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Quality comparison of camellia (*Camellia oleifera* C.Abel) seed oil with different extraction methods

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Abstract

The quality of camellia seed oil (CSO) varies with the oil extraction methods. In the present work, the oil yield, physicochemical properties, bioactive compounds, fatty acid composition, and Fourier transform infrared spectra of CSOs prepared by supercritical fluid, aqueous, pressing, and solvent extraction were explored systematically. Additionally, the microstructure of camellia seed cake after oil extraction was observed by scanning electron microscopy. Results showed that supercritical fluid extraction had the highest oil yield (92.42%), and the extracted oil was also superior to the other methods in the contents of polyphenol, β-sitosterol, and squalene, which were 89.34, 3173.23, and 6.20 mg/kg, respectively. Moreover, CSO extracted by supercritical fluid extraction had lower peroxide value and better colour indexes. In terms of fatty acid composition, CSOs extracted by supercritical fluid, pressing, and solvent extraction were similar, while CSO extracted by aqueous extraction had higher saturated fatty acid contents and lower unsaturated fatty acid contents than the other samples. Fourier transform infrared spectra analysis showed that the extraction methods had no significant effect on the chemical functional groups of CSOs. Scanning electron microscopy revealed that supercritical fluid extraction and solvent extraction could more effectively promote the release of oil from camellia seeds. In general, the quality of CSOs extracted by different methods had significant differences, and supercritical fluid extraction could be a promising extraction method for CSO.

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Introduction

Camellia oleifera C.Abel is a woody oil tree species endemic to China, mainly planted in Hunan, Jiangxi, Guangxi, Guangdong, Fujian, Zhejiang, and other southern provinces (Han *et al.*, 2020). Camellia seed oil (CSO) extracted from camellia seed is one of the four major woody edible oils in the world, and enjoys the reputation as "Asian olive oil" (Liang *et al.*, 2017). CSO has abundant oleic acid and bioactive compounds (polyphenols, sterols, tocopherol, squalene, *etc.*) (Shi *et al.*, 2020). Long-term consumption of CSO has various health effects including preventing cardiovascular sclerosis, lowering blood pressure, lowering blood lipids, antioxidant, and anticancer (Teixeira and Sousa, 2021). With the improvement of living standards, people's awareness of health care is gradually enhanced, and CSO is favoured by consumers as a kind of edible oil with both dietary and therapeutic benefits.

Oil extraction is an essential step in edible oil processing which directly determined the quality and quantity of oil (Nde and Foncha, 2020). The traditional extraction methods of CSO are mainly pressing extraction (PE) and solvent extraction (SE) (Zhou *et al.*, 2019). The extraction technique of PE is simple, and produces good tasting oil. However, the oil yield of PE is relatively low, and the extracted oil may have polycyclic aromatic hydrocarbons due to the high temperature (Lee *et al.*, 2020). The extraction technique of SE is another traditional technology

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developed after the 1970s, and has the advantages of low production cost, and high oil extraction rate, but has the problem of residual organic solvents (Xu *et al.*, 2021). Organic solvents not only can cause pollution to the environment, but can also cause harm to the human body. Therefore, some extraction methods using green and non-toxic solvents have been gradually developed, such as aqueous extraction (AE) and supercritical fluid extraction (SFE). The extraction technique of AE is a method of extracting oil by immersing water into the oilseed cells to replace the oil which has the advantages of a simple extraction process, and low equipment requirements. Yu *et al.* (2013) developed a salt-assisted aqueous extraction to prepare CSO, and optimised the extraction process. Their results showed that the oil yield of this method could reach 88.8% under the optimal conditions. The extraction technique of SFE is another emerging extraction method that separates oil from oilseeds by the supercritical fluid at a lower temperature which can obtain higher oil yields, and better retain the thermosensitive compounds of oil. Wang *et al.* (2019) showed that CSO extracted by supercritical CO_2 extraction was rich in phenols, and had good oxidative stability and antioxidant activity.

In recent years, there have been some comparative studies on the effect of emerging and traditional extraction methods on the quality of vegetable oil. Fernandes *et al.* (2019) systematically studied the effects of pressing, solvent, and supercritical CO_2 extraction on the quality indexes of chia seed oil. Similar studies have been done on walnut, almond, and yellow horn seed oils (Gao *et al.*, 2018; Qi *et al.*, 2019; Gu *et al.*, 2019). However, there are few systematic studies on the effects of different extraction methods on CSO quality. Therefore, the present work systematically explored the effects of different extraction methods (SFE, AE, PE, and SE) on the oil yield, physicochemical indexes, nutritional indexes, and fatty acid composition of CSO. In addition, through detailed comparative analysis, the present work aimed to put forward a high-efficiency and high-quality extraction method of CSO, and promote the development of the CSO industry.

