Quality Assessment of Five Mono-cultivar Virgin Olive Oils Produced in Longnan (China) from 2013 to 2017

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Abstract: The aim of this work was to evaluate the quality of the five mono-cultivar (Frantoio, Leccino, Picholine, Coratina and Ezhi-8) virgin olive oils (Mc-VOOs) produced in Longnan (China) from 2013 to 2017 through analysing the organoleptic quality, physicochemical properties, phenolic contents, antioxidant activity and fatty acid composition. The leading principal components for assessing the quality of Mc-VOOs were extracted by principal component analysis (PCA). The results indicated that the five Mc-VOOs showed obvious differences ($p < 0.05$) in flavour and substance composition with the variation of cultivar and production year; however, the same cultivar of VOO displayed certain homogeneity in five consecutive years of assessment. The five Mc-VOOs were rich in phenolic compounds and unsaturated fatty acids such as oleic acid. The quality of VOO was mainly determined by the genetic characteristics of olive cultivar, meanwhile, fruit maturity, soil and climate factors also affected its quality. The content of phenolic compound, DPPH· scavenging rate, proportion of unsaturated fatty acids and iodine value of Coratina were the highest, on the contrary, Ezhi-8 was the lowest in general. The results of PCA showed that the five leading principal components to evaluate the quality of Mc-VOOs were oleic acid, linoleic acid, acid value, total phenol and trace components (such as C20:1 and squalene) successively. In conclusion, the five Mc-VOOs from Longnan show excellent quality and have certain uniformity in different production years.

Key words: quality assessment, mono-cultivar, virgin olive oil, principal component analysis, Longnan

1 Introduction

As an important woody oil crop, olive is widely planted in the Mediterranean. Olive oil is a high quality edible vegetable oil rich in unsaturated fatty acids, antioxidant polyphenols, tocopherol and other bioactive substances, such as hydroxytyrosol and oleuropein\(^1\). With the enlarging consumption of olive oil globally, many countries far away from the Mediterranean begun to introduce and cultivate olive\(^2\).

The history of olive introduction and cultivation in China is more than 60 years. After long-term trials, agronomists generally agreed that the hot and dry valley in southwest China is more suitable for olive cultivation, especially in Sichuan (Guangyuan), Yunnan (Lijiang), Chongqing (Fengjie), and Gansu (Longnan) provinces\(^3\). Due to superior geographical location, suitable climate and environmental conditions, and the strong support of the local government, Longnan city of Gansu province takes the lead in developing olive cultivation and processing industry, and has become the largest olive base in China. However, compared with the origin of Mediterranean, the development level of olive industry in Longnan city is still relatively low.

Abbreviations: Mc-VOOs, mono-cultivar virgin olive oils; VOO, virgin olive oil; PCA, principal component analysis; PC, principal component; DPPH·, 1,1-diphenyl-2-picrylhydrazyl radical; AV, acid value; PV, peroxide value; IV, iodine value; TPC, total polyphenols content; FFA, free fatty acid; FACs, fatty acid compositions; RSA, radical scavenging activity; IOOC, International Olive Oil Council; SSFA, saturated fatty acids; SUFA, the sum of unsaturated fatty acids; SMUFA, the sum of monounsaturated fatty acids; SPUFA, the sum of polyunsaturated fatty acids; SSFA, the sum of saturated fatty acids.

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backward. More urgently, there is a lack of in-depth investigation on the quality of olive oil produced in Longnan as it is the world’s emerging olive oil production area.

Because of the difference from the cultivation environment of original place, the research on the quality of virgin olive oil (VOO) produced in the area beyond origin has been widely concerned and studied. The genotype of olive cultivar as well as the growing environment and climate have very important impacts on the quality of VOO5. Kosma et al.5 evaluated the quality of VOO extracted from five less well-known Greek olive cultivars and found that the VOO quality differed significantly in cultivars. The quality of seven monovarietal VOO from five different regions of Chile was appraised by the determination of phenolic compounds and volatiles, and the results suggested that the amount of some of characteristic compounds in VOO was related to the location of olive orchards6. The characterisation of VOO from the main Italian varieties (Frantoio and Leccino) showed that there were significant differences between the oils extracted from both cultivars when planted in the environments at different altitudes7. Kharazi et al.8 described the physicochemical properties of Iranian VOO (Zard, Mari and Phishomi) cultivated in Roodbar, Gilan, and they found that most of the parameters showed statistically significant differences. Maier et al.9 reported that the growing region, cultivar and harvest time influence on the quality of olive oil, especially the growing region of some varieties could affect the fatty acid composition.

Xiang et al.10 detected the quality, chemical composition and antioxidant activity of VOO extracted from cultivars of Barnea, Coratina, Koreniki, and Manzanilla in Liangshan (Sichuan province in China). They found that the tested parameters were different from the variety of VOO and the quality of Coratina and Manzanilla was relatively excellent in terms of fatty acid composition and peroxide value.

Considering the changes of climate and environmental factors after the introduction and planting of olive in China, we are very concerned about the quality of VOO produced in Longnan where far from the origin of olives. Therefore, in this work, the quality of five mono-cultivar extra virgin olive oils (Mc-VOOs, Frantoio, Leccino, Picholine, Coratina, and Ezhi-8) produced in Longnan from 2013 to 2017 was detected and evaluated. We hope that this effort will contribute to understanding the quality of VOO produced in Longnan.

2 Materials and Methods

2.1 Chemicals and materials

Methanol, n-hexane, isooctane, potassium hydroxide (KOH), sodium carbonate (Na₂CO₃), ascorbic acid (Vc) and other chemicals were purchased from local companies in China. Folin-Giocalteu’s phenol (biological reagent) and gallic acid (≥ 98%) were purchased from Shanghai Yuanye Biotechnology Co., LTD. 1,1-diphenyl-2-picyrylhydrazyl radical (DPPH·) was purchased from Sigma-Aldrich.

