Original paper

Physicochemical Properties and Lipid Composition of Camellia Seed Oil (Camellia oleifera Abel.) Extracted Using Different Methods

Xuezhi Fang¹²*, Menghao Du², Fan Luo² and Yongfeng Jin¹*

¹College of Life Science, Zhejiang University, 866 Hangtang Road, Hangzhou 310051, China
²Research Institute of Subtropical Forestry, CAF, 73 Daqiao Road, Fuyang 311400, China

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To understand the influence of different extraction methods on properties of Camellia seed oil, the physicochemical properties, fatty acid composition, bioactive compounds content of camellia seed oil extracted by aqueous enzymatic extraction, expeller pressing, organic solvent extraction and supercritical CO₂ extraction were investigated. No significant differences were found among acid values of extracted oils. The peroxide value of hexane-extracted oil and expeller-pressed oil was significant higher than aqueous enzymatic-extracted oil and supercritical CO₂-extracted oil. The major fatty acids present in camellia seed oil were palmitic acid, stearic acid, oleic acid, and linoleic acid. No significant differences were found in the amounts of the major fatty acids in the oils. The aqueous enzymatic-extracted oil had a higher content of total monounsaturated fatty acids, α-tocopherol, β-carotene, squalene and phytosterol than hexane-extracted and expeller-pressed oils. Nine phenolic acids were detected in camellia seed oils, and 3-hydroxytyrosol, benzoic acid, catechins, 4-hydroxybenzoic acid and chlorogenic acid were the predominant compounds. The content of phenolic compounds in the aqueous enzymatic-extracted camellia seed oil was lower than that of other extracted oils. The phospholipid content of the aqueous enzymatic-extracted oil and the supercritical CO₂-extracted oil was significantly lower than that of hexane extracted oil and expeller pressed oil.

Keywords: Camellia seed oil (Camellia oleifera Abel.), aqueous enzymatic extraction, expeller pressing, organic solvent extraction, supercritical CO₂ extraction, physicochemical properties, lipid composition

Introduction

Camellia (Camellia oleifera Abel.) is a common oil-bearing woody plant in southern China, and camellia seed oil has been used extensively in China for cooking over 1,000 years (Zhong et al., 2007) and also as a traditional medicine for stomachaches and burns (Lee et al., 2006). At present, the planting area of the Camellia plant is more than 4 million hectares, and camellia seed oil production is approximately 250 million kilograms (Zhuang, 2008). Camellia seed oil is important edible oil that is pleasant-tasting and has a fatty acid profile similar to that of olive oil, with oleic acid as the predominant fatty acid (74 – 85%). Oils high in oleic acid have been demonstrated to be very stable, even at high temperatures such as those used to fry food (Abdulkarim et al., 2005; Warner et al., 1997).

Hydraulic pressing, expeller pressing and organic-solvent extraction are the most common methods for recovering oil from seeds (Rosenthal et al., 1996). At present, camellia seed oil is obtained either through hexane extraction or a process that combines expeller pressing and hexane extraction (Zhang et al., 2012). These methods are very effective, providing an oil yield greater than 95% (Xie et al., 2011). However, considering the safety and environmental issues associated with the use of hexane, methods other than hexane-based extraction have been investigated. As alternative processes, aqueous-enzymatic
extraction and supercritical-CO$_2$ extraction have been conducted in the laboratory and at the pilot industrial scale (Dominguez et al., 1994; Galvao et al., 2013; Rosenthal et al., 1996). As an important edible oil in China, new extraction method such as aqueous enzymatic extraction for camellia seed oil have been used in pilot scale in Hunan province. Currently, aqueous-enzymatic extraction (Yu et al., 2013; Zhang et al., 2012) and supercritical-CO$_2$ extraction (Wang et al., 2011) are being applied to recover oil from camellia seeds. Some studies have optimized the extraction conditions, and some have focused on the physicochemical characteristics of oils (Fang et al., 2014; Li et al., 2011; Wang et al., 2011; Xie et al., 2011). However, few reports have focused on the effects of different extraction methods on the physicochemical properties and lipid composition of camellia seed oil, which affect the quality and nutritional value of the oils and determine the nature of the subsequent refining process.

