Physicochemical Characteristics of Chinese Qingjinju (Citrus microcarpa) Seed Oils Isolated by Different Extraction Techniques

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Abstract

During Qingjinju processing, a large quantity of seeds is produced as agro-industrial waste. However, these seeds contain oil that may be a potential source of valuable compounds. This work describes the physicochemical properties, nutrient substances, and volatile compounds of Qingjinju seed oils obtained using different extraction methods. The main volatile compounds of Qingjinju seed oils were esters, aldehydes, hydrocarbons, acids, and alcohols. The seven major volatile compounds were found across all extraction techniques. The extraction methods were found to influence the quality of Qingjinju seed oils. Solvent extraction oils possessed superior physicochemical properties, such as lower acid and peroxide values and were lighter in color. Screw press extraction and supercritical CO2 extraction oils contained more nutrient substances, such as limonin and nomilin. These findings suggest that varying the extraction technique can prepare Qingjinju seed oils for targeted applications in the food, cosmetic, detergent, and pharmaceutical industries.

INTRODUCTION

Qingjinju (*Citrus microcarpa*), a cultivar related to citrus, is a hybrid between *Citrus reticulata Blanco* and *Fortunella*spp.[1-2] The fruit is primarily distributed across Southeast Asian regions including Hainan, Taiwan, Malaysia, Indonesia, Thailand, and the Philippines. Across these regions, Qingjinju is known by a variety of names: calamondin and calamansi in Taiwan[3], 'jeruk kasturi' in Indonesia[4], kalamondin and limonsito in the Philippines[1], musk lime in Malaysia, and ma-nao-wan in Thailand[4].

More than 90% of Qingjinju are used for beverage production, with the pulp and seeds discarded after removal of the juice. As Qingjinju seeds comprise approximately 13%[4] of the fruit, the juicing process results in a substantial quantity of waste. For example, in the Hainan province of China alone, approximately 23,000 tons of Qingjinju seeds remain following juice production. Although these seeds are generally regarded as agro-industrial waste, studies have found that citrus seeds contain fiber, protein, and oil, as well as a variety of physiologically active substances, including limonoids[5], flavonoids[6], phenolic compounds[7], and tocopherols[8], making them a potential source of medically and industrially valuable compounds. Citrus seed oil is particularly important, owing to its potential applications in the food, cosmetic, detergent, and pharmaceutical industries[9-10].

There have been an increasing number of studies focused on seeds from various *Citrus* spp. Multiple reports describe the fatty acid composition and physical and chemical properties of lemon (*C. limon L* .)[11-14], grapefruit (*C. parpadisi*)[15-16], mussami (*C. sinensis*)[15-16], sour orange (*C. aurantium*)[11, 16], lime (*C. aurantifolia*)[11], kinnow (*C. reticulata*)[15], and mandarin (*C. deliciosa*)[17]seed oils. In general, citrus seed oil content ranges between 20 and 40% (by weight) and is a good source of unsaturated fatty acids, including linoleic (18:2 n-6), palmitic (16:0), oleic (18:1 n-9), α-linolenic (18:3 n-3), and stearic (18:0) acid. The total tocopherol, sterol, and phenolic contents in the seed oils are 0.8-21.0 mg/100g, 1310.54-3986.58 mg/kg, and 209.90-287.2 mg gallic acid equivalent (GAE)/kg, respectively[18-19]. The physicochemical characteristics of

citrus seed oils greatly vary with the particular citrus variety: iodine values from 91.4 to 110.0 g I_2/100g oil, refractive index (40 °C) from 1.4639 to 1.4681, density (24 °C) from 0.920 to 0.941 g/mL, saponification values from 180.9 to 198.9 mg KOH/g oil, unsaponifiable matter from 0.3% to 0.5%, and acid values from 0.21 to 2.2 mg KOH/g of oil.

Few studies have investigated green kumquant seed oils. Only Manaf et al.[4] have studied the musk lime (*C. microcarpa*) seed oils in Malaysia. The iodine values, saponification values, unsaponifiable matter, and free fatty acid content of the seed oils were 118.0 g I_2/100 g oil, 192.6 mg KOH/g oil, 22 mg/g oil, and 18 mg oleic acid/g oil respectively. The predominant fatty acids of the musk lime seed oils were linoleic, oleic and palmitic acid. To the best of our knowledge, no such quantitative study on the physicochemical characteristics (e.g., refractive index, density) or nutrient substances (e.g., limonin, flavonoids, total phenolic content, β-sitosterol) have been reported for Qinjinju seed oils, nor has there been a thorough qualitative evaluation of its volatile compounds.