Materials and methods

Materials

Camellia seeds were harvested from Zengcheng Teaching and Research Bases of South China Agricultural University (Guangzhou, Guangdong, China) in October 2020. All camellia seeds were dried to a moisture content of 5% for further use.

Chemicals

Squalene, tocopherol (α-, β-, γ-, δ-), and fatty acid methyl ester standard mixture were of HPLC grade, and purchased from Sigma-Aldrich (St. Louis, MO, USA). β-sitosterol and gallic acid were of HPLC grade, and purchased from Yuanye Biotechnology Co., Ltd. (Shanghai, China). Span 20 and Folin-Ciocalteu reagent were of analytical grade, and purchased from Macklin Biochemical Co., Ltd. (Shanghai, China). The other chemicals were purchased from Guangzhou Chemical Reagent Factory (Guangzhou, China).

Oil extraction process

Supercritical fluid extraction

Camellia seed powders (0.18 mm < particle size < 0.45 mm) were prepared by a pulveriser (JYL-C19V, Joyoung Co., Ltd., Jinan, China) and 40- and 80-mesh standard sieves.

A certain amount of camellia seed powders was weighed in the extraction kettle, and the CSO was obtained using a supercritical fluid extraction device (SFE-1 L, Guizhou Aerospace Wujiang Mechanical and Electrical Equipment Co., Ltd., Zunyi, Guizhou). The extraction method was performed following Lin *et al.* (2018) with some modifications. Carbon dioxide was used as the supercritical fluid. The extraction conditions were 30 MPa, 40°C, and 2.5 h. The mixture was put into the separation kettle when the extraction was completed. The separation temperature and pressure were 40°C and 10 MPa, respectively. The separated oil was stored at 4°C for further analysis.

Aqueous extraction

The aqueous extraction was performed following Geng *et al.* (2020). Firstly, a solution of 1.2 mol/L sodium chloride was prepared. Then, using the equipped sodium chloride solution as the solvent, 1.6 g/100 g Span 20 solution was prepared as the aqueous extraction solution. Subsequently, the camellia seed powders (0.18 mm < particle size < 0.45 mm) were mixed with extracted solution (material-to-liquid ratio, 1:4) and was extracted for 60 min at 85°C by a DFS-101s magnetic stirrer (Shanghai Lichen Instrument Technology Co., Ltd., Shanghai, China). The oil sample was finally obtained by centrifugation

(6,790 *g*, 15 min) and stored at 4°C for further analysis.

Pressing extraction

CSO was obtained using a screw press (ZYJ-904, Belston Co., Ltd., Berlin, Germany). The oil sample was finally obtained by centrifugation (6,790 *g*, 15 min) and stored at 4°C for further analysis.

Solvent extraction

A certain amount of camellia seed powders (0.18 mm < particle size < 0.45 mm) was mixed with *n*-hexane (material-to-liquid ratio, 1:7), and the mixture was extracted for 4 h at 45°C using a BTS-100R constant temperature shaker (Jingqi Scientific Instrument Co., Ltd., Shanghai, China). Subsequently, the extracted mixture was filtered, and *n*-hexane was recovered through RE-52AA rotary evaporator (Yarong Biochemical Instrument Factory, Shanghai, China). The oil sample was finally obtained by centrifugation (6,790 *g*, 15 min) and stored at 4°C for further analysis.

Determination of oil yield

Following the GB 5009.6-2016 standard method (National Standards of the People's Republic of China, 2016a), the Soxhlet extraction method was used to determine the total oil concentration in camellia seeds. The oil yield of different extraction methods was calculated using Eq. 1:

$$\text{Oil yield (\%)} = \frac{\text{W1 (weight of oil extracted by one method)}}{\text{W2 (weight of oil extracted by Soxhlet extraction method)}} \quad \text{(Eq. 1)}$$

Determination of physicochemical properties

The determination of acid value (AV) and peroxide value (POV) of CSOs was performed following GB 5009.229-2016 and GB 5009.227-2016 standard methods, respectively (National Standards of the People's Republic of China, 2016b; 2016c). The colour indexes (L*, a*, b*) of CSOs were determined using a CS-580A colorimeter (Hangzhou Color Spectrum Technology Co., Ltd., Zhejiang, China).

