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ORIGINAL PAPER

Physicochemical characterization and antioxidant activities of Chongqing virgin olive oil: effects of variety and ripening stage

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Abstract

This study characterized the quality, composition and antioxidant activities of olive oil from four olive varieties grown in Chongqing, southwest of China. With the fruit skin turned from green to semi black, the percentage of oleic acid decreased while the percentage of linoleic acid increased. The highest concentrations of total phenolic content, total flavonoid content and total antioxidant activity were found in the Coratina olive oils, in which the lowest content of oleuropein was also observed. Significant differences of fatty acid composition at sn-2 position were observed among the varieties, with the percentage of oleic acid at sn-2 position in Picual olive oils were significantly higher than that of other varieties ($p < 0.05$). In addition, the different varieties of olive oil samples were divided into three categories by principal component analysis (PCA), and the quality of Coratina olive oils were greatly affected by fruit ripening stage. With the fruit ripening, the levels of hydroxytyrosol, rutin, total phenolics, total flavonoids and antioxidant activities in Coratina olive oil all declined. This work can provide guidance for the harvesting time and processing of olives in Chongqing.

Keywords Antioxidant activity · Chemical composition · Principal component analysis · Virgin olive oil

Introduction

Extra virgin olive oil (EVOO) is extracted from the olive fruit and consists mainly of monounsaturated and polyunsaturated fatty acids, along with other minor components (vitamin E, sterols, phenolics and volatile compounds). The health benefits of olive oil are related to its high content of unsaturated fatty acid and phenolic compounds [1]. Phenolic compounds are the main antioxidants in EVOO, broadly classified into hydrophilic and lipophilic phenolics [2]. Most of the lipophilic phenolics in olive oil are α-tocopherol [3]. The hydrophilic phenolic compounds are a class of secondary plant metabolites with unique sensory and health properties that are not generally present in other vegetable oils [4].

The composition and quality of EVOO are impacted by several factors in olive fruit growing. The environmental temperature during fruit growth affects the activities of endogenous enzymes. For example, oleic acid desaturase activity decreases with decreasing environmental temperature, resulting in an increase in the content of oleic acid [5, 6]. The latitude in which the fruit grows also affects the fatty acid composition of olive oil, with the content of oleic acid and the ratio of unsaturated fatty acids (UFA) to saturated fatty acids (SFA) increasing in line with the increase in latitude [7]. In addition, proper irrigation is reported to reduce the bitter taste of the oil, without reducing the oil production [8, 9].

The variety and ripening stages of olives are the two main factors determining the composition of olive oil. Significant differences have been reported in the triglyceride composition [10], volatile compounds [11], fatty acid composition [7] and phenolic compounds [3] in olive oils from different varieties of olives. Moreover, due to the action of oleic acid desaturase, the content of oleic acid in olive oil decrease and the content of linoleic acid increase with fruit ripening [12]. The content of phenolic compounds becomes higher when the skin of the fruit begins to darken, but gradually decreases thereafter [13].

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Olives are grown in many non-Mediterranean countries. Chongqing, located in the southwest China, has become one of most important regions for olive cultivation in China, as it features Mediterranean-like climatic patterns, including a warm winter, hot summer and cool autumn. However, numerous studies of quality and chemical composition have paid attention to Mediterranean olive oil [14, 15], few reports are available on the physicochemical properties and antioxidant activity of EVOO grown in south-west of China. The distribution of fatty acids according to the stereospecific numbering (sn) system is another important factor in determining the quality of olive oil and fatty acids at the sn-2 position have been found to be more bioavailable than those at the sn-1,3 positions [16]. To our best knowledge, effects of variety and ripening stage on fatty acids at sn-2 position of olive oil have not yet been reported in literatures. Considering the nutritional value of virgin olive oil and the environment which differs from most Mediterranean countries, the aim of this study was, therefore, to determine the effects of variety and ripening stage on the physicochemical characterization and antioxidant activities of virgin olive oils from introduced olive varieties in Chongqing.

Materials and methods

Plant materials and reagents

Olive fruit from Arbequina, Picual, Ezhi-8 and Coratina cultivars in Chongqing (longitude: E106° 06′ 50.65″, latitude: N30° 09′ 58.48″) were harvested in three different maturity stages with their skin color in green, semi-black and black, between mid-October and early November 2018. The olive maturity index (MI) was calculated according to International Olive Oil Council (IOOC) (2011) from 0 (skin deep green) to 7 (skin and flesh all black).

Lipase (porcine pancreas, 30,000 u/g), hydroxytyrosol, tyrosol, rutin and oleuropein standards (≥ 98%) were purchased from Shanghai Yuanye Bio-Technology Co., Ltd (Shanghai, China). 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), gallic acid, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), catechin standards (≥ 98%), and HPLC-grade methanol (≥ 99.9%) were purchased from Shanghai Macklin Biochemical Co., Ltd (Shanghai, China). 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-Tris (2-pyridyl)-s-triazine (TPTZ) and HPLC-grade isooctane (≥ 99%) were purchased from Shanghai Aladdin Bio-chem Technology Co., Ltd (Shanghai China). All the reagents were analytical grade.

Oil extraction

Olive fruits were harvested by hand from ten trees randomly of each variety with triplicate. Olive oils were extracted within 24 h of harvest. The fresh olives (10 kg) were crushed and then slowly mixed for 30 min. The resulting paste was centrifuged to separate the oil. All samples were placed in amber glass bottles and stored in the dark at 4 °C until analysis.

Quality indices

The free acidity, the peroxide value (PV) and UV spectrophotometric indices (K_{232} and K_{270} measurements) were determined according to the ISO 660-2009, ISO 3960-2001 and ISO 3656-2002 methods.

Fatty acid composition

According to the ISO 5009-2000 method, fatty acids were transformed into methyl esters using potassium hydroxide in methanol solution and analyzed by gas chromatography (GC) (7890A, Agilent technologies, California, USA) equipped with a flame ionization detector (FID). The capillary column was Agilent HP-88 (100 m × 0.25 mm; the film thickness 0.20 μm). Detection conditions were as follows: injector temperature was 270 °C; flame ionization detector temperature was 280 °C; column temperature started at 100 °C for 13 min, increased at 10 °C/min to 180 °C and held for 6 min, then, increased at 1 °C/min to 200 °C and held for 20 min, then, increased at 4 °C/min to 230 °C and held for 10.5 min; the split ratio was 1/100; and the carrier gas was N_2.