In this study, four types of extraction methods, including aqueous-enzymatic extraction, expeller pressing, hexane extraction, and supercritical-CO$_2$ extraction, were applied to extract oil from camellia seeds. The objective of this study was to investigate the effects of the various extraction methods on the physicochemical properties, fatty acid composition and content of bioactive compounds.

Materials and Methods

Materials Camellia seeds (Camellia Oleifera Abel.) were purchased at a local forest farm (Jiangde City, China). The seeds were air-dried and were ground using an electric grinder, and then sieved through a mesh for proximate analysis, hexane extraction, supercritical-CO$_2$ extraction and aqueous-enzymatic extraction. Trichoderma reesei protease, standard extraction materials and fatty acid methyl esters were purchased from Sigma-Aldrich Co. (Shanghai, China). All of the other solvents and reagents were of analytical grade and were purchased from Huadong Medicine Co. (Hangzhou, China).

Methods

Extraction of camellia seed oil using expeller pressing (EP) For expeller pressing, the seeds were dried (moisture content < 8%) and pressed in a local factory using an expeller (6Y-58A, Panfeng Co., Zhejiang, China). The pressing temperature was 120°C. The camellia seed oil was brought to the laboratory and stored in a refrigerator at -4°C until use.

Extraction of camellia seed oil using hexane (HE) For the hexane extraction, 10 g of ground seeds was weighted and placed in a cellulose paper cone, and then extracted using hexane in a Soxhlet extractor (B-811, Buchi Inc. Switzerland) for 8 hours. The solvent was removed from the extract by drying it using nitrogen (N$_2$).

Extraction of camellia seed oil using the aqueous-enzymatic method (AEE) The procedure for aqueous-enzymatic extraction of camellia seed oil was as follows: (1) Place 5 g of ground camellia seeds into a 55-mL screw-capped plastic tube; (2) add 30 mL of distilled water and homogenize two times for 1 min per time using a homogenizer (T10, IKA, Germany); (3) adjust the pH to 5.0 (using 1 M H$_2$SO$_4$ and 0.5 M NaOH) and add 1% enzyme (V/W); (4) conduct the enzymatic hydrolysis at 50°C for 4 h with horizontal shaking on a rotary shaker; (5) centrifuge at 8000 rpm for 30 min using a Beckman centrifuge; (6) carefully remove the top oil layer and place it in a glass tube.

**Extraction of camellia seed oil using supercritical-CO$_2$ extraction (SFE)** Supercritical-CO$_2$ extraction was conducted using a supercritical-fluid extractor (SFE-2, Applied Separation Inc., PA, USA); 10 g of camellia seed powder was loaded into a 50-mL stainless steel extractor and eluted at 3 mL/min using liquefied CO$_2$. The extraction was initiated when the appropriate pressure (35 Mpa) and temperature (45°C) were reached. The extract was separated from the CO$_2$ phase and collected.

**Proximate analysis of camellia seed oil** The analysis was performed using the AOAC methods (Horwitz, 1995) as follows: the oil content was measured using the Soxhlet method (945.48); the moisture content was measured using method 930.15; the total protein content was measured using the Kjeldahl method (955.04), where a factor of 5.595 was used to calculate the content of crude protein; the fiber content was measured using method 962.09; the ash content was measured using method 945.46; and the starch content was measured using method 996.11. The total carbohydrate content was determined by difference.

**Acid, peroxide and iodine values and fatty acid composition** The acid, peroxide and iodine values of the oil samples were determined using AOCS official methods Cd 3-25, Cd 3d-63, and Cd 1-25, respectively (Firestone, 1999).

The fatty acid composition was determined using gas chromatography. Prior to analysis, the oils extracted were converted to their fatty acid methyl esters by the methods of Zhang et al. (2010). The fatty acids were separated using an Agilent 6890N GC system (Agilent, Santa Clara, CA, USA) equipped with a FID detector and a Nuuk capillary column bonded Polyethylene glycol (Omegawax, 30 m × 0.32 mm × 0.25 m), (Supelco, Bellefonte, PA, USA). The inlet temperature was 220°C, and the detector temperature was 220°C, with a split ratio of 1:10. The column temperature was programmed as follows: 150°C for 1 min, increasing by 5°C per minute to 190°C and holding for 20 min.

