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# Quality, composition, and antioxidant activity of virgin olive oil from introduced varieties at Liangshan

Chunrong Xiang [a, 1], Zhou Xu [a, 1], Jie Liu [a], Tian Li [b], Zeshen Yang [c], Chunbang Ding [a, *]

[a] College of Life Science, Sichuan Agricultural University, Ya'an 625014, P. R. China

[b] College of Agronomy, Sichuan Agricultural University, Chengdu 611130, P. R. China

[c] Liang Shan Zhong Ze New Tech Development Co., Ltd., Xichang 615000, P. R. China

[*] Corresponding author. Tel.: +86 835 2885560

E-mail address: dcb@sicau.edu.cn (C. Ding)

[1] These authors contributed equally to this work.

**Abstract:** Virgin olive oil from introduced varieties has attracted a lot of attention because it can grow in environments different from where it originated. We used Acid-base titration, oxidation-reduction titration, high performance liquid chromatography (HPLC), and gas chromatography - mass spectrometry (GC-MS) to evaluate the quality, composition, and antioxidant activities of four virgin olive oils from introduced varieties at Liangshan. The composition and quality index were comparable to an extra virgin olive oil (EVOO) reference, but the free fatty acid and peroxide values were lower than the EVOO reference. Palmitic acid, oleic acid, and linoleic acid were the major fatty acids that conformed to standards, while Koreniki exhibited a high percentage of oleic acid. We determined the contents of α-tocopherol, stigmasterol, and β-sitosterol and found that Koreniki had the highest α-tocopherol content, Barnea had the highest stigmasterol content, and Manzanlilla had the highest β-sitosterol content. The total polyphenol ranged from 55.41 to 180.21 mg/kg oil. The polyphenol extracts of oil had excellent anti-oxidation properties. Principal component analysis (PCA) also showed that Manzanilla had the highest score.

**Key words:** introduced varieties; virgin olive oil; quality; composition; antioxidant activity

## 1. Introduction

Olives, an important woody oil crop, are widely distributed in the Mediterranean. In 1956, olives were introduced to China, and were mainly cultivated along the Jinsha River in the hot-arid valley. Olive oil is a kind of vegetable oil extracted from fresh fruit via physical cold pressing, which is the best method to reduce the loss of its active compounds, and is popular throughout the world because of its rich nutritional ingredients, such as unsaturated fatty acids (UFA), α-tocopherol, polyphenol, sterol, and a variety of minor elements (Capriotti et al., 2014).

Olive oil is unique not only for its high content of oleic acid that can adjust the ratio of low density lipoproteins and high density lipoproteins in organisms (Aguilera et al., 2005; Khaleghi et al., 2015; Ranalli et al., 2008), but also for its suitable proportions of linoleic acid and linolenic acid that benefit hypertensive patients (Maggio et al., 2009). Polyphenol, which is responsible for the bitterness in oil, extends the shelf life of olive oil (Flores et al., 2012), prevents oxidation reactions (Romero-Segura et al., 2012; Tura et al., 2007), and contributes to the satisfactory organoleptic characteristics of oil, e.g. aroma and flavor (Baccouri et al., 2008; Servili et al., 2004). Sterol, a major unsaponifiable fraction of olive oil, significantly contributes to the nutritional value of olive oil (Cañabate-Díaz et al., 2007) and has anti-inflammatory and anti-carcinogenic effects. Oil consumption has been associated with lower incidence rates of coronary heart disease and cancer onset, because olive oil has a wide range of biological functions, such as lowering cholesterol levels, improving digestion functions, and preventing brain aging (Beltrán et al., 2005).

Currently, olives gradually grow in many non-Mediterranean regions with variable altitudes (Gutierrez et al., 2009). The Liangshan district of the Sichuan province is a remarkable representation of olive introduction and cultivation in China. The Liangshan district is located in the Anling river plain hinterland of the western Sichuan plateau, and has a typical dry valley climate zone, with rainy, torrid summers and dry, warm winters. Nevertheless, cultivar and altitude are important factors in relation to composition and olive quality (Jemai et al., 2009; Vinha et al., 2005). Considering the nutritional value of virgin olive oil and the different environments in which it can grow outside of its country of origin, we conducted a study on the characterization of virgin olive oil from introduced varieties to breed cultivars with high quality oil that were well-adapted to the Liangshan environment.

## 2. Materials and methods

### 2.1 Plant materials and reagents

Barnea, Coratina, Koreniki, and Manzanilla which were introduced to Liangshan over 8 years originated from Israel, Italy, Greece and Spain, respectively. We harvested Purple fresh fruits at mid-September 2015.

We purchased hydroxytyrosol, *p*-hydroxybenzoic acid, caffeic acid, epicatechin, *p*-coumaric acid, ferulic acid, rutin, quercetin, α-tocopherol, stigmasterol, β-sitosterol, and 2,2-Diphenyl-1-picryl-hydrazyl (DPPH) from Sigma Chemical Co. (St. Louis, MO, USA). We purchased hexane, methanol, alcohol, sodium chloride, petroleum ether, isopropyl alcohol, ferrozine, gallic acid, potassium hydroxide, sodium salicylate, ferrous sulfate, and hydrogen peroxide from Chengdu Kelong Chemical Factory (Chengdu,China). All the reagents were analytical grade except that mobile phase was chromatographic grade.

66 2.2 Oil extraction

67 We extracted olive oil according to the method reported by Beltrán et al., (2005). We crushed 3 kg
68 olive fruits with a hammer mill, and slowly mixed for 30 min, and centrifuged to separate the oil. We
69 transferred the oil into dark plastic bottles and stored it in dark at 4 ℃ for future study.

