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Changes in Quality and Antioxidant Properties of Virgin Olive Oil of '*Cornicabra*' According to Fruit Maturation in Longnan, China

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Abstract: This work aims to study the influence of olive fruit maturity on physicochemical properties and antioxidant activity which determine the quality of virgin olive oils (VOO). According to the results, the values of all parameters were within the range specified by the Codex Alimentarius (2017). With the increase of fruit maturity, the oil content continued to increase until reached the maximum value (20.05%) in the 7th maturity (M7). K_{232}, K_{270} and peroxide value (PV) decreased with the increase of maturity, while ΔK increased linearly with the increase of maturity. Free fatty acidity (FFA) first decreased and then increased, until reached the maximum value of (0.52 ± 0.03) % in M7. The total polyphenols (TP) and total flavonoids (TF) that characterized the antioxidant properties of olive oil increased with the increase of fruit maturity, which indicated that the oxidative stability (OS) of VOO of '*Cornicabra*' increased with the increase of fruit maturity. The oleic acid (C18:1) content remained above 70 % and reached the maximum of (76.68 ± 0.17) % at M7. The values of monounsaturated fatty acids (MUFA) / polyunsaturated fatty acids (PUFA) and oleic acid (C18:1) / linoleic acid (C18:2) showed a decreasing trend with the maturity stage. Principal component analysis (PCA) showed that the quality of FFA, PV, K_{232}, K_{270}, TP, TF and OS were higher at the 5th maturity (M5), the quality of fatty acid were higher at M7. It can be seen from the analysis that the olive fruit maturity was an important parameter to characterize and distinguish olive oil.

Key words: *Cornicabra*, virgin olive oil, maturity, quality, antioxidant properties

1 Introduction

The olive tree (*Olea europaea* L.) has always been one of the main crops in the Mediterranean area, such as Spain and Greece. Virgin olive oil (VOO), without refining[1], shows unique nutritional and sensory properties. Its chemical composition is very beneficial to health, such as, high content of oleic acid can reduce the risk of cardiovascular disease[2, 3]. phenolic compounds have anti-tumor[4], antioxidant activity[5], antimicrobial effects[6], hypolipidemic action[7], etc. Nowadays, VOO is being appreciated by more and more consumers, and the cultivation and processing of olives has expanded from the Mediterranean region to all over the world.

In 1975, Longnan city, one of the best suitable areas for olives cultivar in China[8, 9], began to introduce and cultivate olive tree from the Mediterranean area and achieved success[10]. In 1998, it was included in the World Olive Oil Distribution Map, and it is also a 'national oil olive demonstration base' and a 'hometown of oil olives in China'. For over 60 years, Longnan city has introduced 126 varieties of *Olea europaea* at home and abroad, and has established a gene bank of germplasm resources with the most varieties of *Olea europaea* in Asia. The planting area reaches 41860 ha. The cultivation and processing of olives has also become an important part of local agricultural economic development. Now more and more varieties have been studied and found to be suitable for planting in Longnan area, and the quality characteristics are closely related to the local environment[11].

The '*Cornicabra*' olive cultivar is native to Spain and is the second most planted olive cultivar in Spain. The olive oil squeezed from it has excellent sensory properties, good stability, high oil content and good oil quality. But the quality and composition of oils from different cultivars and

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regions are different[12]. Therefore, the analysis and characterization of the chemical composition of the oil during the fruit ripening process is the prerequisite for establishing the variety specificity, determining the optimal harvesting time and ensuring the quality of olive products[13]. In 1988, China introduced this species from Spain for the first time. In 2011, it was first introduced and planted in Longnan city (Gansu, China) by Yu Deng and Dongsheng Zhang[14].

At present, many researches that on the quality characterization or analysis of the VOO of '*Cornicabra*' were mainly concentrated in the Mediterranean region, and the researches included the influence of extraction system, crop year and region on oil quality[15]; the triglycerides, total and 2 - position fatty acid composition in the VOO of '*Cornicabra*' compared with other Spanish varieties[16]. VOO is related to many factors, such as cultivar, geographic region, climate, agronomic technology, harvesting system, processing technology, and maturity is one of the most important factors[17]. And maturation has been extended for several months, many metabolic processes and transformations have taken place in the olives, with consecutive and remarkable changes in phenolic and chemical composition of olive oil throughout the period[18, 19]. However, there is no detailed study on the effect of the fruit maturity on the chemical composition and quality assessment of '*Cornicabra*' olive cultivar grown in China.

