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Profiling and quantification of phenolic compounds in *Camellia* seed oils: Natural tea polyphenols in vegetable oil

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ABSTRACT

In Asia, tea seed oils (seed oils from *Camellia oleifera*, *C. chekiangoleosa*, and *C. sinensis*) are used in edible, medicinal, and cosmetic applications. However, these oils differ in their fatty acid contents, and there is little known about their phenolic compounds. Here we analyzed the phenolic compounds of seed oils from three species gathered from 15 regions of China. Twenty-four phenolic compounds were characterized by HPLC-Q-TOF-MS, including benzoic acids (6), cinnamic acids (6), a hydroxyphenylacetic acid, flavanols (4), flavonols (3), flavones (2), and dihydroflavonoids (2). Some of these phenolic compounds had not previously been reported from *C. sinensis* (20), *C. oleifera* (15), and *C. chekiangoleosa* (24) seed oils. Quantification was done by HPLC-QqQ-MS using 24 chemical standards. The total concentrations in the studied samples ranged from 20.56 to 88.56 μg/g. Phenolic acids were the most abundant class, accounting for 76.2–90.4%, with benzoic acid, found at up to 18.87 μg/g. The concentration of catechins, typical of tea polyphenols, ranged between 2.1% and 9.7%, while the other flavonoids varied from 4.2% to 17.8%. Although the cultivation region affected the phenolic composition of the *Camellia* seed oils, in our hierarchical clustering analysis, the samples clustered according to species. The phenolic composition of the seed oils from *C. oleifera* and *C. chekiangoelosa* were similar. We found that the phenolic categories in *Camellia* seed oils were similar to tea polyphenols, thereby identifying a source of liposoluble tea polyphenols and potentially accounting for some of the reported activities of these oils. In addition, this work provides basic data that allows distinction of various *Camellia* seed oils, as well as improvements to be made in their quality standards.

1. Introduction

Camellia L. plants originated from the Yunnan-Guizhou Plateau in China and are mainly distributed in eastern and southeastern Asia. These are important economic plants in this genus. For instance, tea is produced from leaves of *C. sinensis* (L.) O. Ktze, which is considered a healthy drink because of its tea polyphenols content. Tea polyphenols are reported to have antioxidant, antifungal, antibacterial, and anticancer activities (Bashir et al., 2014; Lee & Yen, 2006; Li et al., 2008; Sabaghi, Maghsoudlou, Khomeiri, & Ziaiifar, 2015). Moreover, *C. sinensis* seeds have been developed in recent years as edible oil resources; they contain about 30% oil, with 46.3–56.3% oleic acid, and 22.7–29.4% linoleic acid (Wang, Zeng, Verardo, & Contreras, 2017). Tea trees are grown in over 60 countries all over the world, and the area totals about 4.4 million ha so that the potential of oil production is large. In addition, there are two major oil crops, *C. oleifera* Abel and *C. chekiangoleosa* Hu, which have been cultivated widely in the south of China for thousands of years. The oil content of *C. oleifera* and *C. chekiangoleosa* seeds ranges from 40 to 60%. According to the latest official data from the State Forestry Administration in 2014, the cultivation area of *C. oleifera* and *C. chekiangoleosa* was 3.65 million ha and produced 2.02 million tons of seeds. These two oil crops are also cultivated in other Asia countries (e.g., Vietnam, Japan, and Thailand). The seed oils from these two species have similar fatty acid compositions (76.0–79.5% oleic acid) (Wang et al., 2017), and are referred to as eastern olive oil. These two seed oils are not clearly differentiated and share the same quality standard in China. They are exported from China to France, USA, and Japan for use as cosmetic materials. Interestingly, the seed oils from the three above-mentioned species differ in their fatty acid composition, but all have been used as medicine or cosmetics in many Asia countries, which might be related to their antioxidant, antimicrobial, and anti-inflammatory activities (Fazel, Sahari, & Barzegar,

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2008; Lee & Yen, 2006; Shen, Zhang, Tian, & Hua, 2012).