**Determination of the contents of tocopherol, β-carotene and squalene, and phytosterol** The tocopherol content of the extracted oils was determined using an HPLC method with a fluorescence detection (294 nm excitation, 326 nm emission), as previously described by Moreau (Moreau et al., 2011). The content of β-carotene was determined using the method reported by Dietz (Dietz et al., 1988). The content of squalene was determined using the method reported by Lanzon (Lanzon et al., 1995).

The phytosterols present in the extracted oils were identified and quantified using GC. The procedures for saponification and for
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Table 1. Effect of different extraction methods on the oil yield and acid, peroxide and iodine value of camellia seed oil

<table>
<thead>
<tr>
<th>Extraction method</th>
<th>HE</th>
<th>EP</th>
<th>AEE</th>
<th>SFE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oil yield (%)</td>
<td>100a</td>
<td>94.37 ± 1.71a</td>
<td>82.51 ± 2.11b</td>
<td>73.25 ± 1.27c</td>
</tr>
<tr>
<td>Acid value (mg NaOH/g)</td>
<td>0.43 ± 0.01a</td>
<td>0.56 ± 0.02a</td>
<td>0.45 ± 0.01a</td>
<td>0.41 ± 0.02a</td>
</tr>
<tr>
<td>Peroxide value (meq/kg)</td>
<td>4.45 ± 0.15a</td>
<td>5.77 ± 0.24a</td>
<td>2.12 ± 0.08b</td>
<td>2.28 ± 0.11b</td>
</tr>
<tr>
<td>Iodine value g/100 g</td>
<td>87.00 ± 2.40a</td>
<td>88.30 ± 1.80a</td>
<td>86.70 ± 1.60a</td>
<td>86.10 ± 2.70a</td>
</tr>
</tbody>
</table>

The data shown are the mean value ± SD (n = 3)
Different letters in the same row indicate significant differences at the 5% level.

Results and Discussion

Proximate analysis of camellia seeds The crude oil content of camellia seeds is approximately 40 – 50% (Zhong et al., 2007). The results of this study indicated that the camellia seeds used consisted of 40.62 ± 2.11% crude oil, 10.34 ± 0.23% moisture, 9.87 ± 0.51% crude protein, 16.85 ± 0.53% starch, 3.67 ± 0.07% crude fiber, 2.15 ± 0.09% ash and 10.63 ± 0.37% total carbohydrates.

Acidity varies according to the cultivar and the ripeness of the seeds. Sofou is a traditional food of African people in the Guinea gulf region. Sofou oil has beneficial for nursing mothers and infant growth. Kolo oil was extracted from fruits of Raphia sese palm tree. It was previously reported that enzymatically extracted Kolo oil had a higher acid value than organic solvent-extracted Kolo oil, whereas the contrary was true for Safou oil (Dzondo-Gadet et al., 2004). The acid value of the expeller pressed-oil was 0.56 ± 0.02 mg NaOH/g, and that of supercritical-CO₂ extracted oil was 0.41 ± 0.02 mg NaOH/g. There is no significant difference among acid values of camellia seed oils extracted by four kind of methods (p > 0.05) (Table 1).

In contrast, in the present study, the enzymatically extracted oil had the lowest peroxide value, but there is no significant difference between aqueous enzymatic-extracted oil and supercritical-CO₂ extracted oil. The peroxide value was highest for the expeller-pressed oil, but there is no significant difference between hexane extracted- and expeller pressed-oil, possibly because the high-temperature pretreatment required for expeller pressing and hexane extraction caused the oxidation of endo-antioxidant compounds such as tocopherol and β-carotene. No significant differences (p > 0.05) were observed in the iodine value of the camellia seed oils.