Fatty acid composition at the sn-2 position

Fatty acid composition at the sn-2 position was measured according to the ISO 6800-1997 method. Briefly, purification of a solution of 5 g of oil in 25 mL of hexane by column packed with alumina (15 g of activated alumina and 50 mL of hexane mixed and slowly poured into the column). Then, 0.1 g of the purified oil samples were mixed with 20 mg lipase and 2 mL of Tris–HCl buffer (1 mol/L, pH 8.0), followed by 0.5 mL of 0.1% sodium cholate and 0.2 mL of 22% $CaCl_2$. The mixture was incubated in a water bath at 40 °C for 3 min with shaking. Then, 1 mL HCl (6 mol/mL) and 1 mL diethyl ether were added to stop the hydrolysis reaction. The hydrolytic product was subsequently separated on silica gel plates, and the developing solvents included hexane, diethyl ether, and formic acid (70:30:1; v/v/v). The

2-monoglycerides band (R_f about 0.035) were collected and fatty acid methyl ester of the 2-monoglycerides was prepared and analyzed as described in Sect. 2.4.

Extraction of the phenolic fraction

Phenolics were extracted according to the method described by Xiang et al. [17] with slight modifications. Five grams of oil was dissolved in 10 mL of MeOH/H_2O (80/20, v/v). The mixture was shaken and centrifuged at 8000 rpm for 10 min. The polar fraction was transferred in a 25 mL volumetric flask and the volume was made up to 25 mL with MeOH/H_2O (80/20, v/v).

Determination of the phenolic fraction

Phenolic compounds were qualitatively and quantitatively analyzed by HPLC (2695, Waters, Milford, USA) coupled with column Hypersil GOLD C18 (250 mm × 4.6 mm, 5 µm, Thermo, Waltham, USA) [17]. The chromatographic conditions were as follows: injection volume was 50 µL; detector wavelength was 280 nm; the column temperature was 25 °C; and the mobile phase consisted of water (A) and methanol (B). Gradient elution procedure: 90% A and 10% B (0 ~ 5 min), 60% A and 40% B (5 ~ 30 min), 40% A and 60% B (30 ~ 45 min), 40% A and 60% B (45 ~ 50 min), 90% A and 10% B (50 ~ 60 min). Equilibrate the column with 10% methanol for 5 min before the next run. The mobile phase flow rate was 1 mL/min.

Total phenolics and total flavonoids contents

Total phenolics contents were determined according to the Folin-Ciocalteu method [14]. 20 µL of Folin-Ciocalteu and 20 µL of H_2O were added to 20 µL of phenolic extract or gallic acid standard solution (6.25, 12.5, 25, 50, 100 or 200 mg/L). After 6 min, 140 µL of sodium carbonate solution (7%) was added. After 2 h of incubation in the dark, the absorbance was read at 760 nm. The total phenolics contents were expressed as mg gallic acid equivalents per kilogram of oil (mg GAE kg^{-1}).

The total flavonoid content was determined according to the method described by De Souza et al. [18]. 20 µL of 5% $NaNO_2$ and 20 µL of 10% $AlCl_3$ were add to 40 µL of extract or catechin standard solution (6.25, 12.5, 25, 50, 100 or 200 mg/L). After 10 min, 100 µL of NaOH (1 mol/L) was added. The solution was mixed and the absorbance was measured at 510 nm against the prepared blank reagent. The total flavonoid contents were expressed as mg catechin equivalents per kilogram of oil (mg CE kg^{-1}).

Antioxidant activities

ABTS radical scavenging activity

The ABTS radical scavenging activity was determined according to the method described by Wu et al. [19] with slight modifications. ABTS (7 mmol/L) was mixed with potassium persulfate solution (2.45 mmol/L) in a volume ratio of 1:1, and incubated at room temperature for 12 ~ 16 h in the dark to produce a stock solution of radical cation ($ABTS^{\cdot+}$). The $ABTS^{\cdot+}$ stock solution was diluted with ethanol to an absorbance of 0.700 (±0.020) at 734 nm before analysis. Then 180 µL of $ABTS^{\cdot+}$ stock solution mixed with 20 µL of phenolic extract and the absorbance was recorded at 734 nm after 10 min. The radical scavenging activities were expressed as µmol Trolox/kg of oil (µmol TE/kg).

DPPH radical scavenging activity

The DPPH radical scavenging activity was determined according to the method described by Flores Garcia et al. [20] with slight modifications. 180 µL of DPPH ethanol solution (0.2 mmol/L) mixed with 20 µL of phenolic extract and the absorbance was measured at 517 nm after 30 min. The radical scavenging activities were expressed as µmol Trolox/kg of oil (µmol TE/kg).

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was determined according to the method described by Benzie and Strain [21]. The FRAP reagent was mixed in a volume ratio of 10:1:1 with 0.3 mol/L sodium acetate buffer solution (pH 3.6), 10 mmol/L of TPTZ and 20 mmol/L $FeCl_3 \cdot 6H_2O$. Then, 180 µL of FRAP reagent mixed with 20 µL of phenolic extract and the absorbance was measured at 593 nm after 30 min at 37 °C. The results were expressed as µmol Trolox/kg of oil (µmol TE/kg).

Statistical analysis

All the results were reported as means ± standard deviation (SD), the data were analyzed by one-way analysis of variance (ANOVA) and Tukey tests were performed to compare mean values ($p \leq 0.05$). Principal component analysis (PCA) was applied to ascertain the influence of fruit variety and ripening stage on the quality of olive oil, according to the fatty acid composition, phenolic compounds and antioxidant activity of oils. Statistical analysis was conducted using SPSS software 19.0 (SPSS Inc., Chicago, IL, USA).

Results and discussion

Quality indices

Olive fruit in green (MI 0–1), semi-black (MI 1.5–3.1), and black (MI 3.7–4.8) stages of maturity were submitted to the extraction of olive oil. Free fatty acidity is traditionally considered an important quality indicator and is widely used as a standard for the classification of olive oil [14]. As shown in Table 1, the free fatty acidities of all olive oils in this assay were within the upper limit of 0.8%, established by the International Olive Oil Council (IOOC) (2003), and classified as Extra Virgin Olive Oil (EVOO). The highest value of free fatty acidity among the four varieties was found in the Arbequina Semi black olive oil. When the olive fruit skin turned from green to black, the free acidity of Picual olive oils increased by 0.09%, while those of Ezhi-8 olive oils decreased by 0.06%. Arbequina and Coratina olive oils showed no significant differences in their free acidity levels at the different ripening stages.