The five Mc-VOOs (Frantoio, Leccino, Picholine, Coratina and Ezhi-8) samples were provided by Longnan Garden City Olive Technology Development Co., LTD. Among the five Mc-VOOs, Frantoio, Leccino and Coratina were originated and introduced from Italy; Picholine was introduced from France; Ezhi-8 was bred by the Hubei Institute of Botany (China). The olive fruits used for VOO extraction were collected from Zazipo orchard (Altitude: 1200-1300 m, Northern latitude: 33°26' 47", East longitude: 104°46' 38", sunny side sloping field) in Liangshui Town (Wudu district, Longnan city, Gansu province, China), and the average age of the olive trees was 14 to 15 years. The olive orchard was irrigated by drip irrigation. The maturity index (MI) at harvest time ranged from 5 to 6. The VOO extraction process was performed in an industrial scale within 24 h after fruit samples were harvested. About 750 kg of olive fruits were sorted, cleaned, milled and mixed 45 min at 25-27°C. After that, the obtained paste was centrifuged at 5400 rpm using a three-phase separator (Alfa Laval, Sweden). VOO samples were prepared after the crude oil was precipitated and vacuum filtered with double filter paper and stored at 4°C. The analyses of VOO samples collected from three production batches annually were carried out within 2 months.

2.2 Evaluation of organoleptic quality of Mc-VOOs

The absorbance of Mc-VOOs was measured at the wavelength of 410 nm and 600 nm with a UV-spectrophotometer (UV 1800, Shanghai). Three parallel tests were performed to compare and analyse the colour and turbidity of Mc-VOOs. According to the method recommended by the China National Standard (GB 23347-2009, Olive oil and olive-pomace oil), the trained volunteers were invited to evaluate the organoleptic quality of Mc-VOOs.

2.3 Determination of physico-chemical properties of Mc-VOOs

Acid value (AV) and peroxide value (PV) of Mc-VOOs samples were determined according to the national standards of China, GB/T 5530-2005 and GB/T 5538-2005, respectively.

2.4 Extraction and determination of total polyphenols content from Mc-VOOs and antioxidant activity assay in vitro

The total polyphenols content (TPC) in Mc-VOOs was extracted through the modified method from Tasioula-margari and Okogeri. About 5.0 g of Mc-VOOs was added in a centrifuge tube; 5.0 mL n-hexane and 5.0 mL 80% (v/v) methanol water solution were added to extract TPC. After 2 min of violent oscillation, centrifugation was conducted
at 3000 r/min for 5 min, and the methanol phase was collected. Then, the extraction was repeated twice. The obtained methanol extract sample (MES) was incorporated and for further assay.

In the determination of TPC, 2.5 mL of Folin-Ciocalteu’s phenol and 2 mL of sodium carbonate (7.5%) were added into 0.5 mL of MES, then 5 mL of distilled water was added and shake well. The absorbance at 760 nm was detected into 0.5 mL of MES, then 5 mL of distilled water was added and for further assay.

The radical scavenging activity (RSA) was evaluated according to the method reported by Brand-Williams\(^\textsuperscript{23}\). The scavenging rate of DPPH\(^\cdot\) was monitored at 517 nm and room temperature for 30 min in darkness.

The RSA toward DPPH was expressed as DPPH\(^\cdot\) scavenging rate (\%) = \((1 - (A_s - A_b)/A_c) \times 100\)%, where \(A_s\) is the absorbance of 2.9 mL DPPH + 100 \(\mu\)L MES, \(A_b\) is the absorbance of 2.9 mL methanol + 100 \(\mu\)L MES, and \(A_c\) is the absorbance of 2.9 mL DPPH + 100 \(\mu\)L 80% (v/v) methanol. The equivalent concentration of MES was expressed as the concentration of \(V_c\); and the regression equation is 

\[
Y = 0.1552X - 1.0775 \quad (R^2 = 0.9965),
\]

where \(Y\) is \(V_c\) (\(\mu\)g) and \(X\) is DPPH\(^\cdot\) scavenging rate (\%).

2.5 Analysis of fatty acid compositions (FACs)

The FACs and their relative percentages of Mc-VOOs samples were analysed by GC-MS (Agilent 7890B-5977A) after methyl esterification by the methods of KOH-methanol according to \(\textit{GB/\text{T} 17376-2008}\). The detailed analysis method and conditions of GC-MS was the same as our previous work\(^\textsuperscript{24}\).

The calculation of different fatty acid ratios and theoretical iodine values (IV) was referred to Maestri\(^\textsuperscript{15}\). Iodine value (IV, g/100 g) = palmitoleic acid (\%) \times 1.001 + oleic acid (\%) \times 0.899 + linoleic acid (\%) \times 1.814 + linolenic acid (\%) \times 2.737.

2.6 Data statistics and analysis

All tests were performed in triplicate. SPSS Statistics 17.0 was applied for significant difference, correlation analysis, and principal component analysis (PCA). PCA was performed by the dimensionality reduction technology. Values are expressed as Mean ± SD (\(n = 3\)). Different lowercase letters in the same line are significantly different at \(p < 0.05\) (Tukey HSD) among the five cultivars in the same year; capital letters in the same column are significantly different at \(p < 0.05\) among the five years in the same cultivar.

3 Results and Discussion

3.1 Organoleptic quality of the five Mc-VOOs

The colour and transparence of the five Mc-VOOs from 2013 to 2017 are shown in Table 1. The colour of the five Mc-VOOs samples was evaluated by measuring the absorbance at the wavelength of 410 nm (\(A_{410}\)), and the turbidity and transparence of the samples were expressed as the absorbance at 600 nm (\(A_{600}\)). The colour values of \(A_{410}\) ranged from 0.69 to 2.08, while the values of \(A_{600}\) ranged from 0.02 to 0.46, and all values varied with olive cultivar and production year (\(p < 0.05\)). The actual appearance of the five Mc-VOOs reflected that the filtered VOO were all clear, but the colour had remarkable difference due to variety; specifically, the colour of Coratina was the darkest and greenest, followed by Picholine, and the other three varieties (Frantoio, Leccino and Ezhi-8) were yellow-green in colour. As olive cultivar, origin, maturity stage of fruit, storage