The industrial application of citrus seed oils depends on their fatty acid composition, presence and quantity of nutrient substances, flavor component, sensory properties, and processing yield. Studies have found that, along with the citrus variety[17] and cultivation area[16, 20], the method of oil extraction[14, 21] greatly influences these properties.

In this work, we evaluated the physicochemical properties (e.g., iodine value, refractive index, density, saponification value, unsaponifiable matter, acid value, color, and fatty acid composition), nutrient substances (e.g., limonin, nomilin, and total flavonoid, phenolic, β-sitosterol, and α-tocopherol content) and volatile compounds in Qingjinju seed oils. In addition, we evaluated and compared the characteristics of oil isolated by screw press, solvent, and supercritical CO_2 extraction.

2. MATERIALS and METHODS

2.1 Materials

Qingjinju (*C. microcarpa* (Bunge), Rutaceae) seeds were gifted by Hainan Qingjinju Food Technology Co., Ltd. (Hainan, China) during the 2017-2018 harvest season. Approximately 10 kg of the seeds were washed, drained, and oven-dried (DAIHAN SOF-155, DAIHAN Scientific Co., Ltd., Wonju-si, Korea) at 50 °C for 5-8 h. The seed moisture content was monitored and maintained at 4.0%. Seeds were stored in a refrigerator at 3 °C prior to use.

All chemicals used in the study were of analytical and/or chromatographic grade and were purchased from Shanghai Yuanye Bio-Technology Co., Ltd. (Shanghai, China). Standards used for chromatographic analyses were purchased from ANPEL Laboratory Technologies (Shanghai) Inc. (Shanghai, China).

2.2 Screw pressing of Qingjinju seeds

Qingjinju seeds were pre-roasted in a DAIHAN SOF-155 oven at 170 °C for 8 min with frequent agitation. Approximately 1000 g of the pre-roasted seeds were immediately pressed using a laboratory mechanical screw press (Bestday ZYJ9018, Jiangmen BestdayBear Electric Co., Ltd., Jiangmen, China). The seed oils were centrifuged (Heal Force Neofuge 15R, Shanghai Lishen Scientific Equipment Co., Ltd., Shanghai, China) at 4000 rev/min for 10 min and transferred to brown reagent bottles. This process was repeated three times. The oil samples were stored at 3 °C in a refrigerator.

2.3 Solvent extraction of Qingjinju seeds

Qingjinju seeds were ground (model YC-04B, Shanghai Jinben Machinery Co., Ltd., Shanghai, China) for 30 s and mixed with hexane in a ratio 1:7 (w/v). The mixture was transferred to a closed glass reaction kettle and stirred for 2 h at 55 °C in a water bath (W-O, Shanghai Shengshun Biotechnology Co., Ltd., Shanghai, China). The solution was decanted and the extraction was repeated three times. The solutions were then evaporated under reduced pressure at 60 °C and stored at 3 °C in a refrigerator.

2.4 Supercritical CO_2 extraction of Qingjinju seeds

Qingjinju seeds were first ground (model YC-04B) for 30 s. Approximately 1000 g of the ground seeds were transferred into a supercritical CO_2 extraction apparatus equipped with a 5 L extraction kettle (HA220-50-06, Nantong Huaan Supercritical Extraction Equipment Co., Ltd., Nantong, China). The instrument was operated using the following parameters: extraction temperature, 40 °C; extraction pressure, 35 MPa; analytical pressure, 12 MPa; analytical temperature, 55 °C; CO_2 flow rate, 15 L/h; extraction time, 3 h. The seed oils were stored at 3 °C in a refrigerator.

2.5Physicochemical analysis of Qingjinju seed oils

The specific gravity, refractive indices, and color values of the oil samples were measured in accordance with the People's Republic of China (PRC) National Standards GB 5526-1985[22], GB/T5527-2010[23], and GB/T22460-2008[24], respectively. The acid values, peroxide value, and iodine number were determined using the PRC National Standards GB/T 5530-2005[25], GB/T 5538-2005[26], and GB/T5532-2008[27], respectively. The saponification numbers and unsaponifiable matters were evaluated using the PRC National Standards GB/T5534-2008[28] and GB/T5535.1-2008[29], respectively.