Thus, *Camellia* seed oils might have similar bioactivities to tea. In fact, several tea polyphenols have been reported in *Camellia* seed oils. For instance, (+)-catechin, (−)-epigallocatechin-gallate, (−)-epigallocatechin, (−)-epicatechin-gallate, and (−)-gallocatechin have been reported in *C. sinensis* seed oil in Iran (Fazel et al., 2008). In addition, (−)-epigallocatechin was also reported in the seed oil of *C. oleifera* (Fang, Du, Luo, & Jin, 2015). As for *C. chekiangoleosa*, few findings have been reported, and it is plausible that its seed oil might have been mistaken for the seed oil from *C. oleifera*. Therefore, the reports about the distinction between the three types of tea seed oil are limited. Nevertheless, the characterization of the phenolic compounds in different *Camellia* seed oils is necessary to enable further research about their activities, to distinguish between these oils, to improve their quality standards, and help producers respond to market trends for healthy edible oil.

The coupling of high performance liquid chromatography (HPLC) to accurate mass spectrometry (MS) in qualitative analyses has accelerated the identification of natural products (M. J. Lee, Park, Choi, & Jung, 2013; Li et al., 2017; Liu, Zhuang, Song, Lu, & Xu, 2017). The use of a quadrupole-time-of-flight (Q-TOF) mass analyzer has enabled the characterization of hundreds of phenolic structures in plant foods (Abu-Reidah, Contreras, Arraez-Roman, Fernandez-Gutierrez, & Segura-Carretero, 2014; Ammar, del Mar Contreras, Belguith-Hadrich, Segura-Carretero, & Bouaziz, 2015). This facilitates distinction among isobaric ions with high sensitivity and selectivity and provides the molecular formula to aid in the identification of compounds. In addition, accurate mass analysis of product ions in the MS/MS mode helps with structural elucidation and identification (J. Lee, Ebeler, Zweigenbaum, & Mitchell, 2012). Meanwhile, HPLC coupled with a QqQ-MS analyzer is widely applied in quantitation of single polyphenols using the multiple reaction monitoring (MRM) mode. A QqQ-MS analyzer first selects compound-specific precursor ions, then fragments these in the collision cell, and further scans the desired product ions by the last quadrupole for MS detection (Engstrom, Palijarvi, & Salminen, 2015).

Therefore, here we aimed to determine the phenolic profiles in the seed oils of the three above-mentioned species, which were obtained from 15 representative samples from the main production regions distributed around China. The phenolic compounds were analyzed qualitatively by HPLC-Q-TOF-MS and quantitatively by HPLC-QqQ-MS. The data presented here will provide a basis to further explore the activities of these seed oils, will help to distinguish between the seed oils of these three species, and supplement their quality standards with phenolic compound data.

2. Materials and methods

2.1. Reagents and standards

The solvents methanol and acetonitrile were of HPLC grade (Sigma-Aldrich, St. Louis, MO, USA). The standards benzoic acid, *p*-hydroxybenzoic acid, protocatechuic acid, gallic acid monohydrate, phthalic acid, *p*-hydroxyphenylacetic acid, vanillic acid, cinnamic acid, *p*-coumaric acid, caffeic acid, ferulic acid, sinapic acid, chlorogenic acid, epicatechin, apigenin, kaempferol, luteolin, quercetin, myricetin, naringenin, and taxifolin were purchased from Aladdin Co., Ltd. (Shanghai, China). Catechin, epigallocatechin, and epigallocatechin gallate were purchased from Sigma-Aldrich.