| Items       | Year | Frantoio | Leccino | Picholine | Coratina | Ezhi-8 |
|-------------|------|----------|---------|-----------|----------|--------|
| Colour \(A_{410}\) | 2013 | 1.8 ± 0.01aB | 1.5 ± 0.05bB | 1.5 ± 0.06bcC | 1.6 ± 0.06bB | 1.1 ± 0.03cD |
|            | 2014 | 1.6 ± 0aD  | 0.9 ± 0.01dD | 1.4 ± 0bC  | 1.6 ± 0aB  | 1.2 ± 0.04cC |
|            | 2015 | 1.5 ± 0.01bE | 1.4 ± 0cC  | 1.5 ± 0.01aB | 1.5 ± 0aB  | 1.5 ± 0bB  |
|            | 2016 | 2.1 ± 0aA  | 1.7 ± 0.02cA | 1.4 ± 0.01dC | 1.7 ± 0cA  | 1.8 ± 0.03bB |
|            | 2017 | 1.7 ± 0cC  | 0.7 ± 0.01eE | 1.8 ± 0aA  | 1.7 ± 0.01bA | 0.7 ± 0dE  |
| Transparence \(A_{600}\) | 2013 | 0.16 ± 0.04AB | 0.13 ± 0.03bcA | 0.17 ± 0.01bA | 0.46 ± 0.03aA | 0.06 ± 0.01bcC |
|            | 2014 | 0.12 ± 0.01bB | 0.07 ± 0.01bcB | 0.11 ± 0.01bB | 0.18 ± 0.03aB | 0.09 ± 0.01cB |
|            | 2015 | 0.19 ± 0.01aA | 0.06 ± 0bC  | 0.15 ± 0aB  | 0.16 ± 0bB  | 0.16 ± 0bA  |
|            | 2016 | 0.12 ± 0aB  | 0.08 ± 0.02bB | 0.03 ± 0.01cC | 0.02 ± 0cC  | 0.03 ± 0.01dC |
|            | 2017 | 0.04 ± 0aC  | 0.02 ± 0.01bC | 0.03 ± 0.01abC | 0.02 ± 0bc  | 0.04 ± 0aD  |

Note: Different lowercase letters in the same line are significantly different at \(p < 0.05\) (Tukey HSD) among the five cultivars in the same year; capital letters in the same column are significantly different at \(p < 0.05\) among the five years in the same cultivar.

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conditions of fruit, and olive fruit processing all influence the exterior and flavour of olive oil, thus its organoleptic quality includes colour, taste and aroma\(^{[46]}\). Our results also suggested that the olive cultivar has a significant influence on the sensory quality of VOO.

The special flavour of olive oil is due to the evolution and development of volatile compounds in oil extracted from olive fruit\(^{[1]}\). A specific vocabulary also has been developed for virgin oil sensory descriptors. The positive attributes of virgin olive oil are explained as fruity, bitter and pungent for virgin oil sensory descriptors. The positive attributes of characteristics of virgin oil; however, the taste varies significantly according to different varieties. Coratina had a pungent and bitter aftertaste, followed by Picholine and Frantoio; Leccino and Ezhi-8 had a relatively light taste. The results of comparative analysis on the five VOO samples in five consecutive years showed that there were differences in data level, yet such differences were relatively small in visual sensory and flavour evaluation, which indicates that the VOO of the same variety shows certain homogeneity in organoleptic properties in different production years. Those quality differences in vision, aroma and taste are largely determined by the olive variety, as well as geographical and climatic conditions, the harvesting time, fruit storage condition and processing techniques in the production year of VOO\(^{[16, 17]}\).

Wudu district of Longnan city, where the olive orchard is located, is in the middle reaches of Bailongjiang river basin. Wudu district is a transition zone from the semi-humid climate of the north subtropics to the semi-arid climate of the warm temperate zone. The annual average temperature in Wudu district is about 14.7°C, the annual sunshine hours are 1782 h, and the annual precipitation is around 467 mm\(^{[16]}\). It mainly belongs to the semi-humid climate of the north subtropics, which is different from the Mediterranean basin, where summers are hot and dry and winters are mild and rainy. The cultivation of the same variety of olive in different geographical and climatic conditions will affect the quality of VOO. Our results indicated that olive cultivar played a decisive role in the sensory quality of VOO in the same orchard, while the climate, agronomic management, fruit maturity and processing in different production years also may affect the quality of VOO.

### 3.2 Physicochemical properties of the Mc-VOOs

The International Olive Oil Council (IOOC) has defined the quality of olive oil based on parameters like free fatty acid (FFA) content, peroxide value (PV) and sensory score (taste and aroma). In particular, the quantity of FFA is an important factor for classifying olive oil into commercial grades\(^{[18]}\). As shown in Table 2, the PV and acid value (AV) of the five Mc-VOOs varied with varieties and production years (\(p<0.05\)). During the continuous five years of testing, the PV of the five Mc-VOOs ranged from 2.39 mmol/kg to 9.65 mmol/kg, and the AV ranged from 0.07 mg/g to 0.82 mmol/kg. All the PV and AV meet the requirements of IOOC (2013) and China National Standards (GB 23347-2009) on the quality standards of extra VOO in general. Moreover, there were significant differences in PV and AV among different VOO varieties produced in the same year, as well as the same VOO produced in different years. For instance, during the five-year period, the PV and AV of Frantoio were 4.64-9.05 mmol/kg and 0.26-0.78 mg/g, respectively; the PV and AV of Ezhi-8 ranged within 2.39-8.17 mmol/kg and 0.09-0.34 mg/g, respectively. The results also indicated that the cultivar, production year, maturity degree and other factors of olive fruit would lead to the differences in VOO physicochemical parameters (PV and AV). Although the differences were significant at the sta-