This article examined the variation of the quality characteristics and antioxidant properties of '*Cornicabra*' VOO grown in Longnan with the change of fruit maturity. The detection indicators were several quality indices as defined by Codex Alimentarius (FFA, PV, fatty acid, K_{232}, K_{270} and ΔK); parameters related to the oxidation processes (OS, TP and TF) and oil content.

2 Materials and Methods

2.1 Location and plant material

According to the color of the olive fruit, 2.50 kg of healthy olive fruits (*Olea europaea* L), from 9 year-old trees of '*Cornicabra*' cultivars cultured in Longnan city (33° 24′ 03″ N, 104° 53′ 30″ E; altitude 1036 - 1048 m; average temperature 15.30℃, highest temperature 38℃, lowest temperature -7℃; relative humidity 56.60%, annual precipitation 468.00 mm, sunlight hours 1871 h; sandy soil, pH 7.90) of Gansu province in China, were randomly selected at each picking date at the ripening stage, which were maintained without any artificial irrigation. The sampling date was from October to December 2018 (the specific time were the 10th, 20th and 30th of each month in October and November, and the 10th and 20th of December). The traditional method of evaluating olive maturity was adopted: the same evaluator made a subjective evaluation based on the color change of the peel and flesh when the olive fruit was ripe, and divided the olive fruit maturity into 8 stages[20] (M1, bright-green peel; M2, green-yellowish peel; M3, green peel with reddish spots; M4, reddish-brown peel; M5, black peel with white flesh; M6, black peel with < 50.00% purple flesh; M7, black peel with ≥ 50.00% purple flesh; M8, black peel and purple flesh).

2.2 Analytical methods

2.2.1 Oil extraction

Oil was extracted using an Abencor laboratory mill, which reproduces the industrial process. The extraction process consisted of the following steps: 800.00 g of fresh olive fruits crushing and malaxation for 60 min at 30℃, two rounds of centrifugation at 25℃, 60 s each at 5000 r/min, with 50.00 mL water added between rounds. The oil phase and water phase after centrifugation were collected in a 250.00 mL graduated cylinder, and diluted to the mark with 25℃ deionized water. After standing for 30 min, the volume of oil phase was read, the data was recorded, and the oil phase was removed into the collection bottle, sealed and stored at low temperature. The number of independent oil extraction for each maturity index was 3 times. The oil yield (% wet weight) was calculated according to Equation (1), where the constant of 0.915 is the relative density of olive oil, "Initial pulp mass" refers to the mass of olive pulp.

$$\text{Oil yield} = \frac{\text{The volume of oil}\,(\text{cm}^3) \times 0.915}{\text{Initial pulp mass}\,(\text{g})} \times 100\% \quad (1)$$

2.2.2 Determination of total polyphenols (TP)

The extraction of TP from VOO was based on the procedure described previously by Alarcón Flores *et al.*[21]. Then the Folin - Cioucalteu method was used to determine the content of TP[22]. The content of TP in olive oil was calculated according to the below regression equation. The regression equation is $Y = 7.3741\ X$ - $0.005\ (R = 0.999\ 3)$, where X is the absorbance. The results expressed as mg of gallic acid equivalent (GAE) per kilogram of olive oil (mg GAE / kg).

2.2.3 Determination of total flavonoids (TF)

The extraction method of TF was the same as that of TP. The TF content was determined by the aluminum trichloride color method[23]. The content of TF in olive oil was calculated according to the below regression equation. The regression equation: $Y = 31.0967\ X$ - $0.3414\ (R = 0.999\ 2)$, where X is the absorbance. The results expressed as mg of rutin equivalent (RE) per kilogram of olive oil (mg RE / kg).