70 2.3 Free acidity and peroxide value determination

71 We determined free acidity and peroxide values in accordance with the ISO 660-1996 and ISO
72 3960-2001 methods.

73 2.4 Fatty acid composition analysis

74 The analytical method used for fatty acid composition conformed to the ISO 5508-1990 method. The
75 oil was briefly pre-methylated with methanol. Then, we analyzed the pretreated sample using GC-MS
76 (QP2010 Shimadzu, Japan) with a capillary column (30 m×0.25 mm; 0.25 μm). Detection conditions
77 were as follows: injector temperature was 245°C; flame ionization detector temperature was 230°C;
78 column temperature started at 60°C for 5 min, increased at 15°C/min to 150°C and held for 1 min, and
79 finally increased at 3°C/min to 230°C and held for 7 min; the split ratio was 1/10; the mass range
80 scanned was 45-550 *m/z*; and the carrier gas was He.

81 2.5 Stigmasterol and β-sitosterol analysis

82 We used the HPLC method to perform qualitative and quantitative analyses of stigmasterol and
83 β-sitosterol. The experiment sample was briefly saponified, according to the method Sivakumar et al.,
84 (2006) described. Then, we used Agilent 1260 HPLC (Agilent Technologies, USA), coupled with
85 ZORBAX Eclipse plus C18 column (4.6×150 mm; 5.0 μm), to analyze the pretreated sample.
86 Detection conditions were as follows: injection volume was 10 μL; determined wavelength was 210

nm; determined temperature was 50°C; and the mobile phase was 98.5% methanol aqueous solution at 1 mL/min.

2.6 α-tocopherol determination

We also used the HPLC method to perform qualitative and quantitative analyses of α-tocopherol. Firstly, α-tocopherol was extracted using the method adapted from Carpenter et al., (1979). Then, we used the Agilent 1260 HPLC to analyze the sample. Detection conditions were as follows: injection volume was 10 µL; determined wavelength was 295 nm; determined temperature was 35°C; and the mobile phase was methanol at 1 mL/min.

2.7 Extraction of polyphenol fraction

We extracted a polyphenol fraction according to the method previously described by Bouarroudj et al., (2016) with slight modifications. We dissolved 20 g oil in 20 mL 80% methanol aqueous solution. We shook and centrifuged the mixture at 4000 rpm for 15 min, and we dried the extraction thrice using the rotary evaporator at 40°C to make up to 25 mL.

2.8 Total polyphenol content determination

We used the Folin-Ciocalteau method to determine the total polyphenol content (Singleton et al., 1999). We added 80 µL of a sodium carbonate solution (10%) to 100 µL polyphenol extracts, and a 20 µL Folin-Ciocalteau reagent was added after 5 min. We used a microplate reader (Spectramax M2, USA) to read the absorbance at 765 nm after incubation in the dark for 1 h. The total polyphenol content was expressed in the mg equivalent of gallic acid per kilogram of oil (mg GAE/kg).

2.9 Chromatography analysis of a polyphenol fraction

We used HPLC with column ZORBAX SB-C18 (4.6×150 mm; 5.0 µm) to achieve qualitative and quantitative analysis of a polyphenol fraction. Detection conditions were as follows: injection volume

was 10 µL; determined wavelength was 280 nm; determined temperature was 35°C; and the mobile phase were water (A, contained 0.5% acetic acid), methanol (B), and isopropyl alcohol (C). Gradient elution: 92% A-4% B-4% C (0-14 min), 82% A-9% B-9% C (14-45 min), 70% A-15% B -15% C (45-60 min); and the mobile phase flow rate was 1 mL/min.

2.10 Antioxidant activity

2.10.1 DPPH radical scavenging activity

To investigate the radical scavenging activity of olive oil, we determined the DPPH scavenging activity using previous methods (Rapisarda et al., 1999) with slight modifications. We briefly diluted the polyphenol extraction to 2-40 times. We added 50 µL diluent to 100 µL of freshly prepared DPPH ethanol solution (0.2 mM). We measured absorbance at 517 nm after 30 min reaction. The DPPH radical scavenging effect was calculated using the following formula:

DPPH scavenging effect (%) = $(1 - \frac{As}{Ac}) \times 100$ ,

where *As* was the absorbance of DPPH solution with the test sample and *Ac* was the replacement ethanol.

2.10.2 Hydroxyl radical scavenging activity

We determined the hydroxyl radical scavenging activity of polyphenol extraction using a previous method (Smirnoff & Cumbes, 1989) with slight modifications. We briefly diluted the polyphenol extraction to 1-8 times. We mixed 50 µL diluent with 50 µL $FeSO_4$ (9 mM), 50 µL $H_2O_2$ (6 mM), and 50 µL sodium salicylate (9 mM), then measured the absorbance at 510 nm after incubation at 37°C for 30 min. We used the following formula to calculate the hydroxyl radical scavenging effect:

Hydroxyl scavenging effect (%)= $(1 - \frac{As}{Ac}) \times 100$ ,

where *As* was the absorbance of the hydroxylated salicylate complex with the test sample and *Ac* was the replacement distilled water.