Peroxide values (PV) and UV characteristics are mainly used to evaluate the state of oxidation in olive oil as another important quality indicator [14]. As shown in Table 1, the PV of all olive oils were within the upper limit of 20 meq O_2/kg established by the IOOC (2003). In oils from fruit whose skin had turned from green to black, the PV of Arbequina, Picual, Ezhi-8 and Coratina olive oils decreased by 2.95, 2.47, 6.83 and 1.35 meq O_2/kg, respectively. The PV of Coratina olive oils were comparatively the lowest, while the Ezhi-8 olive oils had the highest PV. The evolution of this quality parameter may be related to the enzymatic activity of lipoxygenase. In fact, some studies have reported the relation between PV and lipoxygenase. When its activity is low, PV decreases. However, high lipoxygenase activity produces an increase in PV [13, 22]. The behavior of this enzyme previously described in the literature could explain the result of this work. PV and K_{232} were used as indicators of olive oil primary oxidation, while K_{270} was considered as an indicator of secondary oxidation [23]. Here, the K_{232} and K_{270} of all olive oils were within the range specified for EVOO (2.5 and 0.2, respectively; Table 1), with the exception of the K_{232} of Ezhi-8 green (2.53 ± 0.01). The K_{232} values of Picual olive oil samples ranged from 1.45 ± 0.09 to 1.96 ± 0.03 and were the lowest of the four varieties, followed by Coratina olive oils, which ranged from 1.92 ± 0.00 to 2.03 ± 0.03. Moreover, as the skin color of the fruit turned black, the K_{270} values of four varieties of olive oil samples showed a slight decrease, indicating that secondary oxidation did not occur during the ripening of the olive fruit [23].

Fatty acid composition

The fatty acid composition of olive oils comprises mainly palmitic acid (C16:0), oleic acid (C18:1) and linoleic acid (C18:2) with small amounts of palmitoleic acid (C16:1), stearic acid (C18:0) and linolenic acid (C18:3). The analyzed olive oils showed fatty acid composition (Table 2) mostly in compliance with established limits by IOOC (2003). The major fatty acid in all olive samples was found to be oleic acid, ranging from 58.35 ± 0.59 to 73.15 ± 0.88%. During the fruits' ripening, the content of oleic acid in the Arbequina, Picual and Coratina olive oils decreased by 3.66%, 3.04% and 0.9% respectively, while the content of linoleic acid increased by 2.02%, 0.95% and 1.03%, respectively. This

Table 1 Free fatty acids, peroxide value, K_{232} and K_{270} of olive oil samples

Variety	Ripening stage	Free fatty acids (% oleic acid)	Peroxide value (meq O_2/kg)	K_{232}	K_{270}
Arbequina	Green	$0.16 \pm 0.03^{A,a}$	$13.38 \pm 0.53^{B,a}$	$2.41 \pm 0.06^{A,a}$	$0.18 \pm 0.01^{A,B,a}$
	Semi black	$0.18 \pm 0.01^{A,a}$	$10.63 \pm 0.88^{B,b}$	$2.19 \pm 0.01^{B,b}$	$0.15 \pm 0.00^{B,b}$
	Black	$0.15 \pm 0.04^{B,a}$	$10.43 \pm 0.95^{A,b}$	$2.04 \pm 0.02^{A,c}$	$0.14 \pm 0.01^{A,B,b}$
Picual	Green	$0.12 \pm 0.02^{B,C,b}$	$12.75 \pm 0.71^{B,a}$	$1.45 \pm 0.09^{C,c}$	$0.16 \pm 0.02^{B,a}$
	Semi black	$0.13 \pm 0.02^{B,b}$	$10.88 \pm 0.18^{B,b}$	$1.96 \pm 0.03^{C,a}$	$0.17 \pm 0.02^{A,B,a}$
	Black	$0.21 \pm 0.02^{A,a}$	$10.28 \pm 0.53^{A,b}$	$1.82 \pm 0.01^{D,b}$	$0.15 \pm 0.01^{A,a}$
Ezhi-8	Green	$0.16 \pm 0.02^{A,B,a}$	$14.43 \pm 0.25^{A,a}$	$2.53 \pm 0.01^{A,a}$	$0.19 \pm 0.01^{A,a}$
	Semi black	$0.10 \pm 0.02^{B,b}$	$12.85 \pm 1.77^{A,a}$	$2.47 \pm 0.00^{A,b}$	$0.18 \pm 0.01^{A,b}$
	Black	$0.10 \pm 0.02^{C,b}$	$7.6 \pm 0.49^{B,b}$	$1.92 \pm 0.01^{C,c}$	$0.12 \pm 0.01^{B,c}$
Coratina	Green	$0.12 \pm 0.01^{D,a}$	$9.5 \pm 0.71^{C,a}$	$2.03 \pm 0.03^{B,a}$	$0.17 \pm 0.02^{A,B,a}$
	Semi black	$0.10 \pm 0.02^{B,a}$	$9.3 \pm 0.28^{B,a}$	$1.92 \pm 0.00^{D,b}$	$0.12 \pm 0.02^{C,b}$
	Black	$0.13 \pm 0.02^{B,C,a}$	$10.85 \pm 1.77^{A,a}$	$2.00 \pm 0.01^{B,a}$	$0.15 \pm 0.01^{A,a,b}$

Results are presented as the mean value ± standard deviation, n = 3

[a–c]Different letters in the same variety indicate significantly different values ($p < 0.05$)

[A–D]Different letters in the same ripening stage indicate significantly different values ($p < 0.05$)