2.6 Fatty acid composition of Qingjinju seed oils

The oil samples were first converted to fatty acid methyl esters (FAMEs) using the method described by Carreau and Dubacq[30]. The FAMEs were then quantified by gas chromatography-mass spectrometry (GC-MS) using a Trace1310 ISQ (Thermo Scientific, Waltham, MA, USA) equipped with a flame ionization detector (FID) and TG-5MS capillary column (30m × 0.25mm i.d., 0.25μm film thickness). The GC temperature program was as follows: initial temperature, 80 °C for 1 min; increased to 200 °C at 10 °C/min; increased to 250 °C at 5 °C /min; increased to 270 °C at 2 °C/min and held for 3 min. The analytical conditions were: detector temperature, 290 °C; injection volume, 1 μL; flow rate of carrier gas, 1.2 mL/min; ionization voltage, 70eV; scanning range, 30-400 amu. To identify and quantify the FAMEs, FAME mixture standards (35 components, C8-C24) were injected into the instrument under the same conditions. The FAMEs were considered positively identified if their mass spectra and retention indices were comparable to the FAME mixture standards. Quantification of the samples was performed using the data handling software of the GC/MS system.

2.7 Volatile compounds of Qingjinju seed oils

A measured quantity of oil samples were transferred to 10 mL vials containing 3 mL saturated sodium chloride solution and balanced at 37 °C for 30 min. (SPME) was performed using a 100 μL polydimethylsiloxane (PDMS) fiber (Supelco Co., Bellefonte, PA, USA), previously tested by Carasek and Pawliszyn[31]. The PDMS fiber was exposed to the volatiles present in the headspace for 30 min at 37 °C to allow for efficient adsorption and then inserted into the GC-MS injector for 5 min to achieve desorption of the volatiles.

The volatiles were analyzed using an Agilent 6890/5975 GC-MS (Agilent Technologies, Santa Clara, CA, USA) equipped with an HP-5MS capillary column (30m × 0.25mm i.d., 0.25 μm film thickness). The GC temperature program was as follows: initial temperature, 40 °C for 5 min; increased to 160 °C at 3 °C/min and held for 2 min; increased to 250 °C at 8 °C/min and held for 3 min. The analytical conditions were: flow rate of carrier gas, 1.0 mL/min; injector temperature, 250 °C; injection mode, splitless; ion source temperature, 230 °C; quadrupole temperature, 180 °C; ionization voltage, 70 eV; scanning range, full-scan mode. The volatiles were authenticated by comparing their MS spectra with those from the NIST mass spectral library of the GC/MS system. The relative percentage of the volatiles was measured by area normalization.

2.8 Nutrient substances of Qingjinju seed oils

The total phenolic content of the oil samples was measured using the PRC National Standards for the grain industry LS/T 6119-2017[32].

The limonin and nomilin content was determined using high-performance liquid chromatography (HPLC) following the method described by Chinapongtitiwat et al.[33], with minor modifications. A measured quantity of oil samples was mixed with dichloromethane (60 mL) and reflux extracted at 50 °C for 1 h. The content

was then vacuum filtered through Whatman No. 1 filter paper and concentrated at 50 °C under reduced pressure using a rotary evaporator (RE-2000B, Shanghai Zengsen Instrument Technology Co., Ltd, Shanghai, China). The residues were dissolved in acetonitrile (5 mL) and filtered through a 0.45 μm syringe filter. A portion of the filtered solution (10 μL) was then analyzed on a C18 HPLC column (4.6 mm × 250 mm, 5 μm) using an Agilent 1260 Infinity II HPLC equipped with a diode array detector (DAD). The analytical HPLC conditions were as follows: mobile phase, acetonitrile:water (45:55, v/v); flow rate, 1.0 mL/min; column oven temperature, 25 °C; DAD detection, 210 nm. The limonin and nomilin content was quantified by co-chromatography using mixture standards (Sigma-Aldrich).

The total α-tocopherol content was determined using HPLC following the method described by Anwar el at.[15], with modifications. Oil samples were saponified by mixing the oil (0.5 g) with 2% ascorbic acid-ethanol solution (100 mL) and 50% aqueous KOH solution (25 mL) and heating at 70 °C for 30 min. The saponification liquid was extracted twice with petroleum ether (50 mL) and the combined organic phases were washed with deionized water (100 mL) until neutral. The organic layers were filtered through anhydrous sodium sulfate and evaporated to dryness under a stream of nitrogen. The extracts were re-dissolved in methanol (10 mL) and filtered through a 0.22 μm syringe filter. A portion of the filtered solution (10 μL) was then analyzed on a C18 HPLC column (4.6 mm × 150 mm, 5 μm) using a Thermo Scientific Ultimate 3000 HPLC equipped with a diode array-fluorescence detector (DAD-FLD). The analytical HPLC conditions were as follows: mobile phase, methanol:water (98:2, v/v); flow rate, 1.0 mL/min; column oven temperature, 20 °C; excitation/emission wavelengths, 294/328 nm. The total α-tocopherol content of the samples was quantified by co-chromatography with pure standards (Sigma-Aldrich).

