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Quality Assessment of *Camellia oleifera* Oil Cultivated in Southwest China

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Abstract: *Camellia oleifera* oil has attracted increasing attention due to its well-balanced composition. In this study, we evaluated the oil content and chemical composition of *C. oleifera* oil cultivated in southwest China. The results showed that the acid and peroxide values were in line with the optimal quality index of the national standard of China. Oleic acid was the most predominant and important fatty acid, which accounted for 80.34–86.18%. The α-tocopherol, polyphenols and squalene ranged from 112.36 to 410.46 mg/kg oil, 14.22 to 53.63 mg/kg oil and 14.80 to 52.49 mg/kg oil, respectively. Principal component analysis (PCA) results showed that the synthesis score of introduced cultivars ('Changlin 3', 'Changlin 4' and 'Changlin 18') was higher that the local cultivars ('Chuanya 21' and 'Chuanlin 2'). This research demonstrated that the introduced *C. oleifera* could adapt to the environment and climate of southwest China and large-scale plant of these introduced cultivars. In addition, the *C. oleifera* oil rich in unsaturated fatty acid has enormous potential to become a kind of functional oil and possesses great prospects for pharmaceutical and industrial applications.

Keywords: *C. oleifera* oil; introduced cultivars; chemical composition; principal component analysis

1. Introduction

Camellia oleifera Abel. is the evergreen shrub or small tree plant of the *Camellia* of Theaceae and has been primarily distributed in the south of China for approximately 2300 years cultivation. In addition, Vietnam, Myanmar, Thailand, Malaysia and Japan have a small distribution [1]. *C. oleifera* oil, a kind of healthy and high-quality edible oil, is extracted from *C. oleifera* seed.

C. oleifera oil has both effects in nutrition and medicine owing to its rich beneficial ingredients, such as unsaturated fatty acid (UFA), vitamin E, phytosterols, polyphenols, squalene and a number of trace elements [2]. It is also known as "eastern olive oil" because of the fatty acid composition similar to olive oil [3]. Interestingly, the level of UFA in *C. oleifera* oil reaches more than 90%, principally including oleic acid and linoleic acid, and higher than that of olive oil [4]. Oleic acid is beneficial to people health through reducing the levels of low-density lipoprotein cholesterol, total cholesterol and glycemic index in the blood [5,6].

Linoleic acid, an essential polyunsaturated fatty acid (PUFA), can decrease the risk of cardiovascular and coronary heart disease [7]. The vitamin E (tocopherols) mainly exists in *C. oleifera* oil in the form of α-tocopherol and the content is twice as much as that of olive oil [8]. Polyphenols of *C. oleifera* oil contribute to the health effect on people and also are very significant for the oxidative stability of the polyunsaturated fatty acids in oil [9]. Moreover, squalene has a particular benefit for vascular health [10]. The trace element concentration in vegetable oil is significant because of the toxicological and nutritional characteristics [11].

Above all, *C. oleifera* oil is recommended as healthy edible oil by Food and Agriculture Organization of the United Nations (FAO) [12]. It has been applied to treat intestinal disorder and stomachache and to protect the organism against ulcerogenesis, oxidation and inflammation [13]. Many studies showed that long-term intake of *C. oleifera* oil could decrease cholesterol level, blood pressure, blood lipid, delay atherosclerosis and prevent decline of neurological function as well as protect liver to resist damage induced by CCl_4 [14,15]. In addition, *C. oleifera* oil can be used as a raw material for production natural skin care cosmetic [16].

The main cultivars of *C. oleifera* grown in southwest China (Sichuan) are the 'Chuanya' and 'Chuanlin' series; these cultivars have low yield and oil content. Therefore, we investigated the 'Changlin' series from east China. The 'Changlin' series of *C. oleifera* cultivars are excellent clones with high and stable yield, high oil content and strong disease resistance. There have been studies on 'Changlin' series cultivated in different environments. For example, Lin and colleagues used SRAP molecular markers for the identification and genetic analysis of *C. oleifera* 'Changlin' series superior clones [17].

The growth adaptability, biological characteristics, main economic characters, oil contents and fatty acid compositions were investigated and analyzed [18,19]. In order to breed cultivars with high quality oil that were well-adapted to the southwest China environment, we collected the fruits from introduced cultivars ('Changlin' series) and local cultivars ('Chuanya 21' and 'Chuanlin 2') cultivated in southwest China, and extracted *C. oleifera* oil, then comprehensively estimated oil content and quality characteristics of *C. oleifera* oil. This can provide some theoretical knowledge for breed selection and more in-depth research on *C. oleifera* oil quality functions.

2. Materials and Methods

2.1. Plant Materials and Reagents

The *C. oleifera* 'Changlin 3', 'Changlin 4', 'Changlin 18', 'Changlin 23', 'Changlin 26', 'Changlin 27', 'Changlin 53' and 'Changlin 166' cultivars were introduced to Ya'an (southwest China, latitude 29°93′ N, longitude 103°09′ E and 1030 m higher than sea level, with an average annual rainfall of 1500 mm, annual average air temperature of 15.4 °C, and relative humidity of 82%) from Jiangxi (east China) at 2012, and the local cultivar ('chuanya 21'and 'chuanlin 2'). In orchards, the *C. oleifera* trees were 7 years old, at a spacing of 3 × 3 m, on the consistent cultivation conditions. About 3 kg of *C. oleifera* fruits were collected from three trees with uniform characteristics were chosen for sampling. *C. oleifera* fruits without damage were collected by manual picking.

The tocopherols (>98%) and gallic acid (≥99%) were from Sigma Chemical Co. (St. Louis, MO, USA). Squalene (>98%) was from Aladdin Biotechnology Co., Ltd. (Shanghai China). Hexane, methanol, alcohol, sodium chloride, petroleum ether, isopropyl alcohol, potassium hydroxide and hydrogen peroxide were obtained from Chengdu Kelong Chemical Factory (Chengdu, China). All the reagents were analytical grade except that methanol and acetonitrile were chromatographic grade.

2.2. Oil Content Assay

The oil content of *C. oleifera* was determined according to the international standard (ISO 659-2009) [20]. In short, the soxhlet extraction method was used. Petroleum ether was the extraction solvent, the extraction temperature was 85 °C, the time was 10 h, and each sample was repeated three times.

2.3. Oil Extraction Assay

The oil extraction was performed as described previously with some modifications [21]. Briefly, the *C. oleifera* seeds were dried in an oven at 60 °C, and then the dried *C. oleifera* seed was ground to fine powder. The ratio of powder to the water was 1:4.5 (g/mL). Then, the mixture was shaken at 75 °C (160 rpm) for 150 min in a thermostat water bath. The

mixture was centrifuged to separate the oil and stored in the dark at 4°C for the following assays.

