇O           | (E,E)-2,4-Heptadienal | 0.95   |
| 9   | 14.52             | C₁₀H₁₄            | α-Cymene      | 0.70         |
| 10  | 14.69             | C₁₀H₁₆            | D-Limonene    | 2.94         |
| 11  | 15.17             | C₁₀H₂₀            | Benzyl Alcohol | 0.72         |
| 12  | 15.35             | C₁₀H₂₀            | Phenylacetaldehyde | 3.68   |
| 13  | 15.99             | C₁₀H₂₀            | (E)-2-Octenal | 1.81         |
| 14  | 16.32             | C₁₀H₂₀            | Acetophenone  | 1.46         |
| 15  | 16.56             | C₁₀H₂₀            | (E,E)-3,5-Octadien-2-one | 1.19    |
| 16  | 16.66             | C₁₀H₂₀O          | Octanol       | 1.44         |
| 17  | 17.97             | C₁₀H₂₀O          | Nonanol       | 7.73         |

Table 3 Volatiles of Zhejiang C. oil
4. Conclusion
The physico-chemical property of C. oleifera oil were analyzed by Soxhlet method, GC and SPME/GC-MS. The results showed that the oil content were 42.28% and 35.84%, respectively, from Yunnan and Zhejiang provinces. The main FA composition of C. oleifera oil were composed of palmitic, stearic, oleic and linoleic acids. In addition, volatiles of the two C. oils mainly be aldehydes, alkenes and acids by SPME/GC-MS, and aldehydes content was the highest.

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