The total flavonoid content was determined using the method described by Marinova et al.[34], with modifications. Either oil samples (25 μL) or a standard solution of rutin was added to a 25 mL volumetric flask and mixed with 5% $NaNO_2$ (1 mL) for 10 min. A 10% $Al(NO_3)_3$ (1 mL) solution was then added and the mixture was incubated for an additional 10 min. Finally, 4% NaOH (10 mL) was added and the volume was brought to 25 mL with ethylene glycol. The solution was mixed and incubated for 15 min, after which the absorbance was measured at 510 nm against a prepared reagent blank. The total flavonoid content of the oils is expressed as mg rutin equivalents (RE)/100 g oils.

The β-sitosterol content was determined by HPLC using the method reported by Maria et al.[35]

2.9 Statistical analysis

All values are presented as the mean ± standard deviation of triplicate measurements. The data were analyzed using one-way analysis of variance (ANOVA) procedures and the means were compared using Tukey's test (SPSS 17.0) at a significance level of $\alpha = 0.05$.

3. RESULTS and DISCUSSION

3.1 Physicochemical analysis of Qingjinju seed oils

Table 1 details the physicochemical properties of the Qingjinju seed oils isolated by screw press, solvent, and supercritical CO_2 extraction and should be interpreted with respect to these specific techniques. The yield of oil significantly varied based on the extraction process, with solvent extraction providing the greatest quantity (35.07%), followed by screw press (31.04%), and supercritical CO_2 extraction (27.69%). These results disagree with those reported by Pradhan et al[36], which may reflect differences between the initial oil content of the seed varieties.

Both the specific gravity (20 °C) and the refractive index (25 °C, cP) varied across the different extraction techniques; however, they showed similar patterns with screw press extraction oil (SPO) > supercritical CO_2 extraction oil (SCO) > solvent extraction oil (SO). The specific gravity of the oils was significantly different, with values ranging between 0.903 and 0.934 across the different extraction techniques. The high specific gravity of SCO is likely due to the inclusion of seed particulate mixed into the oils during the pressing process. The refractive indices ranged from 1.4660-1.4687, which is in good agreement with the findings of Kiralan et al.[37]

The distinct extraction methods resulted in notable color differences between the Qingjinju seed oils. The color of the oils processed by screw press, solvent, and supercritical CO_2 extraction was atrovirens, reseda and faint yellow, respectively. SPO had a deep color, with a value of 4.0 in the red scale, 74.1 in the yellow scale, and 7.0 in the blue scale, which may indicate that screw pressing allows for the extraction of chlorophyll[21].

The average acid and peroxide values of the Qingjinju seed oils ranged from 0.85-1.02 mg KOH/g oil and 4.38-11.94 meq O_2/kg oil, respectively. These values are lower than those obtained by Yilmaz et al[14] in their examination of lemon seed oil. The method of extraction played a role in the acid and peroxide levels of Qingjinju seed oils, with SPO displaying higher acid (1.02) and peroxide (11.94) values as compared to SO and SCO. These findings were in contrast to those reported by Pradhan et al.[36], Moreno et al.[21] and Ixtaina et al.[38] These differences likely result from the high temperature generated during screw press extraction, which can lead to decomposition of the triglycerides into fatty acids and contribute to the formation of peroxides[39].

The iodine number of the Qingjinju seed oils ranged between 107.85 and 118.84 g I_2/100 g, which was in good agreement with the values reported by Manaf el at.[4] While the iodine numbers of SPO and SO were similar, they were significantly higher than for SCO. As the iodine value is a direct measure of the number of double bonds, the higher iodine number for SPO and SO indicates that these oils contain a greater amount of unsaturated compounds. This conclusion is also supported by the GC-MS analysis of the fatty acids performed in this work.

The saponification values of SPO and SCO were 206.16 mg and 207.69 mg KOH/g oil, respectively. These values are slightly higher than those reported by Anwar el al.[15] for citrus seed oils from four different *Citrus* spp. (180.9 to 198.9 mg KOH/g oil). However, SO had a significantly lower saponification value (170.01 KOH/g oil), indicating that the different extraction methods influence the saponification values, which agrees with the findings of Moreno et al.[21]

The unsaponifiable values of the Qingjinju seed oils ranged from 0.37% to 0.62% and were significantly different across all three extraction methods. These values are notably less than the unsaponifiable values of limonin seed[14] and musk lime seed oils[4].

3.2 Nutrient substances of Qingjinju seed oils

Qingjinju seed oils can be utilized as a source of oil for human consumption. Table 2 shows the nutrient substances of the Qingjinju seed oils obtained by different extraction methods.