![Table 2](https://example.com/table2)

| Items | Year | Frantoio | Leccino | Picholine | Coratina | Ezhi-8 |
|-------|------|----------|---------|-----------|----------|--------|
| PV (mmol/kg) | | | | | | |
| 2013 | 5.6 ± 0.3bBC | 8.0 ± 0.2aB | 6.3 ± 0.81bAB | 5.6 ± 0.1bA | 6.1 ± 0.2bB |
| 2014 | 9.1 ± 0.6aA | 9.7 ± 0.5aA | 4.8 ± 0.8cC | 5.5 ± 0.3cA | 7.6 ± 0.3bB |
| 2015 | 4.6 ± 0.6bcC | 6.9 ± 0.4aC | 5.5 ± 0.4bB | 4.1 ± 0.1cB | 2.4 ± 0.3dC |
| 2016 | 6.6 ± 0.2bB | 6.5 ± 0.2bC | 6.7 ± 0.5bAB | 5.4 ± 0.3cA | 8.0 ± 0.4aA |
| 2017 | 6.3 ± 0.4bcB | 6.0 ± 0.4bcC | 7.6 ± 0.5aA | 5.6 ± 0.3cA | 8.2 ± 0.5aA |
| AV (mg/g) | | | | | | |
| 2013 | 0.78 ± 0.09bA | 0.82 ± 0.02aA | 0.50 ± 0.02cA | 0.52 ± 0.02cA | 0.24 ± 0.02dAB |
| 2014 | 0.48 ± 0.07aB | 0.30 ± 0.06bB | 0.24 ± 0.09cB | 0.21 ± 0.03bB | 0.28 ± 0.06bA |
| 2015 | 0.45 ± 0.01aB | 0.43 ± 0.03aB | 0.37 ± 0.07bB | 0.45 ± 0.01aA | 0.34 ± 0.01bA |
| 2016 | 0.28 ± 0.06aC | 0.13 ± 0.03bcC | 0.07 ± 0.03cD | 0.24 ± 0.06abB | 0.09 ± 0.03cC |
| 2017 | 0.26 ± 0.03aC | 0.13 ± 0.03bC | 0.11 ± 0.06bD | 0.19 ± 0.06abB | 0.21 ± 0.03abB |

Note: Different lowercase letters in the same line are significantly different at \(p < 0.05\) (Tukey HSD) among the five cultivars in the same year; capital letters in the same column are significantly different at \(p < 0.05\) among the five years in the same cultivar.
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3.3 Total polyphenols content and antioxidant activity of the Mc-VOOs

Polyphenols derived from olive fruit are one of the most important characteristic components in the evaluation and identification of VOO quality, which directly affect the flavour of VOO. As shown in Fig. 1, the TPC of the five Mc-VOOs varied with olive cultivars and production years from 2013 to 2017. The results indicated that the TPC in the tested oil samples ranged from 26.7 μg/g (Ezhi-8-2013) to 436.8 μg/g (Coratina-2015). Moreover, there were significant differences among different years and olive varieties (p < 0.05), indicating that the two factors had a significant effect on the TPC of Mc-VOOs.

The results also showed that olive variety played a leading role in determining phenolic compounds to some extent. For instance, Coratina was the highest in TPC (except 2017), but Ezhi-8 was the lowest (except 2016). The five-year average TPCs (μg/g) of Frantoio, Leccino, Picholine, Coratina and Ezhi-8 was 185.6, 110.9, 184.5, 228.4 and 102.6, respectively, which indicated the dominance of olive variety in determining the content of bioactive components like polyphenols.

The DPPH model has been used to evaluate the antioxidant activity of other monocultures of VOO which showed certain uniformity in different years. Researches have confirmed that Coratina was noteworthy for its high content of phenolic compounds and excellent quality of the Mc-VOOs produced in Longnan and were related to the ripeness of olive fruit, storage time and oxidation degree of VOO.

The five-year average TPCs of Frantoio, Leccino, Picholine, Coratina and Ezhi-8 were 57.2 ± 11.1, 72 ± 17.7, 73 ± 15.5, 115 ± 8.1 and 52 ± 4.5 μg/g, respectively, which also implied that the quality of Mc-VOOs from the same producing area and variety showed certain uniformity in different years.

AV and PV are the most commonly used parameters to evaluate the quality of edible oil. Xiang et al. reported that the free acidities of four olive oils introduced to and cultivated at Liangshan in Sichuan ranged from 0.14 to 0.28% and the PV ranged from 0.98 to 2.46 meq O2/kg. Both the free acidity and PV did not exceed the upper limit prescribed in the EVOO. The relatively low AV and PV showed the excellent quality of the Mc-VOOs.

The DPPH model has been used to evaluate the antioxidant activity of other monocultures of VOO. Previous studies have shown that the TPC determined by the Folin reagent was better correlated with antioxidant activity than each tested polyphenol or polyphenols groups such as o-diphenols, which is similar to our results.

The main antioxidant phenolic compounds in VOO

| Item                  | Year | Frantoio | Leccino | Picholine | Coratina | Ezhi-8 |
|-----------------------|------|----------|---------|-----------|----------|--------|
| DPPH scavenging capacity (Vc μg/g olive oil) | 2013 | 57 ± 11B | 72 ± 17B | 73 ± 15B | 115 ± 8A | 52 ± 4BC |
|                       | 2014 | 69 ± 8B  | 72 ± 17B | 72 ± 13B | 121 ± 7A | 54 ± 7B  |
|                       | 2015 | 103 ± 5aA| 92 ± 8A  | 88 ± 17B | 121 ± 11A| 84 ± 8A  |
|                       | 2016 | 52 ± 12cB| 68 ± 12B | 79 ± 5B  | 112 ± 8A | 62 ± 5bcB|
|                       | 2017 | 63 ± 7B  | 43 ± 4cC | 90 ± 5A  | 106 ± 10A| 35 ± 8cC |

Note: Different lowercase letters in the same line are significantly different at p < 0.05 (Tukey HSD) among the five cultivars in the same year; capital letters in the same column are significantly different at p < 0.05 among the five years in the same cultivar.
mainly include phenolic acids, hydroxytyrosol and oleuropein. Xiang et al. found that the total polyphenol contents of the four olive oils produced in Lianshan ranged from 55.4 mg/kg to 180.2 mg/kg, and the main compounds were hydroxy tyrosol, p-hydroxybenzoic acid, rutin, and quercetin, which exhibited excellent anti-oxidation properties. The concentration of healthy phenols in VOOS is directly related to the cultivar of olive oil and the geographical conditions. In addition, the harvesting system and extraction process (as well as olive fruit ripeness and olive maturity) can affect the fatty acid composition variations carried out at atmospheric pressure. The five VOOs produced in Longnan are rich in phenolic compounds, which also show their good nutritional and health potential. The phenolic profiles and functions of these VOOS are being further studied.