2.4. Determination of Acid, Peroxide and Iodine Values

The acid, peroxide and iodine values of *C. oleifera* oil were determined according to the international standards. (ISO 660-2009, ISO 3960-2007 and ISO 3961-2018, respectively) [22–24].

2.5. Determination of Fatty Acid Composition

The fatty acid composition was established by gas chromatography mass spectrometry (GC-MS) after transesterification. The fatty acids were methyl esterified (FAMEs) by potassium hydroxide/methanol according to previous method [25]. About 100 mg of oil was transferred into a ground glass stoppered test tube, treated with 2 mL 1 mol/L NaOH/methanol. The sample was mixed on vortex mixer, shook for 30 min at 40 °C. The methyl esters were extracted by 2 mL n-hexane, and the aqueous phase was discarded. The n-hexane extract was washed with distilled water and dried by anhydrous sodium sulfate, filtered for later analysis by GC-MS.

Then, the GC-MS analysis for the FAMEs were conducted by Agilent 7890A gas chromatograph and 5977C mass spectrometry (Agilent Technologies, Santa Clara, California, USA) and equipped with a capillary column HP-5MS (30 m × 0.25 mm; 0.25 μm). The oven temperature programmed from 60 °C hold of 2 min, increasing at 15 °C/min to 150 °C hold of 2 min, then 15 °C/min increasing to 280 °C hold of 3 min. The carrier gas was helium at a flow rate of 0.6 mL/min, the split ratio 100:1. The injector temperature was 240 °C, and the detector temperature was 260 °C. EI ion source, and mass scan ranged from 50 to 500 m/z, and the solvent was delayed 3 min. The FAMEs profiles were identified by comparing the database (National Institute of Standard Technology Library, NIST). The individual fatty acid content was expressed as a percentage of total fatty acids [26].

2.6. Determination of Tocopherols

Qualitative and quantitative analysis of tocopherols in *C. oleifera* oil were determined by high performance liquid chromatography (HPLC) [27]. The analysis was performed by an Agilent 1260 HPLC (Agilent Technologies, Santa Clara, California, USA) equipped with ZORBAX SB-C18 column (150 × 4.6 mm, 5.0 μm) and a fluorescence detector. The excitation wavelength was 295 nm, and the emission wavelength was 325 nm. Methanol was the mobile phase with a flow rate of 0.8 mL/min, and the column temperature was 35 °C.

2.7. Determination of Polyphenols

The polyphenol extraction was performed according to the previous method with some modifications [28]. The 0.5 g oil sample was added to 5 mL n-hexane. 2 mL aqueous methanol (80%, *v/v*) was applied to extract oil for three times. The extract was stored at room temperature overnight. The residual oil was removed by n-hexane. The extract was concentrated by rotary evaporator, and adjusted to 10 mL with methanol. Then, 0.1 mL polyphenols extract was added with 0.02 mL Folin–Ciocalteu and 0.08 mL 10% sodium carbonate solution for 5 min, and then we added 0.8 mL distilled water. The absorbance of mixture was read at 765 nm by Spectra Max M2 microplate reader (Molecular Devices Corp., Silicon Valley, California, USA) after incubation in dark for 1 h. The polyphenols quantities were given as milligram gallic acid equivalents per kilogram.

2.8. Determination of Squalene

The squalene was evaluated by HPLC. Firstly, the oil was saponified as previous method [29]. About 1.0 g of oil was transferred into 250 mL separatory funnel, treated with 50 mL of 2 mol/L potassium hydroxide/ethanol solution. The sample was mixed on vortex mixer, and saponified for 60 min at 80 °C in water bath. After cooling to room temperature,

we added 50 mL of petroleum ether and extracted three times. The petroleum ether phase was washed to neutrality by distilled water and concentrated using a rotary evaporator at 40 °C and dissolved with acetonitrile (1 mL). Subsequently, the sample was analyzed by Agilent 1260 HPLC (Agilent Technologies, Santa Clara, California, USA). The detection conditions were as follow: ZORBAX SB-C18 column (150 × 4.6 mm, 5.0 μm) with a diode array detector. The wavelength was 325 nm, column temperature was 30 °C and mobile phase was methanol/acetonitrile (60/40, v/v) at a flow rate of 1.0 mL/min.

2.9. Determination of Trace Elements

The trace elements were analyzed by inductively coupled plasma mass spectrometry (ICP-MS) [30]. We placed 0.3 g oil sample into a digestion vessel, and then 5 mL HNO$_3$ (5%, v/v) and 1 mL H$_2$O$_2$ solution were added and fully digested in a microwave digester (Anton Paar Co., Graz, Austria). Excess acid was removed under 120 °C, and the residual was diluted to 25 mL with ultrapure water. The trace elements were detected on a PerkinElmer NexION350D system (PerkinElmer Co., Waltham, Massachusetts, USA). The RF power was 1500 W, scanning mode was peak hopping, nebulizer flow rate was 0.8 L/min, coolant gas flow was 15 L/min, replicates were three times, sampling depth was 10 mm and isotopes were selected as ^{24}Mg$^+$, ^{43}Ca$^+$, ^{53}Cr$^+$, ^{55}Mn$^+$, ^{57}Fe$^+$, 63Cu+, ^{66}Zn$^+$, ^{75}As$^+$, ^{111}Cd$^+$ and ^{208}Pb$^+$.

2.10. Statistical Analysis

The results were expressed as the mean ± standard deviation (SD) and were carried out in triplicate. Analysis of variance (ANOVA), DUNCAN test and principal component analysis (PCA) were performed using the IBM SPSS Statistics version 20.0 (SSPS Inc., Chicago, IL, USA). *P*-values were reported based on 5% probability and 95% confidence level.

3. Results and Discussion

3.1. Oil Content

The oil content of the C. oleifera oil is presented in Figure 1, and the oil content of all introduced cultivars was higher than the local cultivars ('chuanya 21' and 'chuanlin 2'). In addition, the oil content of 'changlin 166' was the highest and reached 51.92%. The oil contents of 'changlin 3', 'changlin 4', 'changlin 18', 'changlin 23' and 'changlin 166' were higher than the excellent tree standard (45%) established by the People's Republic of China Forestry Standard (LY/T 1730.1-2008) [31]. The introduced cultivars were adapted to the local climate and had high oil contents.