Fatty acid composition The fatty acid compositions of the oils extracted using the various methods were qualitatively and quantitatively analyzed (Table 2). Seven main components, including two saturated fatty acids, three monounsaturated fatty acids and two polyunsaturated fatty acids, were identified. The content of oleic acid in oils was over 80%, but there were no significant differences (p > 0.05) in the amounts of the major fatty acids in the oils extracted using the different methods. Vegetable
The content of phenolic compounds in the aqueous enzymatic-extracted camellia seed oil was lower than that in the oils extracted using the other methods. The content of endogenous antioxidant materials in oils depends not only on the cultivar and state of ripeness but also on the extraction and refining processes utilized. The hydrolysis of the camellia-seed cell wall by the enzymatic treatment would enable the release of a larger amount of antioxidant compounds, resulting in oil with a higher availability of these bioactive components. Moreover, enzymatic hydrolysis can reduce the extent of the interaction of the antioxidants with the polysaccharides, proteins and pectins in seeds, facilitating their release into the oils (Jiao et al., 2014).

Phenolic compound content Phenolic compounds are a group of polar components that contain one or more aromatic hydroxylated rings (Cert et al., 2000), and they are not only antioxidant molecules but also responsible for bitter taste of vegetable oils. The content of phenolic compounds in the camellia seed oils extracted using the various methods in the present study are given in Table 3. Nine phenolic acids (3-hydroxytyrosol, benzoic acid, catechins, 4-hydroxybenzoic acid, chlorogenic acid, vanillic acid, (-) – epigallocatechin, p-coumaric acid and ferulic acid) were detected, and 3-hydroxytyrosol, benzoic acid, catechins, 4-hydroxybenzoic acid, and chlorogenic acid were the predominant compounds. In virgin olive oil and crude grape-seed oil, the total phenolic content is greater than 100 μg/g, which is higher than that of camellia seed oil in the present study, which was less than 10 μg/g (Table 3).

The content of phenolic compounds in the aqueous enzymatic-extracted camellia seed oil was lower than that in the oils extracted using the other methods. The water phase used in aqueous-enzymatic extraction absorbs phenolic compounds, resulting in a lower concentration of phenolics in the enzymatically extracted oil than in the oils produced using the other methods.
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Content of total phospholipids  Phospholipids (PLs) are important constituents of crude vegetable oil because they affect its stability and quality. Phospholipids affect the stability of oil by chelating metals and decreasing the amount of metal ions (Cert et al., 2000). Phospholipids are undesirable in oil because they are responsible for oil discoloration occurring during the deodorization and steam-distillation processes and the loss of neutral lipids during the neutralization process. The ideal phosphorous content of oil is lower than 5 μg/g (Narayana et al., 2002).

The phospholipid content of the camellia seed oils extracted using various methods is shown in Fig 2. In particular, the phospholipid content of the aqueous enzymatic-extracted oil and the supercritical CO₂-extracted oil was lower than 5 μg/g, which was significantly lower ( p < 0.05) than that of the oils extracted using the other methods. The water phase used for the aqueous enzymatic extraction may have precipitated some of the hydratable phospholipids, decreasing the phospholipid content of the oil obtained using this method. Eisenmenger reported that phospholipid content of supercritical CO₂-extracted wheat germ oil is significant lower than that in hexane extracted wheat germ oil. It may due to the low solubility of phospholipids in supercritical CO₂ (Eisenmenger et al., 2008).

Conclusions  Although camellia seed oil is an important vegetable oil in china, few reports have focused on the effects of different extraction methods on the quality of the oil. In the present study, the physicochemical properties, fatty acid composition, bioactive
compound content (vitamin E, β-carotene, squalene and β-stigmasterol), phenolic compound content and phospholipid content of oils that were extracted using aqueous-enzymatic extraction, expeller pressing, hexane extraction, and supercritical-CO$_2$ extraction were investigated.