3.4 Fatty acids compositions and squalene of the Mc-VOOs

The main fatty acids composing olive oil are triacylglycerols (triglycerides or fats) and small quantities of free fatty acids (FFA). Oleic acid (C18:1), a monounsaturated omega-9 fatty acid, accounts for 55 to 83% of olive oil. Palmitic acid (C16:0), a saturated fatty acid makes up 7.5 to 20% of olive oil. Linoleic acid (C18:2), a polyunsaturated omega-6 fatty acid, accounts for about 3.5 to 21% of olive oil. The fatty acid compositions of the Mc-VOOs from 2013 to 2017 are presented in Table 4. The main fatty acids in the five Mc-VOOs were oleic acid (C18:1, 72.5 - 83.8%), palmitic acid (C16:0, 9.3 - 16.3%), linoleic acid (C18:2, 2.73 - 6.55%), stearic acid (C18:0, 1.28 - 3.56%), palmitoleic acid (C16:1, 0.25 - 2.30%), eicosanoic acid (C20:0, 0.21 - 0.37%) and eicosenoic acid (C20:1, 0.16 - 0.35%) in order of content. The ratios of SMUFA/SPUFA and O/L can reflect the oxidative stability of VOOS. The results showed that the main fatty acid types and concentrations of different Mc-VOOs had high similarities. Moreover, the cultivars with higher SFAs were lower in SSFA, while the cultivars with lower SSFA were lower in oxidized value (Ezhi-8). The above parameters related to the proportion of fatty acids had some significant differences in different cultivars, but the same cultivar did not have significant differences among different years (p < 0.05). The results showed that the five Mc-VOOs from 2013 to 2017 are presented in Table 5. The analysis parameters mainly include the sum of saturated fatty acids (SSFA), the sum of unsaturated fatty acids (SUFAs), the sum of monounsaturated fatty acids (SMUFAs), and the sum of polyunsaturated fatty acids (SPUFAs), and the ratio of saturated fatty acids (SSFA) to total fatty acids (SSFA/TFA), respectively. The SFAs of Ezhi-8 were the highest while that of Coratina was the lowest, the SUFAs of Ezhi-8 were the lowest and that of Coratina was the highest in view of cultivar. Correspondingly, the cultivars with higher SFA had higher IV (Coratina), while the cultivars with lower SFA were lower in iodine values (Ezhi-8). The ratios of SFAs, SUFAs, SMUFAs, and SPUFAs showed certain homogeneity in the five consecutive years of evaluation.

The ratios of SMUFAs/SPUFAs and O/L can reflect the oxidation and storage stability of olive oils; the higher the ratios, the better the stability of oils. The results showed that the SMUFAs/SPUFAs and C18:1/C18:2 varied from 11.7 to 32.1% and from 11.3 to 31.3%, respectively. The O/L
Table 4  Fatty acid compositions of the five Mc-VOOs from 2013 to 2017.