3.2. Acid, Peroxide and Iodine Values

As shown in Table 1, the acid value, peroxide value and iodine value of *C. oleifera* oil were ranged from 1.23 to 1.92 mg/g, 0.52 to 1.73 mmol/kg and 83.80 to 94.69 g/100 g, respectively. The acid value of *C. oleifera* oil was less than 2.0 mg/g, and the peroxide value of *C. oleifera* oil was no more than 0.25 g/100g (9.85 mmol/kg). Thus, the acid and peroxide values were up to the optimal quality index of the China national standard (GB/T 11765-2018) [32].
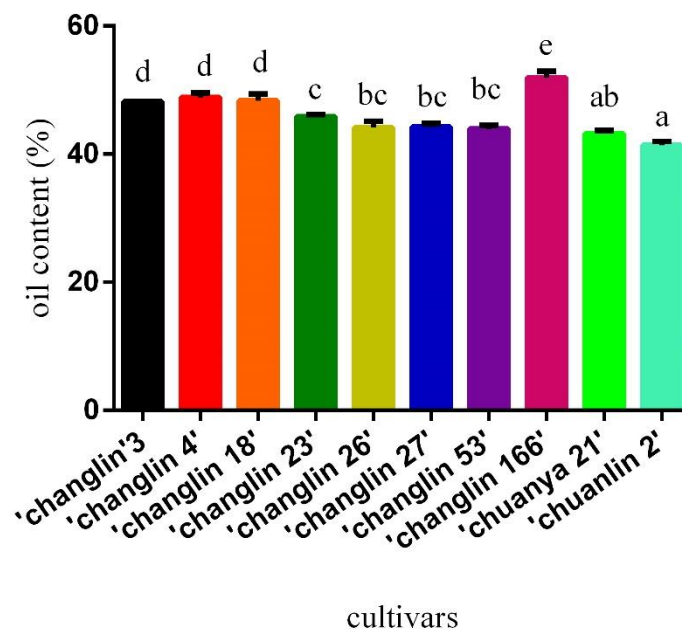
Figure 1. The oil content of *C. oleifera* oil of ten cultivars. The data are expressed as the mean ± standard deviations, n = 3. Different letters indicate significantly different values, *p* < 0.05.

Table 1. Acid, peroxide and iodine values in *C. oleifera* oil from ten cultivars.

	Acid Value (mg/g)	Peroxide Value (mmol/kg)	Iodine Value (g/100g)
'Changlin 3'	1.58 ± 0.11 [a]	0.79 ± 0.04 [b]	88.72 ± 1.69 [bc]
'Changlin 4'	1.61 ± 0.17 [b]	0.52 ± 0.03 [a]	88.92 ± 0.70 [bc]
'Changlin 18'	1.23 ± 0.05 [a]	1.06 ± 0.05 [c]	89.89 ± 0.87 [bc]
'Changlin 23'	1.92 ± 0.19 [b]	0.95 ± 0.03 [b]	85.33 ± 1.01 [a]
'Changlin 26'	1.83 ± 0.10 [b]	1.22 ± 0.07 [d]	88.01 ± 0.54 [b]
'Changlin 27'	1.74 ± 0.22 [b]	1.73 ± 0.03 [f]	88.90 ± 1.01 [bc]
'Changlin 53'	1.60 ± 0.12 [b]	0.72 ± 0.01 [b]	94.69 ± 0.60 [d]
'Changlin 166'	1.24 ± 0.06 [a]	0.55 ± 0.06 [a]	91.17 ± 0.34 [c]
'Chuanya 21'	1.83 ± 0.02 [b]	1.50 ± 0.06 [e]	83.80 ± 0.36 [a]
'Chuanlin 2'	1.89 ± 0.08 [b]	1.61 ± 0.09 [ef]	84.64 ± 0.61 [a]

Data are expressed as the mean ± standard deviations, n = 3. Different letters in the same row indicate significantly different values, *p* < 0.05.

The acid value reflects the amount of free fatty acid, and the peroxide value represents the degree of oxidation of oil and fatty acid [33]. In this study, the acid value and peroxide value was lower than that of previous works [34,35]. The iodine value of local cultivar was lower than introduced cultivars. The iodine value expresses the storage stability of oil. The iodine value was less than 100 g/100 g, indicating that they were non-drying oil [36]. The lower acid, peroxide value and moderate iodine value were related to the *C. oleifera* seed ripeness, processing method, storage condition and time of oil samples and also showed the excellent quality of the *C. oleifera* oil [37].

3.3. Fatty Acid Composition and Content

The major fatty acid composition of the *C. oleifera* oil was shown in Table 2. It could be observed that the fatty acid composition of *C. oleifera* oil included lauric acid (C12:0), myristoleic acid (C14:1), palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2) and eicosenoic acid (C20:1), respectively. The *C. oleifera* oil did not contain erucic acid (C22:1), which is harmful for health. The results of fatty acid composition agreed with other works [38,39]. The oleic acid is a principal and important fatty acid in *C. oleifera* oil.

Table 2. Fatty acid composition in *C. oleifera* oil of ten cultivars (%).

	C12:0	C14:1	C16:0	C18:0	C18:1	C18:2	C20:1
'Changlin 3'	0.01 ± 0.01 [a]	0.01 ± 0.01 [a]	9.19 ± 0.13 [c]	1.16 ± 0.05 [ab]	80.53 ± 0.34 [a]	8.95 ± 0.18 [g]	0.16 ± 0.02 [abc]
'Changlin 4'	-	0.02 ± 0.01 [a]	9.26 ± 0.12 [c]	1.43 ± 0.04 [b]	81.10 ± 0.32 [a]	8.24 ± 0.71 [fg]	0.26 ± 0.03 [c]
'Changlin 18'	0.01 ± 0.01 [a]	0.03 ± 0.03 [a]	8.06 ± 0.32 [b]	1.61 ± 0.13 [c]	82.35 ± 1.00 [ab]	7.75 ± 0.53 [efg]	0.20 ± 0.04 [bc]
'Changlin 23'	0.01 ± 0.00 [a]	-	7.66 ± 0.19 [b]	1.25 ± 0.11 [b]	85.44 ± 0.62 [cd]	5.50 ± 0.35 [ab]	0.13 ± 0.02 [ab]
'Changlin 26'	0.01 ± 0.00 [a]	-	7.55 ± 0.16 [b]	1.25 ± 0.05 [b]	85.67 ± 0.55 [d]	5.40 ± 0.31 [ab]	0.13 ± 0.02 [ab]
'Changlin 27'	0.01 ± 0.01 [a]	0.01 ± 0.01 [a]	8.12 ± 0.08 [b]	1.45 ± 0.03 [bc]	83.36 ± 0.17 [bc]	6.88 ± 0.08 [cde]	0.16 ± 0.01 [abc]
'Changlin 53'	0.02 ± 0.00 [a]	-	7.61 ± 0.25 [b]	0.84 ± 0.27 [a]	85.38 ± 1.28 [cd]	5.97 ± 0.82 [bc]	0.09 ± 0.01 [a]
'Changlin 166'	0.02 ± 0.02 [a]	0.01 ± 0.00 [a]	6.72 ± 0.36 [a]	1.22 ± 0.18 [b]	86.18 ± 1.23 [d]	4.79 ± 0.36 [ab]	0.10 ± 0.01 [ab]
'Chuanya 21'	0.01 ± 0.01 [a]	0.02 ± 0.02 [a]	8.99 ± 0.08 [c]	1.31 ± 0.05 [bc]	85.38 ± 0.49 [cd]	4.19 ± 0.42 [a]	0.10 ± 0.02 [ab]
'Chuanlin 2'	0.01 ± 0.00 [a]	0.02 ± 0.01 [a]	9.01 ± 0.10 [c]	1.65 ± 0.04 [c]	80.34 ± 0.51 [a]	7.35 ± 0.68 [def]	0.14 ± 0.09 [ab]

Data are expressed as the mean ± standard deviations, n = 3. The "-" represents not detected in the identification process. Different letters in the same row indicate significantly different values, $p < 0.05$.