There is no significant difference on acid values of oils extracted by different methods. The peroxide value of hexane-extracted oil and expeller-pressed oil was significant higher than aqueous enzymatic extracted-oil and supercritical CO$_2$ extracted oil. The major fatty acids present in camellia seed oil were palmitic acid, stearic acid, oleic acid, and linoleic acid. No significant differences were found in the amounts of the major fatty acids in the oils. Nine phenolic acids were detected, among which 3-hydroxytyrosol, benzoic acid, catechins, 4-hydroxybenzoic acid, and chlorogenic acid were predominant. The content of phenolic compounds in the aqueous enzymatic-extracted camellia seed oil was lower than that of the oils extracted using the other methods. The main tocopherol compound in the camellia seed oils was α-tocopherol, and β-sitosterol was the only phytosterol that was detected. The aqueous enzymatic-extracted oil had significantly higher contents of α-tocopherol, β-carotene, squalene and phytosterol than the hexane-extracted and expeller-pressed oil. It was reported that the ideal phosphorous content of oil is lower than 5 μg/g (Narayana et al., 2002), the phospholipid content of the aqueous enzymatic-extracted oil and the supercritical CO$_2$-extracted oil was lower than 5 μg/g.

### Table 3. Phenolic acids in camellia seed oil extracted using different methods

<table>
<thead>
<tr>
<th>Phenolic Acid</th>
<th>HE</th>
<th>EP</th>
<th>AEE</th>
<th>SFE</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-Hydroxytyrosol (μg/g)</td>
<td>1.18 ± 0.1a</td>
<td>1.17 ± 0.1a</td>
<td>1.07 ± 0.1b</td>
<td>1.16 ± 0.1a</td>
</tr>
<tr>
<td>Benzoic acid (μg/g)</td>
<td>2.51 ± 0.2a</td>
<td>2.36 ± 0.1b</td>
<td>2.28 ± 0.1c</td>
<td>2.47 ± 0.1ab</td>
</tr>
<tr>
<td>Catechins (μg/g)</td>
<td>1.71 ± 0.1a</td>
<td>1.67 ± 0.1a</td>
<td>0.79 ± 0.0b</td>
<td>1.70 ± 0.2a</td>
</tr>
<tr>
<td>4-Hydroxybenzoic acid (μg/g)</td>
<td>1.29 ± 0.1a</td>
<td>1.29 ± 0.1a</td>
<td>1.05 ± 0.1c</td>
<td>1.16 ± 0.1b</td>
</tr>
<tr>
<td>Chlorogenic acid (μg/g)</td>
<td>0.22 ± 0.0a</td>
<td>0.19 ± 0.0a</td>
<td>0.11 ± 0.0b</td>
<td>0.20 ± 0.0a</td>
</tr>
<tr>
<td>Vanillic acid (μg/g)</td>
<td>0.35 ± 0.0a</td>
<td>0.34 ± 0.0a</td>
<td>0.39 ± 0.0a</td>
<td>0.33 ± 0.0a</td>
</tr>
<tr>
<td>(-)-Epigallocatechin (μg/g)</td>
<td>0.22 ± 0.0a</td>
<td>0.18 ± 0.0b</td>
<td>0.16 ± 0.0b</td>
<td>0.22 ± 0.0a</td>
</tr>
<tr>
<td>p-Coumaric acid (μg/g)</td>
<td>0.36 ± 0.0a</td>
<td>0.33 ± 0.0a</td>
<td>0.27 ± 0.0b</td>
<td>0.35 ± 0.0a</td>
</tr>
<tr>
<td>Ferulic acid (μg/g)</td>
<td>0.10 ± 0.0a</td>
<td>0.10 ± 0.0a</td>
<td>ND</td>
<td>0.11 ± 0.0a</td>
</tr>
<tr>
<td>Total (μg/g)</td>
<td>7.96 ± 0.4a</td>
<td>7.65 ± 0.3a</td>
<td>6.32 ± 0.3b</td>
<td>7.71 ± 0.2a</td>
</tr>
</tbody>
</table>

The data shown are the mean value ± SD (n = 3). Different letters in the same row indicate significant differences at the 5% level. ND: Not detected.

![Fig. 2. Effect of the different extraction methods on the phospholipid content of camellia seed oil](image-url)

Mean values ± SD indicated with different lower-case letters are significantly different ($p < 0.05$).
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References