β-sitosterol is the main sterol in vegetable oils. The β-sitosterol content of the Qingjinju seed oils was 1776-1947 mg/kg, which is higher than observed for oils from *C. aurantifolia* or *C. grandis* [18].

Limonoids are unique substances with highly oxygenated triterpenoid backbones and are only found in the Rutaceae and Meliaceae[40] families. Citrus limonoids have been reported to display anticarcinogenic[41]activity and have been suggested as natural chemo preventatives or nutraceuticals[42]. Limonin and nomilin are the major limonoids and are the primary contributors to the bitterness of citrus fruits and products. The limonin and nomilin content in the Qingjinju seed oils significantly varied across the extraction methods. The limonin content in SO is 722.49 mg/kg, which is 4.22 and 4.90 times less than SCO and SPO, respectively. The nomilin content in SO is 65.02 mg/kg, which is 10.35 and 8.23 times less than SCO and SPO, respectively. This decreased limonoid content may be due to the decomposition of limonin and nomilin during the long exposure to high temperatures in the solvent extraction. Citrus oils are generally characterized by a high percentage of limonin[43-44]. Interestingly, the content of limonin and nomilin in the Qingjinju seed oils was higher than that of other citrus fruits[42].

The flavonoids in the Qingjinju are reported to be 3′,5′-di-C-β-glucopyranosylphloretin, naringin, and hesperidin, and the total flavonoid content in peel ranges from 1168 to 1888 mg/100 g[45]. The total flavonoid content in the Qingjinju seed oils was greatest for solvent extraction (28.66 mg/mL), followed by screw press (26.25 mg/mL) and supercritical CO_2 (24.89 mg/mL) extraction. Notably, the total flavonoid content in the Qingjinju seed oils was higher than that reported for *Moringa oleifera* seed oil (18.24 mg/g)[46], which

has been valued for its medicinal properties. This suggests that the Qingjinju seed oils may also display beneficial antioxidant activity and could be applied for the natural management of various chronic diseases.

The α-tocopherol content of the Qingjinju seed oils ranged from 171.68 to 202.70 mg/kg, which was higher than lemon (155-110 mg/kg)[14], mitha (26.4 mg/kg)[15], and tangerine (116.74 mg/kg)[47] seed oil, but lower than grapefruit (380 mg/kg), mussami (220 mg/kg), or kinnow (557.82 mg/kg)[15]seed oil. The α-tocopherol content varied significantly based on the extraction method, which agrees with the findings of Malacrida et al.[47]

The total phenolic content of the Qingjinju seed oils ranged from 3.83 to 6.25 mg/kg, which was lower than mandarin, orange, lemon, or tangerine seed oils[19, 47]. This difference can be attributed to both the chemical composition of the different *Citrus* spp. and the manner in which the results were obtained. There were significant differences between total phenolic content of the oils obtained using the different extraction methods, which is in agreement with results reported by Mustafa el al.[37] The total phenolic content of SCO was 6.25 mg/kg, which was 1.2 and 1.6 times higher than SPO and SO, respectively. Overall, screw press and supercritical CO_2 extraction outperformed solvent extraction in obtaining nutrient substances from Qingjinju seeds.

3.3 Fatty acid composition of Qingjinju seed oils

The fatty acid composition of the Qingjinju seed oils is detailed in Table 3. Qingjinju seed oils contained a variety of major fatty acids including oleic (34.08-35.49%), linoleic (29.79-30.79%), palmitic (17.17-17.76%), stearic (8.02-8.94%), linolenic (6.50-6.81%), and palmitoleic (1.24-1.53%) acids. This differs from the report by Manaf el at[4], which found that linoleic (31.8%), oleic (29.6%), and palmitic (21.4%) acids were the predominant fatty acids. This is likely due to differences in the production region, as has been observed for grapefruit (*C. paradisi*)[15, 17], mussami (*C. sinensis*)[15, 17], and kinnow (*C. reticulata*)[15, 17].