2.2.4 Determination of oxidation stability (OS)

OS was determined by a Rancimat apparatus (Mod. 892, Metrohm) following the procedures described in Koseoglu *et al.*[24]. The results expressed as the induction time (h).

2.2.5 Determination of FFA, PV, K_{232}, K_{270} and ΔK value

The FFA was determined according to COI/T.20/Doc. No. 34/Rev. 1 – 2017[25]. The PV was determined according to COI/T.20/Doc. No. 35/Rev.1 – 2017[26]. The K_{232}, K_{270} and ΔK

value were determined according to COI/T.20/Doc. No. 19/Rev. 5 – 2019[27].

2.2.6 GC-MS analysis of fatty acid composition and relative content

The fatty acid composition and its relative percentage of extracted olive oils were analysed by GC – MS after methyl esterification by alkaline transmethylation[28, 29]. The mass spectrometry database was the NIST 2011 standard mass spectrometry retrieval library.

2.2.7 Statistical analysis

All the data are in units of measurement. Data processing and mapping were performed using Origin Pro 10.5.36. The data were statistically analyzed by ANOVA, Duncan's multiple range tests and Principal component analysis (PCA) using SPSS 25.0. and SIMCA 14.1. The results are expressed as $(\bar{x} \pm s)$, and $p < 0.05$ which is considered statistically significant.

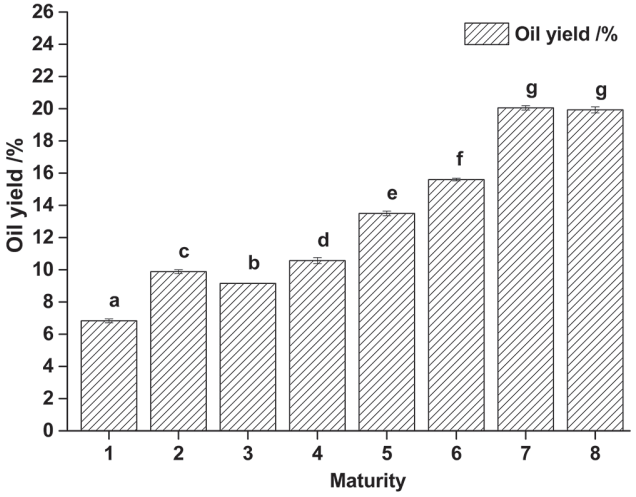

Fig. 1 Variation of oil yield of olive fresh fruit with different fruit maturity. Data were shown in the form of $\bar{x} \pm s\,(n=3)$. Error bars indicate standard deviation. Letters indicate significant differences ($p < 0.05$).

3 Results

3.1 Oil yield

As shown in Fig. 1, with the increase of fruit maturity, the fresh fruit oil yield of *'Cornicabra'* varieties showed an increasing trend. The oil yield of fresh fruits was the lowest (6.83%) at M1 and the highest (20.05%) at M7. The oil yield at M8 was slightly lower than that at M7, which was 19.93%.

3.2 Standard chemical indicators

As can be seen in Table 1, the maximum values of K_{232} and K_{270} both appeared at M2, which were 1.24 ± 0.04 and 0.13 ± 0.01, respectively. The maximum value of PV appeared at M1, which was (8.87 ± 0.04) meq O_2 / kg. In the study of Qarnifa *et al.*, PV also decreased with increasing maturity[30]. All samples were within the limited range of VOO, and ANOVA analysis showed that there were significant differences between the samples.

The FFA values ranged from (0.12 ± 0.03) % to (0.52 ± 0.03) %, which met the standard of FFA (expressed as oleic acid) ≤ 2.0% of VOO stipulated by Codex Alimentarius (2017), and the FFA first decreased and then increased during the maturation process, the maximum appeared at M7. Compared with the data of Salvador *et al.*[31] in the production areas of Toledo and Ciudad Real provinces for five consecutive years, the K_{232} value and the K_{270} value were

Table 1 Quality properties of olive oil samples with different fruit maturity ($\bar{x} \pm s$, $n = 3$, except for ΔK).