Determination of bioactive compounds

Tocopherol

Following the GB 5009.82-2016 standard method (National Standards of the People's Republic of China, 2016d), the reversed-phase high-performance liquid chromatography method was used to determine the tocopherol content in CSOs. Briefly, 1.0 g CSO was mixed with 0.1 g BHT and 10 mL mobile phase. The mobile phase consisted of *n*-hexane and solvent A (90:10, v/v). Solvent A was tert-butyl methyl ether/tetrahydrofuran/methanol (20:1:0.1, v/v/v). A high-performance liquid chromatography system (1260, Agilent Technologies, USA) equipped with a C_{18} column (4.6 × 250 mm × 3 μm) and a fluorescent detector was then performed; column temperature was 30°C, injection volume was 10 μL, and mobile phase flow rate was 0.8 mL/min. In addition, the excitation and emission wavelengths of the fluorescence detector were 294 and 328 nm, respectively. The tocopherol content was calculated by the external standard method.

Squalene

Following the LS/T 6120-2017 standard method (Grain Industry Standard of the People's Republic of China, 2017), a gas chromatography (2010plus, Shimadzu, Kyoto, Japan) equipped with an HP-5 column (30 m × 0.32 mm × 0.15 μm) and a flame ionisation detector was used to identity squalene in CSOs. Briefly, 1.0 g oil sample and 300 μL squalene internal standard solution were mixed with 50 mL ethanol potassium hydroxide solution for saponification. The saponification solution was extracted with *n*-hexane three times. Then, the ethanol was used to wash the organic phase to neutral, and the anhydrous sodium acetate was added to remove water. Finally, the organic solvents were removed through the rotary evaporator. Gas chromatographic conditions were as follows; injection volume was 1 μL at a split ratio of 1:10, injector temperature was 250°C, initial oven temperature was 160°C and increased to 220°C with 15°C/min and held for 2 min, then increased to 280°C with 5°C/min and held for 20 min, and finally increased to 300°C with 5°C/min and held for 2 min. The squalene content was calculated by the internal standard method.

Polyphenol

The polyphenolic content in CSOs was determined by Folin-Ciocalteu method (Wang *et al.*, 2019). Firstly, polyphenols were extracted from CSO (3.0 g) with 5 mL 80% methanol three times. Briefly, 3 mL Folin-Ciocalteu reagent was added to 1 mL sample solution. After 10 min, 2 mL 7.5% sodium carbonate was added, and distilled water was added

to bring the total volume to 10 mL. After a dark reaction of 15 min, the absorbance was measured at 765 nm using a UV-5200 spectrophotometer (Yuanxi instrument Co., Ltd., Shanghai, China). Next, a standard curve was plotted using different concentrations of gallic acid, and the linear regression equation was obtained as $y = 0.017x - 0.0104$ (R^2 = 0.999). The polyphenolic content in CSOs was calculated using the linear regression equation.

β-sitosterol

The β-sitosterol content in CSOs was determined by the colorimetric method of acetic anhydride-concentrated sulphuric acid. Briefly, 1 g CSO was weighed, and the volume was fixed to 10 mL by chloroform. Subsequently, 2 mL sample solution was mixed with 8 mL acetic anhydride and five drops of concentrated sulphuric acid. After 45 min, the absorbance was measured by a UV-5200 spectrophotometer at 662 nm. Next, a standard curve was plotted using different concentrations of β-sitosterol, and the linear regression equation was obtained as $y = 2.4102x + 0.0033$ (R^2 = 0.999). The β-sitosterol content in CSOs was calculated using the linear regression equation.

Fatty acid

Following the GB 5009.168-2016 standard method (National Standards of the People's Republic of China, 2016e), the fatty acid composition of CSO samples was analysed using a gas chromatography (7890B, Agilent Technologies, USA) equipped with a DB-23 column (60 m × 0.25 mm × 0.15 μm). Briefly, the oil sample (60 mg) was thoroughly mixed with 4 mL isooctane and 200 μL 2 mol/L potassium hydroxide-methanol solution. The mixture was thoroughly vortexed for 30 s. Subsequently, 1 g sodium bisulphate was added to the solution to neutralise the excess potassium hydroxide. After the salt was precipitated, the supernatant was used for analysis by gas chromatography. Gas chromatographic conditions were as follows: injection volume was 1 μL at a split ratio of 25:1, carrier gas was nitrogen; initial oven temperature was 50°C and held for 1 min, then increased to 150°C with 25°C/min and held for 4 min, then increased to 180°C with 2°C/min and held for 4 min, then increased to 200°C with 3°C/min and held for 4 min, and finally increased to 230°C with 15°C/min and held for 5 min. Additionally, the injector and detector temperatures were 250 and 280°C, respectively. The fatty acid composition was identified by comparison of retention times to the standard of fatty acid methyl ester. The quantitative analysis was performed using the area normalisation method.