Our results showed that the content of oleic acid ranged from 80.53% to 86.18%, followed by palmitic acid (6.72–9.26%), linoleic acid (4.19–8.95%), stearic acid (0.84–1.65%) and eicosenoic acid (0.09–0.26%). The contents of myristoleic and lauric acids were less than 0.03%. The oleic acid contents of 'Changlin 23', 'Changlin 26' and 'Changlin 166' were higher than local cultivar ('Chuanya 21'), and all introduced cultivars exceeded that of 'Chuanlin 2'. The UFA content of *C. oleifera* oil ranged from 87.85% to 91.44%.

The contents of oleic acid and UFA in *C. oleifera* oil from southwest China were higher than that east China could be attributed to the higher altitude [40,41]. The abundant UFA in *C. oleifera* oil is very important in developing and maintaining the nervous system as well as physiological function in body [19]. The content and composition fatty acid of *C. oleifera* oil at southwest China were consistent with origin that offer a manifestation that the *C. oleifera* has adapted to the southwest China environment and climate.

The previous research results were reported by Su and colleagues [26]. The contents of oleic acid were 74.20% (olive oil), 58.19% (canola oil), 25.90% (corn oil), 79.71% (sunflower oil), 39.46% (peanut oil), 22.40% (soybean oil) and 28.06% (pumpkin oil). In this study, the average content of oleic acid in *C. oleifera* oil was 83.57%. Therefore, the content of oleic acid in *C. oleifera* oil was higher than the common vegetable oils. The oleic acid is a monounsaturated fatty acid. The higher oleic acid of *C. oleifera* oil means it has high stability and digestibility and health-promoting effects that include lowering blood pressure, cholesterol and triglycerides [6].

3.4. Tocopherols Content

As shown in Table 3, the α-tocopherol content of *C. oleifera* oil ranged from 112.36 to 410.46 mg/kg oil. It should be noted that the β-tocopherol, γ-tocopherol and δ-tocopherol were not detected in *C. oleifera* oil. The α-tocopherol content was higher than that of other works in which the α-tocopherol contents were 97.80 mg/kg and 150.66 mg/kg (excepted for the local cultivars) [25,27].

These results suggested that the content of α-tocopherol was closely related to the cultivars and that the content of α-tocopherol from introduced cultivars was higher than the local cultivars. The α-tocopherol is the most bioactive structure in vitamin E [42]. Some previous studies demonstrated that α-tocopherol could facilitate fertility, lower oxidative stress, relief oxidative damage, reduce neuro-inflammation and delay aging [14,43]. In this study, the α-tocopherol content in the *C. oleifera* oil exhibited a higher level. Therefore, *C. oleifera* oil could be regarded as a natural supplement for obtaining α-tocopherol.

Table 3. Content of tocopherols, polyphenols and squalene in *C. oleifera* oil of ten cultivars (mg/kg oil).

	α-Tocopherol	Polyphenols	Squalene
'Changlin 3'	280.58 ± 5.62 [f]	19.47 ± 2.94 [ab]	52.49 ± 5.11 [d]
'Changlin 4'	268.62 ± 8.33 [ef]	14.22 ± 0.19 [a]	26.62 ± 3.78 [b]
'Changlin 18'	195.22 ± 6.79 [c]	28.24 ± 3.76 [c]	37.93 ± 0.30 [c]
'Changlin 23'	271.31 ± 4.34 [ef]	21.27 ± 0.61 [b]	34.33 ± 2.30 [bc]
'Changlin 26'	257.98 ± 4.61 [e]	53.63 ± 2.50 [e]	32.49 ± 3.80 [bc]
'Changlin 27'	410.46 ± 4.20 [h]	49.51 ± 1.48 [de]	14.80 ± 1.54 [a]
'Changlin 53'	351.73 ± 5.82 [g]	47.25 ± 1.87 [d]	33.02 ± 3.66 [bc]
'Changlin 166'	218.28 ± 2.98 [d]	29.04 ± 2.35 [c]	30.95 ± 1.05 [bc]
'Chuanya 21'	140.84 ± 3.24 [b]	45.10 ± 1.77 [d]	33.06 ± 2.37 [bc]
'Chuanlin 2'	112.36 ± 3.81 [a]	30.16 ± 0.91 [c]	16.37 ± 0.66 [a]

Data are expressed as the mean ± standard deviations, n = 3. The "-" represents not detected in the identification process. Different letters in the same row indicate significantly different values, $p < 0.05$.

3.5. Polyphenol Content

Polyphenols are natural antioxidants that scavenging free radicals in vivo and have a clear effect on preventing tumorigenesis, preventing cardiovascular disease and lowering cholesterol [37]. The polyphenol content of *C. oleifera* oil is presented in Table 3. The polyphenol content of 'Changlin 26' was the highest and reached 53.63 mg/kg. The polyphenol contents of 'Changlin 26', 'Changlin 27' and 'Changlin 53' exceeded that of the local cultivars.

These results suggested that the content of polyphenols was related to the cultivars. Furthermore, previous studies indicated that the polyphenol content was affected by the planting soil, environment and processing technology of *C. oleifera* oil [44,45]. In addition, in *C. oleifera* oil, phenolic compounds contribute to *C. oleifera* oil's oxidative stability and can extend the shelf life of *C. oleifera* oil as well as the sensory characteristics, such as its bitter, astringent and pungent taste [46].

3.6. Squalene Content

Squalene has plenty of physiologic functions, such as enhancement of the immune responses and anti-aging, anti-fatigue and anti-tumor effects [47,48]. Thus, we measured the squalene content of *C. oleifera* oil. The squalene content of *C. oleifera* oil was varied with the cultivars (Table 3). In this study, the squalene content of *C. oleifera* oil was lower than that of previous studies, which may be related to the *C. oleifera* cultivars and the process technology of oil [49].

3.7. Trace Elements Concentration

The concentrations of trace elements in *C. oleifera* oil sample are presented in Table 4. The concentrations of trace elements varied significantly and ranged between 20,549.60–37,767.62 μg/kg (Mg), 5329.25–14,781.81 μg/kg (Ca), 2356.90–17,592.39 μg/kg (Mn), 606.46–3039.27 μg/kg (Fe), 12.75–471.67 μg/kg (Zn), 0–0.04 μg/kg (Cu), 0–173.15 μg/kg (Cr), 0 μg/kg (As), 0–0.01 μg/kg (Cd) and 0–0.02 μg/kg (Pb). This is in agreement with the results obtained on *C. oleifera* oil by Cao et al. and Ni et al. [50,51].