Table 2 Fatty acid composition of olive oil samples

Variety	Ripening stage	Fatty acid composition (% of total fatty acids)						UFA%	UFA/SFA	MUFA/PUFA
		Palmitic acid C16:0	Palmitoleic acid C16:1	Stearic acid C18:0	Oleic acid C18:1	Linoleic acid C18:2	Linolenic acid C18:3			
Arbequina	Green	$19.32 \pm 0.74^{B,b}$	$2.69 \pm 0.13^{B,c}$	$1.76 \pm 0.03^{C,a}$	$62.01 \pm 0.68^{B,a}$	$11.63 \pm 0.10^{A,b}$	$1.11 \pm 0.00^{C,a}$	$77.91 \pm 0.68^{C,a}$	$3.58 \pm 0.14^{C,a}$	$5.10 \pm 0.00^{C,a}$
	Semi black	$20.58 \pm 0.16^{A,a}$	$2.98 \pm 0.03^{C,b}$	$1.76 \pm 0.01^{C,a}$	$58.61 \pm 0.16^{D,b}$	$13.57 \pm 0.02^{A,a}$	$1.12 \pm 0.00^{C,a}$	$76.69 \pm 0.14^{D,b}$	$3.33 \pm 0.03^{C,a}$	$4.21 \pm 0.00^{D,b}$
	Black	$20.48 \pm 0.66^{A,a}$	$3.33 \pm 0.11^{B,a}$	$1.77 \pm 0.03^{C,a}$	$58.35 \pm 0.59^{C,b}$	$13.65 \pm 0.16^{A,a}$	$1.08 \pm 0.01^{B,b}$	$76.80 \pm 0.62^{D,b}$	$3.35 \pm 0.12^{C,a}$	$4.21 \pm 0.02^{D,b}$
Picual	Green	$16.59 \pm 0.35^{B,b}$	$2.75 \pm 0.08^{B,c}$	$2.14 \pm 0.02^{A,b}$	$72.86 \pm 0.32^{A,a}$	$3.39 \pm 0.00^{D,c}$	$1.27 \pm 0.02^{B,a}$	$80.47 \pm 0.30^{B,a}$	$4.18 \pm 0.08^{B,a}$	$16.00 \pm 0.07^{A,a}$
	Semi black	$17.83 \pm 0.64^{B,a}$	$3.64 \pm 0.16^{B,b}$	$2.23 \pm 0.04^{A,a}$	$70.13 \pm 0.66^{B,b}$	$4.02 \pm 0.01^{D,b}$	$1.19 \pm 0.01^{B,b}$	$79.19 \pm 0.56^{B,b}$	$3.85 \pm 0.14^{B,b}$	$14.02 \pm 0.00^{A,b}$
	Black	$17.20 \pm 0.50^{B,a}$	$4.25 \pm 0.15^{A,a}$	$2.11 \pm 0.03^{A,b}$	$69.82 \pm 0.54^{B,b}$	$4.34 \pm 0.02^{D,a}$	$1.19 \pm 0.01^{A,b}$	$79.86 \pm 0.43^{C,a,b}$	$4.02 \pm 0.11^{B,a,b}$	$13.20 \pm 0.01^{A,c}$
Ezhi-8	Green	$20.70 \pm 0.26^{A,a}$	$4.97 \pm 0.08^{A,a}$	$1.57 \pm 0.01^{D,b}$	$62.59 \pm 0.27^{B,c}$	$7.38 \pm 0.04^{C,a}$	$1.43 \pm 0.01^{A,a}$	$76.81 \pm 0.24^{D,b}$	$3.36 \pm 0.05^{C,b}$	$7.64 \pm 0.02^{B,c}$
	Semi black	$20.24 \pm 0.35^{A,a}$	$4.98 \pm 0.09^{A,a}$	$1.62 \pm 0.01^{D,a}$	$63.41 \pm 0.37^{C,b}$	$7.14 \pm 0.04^{C,b}$	$1.37 \pm 0.00^{A,b}$	$77.28 \pm 0.32^{C,b}$	$3.45 \pm 0.07^{C,b}$	$7.99 \pm 0.02^{B,b}$
	Black	$16.89 \pm 0.49^{B,b}$	$2.83 \pm 0.10^{C,b}$	$1.66 \pm 0.03^{D,a}$	$69.28 \pm 0.47^{B,a}$	$7.42 \pm 0.01^{C,a}$	$0.87 \pm 0.00^{D,c}$	$80.74 \pm 0.42^{B,a}$	$4.25 \pm 0.12^{B,a}$	$8.72 \pm 0.02^{B,a}$
Coratina	Green	$14.28 \pm 0.12^{D,a}$	$0.65 \pm 0.00^{C,b}$	$1.90 \pm 0.00^{B,a}$	$72.40 \pm 0.10^{A,a}$	$8.54 \pm 0.00^{B,a}$	$0.99 \pm 0.00^{D,a}$	$82.80 \pm 0.11^{A,b}$	$4.94 \pm 0.04^{A,b}$	$7.64 \pm 0.01^{B,a}$
	Semi black	$12.70 \pm 1.15^{C,b}$	$0.67 \pm 0.07^{D,b}$	$1.88 \pm 0.05^{B,a}$	$73.15 \pm 0.88^{A,a}$	$9.37 \pm 0.09^{B,b}$	$0.93 \pm 0.01^{D,b}$	$84.35 \pm 0.95^{A,a}$	$5.58 \pm 0.44^{A,a}$	$7.15 \pm 0.00^{C,b}$
	Black	$14.14 \pm 0.21^{C,a}$	$0.94 \pm 0.02^{D,a}$	$1.83 \pm 0.01^{B,b}$	$71.50 \pm 0.18^{A,b}$	$9.57 \pm 0.01^{B,c}$	$0.92 \pm 0.00^{C,b}$	$83.13 \pm 0.18^{A,b}$	$5.05 \pm 0.07^{A,b}$	$6.91 \pm 0.01^{C,c}$

Results are presented as the mean value ± standard deviation, n = 3

UFA unsaturated fatty acids, *SFA* saturated fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids

[a–c]Different letters in the same variety indicate significantly different values ($p < 0.05$)

[A–D]Different letters in the same ripening stage indicate significantly different values ($p < 0.05$)

could be explained by oleic acid desaturase, in which oleic acid is converted to linoleic acid [24, 25]. Among the four varieties, the oleic acid content was highest in the Coratina Semi black olive oil (73.15 ± 0.88%), and lowest in the Arbequina Black olive oil (58.35 ± 0.59%). In addition, compared with the other three varieties of olive oils, Coratina olive oils had lower levels of palmitic acid, palmitoleic acid and linolenic acid.

The percentage of unsaturated fatty acids (UFA), the ratio of unsaturated to saturated fatty acids (UFA/SFA), and the ratio of monounsaturated to polyunsaturated fatty acids (MUFA/PUFA) in all olive oil samples were calculated (Table 2). The ratios of UFA and SFA ranged from 3.33 ± 0.03 to 5.58 ± 0.44, with little difference among the four varieties. In addition, the ratio of MUFA and PUFA in all olive oils decreased during fruit ripening. This is mainly due to the increase of PUFA in the ripening process, which increases olive oil's susceptibility to oxidation. With presence of some factors such as enzymes and oxygen molecules, PUFA are converted to their corresponding hydroperoxide [23].