2.10.3 $Fe^{2+}$ chelating capability

We determined the $Fe^{2+}$ chelating capabilities of polyphenol extraction using a method previously described by Oyaizu (1986), with slight modifications. We briefly diluted the polyphenol extraction to 1-8 times. We mixed 50 μL diluent with 100 μL $FeSO_4$ (0.125 mM) and 50 μL ferrozine (1.0 mM), and measured the absorbance at 562 nm after incubation for 10 min. We used the following formula to calculate the $Fe^{2+}$ chelating capabilities:

$Fe^{2+}$ chelating capability (%)= $(1 - \frac{As}{Ac}) \times 100$ ,

where *As* was the absorbance of the test sample and *Ac* was the replacement distilled water.

2.11 Statistical analysis

We reported all results as mean ±standard deviation (S.D.). We used IBM SPSS Statistics 20 to apply principle components analysis (PCA), calculation of 50% elimination concentrations ($EC_{50}$), and least significant difference tests to the oil samples.

## 3. Results and discussion

3.1 Free acidity and peroxide value

Free acidity and peroxide values reflect the free fatty acid and active oxygen content of oil, are used as traditional criteria for classifying olive oil, and are considered to be an exclusive and important quality index. As shown in Table 1, the free acidity of four olive oils ranged from 0.14 to 0.28%. Meanwhile, the peroxide values of four olive oils ranged from 0.98 to 2.46 meq $O_2$/kg. Both the free acidity and peroxide values did not exceed the upper limit of EVOO that the International Olive Council (Council,

2013) formulated. The extremely low free acidity and peroxide values might be closely related to short storage time, ripeness, and the oxidation degree of the samples (Hbaieb et al., 2016).

### 3.2 Fatty acid composition

We used GC-MS to identify the fatty acid compositions of four oils, which are shown in Table 2 and Fig. 1. Manzanilla olive oil consisted of 11 kinds of fatty acids, i.e palmitic acid, palmitoleic acid, heptadecanoic acid, heptadecenoic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, arachidic acid, gadoleic acid, and behenic acid. Barnea olive oil contained 10 fatty acids and did not contain heptadecenoic acid; and Coratina olive oil and Koreniki olive oil only contained 9 of 11 fatty acids and did not contain heptadecanoic acid and heptadecenoic acid. Among the detected fatty acids, palmitic acid was the major saturated fatty acid (SFA) and ranged from 12.69 to 14.24%, and oleic acid was the major unsaturated fatty acid (UFA) and ranged from 60.94 to 74.03%. In addition, UFA percentages ranged from 73.23 to 81.3% and SFA ranged from 15.87 to 18.05%, with the Koreniki and Manzanilla oils having the highest percentages, respectively. The ratios of UFA and MFA ranged from 4.05 to 5.12%, and were not greatly different among the four varieties. Moreover, in comparison to the three other oils, Koreniki oil had a significantly lower proportion of 4.61:1 of n-6 PUFA and n-3 PUFA, which was close to the World Health Organization's suggestion for the optimal proportion (4:1) for the human body. Simopoulos (2002) also reported that the optimal n-6/n-3 ratio was supposed to be less than 4:1. Additionally, the population does not consume enough essential dietary nutrients, including n-3 PUFA, which increases the risk of developing various diseases, especially cardiovascular diseases (Trebušak et al., 2014).

### 3.3 Stigmasterol and β-sitosterol contents

Stigmasterol and β-sitosterol, which are minor essential compounds present in olive oil and the

dominant phytosterol, can exist as free or esterified structures with sugar or fatty acid moieties (Breinhölder et al., 2002; Rocco and Fanali, 2009), and help reduce blood cholesterol levels (Temime et al., 2008). Four oil samples had different Stigmasterol content, as shown in Table 3, with Coratina oil having the lowest value at 19.08 mg/kg. Meanwhile, β-sitosterol content also exhibited obvious variation, with Manzanilla oil having the highest value at 846.25 mg/kg and Koreniki oil having the lowest value at 279.14 mg/kg, which was lower than that of EVOO, as reported by Rocco and Fanali (2009).

3.4 α-tocopherol content

α-tocopherol, a version of Vitamin E that occurs in eight natural forms, has the highest vitamin E activity (Fujisawa et al., 2010). The α-tocopherol content varied from 87.09 to 147.06 mg/kg (Table 3). Among the four oils, the Manzanilla oil exhibited the lowest α-tocopherol content at 87.09 mg/kg, while Coratina and Koreniki oil had similar α-tocopherol content. According to Baldioli et al., (1996), the total tocopherol content in good quality oil is generally higher than 100 mg/kg and α-tocopherol accounts for 90% to 95% of total tocopherol. Using these indicators, all of the introduced olive oil that was tested, with the exception of Manzanilla, could be classified as good quality oil. However, they have lower content than EVOO, according to Reboredo-Rodríguez et al., (2016). Moreover, α-tocopherol is a lipid soluble antioxidant that co-localizes with PUFA-enriched phospholipid domains of the cell membrane that are highly susceptible to peroxidation (Atkinson et al., 2010; Lebold et al., 2014). Its specific inhibitory effects have been seen on protein kinase C, on the growth of certain cells, and in regulation of the expression of certain genes (CD36 and collagenase) (Griboff et al., 2014; Lushchak and Semchuk, 2012).