Table 4. Concentration of trace elements in the *C. oleifera* oil of ten cultivars (µg/kg oil).

|	Mg	Ca	Mn	Fe	Zn	Cu	Cr	As	Cd	Pb
'Changlin 3'	37767.62 ± 141.24 [i]	14781.81 ± 369.93 [g]	4223.08 ± 165.36 [c]	3039.27 ± 13.13 [f]	213.84 ± 10.81 [d]	-	105.37 ± 1.82 [b]	-	0.01 ± 0.00	-
'Changlin 4'	34529.46 ± 171.04 [f]	10793.01 ± 325.66 [f]	3416.64 ± 30.60 [b]	880.91 ± 20.89 [c]	375.67 ± 0.00 [e]	-	-	-	-	-
'Changlin 18'	30224.19 ± 123.92 [d]	6016.77 ± 14.39 [b]	4327.17 ± 65.38 [c]	1380.45 ± 78.16 [d]	18.32 ± 0.66 [a]	-	-	-	-	0.01 ± 0.00 [a]
'Changlin 23'	26930.09 ± 180.88 [c]	8194.09 ± 63.57 [c]	3057.80 ± 43.03 [b]	606.46 ± 26.99 [a]	15.51 ± 0.50 [a]	-	-	-	-	0.01 ± 0.00 [a]
'Changlin 26'	24624.36 ± 145.97 [b]	5667.37 ± 157.82 [ab]	17247.78 ± 258.31 [e]	916.49 ± 66.65 [c]	51.82 ± 2.74 [b]	0.04 ± 0.00 [b]	-	-	-	0.02 ± 0.00 [a]
'Changlin 27'	36872.35 ± 521.63 [h]	8864.91 ± 319.01 [de]	17592.39 ± 398.03 [e]	922.38 ± 51.66 [c]	12.75 ± 1.39 [a]	-	-	-	-	0.01 ± 0.00 [a]
'Changlin 53'	37339.69 ± 387.01 [hi]	9290.46 ± 49.84 [e]	12767.51 ± 103.47 [d]	820.84 ± 24.68 [bc]	471.67 ± 13.44 [f]	-	173.15 ± 8.57 [d]	-	-	0.01 ± 0.00 [a]
'Changlin 166'	20549.60 ± 257.41 [a]	5939.70 ± 78.35 [b]	2298.96 ± 47.66 [a]	1345.96 ± 62.36 [d]	47.88 ± 1.17 [b]	-	77.82 ± 4.64 [a]	-	-	0.02 ± 0.01 [a]
'Chuanya 21'	35398.45 ± 261.88 [g]	8347.00 ± 90.15 [cd]	17408.37 ± 153.80 [e]	1992.82 ± 130.67 [e]	103.72 ± 4.61 [c]	0.02 ± 0.00 [a]	143.96 ± 8.31 [c]	-	-	0.02 ± 0.00 [a]
'Chuanlin 2'	33643.24 ± 162.45 [e]	5329.25 ± 69.43 [a]	2356.90 ± 12.81 [a]	689.45 ± 19.46 [ab]	106.53 ± 6.99 [c]	0.02 ± 0.00 [a]	120.02 ± 6.77 [b]	-	-	-

Data are expressed as the mean ± standard deviations, n = 3. The "-" represents not detected in the identification process. Different letters in the same row indicate significantly different values, $p < 0.05$.

The presence of some trace elements, like Mg, Mn, Ca, Fe, Cu, Zn and Cr, could increase the oxidation rate of edible oil [52]. The elements of As, Cd, and Pb in edible oil could exhibit toxic effects on humans when the content exceeds certain boundaries [53]. In our study, the concentrations of harmful elements of Pb, Cd and As in all *C. oleifera* oil samples were lower than 0.02 μg/kg. Among them, As was not detected, and Pb was extremely lower than that the maximum limits of China national standard of GB 2762-2017 (0.1 mg/kg) in all oil samples [54]. These results indicated that the *C. oleifera* oil showed an excellent quality, and the soil of cultivation region (southwest China) did not suffer from contamination. Thus, this region is suitable to establish a standardized cultivation base of *C. oleifera*.