Fatty acid composition at the sn-2 position

Fatty acids at the sn-2 position are more easily digested and absorbed by the human body than fatty acids at the sn-1,3 positions [16]. Therefore, in order to further understand the nutritional value of olive oil to the human body, the composition of fatty acids at the sn-2 position of the olive fruits of different varieties and ripening stages were evaluated in this study. The fatty acid composition at sn-2 position comprised mainly palmitic acid, palmitoleic acid, oleic acid, linoleic acid and linolenic acid. Research has shown that the high content of UFA at the sn-2 position in edible oils is beneficial for digestion and overall health [26]. The fatty acid composition at the sn-2 position of the four varieties of olive oil samples is shown in Table 3. Significant differences in the composition of fatty acids at sn-2 position were observed between the varieties. Palmitic acid (C16:0) is the main SFA at the sn-2 position of olive oil, its distribution was similar in all four olive oil samples. Palmitoleic acid (C16:1), a special omega-7 group fatty acid, is a skin lipid component that may play a moderator role in the process of epidermis regeneration [27]. Here, the palmitoleic acid at the sn-2 position of the Coratina olive oils was measured to be between 0.52 ± 0.04 and 0.78% ± 0.01% during fruit ripening, which was significantly lower than that of the other three varieties. As a monounsaturated omega-9 group fatty acid, oleic acid (C18:1) provides beneficial antioxidant and hypolipidemic effects and can prevent cardiovascular diseases [28]. With the fruit skin turned from green to semi black, the content of oleic acid at the sn-2 position in the Picual olive oils ranging from 88.05 ± 0.82 to 90.76 ± 0.49% were found to be significantly higher than the other three varieties. The lowest sn-2 oleic acid content and the highest sn-2 linoleic acid and linolenic acid contents were observed in Arbequina olive oils. Linoleic acid and linolenic acid are omega-6 and omega-3 group fatty acids, which are both beneficial to human health [29]. In this study, a high percentage of sn-2 linoleic acid (ranging from 4.17 ± 0.61 to 18.62 ± 0.01%) and a small concentration of sn-2 linolenic acid (ranging from 0.73 ± 0.01 to 1.94 ± 0.09%) were detected in all olive oil

Table 3 Fatty acid composition at sn-2 position of olive oil samples

Variety	Ripening stage	Fatty acid composition at sn-2 position (% of total fatty acids)				
		Palmitic acid C16:0	Palmitoleic acid C16:1	Oleic acid C18:1	Linoleic acid C18:2	Linolenic acid C18:3
Arbequina	Green	$1.49 \pm 0.29^{A,a}$	$3.37 \pm 0.18^{B,b}$	$76.56 \pm 0.26^{D,a}$	$16.64 \pm 0.07^{A,c}$	$1.94 \pm 0.09^{A,a}$
	Semi black	$1.41 \pm 0.06^{A,a}$	$3.52 \pm 0.13^{B,b}$	$73.98 \pm 0.20^{D,c}$	$18.62 \pm 0.01^{A,a}$	$1.77 \pm 0.00^{A,a,b}$
	Black	$1.28 \pm 0.17^{A,a}$	$3.80 \pm 0.14^{A,a}$	$75.14 \pm 0.80^{D,b}$	$17.51 \pm 0.91^{A,b}$	$1.71 \pm 0.18^{A,b}$
Picual	Green	$1.29 \pm 0.20^{A,a}$	$2.35 \pm 0.09^{C,c}$	$90.76 \pm 0.49^{A,a}$	$4.17 \pm 0.61^{C,b}$	$0.92 \pm 0.23^{C,a}$
	Semi black	$0.94 \pm 0.05^{B,b}$	$2.94 \pm 0.09^{C,b}$	$89.57 \pm 0.07^{A,b}$	$5.08 \pm 0.05^{C,a}$	$1.11 \pm 0.02^{B,a}$
	Black	$1.08 \pm 0.00^{A,b}$	$3.92 \pm 0.27^{A,a}$	$88.05 \pm 0.82^{A,c}$	$5.43 \pm 0.37^{C,a}$	$1.06 \pm 0.17^{B,a}$
Ezhi-8	Green	$0.97 \pm 0.01^{B,b}$	$4.14 \pm 0.01^{A,b}$	$83.66 \pm 0.01^{C,b}$	$9.53 \pm 0.02^{B,a,b}$	$1.22 \pm 0.00^{B,a}$
	Semi black	$1.41 \pm 0.21^{A,a}$	$4.20 \pm 0.04^{A,a}$	$83.41 \pm 0.49^{C,b}$	$9.06 \pm 0.04^{C,b}$	$1.14 \pm 0.04^{B,a}$
	Black	$1.31 \pm 0.21^{A,a}$	$2.34 \pm 0.03^{B,c}$	$84.45 \pm 0.07^{C,a}$	$10.16 \pm 0.27^{C,a}$	$0.89 \pm 0.04^{C,b}$
Coratina	Green	$1.19 \pm 0.07^{A,B,a}$	$0.52 \pm 0.04^{D,c}$	$87.45 \pm 0.89^{B,a}$	$9.63 \pm 0.81^{B,c}$	$0.82 \pm 0.14^{C,a}$
	Semi black	$1.45 \pm 0.12^{A,b}$	$0.64 \pm 0.01^{D,b}$	$86.46 \pm 0.32^{B,b}$	$10.47 \pm 0.16^{B,b}$	$0.73 \pm 0.01^{C,a}$
	Black	$1.19 \pm 0.01^{A,a}$	$0.78 \pm 0.01^{C,a}$	$85.42 \pm 0.24^{B,c}$	$11.43 \pm 0.20^{B,a}$	$0.81 \pm 0.04^{C,a}$

Results are presented as the mean value ± standard deviation, n = 3

[a–c]Different letters in the same variety indicate significantly different values ($p < 0.05$)

[A–D]Different letters in the same ripening stage indicate significantly different values ($p < 0.05$)

samples. The content of linoleic acid at the sn-2 position in the Picual olive oils ranged from 4.17 ± 0.61 to 5.43 ± 0.37% were the lowest of all four varieties, while the linolenic acid at the sn-2 position in the Coratina olive oils (ranging from 0.73 ± 0.01 to 0.82% ± 0.14%) were significantly lower than that in the other three varieties (which ranged from 0.89 ± 0.04 to 1.94 ± 0.09%). Furthermore, it was noted in general that the fatty acid composition at the sn-2 position did not change significantly during the ripening stages in any of the varieties.