Olive oil samples*	Chemical indicators							
	K_{232}	K_{270}	ΔK	OS(h)	TP (mg GAE / kg)	TF (mg RE / kg)	FFA (% oleic acid)	PV (meq O_2 / kg)
M1	1.22 ± 0.01d	0.10 ± 0.01bcd	0.0012	13.13 ± 0.08d	120.57 ± 1.72f	5.01 ± 0.12c	0.17 ± 0.04d	8.87 ± 0.04e
M2	1.24 ± 0.04d	0.13 ± 0.01e	0.0005	11.89 ± 0.08a	122.60 ± 9.23c	5.03 ± 0.12c	0.18 ± 0.04d	6.60 ± 0.04cd
M3	1.14 ± 0.01c	0.10 ± 0.01bcd	0.0002	12.09 ± 0.19b	149.49 ± 1.23d	5.10 ± 0.56c	0.14 ± 0.03c	7.16 ± 0.06d
M4	1.24 ± 0.00d	0.11 ± 0.00d	0.0003	12.46 ± 0.11c	161.63 ± 1.13e	4.82 ± 0.12c	0.12 ± 0.03a	5.47 ± 0.02a
M5	1.22 ± 0.01d	0.11 ± 0.00d	0.0033	15.53 ± 0.08g	179.05 ± 2.00f	5.15 ± 0.00c	0.13 ± 0.03b	5.57 ± 0.11ab
M6	1.05 ± 0.03a	0.08 ± 0.01ab	0.0030	14.18 ± 0.08e	88.77 ± 2.43a	1.99 ± 0.32a	0.25 ± 0.04e	5.84 ± 0.06b
M7	1.11 ± 0.06bc	0.09 ± 0.01abc	0.0022	14.42 ± 0.11f	114.19 ± 0.83b	2.62 ± 0.32b	0.52 ± 0.03g	6.18 ± 0.04bc
M8	1.07 ± 0.02ab	0.07 ± 0.01a	0.0023	14.12 ± 0.06e	121.80 ± 6.35c	2.70 ± 0.12b	0.51 ± 0.04f	6.52 ± 0.09c
Standard Norm**	≤ 2.60	≤ 0.25	≤ 0.01	no limit	no limit	no limit	≤ 2.0	≤ 20.0

* Olive oil samples with different fruit maturity.

** Standard Norm refer to COI/T.15/NC No. 3/Rev. 12, 2018 and Codex Stan 33-1981, 2017.

a – g: different letters in the same column concerning all samples have significantly different values ($p < 0.05$).

basically close, and the PV of '*Cornicabra*' cultivar in Toledo and Ciudad Real provinces was between 6.50 meq O_2 / kg and 13.00 meq O_2 / kg. The value was different in different years. The content of FFA was between 0.34% and 1.01%, which was consistent with the content change in Longnan area.

3.3 Fatty acid composition

The composition and content of fatty acids in olive oil samples are shown in Table 2. The main fatty acids found in olive oil were C18:1, C18:2, C16:0 and C18:0. The C18:1 content ranged from (71.19 ± 0.23) % to (76.68 ± 0.17) %. The C18:1 content in the samples were within the range of 55.00% - 83.00% determined by Codex Alimentarius (Codex Stan 33-1981, 2017). The C18:1 content of olive oil increased with increasing maturity. The amounts of C18:2, between (2.90 ± 0.01) % and (4.33 ± 0.05) %, were between 2.50% - 21.00% set by IOC (COI/T.15/NC No. 3/Rev. 12, 2018). The contents of linolenic acid (C18:3) in olive oil samples, between (0.63 ± 0.01) % and (0.88 ± 0.02) %, were below the limit set by IOC (≤ 1.00%, COI/T.15/NC No. 3/Rev. 12, 2018). Except for C17:1 and C20:1 fatty acids in the olive oil samples, there were significant differences among other fatty acids ($p < 0.05$). Compared with the five consecutive years of fatty acid data studied by Salvador *et al.*[31] in the production areas of Toledo and Ciudad Real provinces, the content of C16:0 and C16:1 in Longnan was relatively high, and the content of C18:1 and C18:2 was relatively low. The contents of C18:0 and C18:3 were relatively close. Kamoun *et al.*[32], by studying the pomological and chemical characterization of many varieties, confirmed that the same olive variety presents great genetic differences according to the geographical origins, and leading to different olive traits.