| Fatty acid | Year  | Frantoio  | Leccino   | Picholine | Coratina | Ezhi-8  |
|------------|-------|-----------|-----------|-----------|----------|---------|
|            | 2013  | 1.3 ± 0.19abA | 0.8 ± 0.34bcA | 0.92 ± 0.23bcA | 0.35 ± 0.05cAB | 1.8 ± 0.43aA |
|            | 2014  | 1.1 ± 0.37abAB | 1.2 ± 0.73abA  | 0.77 ± 0.30abA  | 0.25 ± 0.10bB | 1.7 ± 0.35aA  |
| C16:1      | 2015  | 0.75 ± 0.11bAB | 1.0 ± 0.35bA  | 0.84 ± 0.51bA  | 0.45 ± 0.26AB | 2.3 ± 0.81aA  |
|            | 2016  | 0.64 ± 0.16bB  | 0.91 ± 0.22bA  | 0.58 ± 0.12bA  | 0.43 ± 0.06AB | 2.2 ± 0.41aA  |
|            | 2017  | 0.87 ± 0.01bAB | 0.88 ± 0.03bA  | 0.68 ± 0.02cA  | 0.61 ± 0.04AB | 1.1 ± 0.03aA  |
|            | 2013  | 12.6 ± 1.2abA | 14.0 ± 1.8abA | 11.2 ± 0.6bAB | 10.7 ± 2.6bA | 15.4 ± 0.7aAB |
|            | 2014  | 13.9 ± 0.6aA  | 12.3 ± 0.8aA  | 10.7 ± 1.1bAB | 9.3 ± 1.1cA | 14.3 ± 0.3aAB |
| C16:0      | 2015  | 13.8 ± 1.0abA | 14.7 ± 1.1abA | 12.6 ± 1.0abA | 10.7 ± 3.2bA | 16.1 ± 1.2aA  |
|            | 2016  | 12.1 ± 1.0bcA | 13.2 ± 1.0bA  | 10.1 ± 0.9cB  | 10.2 ± 0.8cA | 16.3 ± 1.1aA  |
|            | 2017  | 12.5 ± 0.1cA  | 13.1 ± 0.2bA | 12.9 ± 0.1bA  | 11.9 ± 0.1cA | 13.6 ± 0.1aB  |
|            | 2013  | 4.8 ± 0.6aA   | 4.1 ± 0.7aA  | 3.6 ± 1.2aA  | 3.7 ± 0.3aB | 2.7 ± 0.9aC  |
|            | 2014  | 4.8 ± 0.7aA   | 4.9 ± 0.7aA  | 4.2 ± 1.5aA  | 3.2 ± 1.2aA | 3.9 ± 1.4aBC |
| C18:2      | 2015  | 6.6 ± 1.3aA   | 5.2 ± 1.0aA  | 3.2 ± 1.1aA  | 5.7 ± 0.3aB | 3.8 ± 0.1BC  |
|            | 2016  | 6.1 ± 0.4abA  | 5.3 ± 0.5aA  | 3.1 ± 0.2aA  | 5.8 ± 0.4abA | 6.4 ± 0.3aA  |
|            | 2017  | 5.2 ± 0.5aA   | 5.0 ± 0.5aA  | 3.7 ± 0.1aA  | 4.9 ± 0.6abA | 5.5 ± 0.5aAB |
|            | 2013  | 78.6 ± 0.9abA | 79.2 ± 1.2abA | 80.8 ± 0.8abA | 82.7 ± 3.0aA | 78.1 ± 0.3bA  |
|            | 2014  | 78.0 ± 0.9bA  | 78.5 ± 2.7bA | 80.3 ± 1.1abA | 83.8 ± 1.7aA | 77.1 ± 1.4bA  |
| C18:1      | 2015  | 75.7 ± 1.3aA | 76.0 ± 0.8aA | 79.1 ± 0.6bA | 79.6 ± 3.1aA | 75.46 ± 2.4aAB |
|            | 2016  | 78.0 ± 1.7abA | 77.5 ± 1.9abA | 82.1 ± 1.3aA | 80.3 ± 0.8aA | 72.5 ± 2.0cB  |
|            | 2017  | 78.3 ± 0.6aA  | 78.1 ± 0.7abA | 79.1 ± 0.2aA | 79.3 ± 0.6aA | 76.7 ± 0.6bAB |
|            | 2013  | 1.9 ± 0.5bA   | 1.3 ± 0.2cB  | 2.8 ± 0.1aA  | 1.6 ± 0.1bcB | 1.4 ± 0.1bcBC |
|            | 2014  | 2.4 ± 0.5aA   | 2.4 ± 0.3aA  | 3.2 ± 0.4aA  | 2.6 ± 0.4aA | 2.2 ± 0.5aA  |
| C18:0      | 2015  | 2.6 ± 0.2abA  | 2.4 ± 0.21abA | 3.6 ± 0.9aA  | 2.5 ± 0.5abA | 1.6 ± 0.2bBC |
|            | 2016  | 2.4 ± 0.1bA   | 2.5 ± 0.1bA  | 3.4 ± 0.2aA  | 2.3 ± 0.1bAB | 1.8 ± 0.1cAB |
|            | 2017  | 2.3 ± 0.1bA   | 2.2 ± 0.1cA  | 2.6 ± 0.1aA  | 2.3 ± 0.1bcAB | 2.3 ± 0.1bcA  |
|            | 2013  | 0.35 ± 0.11aA | 0.26 ± 0.07aA | 0.22 ± 0.10aA | 0.25 ± 0.02aA | 0.22 ± 0.04aA |
|            | 2014  | 0.35 ± 0.05aA | 0.24 ± 0.12aA | 0.26 ± 0.05aA | 0.30 ± 0.03aA | 0.26 ± 0.10aA |
| C20:1      | 2015  | 0.23 ± 0.10aA | 0.24 ± 0.09aA | 0.18 ± 0.04aA | 0.35 ± 0.08aA | 0.29 ± 0.06aA |
|            | 2016  | 0.20 ± 0.09aA | 0.16 ± 0.07aA | 0.20 ± 0.08aA | 0.33 ± 0.09aA | 0.24 ± 0.06aA |
|            | 2017  | 0.31 ± 0.04abcA | 0.27 ± 0.02cA | 0.33 ± 0.01abA | 0.34 ± 0.02aA | 0.28 ± 0.01bcA |
|            | 2013  | 0.21 ± 0.02aA | 0.24 ± 0.12aA | 0.31 ± 0.13aA | 0.37 ± 0.11aA | 0.23 ± 0.01aA |
|            | 2014  | 0.30 ± 0.15aA | 0.26 ± 0.15aA | 0.30 ± 0.05aA | 0.30 ± 0.03aA | 0.26 ± 0.07aA |
| C20:0      | 2015  | 0.34 ± 0.12aA | 0.28 ± 0.06aA | 0.30 ± 0.08aA | 0.32 ± 0.05aA | 0.29 ± 0.08aA |
|            | 2016  | 0.29 ± 0.06aA | 0.27 ± 0.07aA | 0.32 ± 0.18aA | 0.37 ± 0.11aA | 0.28 ± 0.07aA |
|            | 2017  | 0.34 ± 0.01aA | 0.28 ± 0.02bA | 0.36 ± 0.01aA | 0.35 ± 0.02aA | 0.30 ± 0.01bA |