Since the olive oil samples were obtained from the same source, there was no significant differences in their environmental growth factors, such as sediment and light. Moreover, the progressive ripening stages of the fruits were found to have little effect on the fatty acid composition at the sn-2 position in the olive oils, which, therefore, suggests that the differences in their sn-2 fatty acid composition are directly related to the specific varieties of the olives.

Phenolic composition

The phenolic composition of olive oil is an important factor in quality evaluation. Phenolic compounds can increase the oxidative stability of olive oil and are related to its color and clarity [30]. Such compounds include mainly phenolic alcohols, phenolic acids, secoiridoid derivatives and flavonoids [4, 31]. In this study, four main individual polyphenols in olive oil were identified by high-performance liquid chromatography (HPLC) (Fig. 1), namely oleuropein, hydroxytyrosol, tyrosol and rutin.

Oleuropein is one of the main phenolic glycosides in olive oil and offers anti-oxidant, anti-cancer and hypoglycemic effects. The content of oleuropein in the four varieties of olive oil samples is shown in Fig. 1a. Of the four varieties, Ezhi-8 olive oils had the highest content of oleuropein (ranging from 5.76 ± 0.36 to 7.09 ± 0.45 mg/kg), while Coratina olive oils had the lowest (ranging from 2.87 ± 0.53 to 3.79 ± 0.02 mg/kg). With the fruit skin turned from semi black to black, the oleuropein concentration in the Picual and Ezhi-8 olive oils decreased to 3.86 ± 0.45 mg/kg and 5.76 ± 0.36 mg/kg, respectively. However, the Arbequina and Picual olive oils maintained their oleuropein content at any stage in the ripening process. This may be related to varieties differences in activities of β-glucosidase. On the other hand, the compartmentalization of oleuropein and their degrading enzymes in olive fruit may also be one of the reasons for the maintained oleuropein content in Arbequina and Coratina olive oil.

Hydroxytyrosol and tyrosol are the main phenolic alcohols in olive oil, and also two of its most abundant phenolic compounds [32]. The contents of hydroxytyrosol and tyrosol in the olive oil samples are shown in Fig. 1b, c. During fruit ripening, the content of hydroxytyrosol in the four varieties of olive oil samples decreased. Among them, the hydroxytyrosol contents of both Ezhi-8 and Coratina olive oils were higher than that of the other two varieties (5.90 ± 0.11–7.48 ± 0.65 mg/kg and 3.67 ± 0.04–9.37 ± 0.28 mg/kg, respectively). Picual olive oils had the lowest content of hydroxytyrosol (1.60 ± 0.13–3.69 ± 0.37 mg/kg). The content of tyrosol in the Picual, Ezhi-8 and Coratina olive oils increased by 2.07 mg/kg, 6.28 mg/kg and 1.26 mg/kg, respectively, during the ripening process. The content of rutin in the four varieties, however, showed two different trends: in Arbequina and Coratina olive oil samples, the rutin decreased gradually during the ripening process, while, in the Picual and Ezhi-8 olive oils the trend was for the rutin content to increase. This may be related to the diverse endogenous enzyme activities in the different varieties of the olive fruit.

The phenolic content in olive oil depends on various factors, such as phenolic glycosides amount and enzymatic activities in olive fruit tissues and technological factors during olive oil extraction [33, 34]. The most important class of endogenous enzymes are β-glucosidases and oxidoreductase enzymes, which are responsible for the hydrolysis of phenolic glycosides and oxidation of phenolic compounds, respectively [34]. Oleuropein is hydrolyzed by the action of β-glucosidases to form glucose and the corresponding aglycone [35] and hydroxytyrosol is the main degradation product of oleuropein. The activities of endogenous enzymes in olives depends mainly on the variety and maturation degree of the olive fruit [13, 14], which could also explain differences in phenolic compounds.

Total phenolics and flavonoids contents

Phenolic compounds are responsible for the sensory attributes of bitterness and pungency [2, 36]. When the concentration of phenolics in olive oil is very high, the product's qualities of bitterness and pungency are intense [37]. Here, the total phenol and flavonoid contents of the four varieties of olive oil samples are shown in Fig. 1e, f, in which it can be seen that the total flavonoid content is highly correlated with the total phenolic content ($p < 0.01$) ($r = 0.93$). Coratina is well known for its high content of phenolic compounds [38], the total phenolics and flavonoids contents of the Coratina olive oil samples were found to be significantly higher than that of the other three varieties at every stage of ripening. As the fruit ripened, however, the total phenolics content of the Coratina olive oils decreased. As mentioned above, this may be related to phenolic glycosides amount and enzymatic activities in olive fruit. β-glucosidase is one of the main enzyme determining the total phenolic content of olive oil during olive ripening [39]. During the extraction process, phenolic glycosides, such as oleuropein, are hydrolyzed by the endogenous β-glucosidase to produce secoiridoid