3.5 Polyphenol composition and content

196 The amount of polyphenol in olive oil is a crucial factor used to evaluate its quality, given that
197 polyphenol contributes to olive oil's oxidative stability, and is responsible for its sharp, bitter taste, to a
198 certain extent (Bajoub et al., 2016; Gutierrez et al., 2001). The polyphenol composition and content are
199 summarized in Table 4. The total polyphenol of Barnea, Coratina, Koreniki, and Manzanilla oils were
200 149.32, 58.92, 180.21, and 55.41 mg/kg, respectively. Simultaneously, we used HPLC to identify 8
201 individual polyphenol (Fig. 2), including hydroxyltyrosol, *p*-hydroxybenzoic acid, caffeic acid,
202 epicatechin, *p*-coumaric acid, ferulic acid, rutin, and quercetin, and found it was merely several
203 polyphenols lower than the limit of quantity (LOQ), such as caffeic acid, epicatechin, *p*-coumaric and
204 ferulic. We found Rutin and Quercetin, common flavonoids of olive oil that have scarcely been
205 reported in previous olive oil studies, in four oils. Rutin contents, ranging from 11.21 to 21.74 mg/kg,
206 were significantly higher than the other polyphenol compounds, especially in Koreniki, while
207 quercetin was only partially represented (at about 1 mg/kg). Interestingly, Oleuropein, belonging to
208 secoiridoids, was not present in these oils, which was not in accordance with previous studies on
209 Italian olive oil (Cioffi et al., 2010). This might have been because of lower polyphenol content and
210 injection volume.

211 3.6 DPPH radical scavenging activity

212 The DPPH model is widely used to evaluate the antioxidant capacities of natural products, and it has
213 been used for olive oil and individual antioxidant polyphenol (Espín et al., 2000). The results of DPPH
214 radical scavenging efficiency of four oil extracts are exhibited in Fig. 3A and were evaluated by $EC_{50}$
215 (Table 5). As shown in Fig. 3A, all extracts exhibited a concentration-dependent manner of the DPPH
216 radical scavenging effect. In addition, the $EC_{50}$ of Barnea, Coratina, Koreniki, and Manzanilla oil
217 extracts were 49.66, 21.33, 20.00, and 25.33 μg/mL, indicating that the DPPH radical elimination

capacity of the Koreniki oil extract was the best, as compared to the other oil extracts.

### 3.7 Hydroxyl radical scavenging activity

The hydroxyl radical is one of the most harmful and reactive oxygen species, easily crossing cell membranes and damaging lipids, proteins, and DNA. Hence, hydroxyl radical elimination is necessary to protect living systems (Birben et al., 2012). As shown in Fig. 3B, the hydroxyl radical scavenging efficiency of four oil extracts decreased as dilution ratios increased. Additionally, the $EC_{50}$ (Table 5) contained in Barnea, Coratina, Koreniki, and Manzanilla oil extracts were 63.00, 5.33, 57.00, and 45.00 μg/mL, respectively, indicating that Coratina had a better hydroxyl radical elimination capacity.

### 3.8 $Fe^{2+}$ chelating capability

The iron-coordination mechanism has also been identified as an important component in characterizing polyphenol antioxidant activity (Moran et al., 1997). It has been suggested that an iron complex that was not reduced by cellular reductants to catalytically generate ·OH might root in the iron binding to polyphenol compounds (Perron et al., 2008). As shown in Fig. 3C, four oil extracts exhibited obvious $Fe^{2+}$ chelating activity in a concentration-dependent manner. Additionally, the $EC_{50}$ (Table 5) of four oil extracts were 39.00, 19.00, 83.00, and 16.66 μg/mL. These results indicated that the $Fe^{2+}$ chelating capabilities of polyphenol extracts from different varieties of olive oils were significantly different (Ziogas et al., 2010). Notably, there is no clear correlation between $EC_{50}$, peroxide values, and total polyphenol. The reason for this phenomenon might have been that the peroxide value was extremely low and had different amounts of individual polyphenol.

### 3.9 Principal component analysis

We applied PCA to evaluate the quality of four oils based on their stigmasterol, β-sitosterol, α-tocopherol, total polyphenol, and fatty acid compositions. Figure 4 shows a scatter diagram of 15

indexes. The PCA results indicated that two factors accounted for 84.68% of the total variance (F1: 55.70%, F2: 28.98%), reflecting primary information of original data. The first principal component was positively related to palmitoleic acid, β-sitosterol, heptadecenoic acid, and heptadecanoic acid, and was negatively related to oleic acid, linolenic acid, and α-tocopherol. The second principal component included total polyphenol, stigmasterol, arachidic acid, gadoleic acid, and behenic acid. Principle component scores of four oil samples are shown in Table 6. We used synthesis scores to evaluate the quality of four virgin olive oils, with Manzanilla receiving the highest score and Coratina receiving the lowest score among the tested oil samples.

## 4. Conclusion

We investigated the quality, composition, and antioxidant properties of virgin olive oils from four introduced varieties. The results confirmed that all of the parameters manifested apparent variations between the oil samples. Coratina and Manzanilla were superior to the others, in terms of free fatty acids and peroxide values. Koreniki exhibited a high percentage of oleic acid and the optimal proportion for the human body, at about the ratio of n-6 PUFA and n-3 PUFA. Barnea, Manzanilla, and Koreniki had the highest contents of stigmasterol, β-sitosterol, and α-tocopherol, respectively. Koreniki had the highest content of total polyphenol. All of the oil samples exhibited excellent antioxidation properties. PCA results showed that Manzanilla had the highest score. In general, these results indicated that four virgin olive oils from introduced varieties had good quality, especially Manzanilla, and highlighted the high potential of olive oil as a phytochemical resource and possible functional food.