Note: Different lowercase letters in the same line are significantly different at p < 0.05 (Tukey HSD) among the five cultivars in the same year; capital letters in the same column are significantly different at p < 0.05 among the five years in the same cultivar.
| Items | Year | Frantoio | Leccino | Picholine | Coratina | Ezhi-8 |
|-------|------|----------|---------|-----------|----------|--------|
| SSFA/\% | 2013 | 15.5 ± 1.6aA | 14.2 ± 0.6abBC | 12.7 ± 2.8bA | 17.0 ± 0.7aA |
|       | 2014 | 14.9 ± 1.2abA | 14.2 ± 1.0bBC | 12.2 ± 0.7cA | 16.8 ± 0.6aA |
|       | 2015 | 17.4 ± 1.2aA | 16.4 ± 0.5aA | 13.6 ± 3.0aA | 17.9 ± 1.5aA |
|       | 2016 | 16.0 ± 1.2abA | 13.8 ± 1.2bcC | 12.9 ± 0.9cA | 18.3 ± 1.3aA |
|       | 2017 | 15.6 ± 0.2bA | 15.9 ± 0.1abAB | 14.6 ± 0.1dA | 16.2 ± 0.2aA |
|       | 2013 | 84.3 ± 1.6abA | 85.6 ± 0.5abA | 87.0 ± 2.7aA | 82.9 ± 0.7bA |
|       | 2014 | 84.9 ± 1.3abA | 85.6 ± 1.1abA | 87.6 ± 0.7aA | 82.9 ± 0.7bA |
|       | 2015 | 82.4 ± 1.2aA | 83.3 ± 0.5abB | 86.2 ± 3.0aA | 81.8 ± 1.5aA |
|       | 2016 | 83.8 ± 1.2abA | 85.9 ± 1.20aA | 86.9 ± 0.9aA | 81.4 ± 1.3bA |
|       | 2017 | 84.2 ± 0.2aA | 83.9 ± 0.2cdAB | 85.1 ± 0.1aA | 83.5 ± 0.2dA |
| SMUFA/\% | 2013 | 80.3 ± 1.5aA | 81.9 ± 0.9aAB | 83.3 ± 3.0aA | 80.1 ± 0.7aA |
|       | 2014 | 79.9 ± 2.0aB | 81.4 ± 1.3abAB | 84.4 ± 1.7aA | 79.0 ± 1.3bA |
|       | 2015 | 77.2 ± 0.6aA | 80.1 ± 0.7aB | 80.4 ± 2.8aA | 77.9 ± 1.5aAB |
|       | 2016 | 78.6 ± 1.7bca | 82.9 ± 1.1aA | 81.1 ± 0.7abA | 75.0 ± 1.6cB |
|       | 2017 | 79.2 ± 0.6abA | 80.1 ± 0.2aB | 80.3 ± 0.6aA | 78.1 ± 0.5bAB |
| SPUFA/\% | 2013 | 4.1 ± 0.7aA | 3.6 ± 1.2aA | 3.7 ± 0.3aB | 2.7 ± 0.9aC |
|       | 2014 | 4.9 ± 0.7aA | 4.2 ± 1.5aA | 3.2 ± 1.2aB | 3.9 ± 1.4aBC |
|       | 2015 | 5.2 ± 1.0abA | 3.2 ± 1.1cA | 5.7 ± 0.3abA | 3.8 ± 0.1bBC |
|       | 2016 | 5.3 ± 0.5bA | 3.1 ± 0.2cA | 5.8 ± 0.4aB | 6.4 ± 0.3aA |
|       | 2017 | 5.0 ± 0.5aA | 3.7 ± 0.1bA | 4.9 ± 0.6abAB | 5.5 ± 0.5aB |
| SMUFA/ | 2013 | 20.1 ± 3.3aA | 24.1 ± 7.1aA | 22.4 ± 2.5aAB | 32.1 ± 12.6aA |
| SPUFA | 2014 | 16.4 ± 2.6aA | 20.9 ± 7.2aA | 28.8 ± 10.7aA | 22.3 ± 9.1aAB |
|       | 2015 | 15.1 ± 2.7abA | 26.9 ± 9.4aA | 14.0 ± 0.5bB | 20.4 ± 0.7aAB |
|       | 2016 | 15.0 ± 1.6bA | 27.3 ± 1.9aA | 14.1 ± 1.0bB | 11.7 ± 0.8bB |
|       | 2017 | 16.0 ± 1.8bA | 21.5 ± 0.4aA | 16.7 ± 1.9bAB | 14.4 ± 1.4AB |
| C18:1/C18:2 | 2013 | 19.8 ± 3.3aA | 23.7 ± 7.0aA | 22.3 ± 2.5aAB | 31.3 ± 12.1aA |
|       | 2014 | 16.1 ± 2.7aA | 20.6 ± 7.0aA | 28.6 ± 10.6aA | 21.8 ± 9.0aAB |
|       | 2015 | 14.8 ± 2.6abA | 26.5 ± 9.3aA | 13.9 ± 0.5bB | 19.7 ± 0.9aAB |
|       | 2016 | 14.8 ± 1.6bA | 27.0 ± 1.8aA | 14.0 ± 1.0bB | 11.3 ± 0.8bB |
|       | 2017 | 15.8 ± 1.8bA | 21.2 ± 0.4aA | 16.5 ± 1.9bAB | 14.1 ± 1.4AB |
| IV | 2013 | 79.4 ± 1.8abA | 80.2 ± 1.4abA | 81.5 ± 2.2aA | 77.0 ± 1.3bA |
|       | 2014 | 80.7 ± 0.5aA | 80.7 ± 2.0aA | 81.5 ± 1.0aA | 78.1 ± 1.8aA |
|       | 2015 | 78.8 ± 2.0abA | 77.8 ± 1.4abA | 82.5 ± 3.0aA | 77.0 ± 1.3bA |
|       | 2016 | 80.2 ± 0.7bca | 79.9 ± 1.2bcA | 83.1 ± 1.1aA | 79.1 ± 1.0cA |
|       | 2017 | 80.7 ± 0.3aA | 78.6 ± 0.1cA | 80.7 ± 0.5aA | 80.0 ± 0.5abA |

**SSFA:** sum of saturated fatty acids, **SUFA:** sum of unsaturated fatty acids, **SMUFA:** sum of monounsaturated fatty acids, **SPUFA:** sum of polyunsaturated fatty acids, **SSFA:** sum of saturated fatty acids, IV: iodine value. Note: Different lowercase letters in the same line are significantly different at \( p < 0.05 \) (Tukey HSD) among the five cultivars in the same year; capital letters in the same column are significantly different at \( p < 0.05 \) among the five years in the same cultivar.
ratios of five-year mean values of Frantoio, Leccino, Picholine, Coratina and Ezhi-8 are 14.6%, 16.3%, 23.8%, 19.0% and 19.6%, respectively. The results showed that the higher oleic acid content and the lower linoleic acid content in VOO, the larger the O/L ratio, which also indicated that the five Mc-VOOs had better storage and oxidation stability. The differences of the five Mc-VOOs in fatty acid profiles were attributed to their genetic differences, production year and area, irrigation and water stress, as well as the extraction system.

Squalene, the major olive oil hydrocarbon, is an intermediate compound in the biosynthesis of sterols in plant, microorganism and animal world. It makes up more than 90% of the hydrocarbon fraction ranging from 200 to 12000 mg/kg oil. As shown in Fig. 2, the relative content of squalene in Mc-VOOs from 2013 to 2017 ranged from 0.12 to 0.32%, and the differences among varieties and production years were not significant. The squalene content of 28 olive cultivars from World Olive Germplasm Collection of Cordoba ranged from 1100 to 8390 mg/kg oil, and the high variability observed could be explained by the genetic component. Our results also confirm that the squalene content depends on olive cultivar and oil extraction technology.