There were significant differences between several of the fatty acid profiles due to the method used for extraction. For example, oleic and linoleic acid content significantly varied across all three extraction methods, which is in agreement with results reported by Pradhan et al.[36] The unsaturated fatty acid content in the oils extracted using the different methods ranged from 73.12 to 73.54%, which was higher than *C. sinensis* ,*C. paradisi* , *C. aurantium* , and *C. reticulata*[11]

3.4 Volatile compounds of Qingjinju seed oils

The volatile compounds of the Qingjinju seed oils are described in Table 4. The primary volatile compound classes in the Qingjinju seed oils were esters, aldehydes, hydrocarbons, acids, and alcohols, which agrees with results reported by Kalua el al[48]. The identity and relative concentrations of volatile compounds were significantly different between oils produced by the different extraction techniques, similar to results reported by Moreno et al.[21] A total of 43 volatile compounds were identified in SPO, 42 in SO, and 44 in SCO. The greatest number of compounds in both SPO and SCO were aldehydes, containing 16 and 13, respectively. In SO, the largest number of compounds in SO were hydrocarbons (18). Regardless of the extraction method, seven volatile compounds were observed: 2-methylaminobenzoic acid methyl ester, 2-methylaminobenzoic acid ethyl ester, (*E*)-2-heptenal, (*E* ,*E*)-2,4-heptadienal, (*E* ,*E*)-2,4-decadienal, 2,4-decadienal, and *n* -hexadecanoic acid. These compounds accounted for 40.52%, 46.19%, and 54.76% of the total volatile compounds in SPO, SO, and SCO, respectively. These appear to be the primarily volatile compounds of the Qingjinju seed oils.

The relative contents of volatile compounds in SPO were esters (30.28%), aldehydes (22.61%), and hydrocarbons (26.32%). The volatile compounds in SO were primarily comprised of hydrocarbons (43.74%), esters (25.79%), and aldehydes (19.91%). The volatile compounds in SCO were primarily composed of esters (32.55%), aldehydes (30.95%), and alcohols (12.91%). The relative content of limonene was 13.4 times higher in SPO (24.49%) than SCO, while no limonene was detected in SO. The relative content of limonene could therefore be used as a characteristic indicator to determine the extraction method. The relative contents of octane and 2,2,4,6,6-pentamethylheptane were 9.73% and 9.75% in SO, but were absent from SPO and

SCO. These two volatile compounds may be exogenously supplied by the solvent used during the extraction process. Whether these two volatile compounds are indicative of the solvent extraction technique requires further evaluation.

4. CONCLUTION

It may be concluded that the different extraction methods modify the physicochemical properties, nutrient substances, and volatile compounds of Qingjinju seed oils. The oil yield obtained by solvent extraction (35.07%) was higher than that of screw press (31.04%) and supercritical CO_2 (27.69%) extraction. Specific gravity, refractive indices, acid values, peroxide value, and unsaponifiable matters of SO were lower than that of SPO and SCO, suggesting that SO possessed superior physicochemical properties. The Qingjinju seed oils consisted of major fatty acids including oleic acid (34.08-35.49%), linoleic acid (29.79-30.79%), palmitic acid (17.17-17.76%), stearic acid (8.02-8.94%), and linolenic acid (6.50-6.81%), making the oil a good source of unsaturated fatty acids. SPO and SCO contained limonin (3544.63 mg/kg, 3052.96 mg/kg) and nomilin (534.90 mg/kg, 673.13 mg/kg), which were underrepresented in SO (722.49 mg/kg, 65.02 mg/kg). Supercritical CO_2 and screw press extraction were therefore more beneficial for retaining the limonoid content in Qingjinju seed oils. The volatile compounds of SPO and SCO were primarily aldehydes, while hydrocarbons were the major volatile class in SO. The seven main volatile compounds were identified across the extraction methods. Qingjinju seed oils could provide valuable stock oil for food or chemical industries and different extraction methods can be selected according to their intended use.

ABBREVIATIONS USED

SPO: screw press oil; SO: solvent extraction oil; SCO: supercritical CO_2 extraction oils

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Table 1 Physicochemical properties of Qingjinju seed oils obtained using different extraction methods

Properties Evaluated	SPO	SO	SCO
yield (%)	31.04±0.62*	35.07±0.61*	27.69±0.79*
specific gravity (20 °C)	0.934±0.01*	0.903±0.01*	0.912±0.01*
refractive indices (25 °C, cP)	1.4687±0.0001*	1.4660±0.0002*	1.4686±0.0002*
color (red units)	4.0±0.1*	0.6±0.1	0.9±0.1
color (yellow units)	74.1±0.5*	9.6±0.2*	60.1±0.4*
color (blue units)	7.0±0.1*	0	0
acid values (mg KOH/g of oil)	1.02±0.01	0.85±0.01*	1.01±0.43
peroxide value (meq O_2/kg oil)	11.94±0.11*	4.38±0.09	6.53±0.21
iodine number (g I_2/100g oil)	117.35±0.12	118.84±0.88	107.85±1.15*
saponification numbers (mg KOH/g oil)	206.16±3.50	170.01±1.63*	207.69±2.03
unsaponifiable matters (%)	0.43±0.03*	0.37±0.01*	0.62±0.01*

SPO: screw press oils; SO: solvent extraction oils; SCO: supercritical CO_2 extraction oils. Significant differences ($p < 0.05$) between the values of a given property are indicated by an asterisk (*).