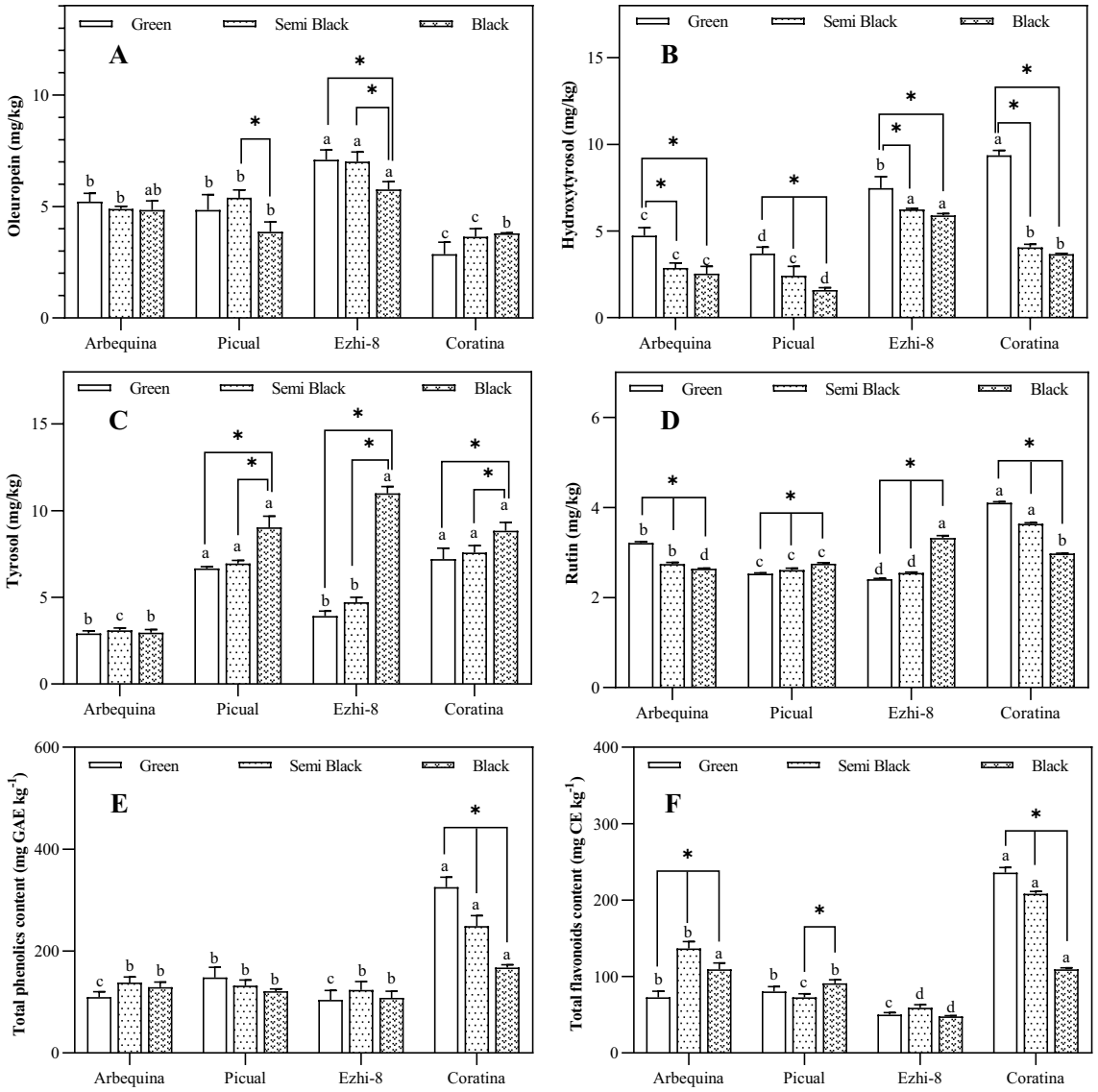

Fig. 1 Contents of individual phenolics, total phenolics and total flavonoids in olive oil samples. **a** Oleuropein, **b** hydroxytyrosol, **c** tyrosol, **d** rutin, **e** total phenolics content and **f** total flavonoids content. Values are means ± standard deviations (n = 3). *In the same variety indicates significantly different values ($p < 0.05$). [a–d]Different letters in the same ripening stage indicate significantly different values ($p < 0.05$)

compounds, which constitute the most important phenolic components of olive oil [34, 40]. In addition, the activities of polyphenol oxidase and peroxidase will also affect the total phenolics contents in olive oil [34].

Antioxidant activities

The antioxidant activity of all olive samples in this study are shown in Fig. 2. Generally, ABTS and DPPH are able to evaluate the radical scavenging activity of compounds, while FRAP is used to determine the capacity of reductants in a sample [41, 42]. The highest ABTS radical-scavenging activities were observed in Coratina olive oil (ranging from 500.73 ± 53.76 to 671.88 ± 17.31 μmol TE/kg), followed by the extracts of Arbequina and Picual olive oils (from 477.95 ± 19.68 to 523.05 ± 10.96 μmol TE/kg and from 408.46 ± 34.82 to 425.97 ± 31.01 μmol TE/kg, respectively). The extracts of Ezhi-8 olive oil had the lowest activities (ranging from 323.30 ± 17.04 to 409.28 ± 32.83 μmol TE/kg). Moreover, the DPPH results varied from 181.22 ± 5.90 to 826.85 ± 6.65 μmol TE/kg. For FRAP, the results ranged from 322.18 ± 18.90 to 1163.52 ± 24.74 μmol TE/kg. The results of both the DPPH radical scavenging activity and the FRAP assay were similar to those of the ABTS, all of which showed that the extracts of Coratina olive oil had the highest

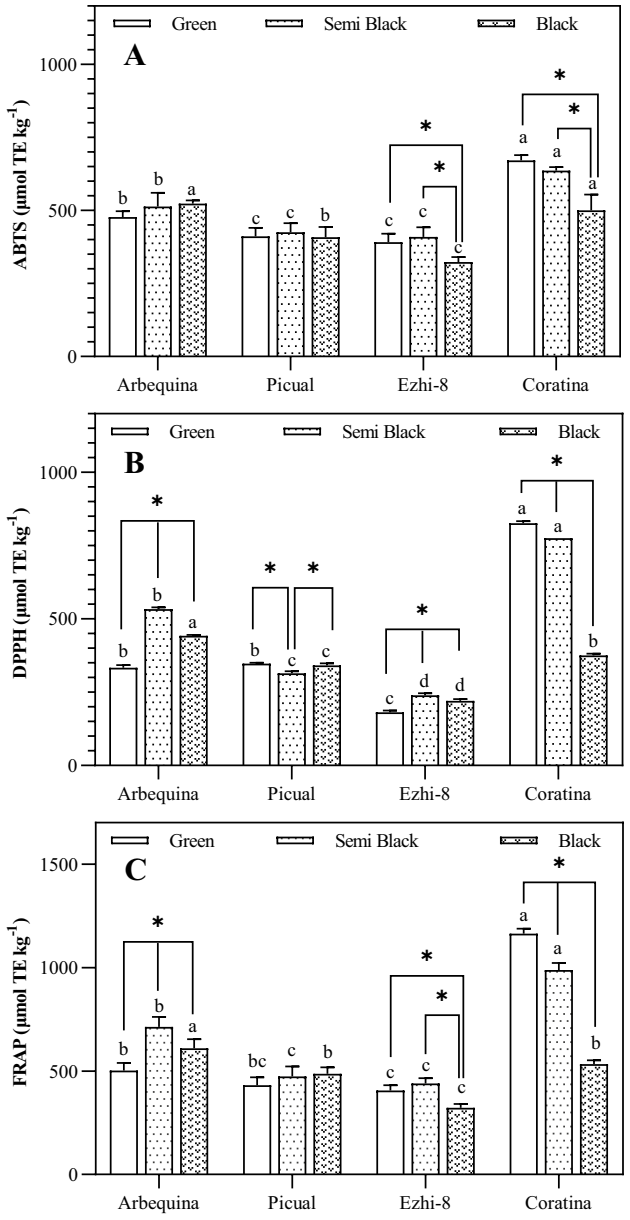

Fig. 2 Total antioxidant activity in olive oil samples. Values are means ± standard deviations (n = 3). *In the same variety indicates significantly different values ($p < 0.05$). [a–d]Different letters in the same ripening stage indicate significantly different values ($p < 0.05$)