Fourier transform infrared analysis

A Fourier transform infrared (FTIR) spectrometer (Vertex70, Bruker Technology Co., Ltd., Germany) equipped with attenuated total reflectance (ATR) sampling device was used to determine the FTIR spectra of CSOs in mid-infrared mode following Han *et al.* (2020) with slight modifications. The scanning range, resolution, and number of scans were 400 - 4000 cm^{-1}, 4 cm^{-1}, and 64, respectively.

Scanning electron microscopy analysis

After oil extraction, the camellia seed cakes of different extraction methods were crushed and dried. The dried seed cake powders were gold-coated with the help of the vacuum coating instrument (EM ACE600, Leica Instrument Co., Ltd., Germany). Subsequently, the microstructure of camellia seed cakes was observed by scanning electron microscopy (SEM) (EVO MA15, Carl Zeiss Co., Ltd., Germany).

Statistical analysis

One-way analysis of variance (ANOVA) was performed by SPSS 26 (IBM Watson Analytics, Beijing, China). Origin 2021 (Origin Lab Corporation, Northampton, MA) was employed to plot the graphs.

Results and discussion

Oil yield of camellia seeds with different extraction methods

As can be seen from Table 1, the order of oil yield of camellia seeds with different extraction methods was SFE (92.42%) > SE (92.3%) > AE (85.74%) > PE (84.79%). The oil yields of SFE and SE were significantly ($p < 0.05$) higher than that of AE and PE. This might have been due to the excellent permeability and extraction capacity of carbon dioxide and *n*-hexane. However, the oil yields of AE and PE were relatively low. The emulsification phenomenon in the extraction process was the main reason for the relatively low oil yield of AE. For PE, the reason for the relatively low oil yield could have been due to the fact that camellia seed cake had certain adsorption to oil during the pressing process,

Table 1. Oil yield and physicochemical properties of camellia seed oils extracted by different methods.

Index	SFE	AE	PE	SE
Oil yield (%)	92.42 ± 0.18[a]	85.74 ± 0.03[b]	84.79 ± 0.30[c]	92.30 ± 0.10[a]
Acid value (mg/g)	3.66 ± 0.04[b]	4.26 ± 0.02[a]	3.23 ± 0.04[d]	3.39 ± 0.1[c]
Peroxide value (g/100 g)	0.055 ± 0.002[d]	0.082 ± 0.002[b]	0.177 ± 0.003[a]	0.072 ± 0.002[c]
L*	54.72 ± 0.39[a]	51.49 ± 0.06[c]	53.11 ± 0.01[d]	52.82 ± 0.12[b]
a*	-3.31 ± 0.02[b]	-3.00 ± 0.01[a]	-3.55 ± 0.23[c]	-3.64 ± 0.02[c]
b*	8.19 ± 0.12[d]	32.34 ± 0.06[a]	29.44 ± 0.46[b]	24.94 ± 0.14[c]

SFE: supercritical fluid extraction; AE: aqueous extraction; PE: pressing extraction; and SE: solvent extraction. Values are mean ± standard deviation of three replicates (n = 3). Means followed by different lowercase superscripts in a row are significantly different at $p < 0.05$.

thus resulting in some oil could not be completely extracted. In addition, the water content of camellia seed was another key factor affecting the oil yield of PE (Martínez *et al.*, 2008). These results were consistent with the study of Jung *et al.* (2012) in which the oil yields of supercritical fluid extraction and *n*-hexane extraction were significantly higher than that of mechanical pressing extraction when extracting perilla seed oil.

Physicochemical properties of camellia seed oils extracted by different methods

The colour indexes, AV, and POV of the CSOs obtained by different extraction methods are presented in Table 1. Colour is considered one of the quality indicators of edible oil, and also plays an essential role in its sensory properties and product market acceptance. The L* value of CSO extracted by SFE was the highest, which indicated that the brightness and light transmittance of oil were higher. The b* values of CSOs extracted by AE and PE were relatively high. This might have been due to the higher extraction temperatures of AE and PE which promoted the degradation of pigment components.