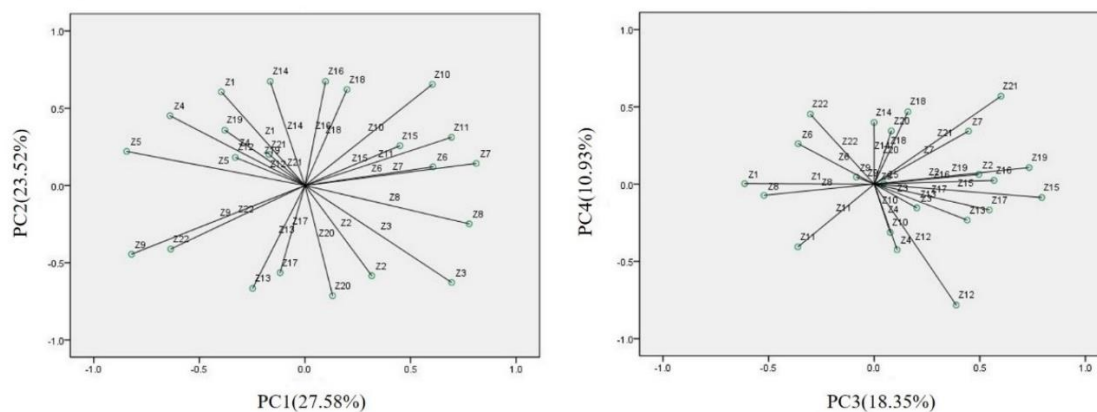### 3.8. Principal Component Analysis

PCA is a statistical tool that can interpret most of the information of inter-correlated raw variables through several uncorrelated linear combinations [55]. We performed a comprehensive evaluation of the quality of *C. oleifera* oil by PCA based on oil content, acid value, peroxide value, iodine value, fatty acid composition, *α*-tocopherol, polyphenols, squalene and trace elements. The results were shown in Figures 2 and 3 and Table 5. The contribution rates of the first four principal components were 27.58, 23.52, 18.35 and 10.93%, and the cumulative contribution rates of first four principal components were 80.38%, which contained all of the information of *C. oleifera* oil and could fully reflect the overall oil quality. Therefore, the first four principal components were selected for analysis.
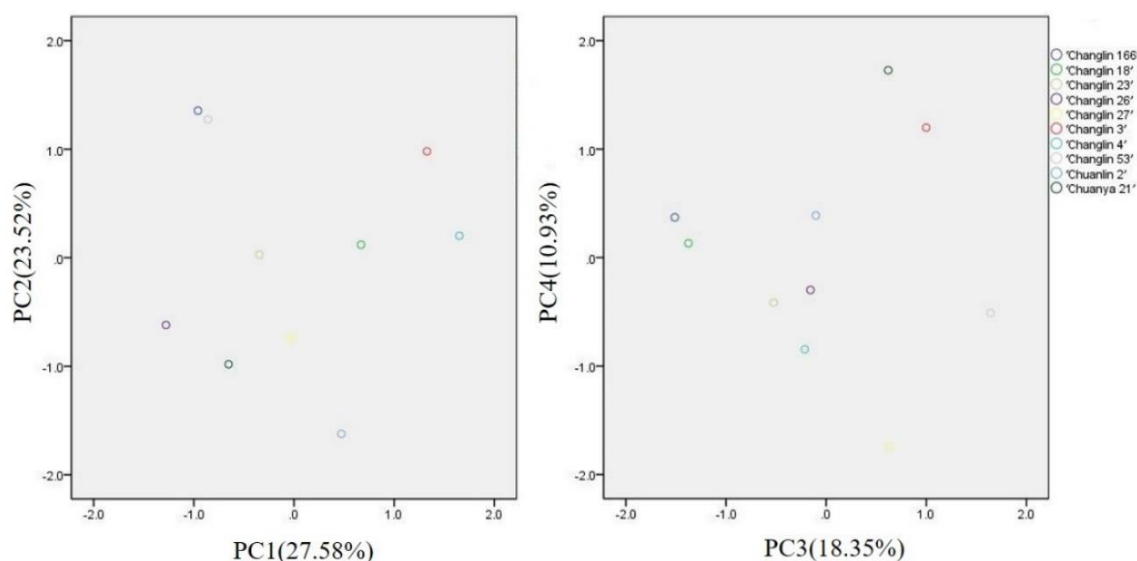
Figure 2. The score plot of oil samples. PC1, principal component 1; PC2, principal component 2; PC3, principal component 3; and PC4, principal component 4.

Table 5. Principal components and synthesis score.

Cultivars	Component 1	Component 2	Component 3	Component 4	Synthesis Score
'Changlin 3'	1.37	4.24	1.56	0.95	1.79
'Changlin 4'	1.76	2.70	−0.42	−1.11	0.89
'Changlin 18'	1.30	1.23	−2.71	−0.26	0.11
'Changlin 23'	−0.63	−0.74	−1.03	−0.27	−0.57
'Changlin 26'	−1.27	−3.35	−0.18	−0.01	−1.17
'Changlin 27'	1.18	−1.42	1.12	−2.97	−0.21
'Changlin 53'	−3.70	0.74	3.15	−0.66	−0.36
'Changlin 166'	−3.83	0.59	−2.89	0.65	−1.36
'Chuanya 21'	0.53	−0.74	−1.03	−0.27	−0.25
'Chuanlin 2'	1.16	−1.57	−0.12	0.82	0.04

Figure 3. The scatter plots of the constituents. PC1, principal component 1; PC2, principal component 2; PC3, principal component 3; and PC4, principal component 4. Z1 to Z22 represent the oil content, acid value, peroxide value, iodine value, lauric acid, myristoleic acid, palmitic acid, stearic acid, oleic acid, linoleic acid, eicosenoic acid, α-tocopherol, polyphenols, squalene, Mg, Ca, Mn, Fe, Zn, Cu, Cr and Pb, respectively.

As shown in Figure 2, the distribution of the oil samples on different principal components was scattered; this indicated that the components of the oil samples from different cultivars varied greatly. As illustrated in Figure 3, the peroxide value, myristoleic acid, palmitic acid, stearic acid, linoleic acid and eicosenoic acid had a higher loading on the first principal component. The second principal component included the oil content, iodine value, linoleic acid, squalene, Ca and Fe.

The third principal component included Mg, Ca, Mn, Zn and Cr. The fourth principal component mainly included myristoleic acid, palmitic acid, squalene, Fe, Cr and Pb. The component score and synthesis score of the four principal components from *C. oleifera* oil re shown in Table 5. The synthesis score of 'Changlin 3' was the highest. The synthesis scores of 'Changlin 3', 'Changlin 4', 'Changlin 18' and 'Changlin 27' were higher than 'Chuanya 21' (local cultivar). 'Changlin 3', 'Changlin 4' and 'Changlin 18' were higher than 'Chuanlin 2' (local cultivar).

The higher the synthesis score, the better the quality of oil. Therefore, the oil quality of 'Changlin 3' was the highest, followed by 'Changlin 4' and 'Changlin 18'. In addition, the results suggested that the introduced cultivars of 'Changlin 3', 'Changlin 4' and 'Changlin 18' were fully adapted to the local climate and superior to the local cultivar.

4. Conclusions

According to the results in the study, the introduced cultivars of *C. oleifera* could adapt to the environment and climate of southwest China. The *C. oleifera* oil expressed unique qualitative characteristics. The chemical properties of *C. oleifera* oil were in accord with the national standard of China. The *C. oleifera* oil was rich in UFA, α-tocopherol, polyphenols and squalene and included some trace elements. However, the concentrations of harmful elements of Pb, Cd and As in *C. oleifera* oil samples were extremely low compared with that the maximum limits of the China national standard. The *C. oleifera* oil cultivated in southwest China demonstrated excellent quality, and the oil qualities of the introduced cultivars 'Changlin 3', 'Changlin 4' and 'Changlin 18' were superior to the local cultivars ('Chuanya 21' and 'Chuanlin 2'). This presents a promising option for the large-scale planting of these introduced cultivars.