Table 2 Nutrient substances of the Qingjinju seed oils obtained using different extraction methods

Nutrient Substances	SPO	SO	SCO
β-sitosterol (mg/kg)	1776.98±1.26*	1947.62±1.3*	1928.55±4.9*
limonin(mg/kg)	3544.63±26.9*	722.49±5.09*	3052.96±9.11*
nomilin (mg /kg)	534.90±3.39*	65.02±0.87*	673.13±2.52*
α-tocopherols(mg/kg)	194.50±1.05*	202.70±0.95*	171.68±1.84*
total flavonoids (mg/mL)	26.25±0.25*	28.66±0.27*	24.89±0.11*
total phenolic (mg/kg)	5.14±0.19*	3.83±0.16*	6.25±0.15*

SPO: screw press oils; SO: solvent extraction oils, SCO: supercritical CO_2 extraction oils. Significant differences ($p < 0.05$) between the values of a given property are indicated by an asterisk (*).

Table 3 Fatty acid compositions of the Qingjinju seed oils obtained using different extraction methods

Fatty acids	SPO (%)	SO (%)	SCO (%)
palmitic (C16:0)	17.17±0.06*	17.33±0.27	17.76±0.30*
palmitoleic(C16:1)	1.53±0.02	1.24±0.05*	1.43±0.04
margaric (C17:0)	0.12±0.01	0.10±0.01	0.96±0.01
stearic (C18:0)	8.09±0.06	8.94±0.05*	8.02±0.12
oleic (C18:1 n-9)	35.49±0.15*	34.73±0.14*	34.08±0.24*
linoleic (C18:2 n-6)	29.79±0.03*	30.31±0.16*	30.79±0.35*
linolenic (C18:3 n-3)	6.50±0.12	6.81±0.06	6.65±0.15
arachidic (C20:0)	0.59±0.01	0.59±0.01	0.48±0.02*
gondoic (C20:1)	0.11±0.01	0.14±0.03	0.09±0.01
behenic (C22:1)	0.12±0.01	0.02±0.01*	0.10±0.01
tetracosanoic (C24:0)	0.40±0.01	0.44±0.04	0.25±0.04*
unsaturated fatty acid	73.54±0.07*	73.27±0.10	73.12±0.07

SPO: screw press oils; SO: solvent extraction oils, SCO: supercritical CO_2 extraction oils. Significant differences ($p < 0.05$) between the values of a given property are indicated by an asterisk (*).

Table 4 Volatile compounds of the Qingjinju seed oils obtained using different extraction methods

Identified compound	SPO (%)	SO (%)	SCO (%)
esters	**30.29**	**25.79**	**32.55**
2-methylaminobenzoic acid methyl ester	26.5	24.52	29.81
2-methylaminobenzoic acid ethyl ester	2.48	1.27	2.74
piperidin-2-one-5-carboxylic acid,5,6-didehydro-, methyl(ester)	0.88	-	-
methyl picolinate	0.43	-	-
aldehydes	**23.27**	**19.91**	**30.95**
(*E*)-2-heptenal	1.69	1.14	3.53
(*E*,*E*)-2,4-heptadienal	0.66	1.01	2.72
2,4-decadienal	0.71	4.28	4.25
(*E*,*E*)-2,4-decadienal	2.93	12.48	10.21
hexanal	2.89	-	3.71
furfural	4.87	-	-
pyrrole-2-carboxaldehyde	0.61	-	-
phenylacetaldehyde	2.54	-	-
(*E*)-2-octenal	0.85	-	1.34
5-methylhexanal	0.37		1.06
(*E*)-2-tridecenal	0.24	-	-
3-ethylbenzaldehyde	0.3	-	-
(*E*,*E*)-2,4-nonadienal	0.3	-	2.13
(*E*)-2-decenal	0.36	-	-
3-ethoxybenzaldehyde	1.77	-	-
(*E*)-2-tridecenal	0.2	-	-
glyoxime	-	0.18	-
heptanal	-	0.63	-
2-hydroxy-4-methylbenzaldehyde	-	0.19	-
5-methylfurfural	1.98	-	0.52
(*E*)-2-nonenal	-	-	0.54
3-methyl-2-butenal	-	-	0.33
5-hydroxymethylfurfural	-	-	2.88
vanillin	-	-	0.62