The AV and POV represent the degree of oxidative rancidity of oil which are vital indexes to evaluate oil quality (Hussain *et al.*, 2018). The AV of CSO extracted by AE was the highest, which might have been due to the fact that AE was carried out in a water system, and the oil was easy to hydrolyse and turn rancid. For POV, that of CSO extracted by PE was the highest, while that of CSO extracted by SFE was the lowest, thus indicating that the extraction temperature had a significant effect on POV of CSOs. Higher POV may be due to the higher amount of peroxide formation and decomposition via fission, dehydration, and the formation of free radicles at higher temperatures (Naik *et al.*, 2021). During the pressing process, the temperature of the material increased under the extrusion and friction of the screw, which accelerates the formation of peroxides in the oil. However, the extraction temperature of SFE was relatively low, which might be that CO_2 and high pressure inhibited the activities of lipoxygenase and peroxidase, so the peroxide of oil was low (Shao *et al.*, 2015).

The content of bioactive compounds of camellia seed oils extracted by different methods

The results of bioactive compounds of CSOs extracted by different methods are shown in Figure 1. Polyphenols are secondary metabolites that exist widely in plants, and have a wide range of health promotion effects including antioxidation, anti-inflammation, anti-atherosclerosis, and anti-mutation (Gorzynik-Debicka *et al.*, 2018). Folin-Ciocalteu spectrophotometric method is usually performed to determine the polyphenolic contents in oil samples. Figure 1A shows that there were significant ($p < 0.05$) differences among the polyphenolic content of CSOs extracted by different extraction methods, and the highest content (89.34 mg/kg) was observed in CSO extracted by SFE.

Squalene is a highly unsaturated hydrocarbon originally found in shark liver oil, hence its name. Squalene has several physiological functions such as liver protection, anti-tumour, anti-cancer, and anti-aging (Lou-Bonafonte *et al.*, 2018). In the present work, the squalene contents in CSO extracted by different methods were determined by gas chromatography. As shown in Figure 1B, the squalene content in CSO extracted by SFE was the highest (6.20 mg/kg), while the CSO extracted by SE was the lowest (5.30 mg/kg). This could have been

due to the fact that carbon dioxide was more capable of extracting squalene than *n*-hexane.

Vegetable oil is rich in phytosterols, and one of the best natural sources of phytosterols in the diet. In recent years, phytosterols had attracted more and more attention due to their effect on reducing blood cholesterol, and their potential contribution in cardiovascular disease prevention (Yang *et al.*, 2019). In the present work, β-sitosterol was one of the major phytosterols found in CSOs. As shown in Figure 1C, when compared with other functional components, the β-sitosterol content in CSOs extracted by different extraction methods was higher; β-sitosterol content in CSO extracted by SFE was the highest (3173.23 mg/kg), and the β-sitosterol content in CSO extracted by the other three methods was relatively close.

Tocopherols, as potential antioxidants, could improve the oxidative stability of oil by breaking the chain reaction in the peroxidation of unsaturated lipids (Yang *et al.*, 2018). Tocopherols contain four kinds of structures namely α-, β-, γ-, and δ-, among which α-tocopherol has the higher biological activity (Shahidi *et al.*, 2021). In the present work, the liquid chromatography method was used to determine the α-, β-, γ-, and δ-tocopherols in CSOs, but only α-tocopherol was found. As shown in Figure 1D, the α-tocopherol content in CSO extracted by PE was significantly ($p < 0.05$) higher than that of the other methods, and α-tocopherol was not detected in CSO extracted by SFE. This indicated that a higher temperature was beneficial to improve the extraction of tocopherols in a certain range.

Altogether, the contents of polyphenols, β-sitosterol, and squalene in CSO extracted by SFE were significantly ($p < 0.05$) higher than those of the other extraction methods. This might have been due to the relatively poor thermal stability of these compounds. Therefore, the low-temperature extraction process of SFE had a particular advantage in protecting certain bioactive compounds. In addition, the good extraction performance of carbon dioxide was also a vital factor.