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References

1. Ma, J.L.; Ye, H.; Rui, Y.K.; Chen, G.C.; Zhang, N.Y. Fatty acid composition of *Camellia oleifera* oil. *J. Verbrauch. Lebensm.* **2010**, *6*, 9–12. [CrossRef]
2. Zhu, M.T.; Shi, T.; Chen, Y.; Luo, S.H.; Leng, T.; Wang, Y.; Guo, C.; Xie, M.Y. Prediction of fatty acid composition in camellia oil by ^{1}H NMR combined with PLS regression. *Food Chem.* **2018**, *279*, 339–346. [CrossRef]
3. Yang, J.Y.; Li, M.; Zou, X.G.; Peng, B.; Yin, Y.L.; Deng, Z.Y. A novel aqueous extraction for camellia oil by emulsified oil-frozen/thawed method. *Eur. J. Lipid Sci. Tech.* **2019**, *121*, 1800431. [CrossRef]
4. Feas, X.; Estevinho, L.M.; Salinero, C.; Vela, P.; Sainz, M.J.; Vazquez-Tato, M.P.; Seijas, J.A. Triacylglyceride, antioxidant and antimicrobial features of virgin *Camellia oleifera*, *C. reticulata* and *C. sasanqua* Oils. *Molecules* **2013**, *18*, 4573–4587. [CrossRef] [PubMed]
5. Yuan, J.; Wang, C.; Chen, H.; Zhou, H.; Ye, J. Prediction of fatty acid composition in *Camellia oleifera* oil by near infrared transmittance spectroscopy (NITS). *Food Chem.* **2013**, *138*, 1657–1662. [CrossRef] [PubMed]
6. Zeb, A. Triacylglycerols composition, oxidation and oxidation compounds in camellia oil using liquid chromatography-mass spectrometry. *Chem. Phys. Lipids* **2012**, *165*, 608–614. [CrossRef] [PubMed]
7. Ramsden, C.E.; Zamora, D.; Leelarthaepin, B.; Majchrzak-Hong, S.F.; Faurot, K.R.; Suchindran, C.M.; Ringel, A.; Davis, J.M.; Hibbeln, J.R. Use of dietary linoleic acid for secondary prevention of coronary heart disease and death: Evaluation of recovered data from the Sydney Diet Heart Study and updated meta-analysis. *BMJ* **2013**, *346*, e8707. [CrossRef]
8. Bai, Y.N.; Song, D.H.; Zhang, F.Q.; Xiao, X.J.; Wang, Q. Comparison of nutritional value of camellia seed oil and olive oil. *China Oil Fat* **2008**, *33*, 39–41.
9. Siger, A.; Nogala-kalucka, M.; Lampart-szczapa, E. The content and antioxidant activity of phenolic compounds in cold-pressed plant oils. *J. Food Lipid* **2007**, *15*, 137–149. [CrossRef]
10. Cayuela, J.A.; García, J.F. Nondestructive measurement of squalene in olive oil by near infrared spectroscopy. *LWT* **2018**, *88*, 103–108. [CrossRef]
11. Nunes, L.S.; Barbosa, J.T.; Fernandes, A.P.; Lemos, V.A.; Santos, W.N.; Korn, M.G.; Teixeira, L.S. Multi-element determination of Cu, Fe, Ni and Zn content in vegetable oils samples by high-resolution continuum source atomic absorption spectrometry and microemulsion sample preparation. *Food Chem.* **2011**, *127*, 780–783. [CrossRef]
12. Wang, X.Q.; Zeng, Q.M.; Verardo, V.; Contreras, M.D.M. Fatty acid and sterol composition of tea seed oils: Their comparison by the "FancyTiles" approach. *Food Chem.* **2017**, *233*, 302–310. [CrossRef] [PubMed]
13. Cheng, Y.T.; Wu, S.L.; Ho, C.Y.; Huang, S.M.; Cheng, C.L.; Yen, G.C. Beneficial effects of camellia oil (*Camellia oleifera* Abel.) on ketoprofen-induced gastrointestinal mucosal damage through upregulation of HO-1 and VEGF. *J. Agric. Food Chem.* **2014**, *62*, 642–650. [CrossRef]
14. Bumrungpert, A.; Pavadhgul, P.; Kalpravidh, R.W. Camellia oil-enriched diet attenuates oxidative stress and inflammatory markers in hypercholesterolemic subjects. *J. Med. Food* **2016**, *19*, 895–898. [CrossRef] [PubMed]
15. Ko, J.; Yeh, W.J.; Huang, W.C.; Yang, H.Y. *Camellia Oleifera* Seed Extract Mildly Ameliorates Carbon Tetrachloride-Induced Hepatotoxicity in Rats by Suppressing Inflammation. *J. Food Sci.* **2019**, *84*, 1586–1591. [CrossRef] [PubMed]
16. Athar, M.; Nasir, S. Taxonomic perspective of plant species yielding vegetable oils used in cosmetics and skin care products. *Afr. J. Biotechnol.* **2005**, *4*, 36–44.
17. Lin, P.; Yao, X.H.; Wang, K.L.; Zheng, T.T.; Teng, J.H. Identification and genetic analysis of camellia oleifera changlin series superior clones by SRAP molecular marker. *J. Agr. Biotech.* **2010**, *18*, 272–279.
18. Du, Y.W.; Deng, X.Z.; Zhou, W.G.; Yao, X.H.; Cheng, J.Y. Introduction and comprehensive evaluation of *Camellia oleifera* 'Changlin' cultivars. *Develop. Tech.* **2014**, *28*, 96–100.
19. Yang, C.Y.; Liu, X.M.; Chen, Z.Y.; Lin, Y.S.; Wang, S.Y. Comparison of oil content and fatty acid profile of ten new *Camellia oleifera* cultivars. *J. Lipid.* **2016**, *10*, 3982486.
20. International Organization for Standardization. *Oilseeds-Determination of Oil Content*; ISO 659-2009; ISO: Geneva, Switzerland, 2009.
21. Guo, Y.B.; Tang, B.; Qiu, A.Y.; Ji, C.G.; Liu, T.S. Technology for aqueous extraction of camellia seed oil. *Tran. CSAE* **2008**, *24*, 249–252.
22. International Organization for Standardization. *Animal and Vegetable Fats and Oils-Determination of Acid Value and Acidity*; ISO 660-2009; ISO: Geneva, Switzerland, 2009.