activity, followed by the extracts of the Arbequina, Picual and Ezhi-8 olive oils, respectively.

The antioxidant activity measured by ABTS, DPPH and FRAP is highly correlated with total phenolic content ($p < 0.01$) ($r = 0.85$, 0.91 and 0.93, respectively). These results reveal that phenolic compounds are the main components of polar extracts of virgin olive oil. In addition, the antioxidant capacity measured by these three methods also show significant correlations ($p < 0.01$) between ABTS and DPPH ($r = 0.95$), FRAP and DPPH ($r = 0.98$), and ABTS and FRAP ($r = 0.96$).

Principal component analysis

Principal component analysis (PCA) was applied in this study to classify the quality of four varieties of olive oil samples based on their contents of oleic acid, oleic acid (sn-2), phenolic compound composition and concentration, total phenolics and flavonoids contents and antioxidant activity. As shown in Fig. 3a, b, the PCA results indicate that two factors accounted for 81.73% of the total variance (F1: 57.66%, F2: 24.07%). The first principal component (PC1) was found to be positively related to the antioxidant activity, total phenolic and flavonoid content, C18:1, the content of hydroxytyrosol, tyrosol and rutin, but it was negatively related to oleic acid (sn-2) and oleuropein. The second principal component (PC2) was positively related to oleic acid, sn-2 C18:1, tyrosol, total phenolic and flavonoid content, rutin, hydroxytyrosol and DPPH, and was negatively related to oleuropein, ABTS and FRAP. The total phenolic content, total flavonoid content and total antioxidant activity accounted for a large proportion in PC1, while the content of oleic acid, oleic acid (sn-2) and tyrosol accounted for a large proportion in PC2. Oleuropein was negatively correlated with both PC1 and PC2. The different varieties of olive oil samples were divided into three categories by PCA. Coratina olive oil samples showed higher PC1 scores than the other three varieties and the olive oil from different fruit ripening stage varied greatly in the PC1 direction. Mainly due to their high phenolic content and total antioxidant activity, which were greatly affected by fruit ripening stage. In fact, with the fruit ripening, the levels of hydroxytyrosol, rutin, total phenolics, total flavonoids and antioxidant activities in Coratina olive oil all declined. In addition, due to the contents of C18:1, sn-2 C18:1 and tyrosol were much higher in the Picual than in the Arbequina oils. The PC2 scores of the Picual oils were significantly higher than those of the Arbequina oils.

Conclusion

This study was performed to determine the effects of variety and ripening stage on the chemical composition, antioxidant activity and quality characteristics of virgin olive oils from four olive varieties (Arbequina, Picual, Ezhi-8 and Coratina) introduced into the Chongqing region in China. The results indicate that when the fruit skin turned from green to black, the percentage of oleic acid in the Arbequina, Picual and Coratina olive oils decreased and the percentage of linoleic acid increased. Coratina was determined to be superior to the other varieties in terms of total phenolic and flavonoid contents and total antioxidant activity and the quality of

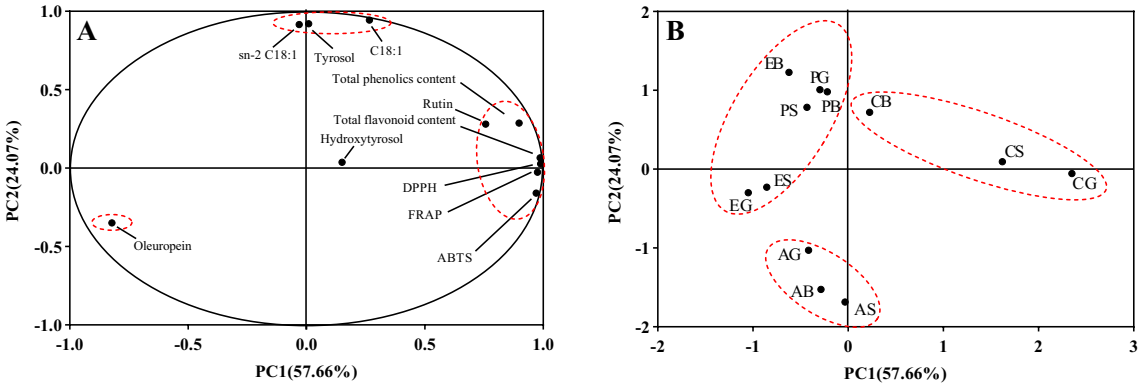

Fig. 3 Principal component analysis (PCA) according to the contents of oleic acid, oleic acid (sn-2), phenolic compound composition and concentration, total phenolic and flavonoid contents, and the antioxidant activity of olive oil samples. **a** Loading plot; **b** Scores plot. AG: Arbequina (green); AS: Arbequina (semi black); AB: Arbequina (black); PG: Picual (green); PS: Picual (semi black); PB: Picual (black); EG: Ezhi-8 (green); ES: Ezhi-8 (semi black); EB: Ezhi-8 (black); CG: Coratina (green); CS: Coratina (semi black); CB: Coratina (black)

Coratina olive oils were greatly affected by fruit ripening stage. With the fruit ripening, the levels of hydroxytyrosol, rutin, total phenolics, total flavonoids and antioxidant activities in Coratina olive oil all declined. In addition, significant differences of fatty acid composition at sn-2 position were observed among the sample varieties, with the content of oleic acid at sn-2 position in Picual olive oils significantly higher than in other varieties. Finally, in this study, the fruits harvested at a lower maturity index seemed to be more suitable for the production of high chemical quality EVOO.

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