2.2. Sampling and sample preparation

The present work was carried out on 15 samples from the major *Camellia* cultivation regions of China, including six *C. sinensis* samples from Henan Xinyang (HNXY), Jiangxi Lushan (JXLS), Shandong Rizhao (SDRZ), Hubei Enshi (HBES), Zhejiang Hangzhou (ZJHZ), and Fujian Quanzhou (FJQZ); six *C. oleifera* samples from Anhui Huangshan (AHHS), Jiangxi Ganzhou (JXGZ), Guangxi Liuzhou (GXLZ), Hunan Chenzhou (HNCZ), Hunan Huaihua (HNHH), and Fujian Shanming (FJSM); and three *C. chekiangoleosa* samples from Jiangxi Dexing (JXDX), Zhejiang Kaihua (ZJKH) and Hunan Loudi (HNLD). Random sampling was conducted, and the seeds were then mixed evenly in each site. After being dried and unshelled, the seeds were crushed using a screw press to obtain oil. The oil samples were placed in glass bottles, which were completely filled to exclude any headspace volume, and stored in the dark at room temperature until further extraction.

2.3. Extraction of phenolic compounds from Camellia seed oil

Oil samples were extracted as follows: 2.5 g of oil was weighed into a centrifuge tube. Then, 5 mL of *n*-hexane and 6 mL of methanol-water (60:40, v/v) were added. The mixture was stirred for 3 min in a vortex apparatus, and subsequently, the tube was centrifuged at 3500*g* for 10 min at 4 °C. The methanol solution was separated, and the operation was repeated three times. The methanolic extracts were combined and evaporated to dryness at 35 °C. The residue was redissolved in 250 μL methanol-water (50:50, v/v), and filtered through 0.2 μm organic membrane filters for further analysis.

2.4. HPLC-ESI-Q-TOF-MS analysis

The phenolic compounds of the extracts were characterized using HPLC (Agilent 1200 series rapid resolution, Agilent Technologies, Santa Clara, CA, USA) coupled to a quadrupole-time of flight (Q-TOF) 6540 ultrahigh definition accurate mass spectrometer. Separation was achieved on a SPURSIL-C18 column (2.1 μm × 150 mm × 2.1 mm) from Dikma (Beijing, China), thermostated at 30 °C, at a flow rate of 0.4 mL/min, using a 5 μL of injection volume. The mobile phase was composed of 0.1% acetic acid in water (A) and 0.1% acetic acid in acetonitrile (B). The gradient elution involved a three-step program: 0–20 min, 0–50% B; 20–25 min, 50–100% B; 25–27 min, 100–0% B. A post-run of 5 min was programmed to equilibrate the column between analyses.

The electrospray ionization (ESI) source was operated in the negative ionization mode. The mass spectrometer parameters were as follows: capillary 4000 V; nebulizer pressure, 30 psi; fragment voltage, 140 V; drying gas flow rate, 9 L/min; gas temperature, 190 °C; N_2 sheath gas temperature, 350 °C; N_2 sheath gas flow rate, 10 L/min. The accurate mass spectra were recorded across the range of m/z 100–1000 in full scan mode. To assure the desired mass accuracy of the recorded ions, continuous internal calibration was performed during analyses with the use of signals at m/z 112.9855 and 1033.9881. Optimal ionization conditions were evaluated by a tuning fluid, and the instrument provided a resolution of at least 100,000. Fragmentation was performed using a collision energy of 10–40 eV. The full-scan data were processed using Agilent Mass Hunter software version and the Qualitative Analysis Software B.05.00.

For the qualitative analysis, an in-house database of phenolic compounds from *Camellia* plants (106 compounds) was set up based on the relative literature and databases, including ChemSpider, Pubmed, SciFinder, and the Chemistry database of CAS (Chinese Academy of Sciences).

2.5. HPLC-QqQ-MS analysis

The quantitative analysis was performed on an Agilent 6460 equipped with a triple-quadrupole (QqQ) mass analyzer, which was operated in the negative ionization mode. The LC separation and mass analyzer parameters were the same as for the HPLC-ESI-Q-TOF-MS. However, the gradient elution program was adjusted as followed: 0–30 min, 0–10% B; 30–50 min, 10–40% B; 50–55 min, 40–0% B. The collision gas was high-purity argon. In addition, the collision energy and cone voltage were optimized for each ion. This means that, in the

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