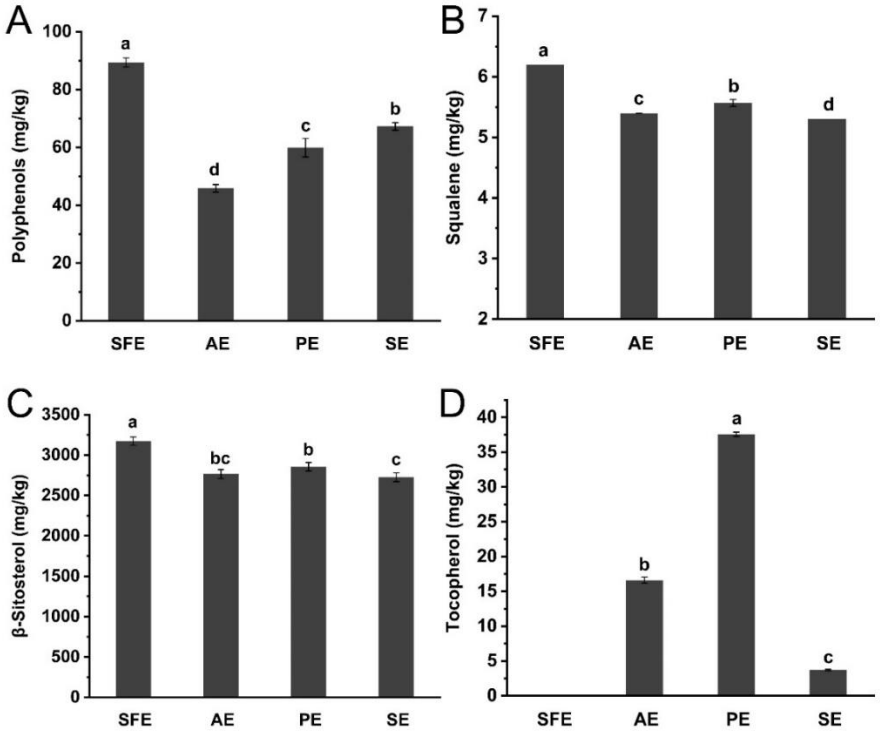

Figure 1. The contents of bioactive compounds of camellia seed oils extracted by different methods. **(A)** Polyphenols, **(B)** squalene, **(C)** β-sitosterol, and **(D)** tocopherol.

Fatty acid composition of camellia seed oils extracted by different methods

The fatty acid compositions of CSOs extracted by different extraction methods are shown in Table 2. A total of six fatty acids were identified by gas chromatography including oleic acid (C18:1), linoleic acid (C18:2), palmitic acid (C16:0), stearic acid (C18:0), linolenic acid (C18:3), and 11-eicosenoic acid (C20:1). Among them, oleic acid (80.42 - 80.93%), linoleic acid (7.61 - 7.69%), and palmitic acid (8.68 - 9.02%) were the three main fatty acids in CSO, which were consistent with the results of Fang

et al. (2015). The contents of oleic acid and monounsaturated fatty acids (MUFA) in CSO extracted by AE were significantly ($p < 0.05$) lower than those of the other samples. On the contrary, the contents of palmitic, stearic, and saturated fatty acids (SAFA) in CSO extracted by AE were significantly ($p < 0.05$) higher than those of the other samples. In addition, there were relatively small differences among the four extraction methods in linoleic, linolenic, and polyunsaturated fatty acids (PUFA). To summarise, the fatty acid compositions of CSOs extracted by SFE, PE, and SE were similar, while there were some differences between CSO extracted by AE and the other methods.

Table 2. Fatty acid composition of camellia seed oils extracted by different methods.

Fatty acid composition (%)	SFE	AE	PE	SE
Palmitic acid (C16:0)	8.82 ± 0.01^{b}	9.02 ± 0.02^{a}	8.68 ± 0.08^{c}	8.68 ± 0.03^{c}
Stearic acid (C18:0)	2.14 ± 0.01^{d}	2.34 ± 0.00^{a}	2.19 ± 0.01^{b}	2.17 ± 0.02^{c}
Oleic acid (C18:1)	80.81 ± 0.03^{b}	80.42 ± 0.03^{c}	80.85 ± 0.09^{ab}	80.93 ± 0.01^{a}
Linoleic acid (C18:2)	7.66 ± 0.01^{ab}	7.61 ± 0.01^{c}	7.69 ± 0.02^{a}	7.63 ± 0.01^{b}
Linolenic acid (C18:3)	0.17 ± 0.00^{b}	0.18 ± 0.00^{a}	0.18 ± 0.01^{b}	0.16 ± 0.01^{b}
11-Eicosenoic acid (C20:1)	0.41 ± 0.01^{b}	0.44 ± 0.00^{a}	0.41 ± 0.01^{b}	0.42 ± 0.01^{b}
SAFA	10.96 ± 0.01^{b}	11.36 ± 0.02^{a}	10.87 ± 0.08^{c}	10.85 ± 0.02^{c}
MUFA	81.21 ± 0.02^{b}	80.86 ± 0.03^{c}	81.27 ± 0.09^{ab}	81.35 ± 0.02^{a}
PUFA	7.83 ± 0.01^{b}	7.78 ± 0.01^{c}	7.87 ± 0.02^{a}	7.79 ± 0.01^{c}

SFE: supercritical fluid extraction; AE: aqueous extraction; PE: pressing extraction; SE: solvent extraction; SAFA: saturated fatty acids; MUFA: monounsaturated fatty acids; and PUFA: polyunsaturated fatty acids. Values are mean ± standard deviation of three replicates ($n = 3$). Means followed by different lowercase superscripts in a row are significantly different at $p < 0.05$.