Identified compound	SPO (%)	SO (%)	SCO (%)
Hydrocarbons	**26.32**	**43.74**	**9.09**
4-methyl-1,4-hexadiene	0.21	-	-
β-phellandrene	0.95	-	-
limonene	24.49	-	1.82
cycloheptane	0.51	-	-
1,3-cyclopentadiene	0.37	-	-
octane	-	9.73	-
2,2,4,6,6-pentamethylheptane	-	9.76	-
1,4-pentadiene	-	0.69	-
1-methylazetidine	-	0.17	-
2,4,4-trimethyl-1-hexene	-	0.18	-
1-fluorooctane	-	0.45	-
dodecane	-	2.82	-
2-methyl-1,5-hexadiene	-	0.32	-
tridecane	-	1.83	-
2-methylpentane	-	0.8	-
2,2-dimethylbutane	-	0.34	-
icosane	-	1.37	
tetracosane	-	1.59	-
3-ethyl-5-methylheptane	-	0.21	-
cyclopropylcyclohexane	-	0.68	-
(*E*)-9-octadecene	-	0.22	-
squalene	-	11.51	-
(*E*)-5-octadecene	-	1.07	-
2,4,6-trimethyloctane	-	-	2.58
1-tetradecyne	-	-	0.62
butylcyclopentane	-	-	0.56
(1-methylethylidene)cyclobutane	-	-	2.39
n-butylcyclohexene	-	-	1.12
Acids	**12.13**	**1.49**	**5.40**
n-hexadecanoic acid	5.55	1.49	1.50
2-amino-4-methylbenzoic acid	1.11	-	-
hexanoic acid	1.82		
(*Z*,*Z*)-9,12-octadecadienoic	3.65	-	-
benzoic acid	-	-	1.95
octanoic acid	-	-	0.58
nonanoic acid	-	-	0.86
6-benzoylhexanoic acid	-	-	0.51
Alcohols	**5.51**	**1.46**	**12.92**
glycolaldehyde dimethyl acetal	0.83	-	-
2,3-butanediol	1.54	-	-
2-octen-4-ol	0.22	-	-
3-fluorobenzyl alcohol	1.49	-	-
(*E*)-3,7-dimethyl-2,6-octadien-1-ol acetate	0.57	-	-
3β-(1-hydroxy-1-methylethyl)-5-methylene-8aβ-methyldecahydronaphthalene	0.25	-	-
phytol	0.61	-	-
1-hexanol,2-ethylene	-	0.71	-
2-methylenecyclopentanol	-	0.75	-
1-hexanol	-	-	0.82
L-arabinitol	-	-	0.44

Identified compound	SPO (%)	SO (%)	SCO (%)
phenethyl alcohol	-	-	1.63
cyclopentanemethanol	-	-	0.44
1,2-heptanediol	-	-	2.02
(*E*,*E*)-4,6-nonadien-8-yn-3-ol	-	-	0.63
2-cyclopentylethanol	-	-	1.69
2-cyclohexen-1-ol	-	-	0.96
2-methylenecyclopentanol	-	-	1.4
Ketones	**1.10**	**0.90**	**4.53**
2-pyrrolidinone	0.73	-	-
7-ethyl-4-nonanone	0.37	-	-
acetophenone	-	0.31	-
2-ethylcyclopentanone	-	-	1.78
3,5-octadien-2-one	-	0.59	-
3,4-dimethyl-4-hexen-2-one	-	-	2.29
γ-octalactone	-	-	0.46
Others	**1.42**	**6.70**	**4.53**
isopropenylpyrazine	0.15	-	-
2,3-dihydrobenzofuran	0.79	-	-
1*H*-imidazole	0.24	1.86	-
2-ethylacridine	0.24	-	-
4-(1-methyl-piperidin-4-yl)-benzene-1,2-diol	-	0.23	-
2-(1-cyclopropyl-ethylidene)-malononitrile	-	0.16	-
N-ethylpropanamide	-	0.22	-
naphthalene	-	1.25	-
2-deoxy-D-erythro-pentose	-	0.59	-
2-methylnaphthalene	-	1.57	-
2-propenamide	-	0.15	-
1*H*-pyrazole	-	0.41	0.42
1,3-dimethylnaphthalene	-	0.26	-
toluene	-	-	1.25
(+)-phendimetrazine	-	-	0.73
3,5-dihydroxytoluene	-	-	1.16
benzothiazole	-	-	0.6
semioxamazide	-	-	0.37
Total identified	100.0	99.99	99.89
Number of compounds identified	43	42	44

SPO: screw press oils; SO: solvent extraction oils, SCO: supercritical CO_2 extraction oils.