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**Figures captions**

**Fig. 1. Fatty acid composition gas chromatogram of four olive oils.**

1-palmitic acid, 2-palmitoleic acid, 3-heptadecanoic acid, 4-heptadecenoic acid, 5- stearic acid, 6-oleic acid, 7-linolei cacid, 8-linolenic acid, 9-arachidic acid, 10-gadolei cacid, 11-behenic acid

**Fig. 2. Liquid chromatograms of individual polyphenol of four olive oils and standard substances.**

1-hydroxy tyrosol, 2-*p*-hydroxybenzoic acid, 3-caffeic acid, 4-epicatechin, 5-*p*-coumaric acid, 6-ferulic acid, 7-rutin, 8-quercetin

**Fig. 3 DPPH radical scavenging effects (A), hydroxyl radical scavenging effects (B) and $Fe^{2+}$ chelating capacity (C) of four oils.**

**Fig. 4 Scatter diagram of constituents of tested oils samples**.

x1-stigmasterol, x2-β-sitosterol, x3-α-tocopherol, x4-total polyphenol, x5-palmitic acid, x6-palmitoleic acid, x7-heptadecanoic acid, x8-heptadecenoic acid, x9- stearic acid, x10-oleic acid, x11-linoleic acid, x12-linolenic acid, x13-arachidic acid, x14-gadoleic acid, x15-behenic acid

389 **Table 1. Free acidity (% oleic acid) and peroxide value (meq $O_2$/kg) of four olive oils**

| | Barnea | Coratina | Koreniki | Manzanilla | EVOO[a] |
|---|---|---|---|---|---|
| Free fatty acids | 0.21±0.01[b] | 0.14±0.01[a] | 0.28±0.01[c] | 0.23±0.01[b] | ≤0.8 |
| Peroxide value | 1.46±0.41[a] | 1.33±0.17[a] | 2.46±0.67[b] | 0.98±0.15[a] | ≤20 |

390 [a] Reference value of extra virgin olive oil formulated by International olive council(COI/T.15.2013).

391 Values are means ± standard deviations (n = 3).

392 Different letters in the same row indicate significantly different values ($p < 0.05$).

393 **Table 2. Fatty acid composition of four olive oils (% m/m methylic esteer).**

| Fatty acids | Barnea | Coratina | Koreniki | Manzanilla | EVOO[a] |
|---|---|---|---|---|---|
| Palmitic acid ($C_{16:0}$) | 12.69±0.09$^{a}$ | 13.40±0.37$^{b}$ | 13.23±0.46$^{bc}$ | 14.24±0.03$^{c}$ | 7.5-20 |
| Palmitoleic acid ($C_{16:1}$) | 1.02±0.02$^{c}$ | 0.42±0.04$^{a}$ | 0.74±0.06$^{b}$ | 1.86±0.03$^{d}$ | 0.30-3.50 |
| Heptadecanoic acid ($C_{17:0}$) | 0.08±0.01 | ND | ND | 0.11±0.01 | ≤0.30 |
| Heptadecenoic acid ($C_{17:1}$) | ND | ND | ND | 0.24±0.01 | ≤0.30 |
| Stearic acid ($C_{18:0}$) | 2.20±0.07$^{b}$ | 1.87±0.16$^{a}$ | 2.61±0.25$^{c}$ | 2.98±0.12$^{d}$ | 0.50-5.00 |
| Oleic acid ($C_{18:1}$) | 66.09±1.69$^{bc}$ | 65.66±6.37$^{bc}$ | 74.03±1.24$^{b}$ | 60.94±6.48$^{a}$ | 55.00-83.00 |
| Linoleic acid ($C_{18:2}$) | 13.41±0.55$^{c}$ | 10.29±0.94$^{b}$ | 5.37±0.58$^{a}$ | 9.23±0.35$^{d}$ | 3.50-21.00 |
| Linolenic acid ($C_{18:3}$) | 0.69±0.03$^{a}$ | 0.84±0.10$^{ab}$ | 0.78±0.07$^{ab}$ | 0.67±0.02$^{a}$ | ≤1.00 |
| Arachidic acid ($_{20:0}$) | 0.43±0.01$^{a}$ | 0.39±0.22$^{a}$ | 0.57±0.06$^{b}$ | 0.55±0.01$^{b}$ | ≤0.60 |
| Gadoleic acid ($C_{20:1}$) | 0.27±0.01$^{a}$ | 0.53±0.02$^{c}$ | 0.38±0.04$^{b}$ | 0.29±0.01$^{a}$ | ≤0.40 |
| Behenic acid ($C_{22:0}$) | 0.12±0.01$^{c}$ | 0.20±0.07$^{b}$ | 0.28±0.01$^{a}$ | 0.17±0.01$^{bc}$ | ≤0.20 |
| Unsaturate fatty acid (UFA) | 81.14±1.97 | 77.74±5.43 | 81.3±0.50 | 73.23±6.11 | |
| Saturate fatty acid (SFA) | 15.52±0.17$^{c}$ | 15.87±0.58$^{bc}$ | 16.69±0.77$^{b}$ | 18.05±0.14$^{a}$ | |
| UFA/SFA | 5.12±0.11$^{b}$ | 4.09±0.46$^{b}$ | 4.87±0.25$^{b}$ | 4.05±0.36$^{a}$ | |
| n-3 PUFA /n-6 PUFA | 13.92±0.02$^{d}$ | 7.51±0.0.02$^{b}$ | 4.61±0.12$^{a}$ | 9.61±0.15$^{c}$ | |

394 [a] Reference value of extra virgin olive oil formulated by International olive council(COI/T.15.2013).