23. International Organization for Standardization. *Animal and Vegetable Fats and Oils-Determination of Peroxide Value*; ISO 3960-2007; ISO: Geneva, Switzerland, 2007.
24. International Organization for Standardization. *Animal and Vegetable Fats and Oils-Determination of Iodine Value*; ISO 3961-2018; ISO: Geneva, Switzerland, 2018.
25. Ren, C.Y.; Zhang, Y.P.; Tang, F.B.; Shen, D.Y.; Mo, R.H. Analysis of main chemical components in camellia oil, olive oil, walnut oil and torreya seeds oil. *J. Food Safe. Qua. Test.* **2015**, *6*, 5011–5016.
26. Su, M.H.; Shih, M.C.; Lin, K.H. Chemical composition of seed oils in native Taiwanese Camellia species. *Food Chem.* **2014**, *156*, 369–373. [CrossRef] [PubMed]
27. Cao, J.; Li, H.Y.; Xia, X.; Zou, X.G.; Li, J.; Zhu, X.M.; Deng, Z.Y. Effect of fatty acid and tocopherol on oxidative stability of vegetable oils with limited air. *Int. J. Food Prop.* **2015**, *18*, 808–820. [CrossRef]
28. Liao, Y.X. *The Study of Polyphenol and Oxidation Stability of Oil from Camellia*; Central South University of Forestry and Technology: Hunan, China, 2013; pp. 9–11.
29. Liu, L.Y. Determination of squalene in camellia oil by High Performance Liquid Chromatography. *Mod. Food* **2017**, *11*, 84–85.
30. Ni, Z.L.; Tang, F.B.; Liu, Y.H.; Shen, D.Y.; Mo, R.H. Multielemental analysis of camellia oil by microwave dry ashing and inductively coupled plasma mass spectrometry. *Anal. Lett.* **2015**, *48*, 1777–1786. [CrossRef]
31. State Forestry Administration. *Regulations of Selection and Breeding for Plus Tree and Superior Clone*; LY/T 1730.1-2008; State Forestry Administration: Beijing, China, 2008.
32. China National Standardization Committee. *Oil-Tea Camellia Seed Oil*; GB/T 11765-2018; China National Standardization Committee: Beijing, China, 2018.
33. Zhu, J.L.; Chai, Z.L.; Wu, C.R.; Huang, Y.T.; Wu, X.Y. Study on the comprehensive characters of *Toona sinensis* and its oil in Zhejiang Province. *J. Chin. Cer. Oil Asso.* **2019**, *34*, 67–73.
34. Chen, Y.H. Effects of five extraction methods on the quality of camellia oil. *Grain Fats* **2019**, *32*, 33–37.
35. Jiang, H. *Study on the Process and Quality of Extracting Camellia Seed Oil by Aqueous Enzymatic Method*; Shanghai Normal University: Shanghai, China, 2013; pp. 15–30.
36. Yang, X.; Yin, S.S.; Bi, H.S. Standard Identification of Camellia Oil and Other Cooking Oils. *J. Hefei Norm. Univ.* **2017**, *35*, 31–35, 53.
37. Nie, M.; Yang, X.H.; Yao, X.H.; Fang, X.Z.; Wang, Y.P. Effects of processing methods on physicochemical property and nutritient component of tea oil. *Fore. Res.* **2010**, *23*, 165–169.
38. Ni, X.R.; Qin, Y.C.; Liu, B.T.; Wang, Y.B.; Ding, M.; Qian, H. Anlysis of oil content and fatty acid composition of Changlin series *Camellia olefera* seeds. *Jiangxi Fore. Sci. Tech.* **2014**, *42*, 18–20.
39. Zhu, G.L.; Zhou, W.G.; Li, S.G.; Wang, K.L.; Yu, Q.F.; Hu, Z.Z. Research on oil content and fatty acid composition of in different Changlin varieties *Camellia oleifera* seeds. *Hubei Fore. Sci. Tech.* **2013**, *42*, 21–23.
40. He, Y.C.; Wu, S.J.; Wang, Y.J.; Zhu, P.L. Active components of twenty different kinds of geographical provenances in Jiangxi Province on the base of *Camellia oleifera* oil by grey relational grade analysis. *South. China Fore. Sci.* **2017**, *45*, 26–32.
41. Zou, Y.J. *Geographical Patterns of Variations in Main Chemical Components of Oil-Tea Camellia Seeds*; Nanchang University: Nanchang, China, 2019; pp. 30–37.
42. Morel, S.; Didierlaurent, A.; Bourguignon, P.; Delhaye, S.; Baras, B.; Jacob, V.; Planty, C.; Elouahabi, A.; Harvengt, P.; Carlsen, H.; et al. Adjuvant System AS03 containing alpha-tocopherol modulates innate immune response and leads to improved adaptive immunity. *Vaccine* **2011**, *29*, 2461–2473. [CrossRef]
43. Giakoustidis, D.; Papageorgiou, G.; Iliadis, S.; Kontos, N.; Kostopoulou, E.; Papachrestou, A.; Tsantilas, D.; Spyridis, C.; Takoudas, D.; Botsoglou, N.; et al. Intramuscular administration of very high dose of alpha-tocopherol protects liver from severe ischemia/reperfusion injury. *World J. Surg.* **2002**, *26*, 872–877. [CrossRef] [PubMed]
44. Chen, Z.J. Determination of phenols in six kinds of camellia oil. *Anhui Agri. Sci. Bull* **2018**, *24*, 30–31, 34.
45. Wang, X.; Zeng, Q.; Del Mar Contreras, M.; Wang, L. Profiling and quantification of phenolic compounds in Camellia seed oils: Natural tea polyphenols in vegetable oil. *Food Res. Int.* **2017**, *102*, 184–194. [CrossRef]
46. Liu, L.; Zhao, Z.H. Study on polar antioxidant in tea seed oil. *J. Chin. Cer. Oil Asso.* **2002**, *3*, 4–9.
47. Reddy, L.H.; Couvreur, P. Squalene: A natural triterpene for use in disease management and therapy. *Adv. Drug Deliv. Rev.* **2009**, *61*, 1412–1426. [CrossRef] [PubMed]
48. Tegenge, M.A.; Von Tungeln, L.S.; Mitkus, R.J.; Anderson, S.A.; Vanlandingham, M.M.; Forshee, R.A.; Beland, F.A. Pharmacokinetics and biodistribution of squalene-containing emulsion adjuvant following intramuscular injection of H5N1 influenza vaccine in mice. *Regul. Toxicol. Pharmacol.* **2016**, *81*, 113–119. [CrossRef] [PubMed]
49. Deng, L.; Deng, Z.Y.; Hu, J.N.; Li, J.; Fan, Y.W. Physical-chemical properties and nutrients of oil-tea camellia seed oil in different refining stages. *Food Sci.* **2015**, *36*, 111–115.
50. Cao, Y.Q.; Yao, X.H.; Ren, H.D.; Wang, K.L. Determination of fatty acid composition and metallic element content of four Camellia species used for edible oil extraction in China. *J. Consum. Prot. Food Saf.* **2017**, *12*, 165–169. [CrossRef]
51. Ni, Z.L.; Tang, F.B.; Qu, M.H.; Liu, Y.H.; Shen, D.Y. Determination of five heavy metals in Camellia Seed Oil by microwave digestion-ICP-MS. *Food Sci.* **2013**, *34*, 165–167.
52. Kara, D.; Fisher, A.; Hill, S. Extraction of trace elements by ultrasound-assisted emulsification from edible oils producing detergentless microemulsions. *Food Chem.* **2015**, *188*, 143–148. [CrossRef] [PubMed]

53. Bakircioglu, D.; Kurtulus, Y.B.; Yurtsever, S. Comparison of extraction induced by emulsion breaking, ultrasonic extraction and wet digestion procedures for determination of metals in edible oil samples in Turkey using ICP-OES. *Food Chem.* **2013**, *138*, 770–775. [CrossRef] [PubMed]

54. China National Food and Drug Supervision and Administration Commission. *National Food Safety Standard-Limits of Contaminants in Food*; GB 2762-2017; China National Food and Drug Supervision and Administration Commission: Beijing, China, 2017.

55. Jolliffe, I.T.; Cadima, J. Principal component analysis: A review and recent developments. *Philos. Trans. A Math. Phys. Eng. Sci.* **2016**, *374*, 1340–1347. [CrossRef] [PubMed]