Fourier transform infrared analysis of camellia seed oils extracted by different methods

To study the effects of different extraction methods on the chemical functional groups of CSOs, an FTIR spectrometer was used, and the results are as shown in Figure 2. It could be observed that the differences among various extracted CSOs were minor, with slight differences at 3474 cm^{-1} could be observed. The characteristic peak at 3474 cm^{-1} corresponded to the hydroxyl group (water), thus indicating that there might be a small amount of water in the CSOs extracted by AE and SE. The characteristic peaks at 3005 and 1655 cm^{-1} corresponded to the stretching vibration of =CH and C=C, respectively, thus related to the abundant unsaturated fatty acids in CSO. The characteristic peaks at 2922 and 2853 cm^{-1} corresponded to the asymmetric and the symmetrical stretching vibration of CH_2, respectively (Han *et al.*, 2020). At 1743 cm^{-1}, the demonstrated peak corresponded to the ester carbonyl functional group of triglycerides (Chauhan *et al.*, 2015). The range between 1500 and 900 cm^{-1} was the "fingerprint region" of oils because it revealed the existence of the main functional groups of fatty acids (Naik *et al.*, 2021). The bands at 1462 cm^{-1} corresponding to the C-H antisymmetric stretching vibration, thus indicating the presence of methyl esters in the sample. The peaks at 1160 and 1095 cm^{-1} corresponded to the asymmetric and symmetrical stretching vibration of C-O-C, respectively, thus indicating the presence of ester groups (Gu *et al.*, 2019). Additionally, the characteristic peak at 722 cm^{-1} corresponded to C-H bond from long-chain alkanes.

Microstructure analysis of camellia seed cakes with different extraction methods

A scanning electron microscope was used to analyse the oil extraction mechanism of different extraction methods (Figure 3). It was apparent that the cell structure of camellia seeds was destroyed in varying degrees after different extraction methods. It could be seen from Figure 3A that camellia seed cells was opened almost completely after SFE, and there was little residual oil on the cell surface, but some other substances. The high-pressure treatment in the SFE process could better destroy the camellia seed cells, rendering the extractant to enter the cells to extract the oil. Moreover, the high oil yield of SFE was also connected with the excellent extraction performance of CO_2 (Wrona *et al.*, 2021). Figure 3B shows the microstructure of camellia seed cake after

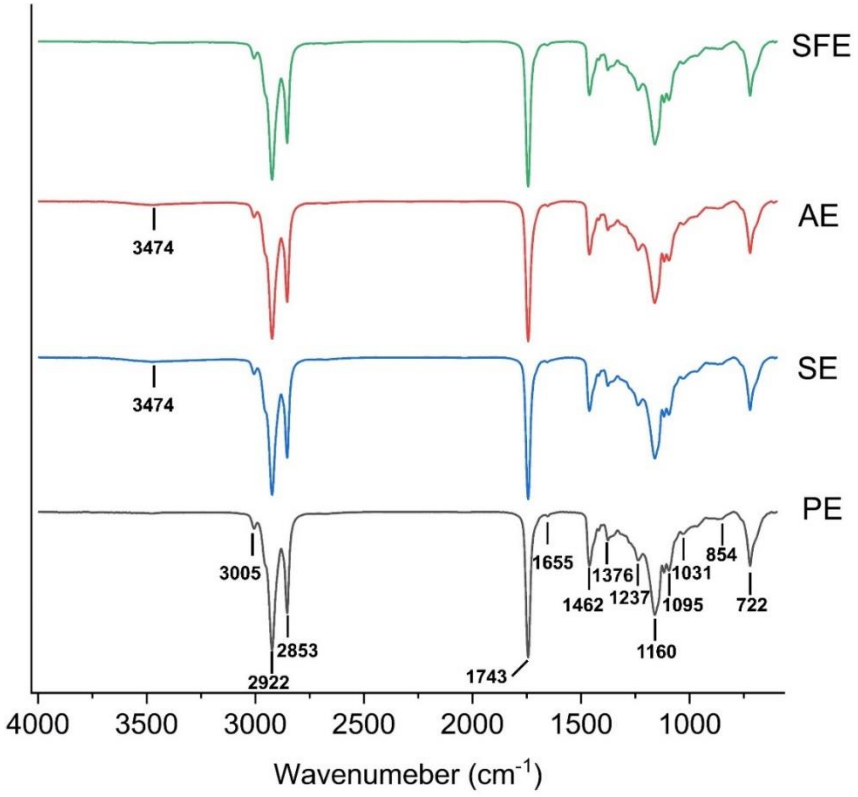

Figure 2. FTIR analysis of camellia seed oils extracted by different methods.