395 ND: not detected .Values are means ± standard deviations (n = 3).

396 Different letters in the same row indicate significantly different values ($p < 0.05$).

397 **Table 3. Stigmasterol, β-sitosterol and α-tocopherol contents (mg/kg) of four olive oils.**

| | Barnea | Coratina | Koreniki | Manzanilla |
|---|---|---|---|---|
| Stigmasterol | 69.79±3.86$^{c}$ | 19.08±1.33$^{a}$ | 59.38±0.54$^{c}$ | 41.52±3.29$^{b}$ |
| β-sitosterol | 561.30±6.78$^{c}$ | 365.47±4.96$^{b}$ | 279.14±6.22$^{a}$ | 846.25±10.73$^{d}$ |
| α-tocopherol | 121.77±7.43$^{b}$ | 139.0±8.75$^{bc}$ | 147.06±6.38$^{c}$ | 87.09±3.64$^{a}$ |

398 Values are means ± standard deviations (n = 3).

399 Different letters in the same row indicate significantly different values ($p < 0.05$)

400 **Table 4. Contents of total polyphenol and individual polyphenol (mg/kg ) in oil samples determined by HPLC.**

| | Barnea | Coratina | Koreniki | Manzanilla |
|---|---|---|---|---|
| Total polyphenol | 149.32±5.89$^{b}$ | 58.92±2.00$^{a}$ | 180.21±1.77$^{c}$ | 55.41±1.35$^{a}$ |
| Hydroxy tyrosol | 1.38±0.01$^{a}$ | 2.39±0.29$^{b}$ | 1.58±0.04$^{a}$ | 3.91±0.29$^{c}$ |
| *p*-hydroxybenzoic acid | 1.55±0.02$^{a}$ | 2.48±0.14$^{b}$ | 3.51±0.04$^{c}$ | 8.92±0.07$^{d}$ |
| Caffeic acid | - | - | 0.16±0.01 | 0.17±0.01 |
| Epicatechin | - | - | - | 1.04±0.04 |
| *p*-coumaric acid | - | - | 0.12±0.01 | 0.07±0.01 |
| Ferulic acid | - | - | 0.45±0.01 | 1.03±0.01 |
| Rutin | 18.84±0.13$^{c}$ | 16.97±0.14$^{b}$ | 21.74±0.23$^{d}$ | 11.21±0.17$^{a}$ |
| Quercetin | 0.42±0.01$^{a}$ | 0.49±0.01$^{b}$ | 0.71±0.05$^{c}$ | 0.89±0.01$^{d}$ |

401 -: Lower than Limit of Quantity. Values are means ± standard deviations (n = 3).

402 Different letters in the same row indicate significantly different values ($p < 0.05$).

403 **Table 5. EC50 values (μg/mL) of four olive oils polyphenol extracts in antioxidant abilities.**

| | DPPH radical scavenging | Hydroxyl radical scavenging | $Fe^{2+}$chelating |
|---|---|---|---|
| Barnea | 49.66±5.13$^{b}$ | 63.00±7.81$^{c}$ | 39.00±7.00$^{b}$ |
| Coratina | 21.33±3.51 | 5.33±0.57 | 19.00±1.00 |
| Koreniki | 20.00±5.19$^{a}$ | 57.00±13.00$^{bc}$ | 83.00±9.64$^{c}$ |
| Manzanilla | 25.33±1.52$^{a}$ | 45.00±1.00$^{b}$ | 16.66±3.78$^{a}$ |

404 Values are means ± standard deviations (n = 3).

405 Different letters in the same line indicate significantly different values ($p$ <0.05).

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407 **Table 6. The principle component score of four olive oils.**

| Score | Barnea | Coratina | Koreniki | Manzanilla |
|---|---|---|---|---|
| Principal component 1 score | 0.12 | -2.03 | -2.13 | 4.04 |
| Principal component 2 score | 1.25 | -2.75 | 1.91 | -0.41 |
| Synthesis score | 0.51 | -2.28 | -0.74 | 2.51 |