3.5 Principal component analysis of characteristic components of the Mc-VOOs

Principal component analysis (PCA) is often used to evaluate food quality, including olive oil. To further explore the main factors that affect the quality of VOO, PCA was conducted on the all measured parameters, and the results are shown in Table 6 and Table 7. The contribution rates of the first five extracted principal components are 30.9%, 22.8%, 10.8%, 8.6%, and 7.2%, respectively, and the cumulative contribution rate is 80.3%. These components contain most of the information of olive oil and can fully reflect the overall quality of Mc-VOO, so the first five principal components can be selected for analysis.

As shown in Table 7, the principal eigenvector can reflect the contribution rate of each index to the principal component (PC). The first PC mainly includes the information of C18:1 (0.96) and MUFA (0.93), which indicates that the first PC can be divided into the proportion of oleic acid in VOO from the eigenvectors. It also indicates that oleic acid and MUFA are the main factors which determine the quality of VOO. The second PC mainly expresses the information of C18:2 (0.86), PUFA (0.86) and IV (0.76), which suggested that the second PC can be divided into the PUFA of VOO. The third PC mainly covers the information of AV (0.88) and turbidity (0.72), which implied that the third PC can be divided into the appearance and physicochemical properties of VOO. The fourth PC mainly reflects the information of TPC (0.58), which showed that the fourth PC can be divided into the phenolic bioactive ingredients of VOO. The fifth PC mainly represents the information of C20:1 (0.66) and squalene (0.50), which indicated

Table 6 Eigenvalues, contribution rates, and cumulative contribution rates of principal components.

| Principal component | Eigenvalues | Contribution rate (%) | Cumulative contribution rate (%) |
|---------------------|-------------|-----------------------|----------------------------------|
| 1                   | 6.49        | 30.9                  | 30.9                             |
| 2                   | 4.80        | 22.8                  | 53.7                             |
| 3                   | 2.26        | 10.8                  | 64.5                             |
| 4                   | 1.81        | 8.61                  | 73.1                             |
| 5                   | 1.52        | 7.25                  | 80.3                             |
| 6                   | 0.96        | 4.59                  | 84.9                             |
| 7                   | 0.75        | 3.59                  | 88.5                             |
| 8                   | 0.73        | 3.49                  | 92.0                             |
| 9                   | 0.61        | 2.90                  | 94.9                             |
| 10                  | 0.35        | 1.65                  | 96.6                             |
that the fifth PC can be divided into the minor component of VOO. The load diagram of PCA is showed in Fig. 3. The results of PCA can reflect the main factors for evaluating the comprehensive quality of VOO, and have good agreement with related norms (IOOC, 2013; GB 23347-2009). The results of PCA show that the comprehensive quality of VOO can be evaluated by determining its fatty acid composition and proportion, sensory characteristics, physicochemical properties and phenolic compound content.

### Table 7  Eigenvectors of correlation matrices of 5 leading principal components.

| Characteristics | Component 1 | Component 2 | Component 3 | Component 4 | Component 5 |
|-----------------|-------------|-------------|-------------|-------------|-------------|
| C18:1           | 0.96        | -0.13       | -0.01       | -0.24       | -0.01       |
| MUFA            | 0.93        | -0.25       | 0.01        | -0.23       | 0.06        |
| C16:0           | -0.90       | -0.28       | 0.17        | 0.22        | 0.11        |
| SFA             | -0.88       | -0.26       | -0.01       | 0.35        | -0.05       |
| C16:1           | -0.77       | -0.43       | 0.08        | 0.20        | 0.19        |
| DPPH            | 0.62        | 0.34        | 0.13        | 0.39        | 0.28        |
| C20:0           | 0.48        | 0.47        | -0.31       | 0.33        | 0.20        |
| PUFA            | -0.48       | 0.86        | 0.01        | -0.09       | -0.06       |
| C18:2           | -0.48       | 0.86        | 0.01        | -0.09       | -0.06       |
| MUFA/PUFA       | 0.51        | -0.84       | -0.02       | 0.14        | -0.01       |
| C18:1/C18:2     | 0.53        | -0.82       | -0.02       | 0.13        | -0.01       |
| IV              | 0.47        | 0.76        | 0.02        | -0.38       | -0.01       |
| Colour          | 0.19        | 0.35        | 0.21        | 0.23        | 0.23        |
| AV              | 0.07        | 0.02        | 0.88        | -0.14       | -0.01       |
| Turbidity       | 0.45        | 0.01        | 0.72        | 0.22        | -0.06       |
| Squalene        | 0.05        | -0.02       | -0.64       | 0.14        | 0.50        |
| PV              | -0.30       | -0.11       | -0.17       | -0.58       | -0.35       |
| TPC             | 0.30        | 0.50        | -0.06       | 0.58        | -0.26       |
| C20:1           | 0.14        | 0.32        | 0.07        | -0.16       | 0.66        |
| C18:0           | 0.41        | 0.14        | -0.53       | 0.24        | -0.54       |

**Fig. 3** Load diagram for principal component analysis (PCA).

In conclusion, the quality of the five leading mono-cultivar (Frantoio, Leccino, Picholine, Coratina and Ezhi-8) virgin olive oils produced in Longnan from 2013 to 2017 was evaluated; the main factors that determine the quality of virgin olive oil were identified by principal component analysis. The AV and PV of the five Mc-VOOs from Longnan were less than 1.0 mg/g and 10 mmol/kg respectively, while the relative percentage of C18:1 ranged from 72.5 to 83.8%. There were significant differences (p<0.05) in the flavours, physicochemical parameters and active ingredients (fatty acid compositions and phenolic compounds) of the five Mc-VOOs; however, the quality of the same cultivar varied slightly in different years. The five Mc-VOOs produced in Longnan were rich in polyphenols and monoun-
saturated fatty acids with antioxidant activity, and varied with varieties. The quality of VOO was determined by its variety, but planting and climatic conditions also affected its quality. The results of PCA showed that the five PCs to evaluate the quality of VOO included oleic acid proportion, PUFA, transparency and acidity, phenolic compounds content, squalene and other trace components. The five Mc-VOOs from Longnan (China) showed certain quality uniformity and homogeneity in the five consecutive years from 2013 to 2017. This work provides important data for the quality evaluation of VOO introduced, cultivated and processed in Longnan, an emerging olive oil production region far from the Mediterranean where olive originates.

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