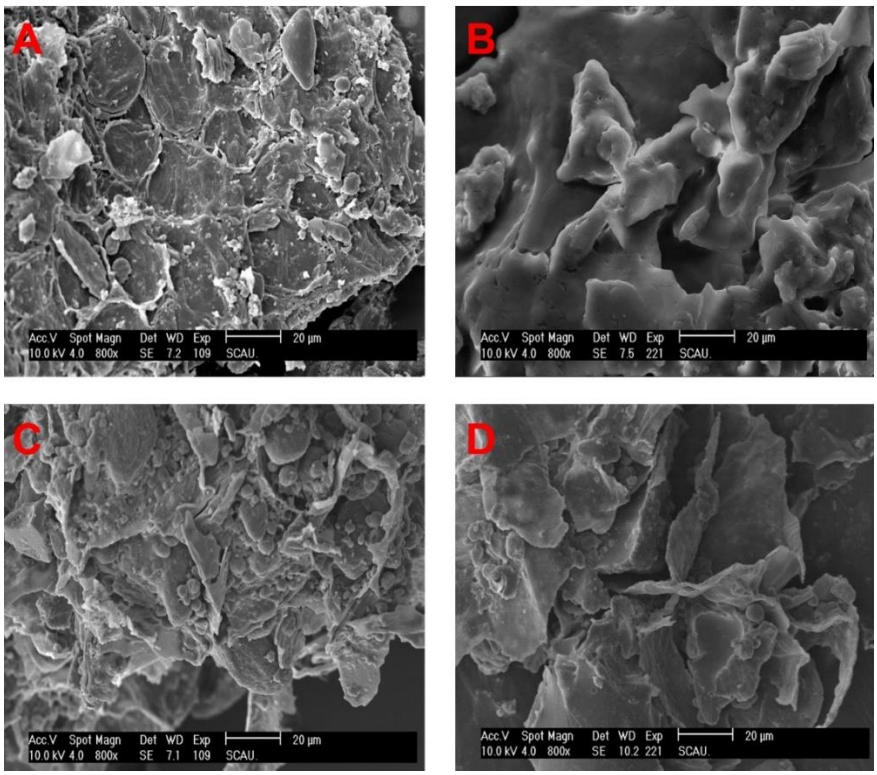

Figure 3. SEM micrographs of camellia seed cakes after different extraction methods. **(A)** Supercritical fluid extraction (SFE), **(B)** aqueous extraction (AE), **(C)** press extraction (PE), and **(D)** solvent extraction (SE).

AE. Some of the components (starch, protein, and oil droplets) in the seed cells could be observed to stick together, which might be because Span 20 remained on the camellia seed cake, and made the cell surface stickier. As shown in Figure 3C, the microstructure of camellia seed cake after PE was loose and broken, and there were a lot of residual oils on the surface. This might be due to the fact that PE extruded the oil from the material with the help of external force, while the oil of the raw materials mainly existed in the cells as a spherical "fat body" with a minimal diameter, and proteins entangled this "fat body", hence the oil was difficult to be entirely extracted by PE. Based on Figure 3D, the surface structure of the camellia seed cake after SE was relatively smooth and complete, and the residual oil was also rather less. This might have been due to the fact that organic solvent was good at dissolving oils without affecting other components in the cells during extraction. In general, when it came to the oil extraction effect, SFE and SE were obviously better than PE and AE, consistent with the analysis results of oil yield.

Conclusion

The oil extraction method had significant influences on the quality of CSO. Results of oil yield and scanning electron microscopy both showed that SFE and SE could better separate the oil from camellia seed. Meanwhile, most of the physicochemical and nutritional indexes of CSO extracted by SFE were better than the other methods. The fatty acid composition of CSOs extracted by SFE, PE, and SE were similar, while CSO extracted by AE had some differences from the other methods. In addition, the chemical functional groups of CSO were little affected by the extraction methods. In general, when compared with AE and the traditional extraction methods, SFE obtained higher oil yield, and better protected the bioactive compounds, which is a high-benefit and high-quality extraction method of CSO. The present work not only provided theoretical guidance for the processing industry of CSO, but also a reference for the extraction of other oilseeds.

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