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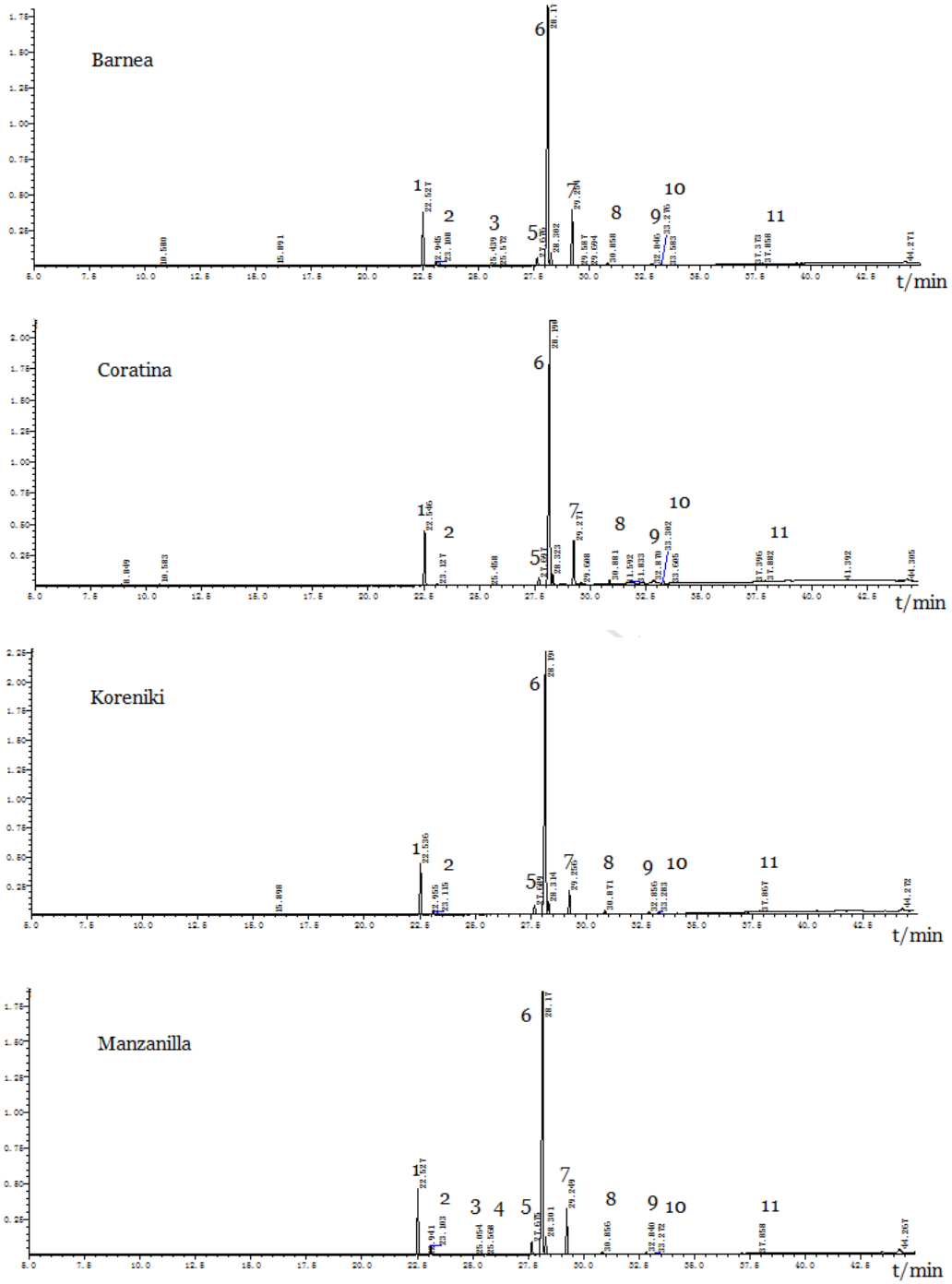


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414 **Fig. 1**

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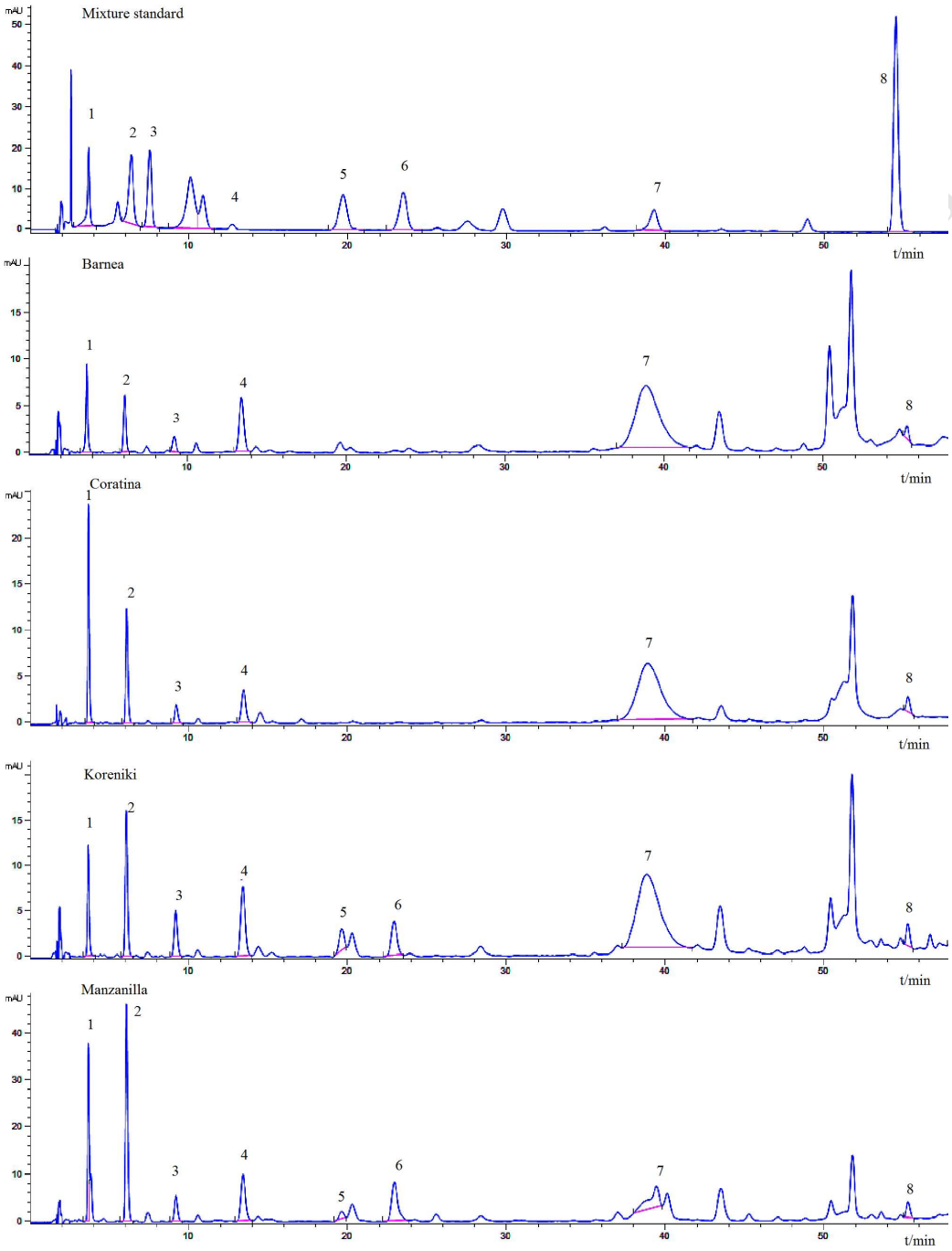


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418 **Fig. 2**

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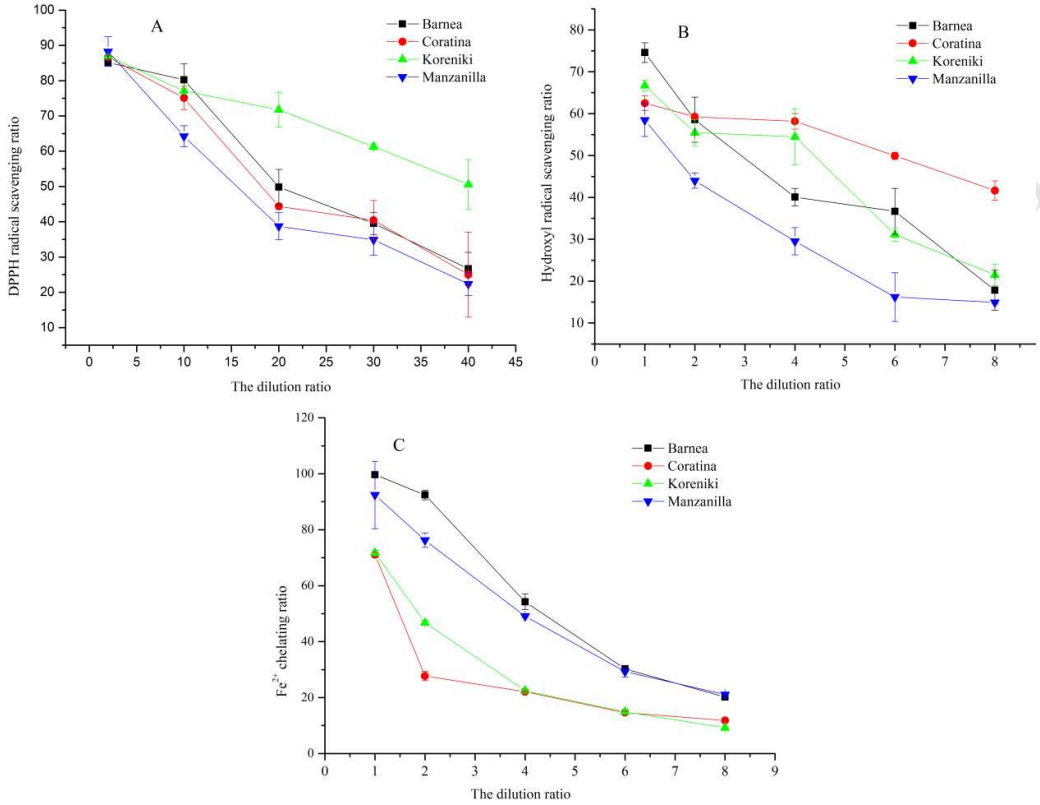


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423 **Fig. 3**

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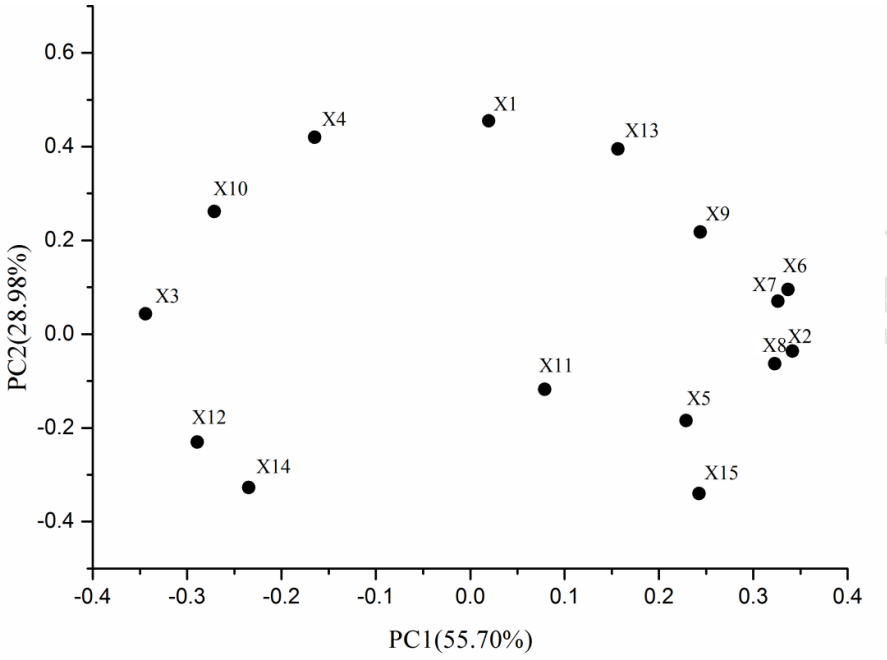


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**Fig. 4**

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Highlights

1-Quality properties of four virgin olive oils from introduced varieties.

2-Composition of four virgin olive oils from introduced varieties.

3-Antioxidant activity of four virgin olive oils polyphenol extracts.

4-Comprehensive assessment on four virgin olive oils quality.