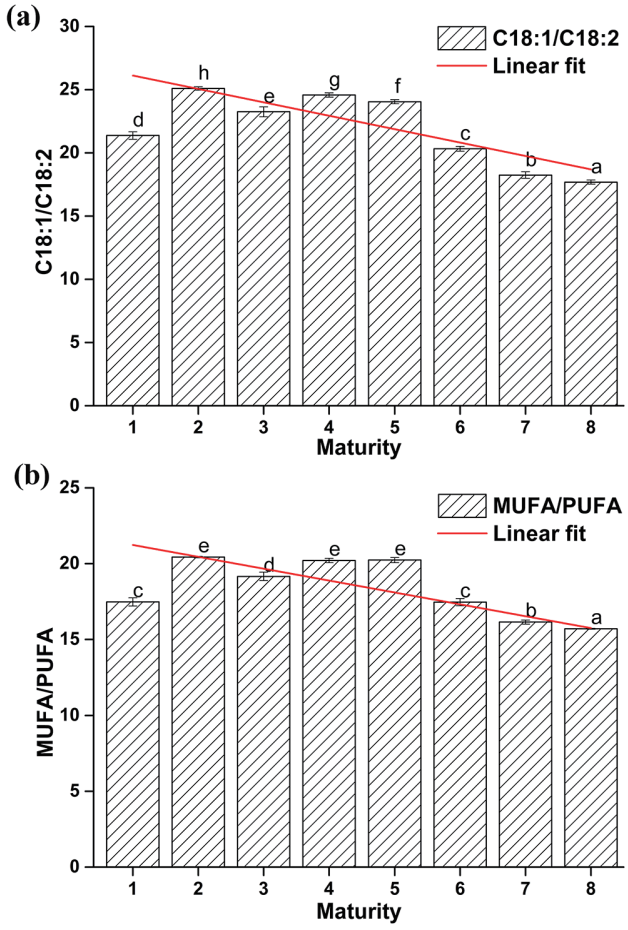

Fig. 2 C18:1/C18:2 and MUFA/PUFA ratios of VOO of '*Cornicabra*' at different fruit maturity. Error bars indicate standard deviation. Letters indicate significant differences ($p < 0.05$).

Table 2 Changes of the fatty acids in olive oil samples with different fruit maturity ($\bar{x} \pm s$, $n = 3$).

Olive oil samples*	Fatty acids									
	C16:0	C16:1	C17:1	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C22:0
M1	15.97 ± 0.16f	1.78 ± 0.02h	0.06 ± 0.04a	3.04 ± 0.05c	72.95 ± 0.43c	3.41 ± 0.03d	0.88 ± 0.02g	0.44 ± 0.01b	0.27 ± 0.01a	0.17 ± 0.02ab
M2	15.04 ± 0.08e	1.57 ± 0.01e	0.10 ± 0.01a	3.18 ± 0.02d	74.22 ± 0.31d	2.96 ± 0.02b	0.77 ± 0.01f	0.45 ± 0.01b	0.26 ± 0.01a	0.21 ± 0.04b
M3	14.04 ± 0.08c	1.50 ± 0.01a	ND	2.87 ± 0.05a	72.38 ± 0.31b	3.11 ± 0.04c	0.74 ± 0.01e	ND	ND	ND
M4	14.31 ± 0.02d	1.65 ± 0.02g	ND	2.96 ± 0.02b	71.19 ± 0.23a	2.90 ± 0.01a	0.71 ± 0.01d	ND	ND	ND
M5	14.19 ± 0.15cd	1.60 ± 0.02f	ND	3.34 ± 0.02e	75.28 ± 0.16e	3.13 ± 0.02c	0.68 ± 0.01c	0.41 ± 0.01a	0.24 ± 0.04a	0.18 ± 0.05ab
M6	12.20 ± 0.02b	1.32 ± 0.03c	ND	3.66 ± 0.02f	72.58 ± 0.07bc	3.57 ± 0.04e	0.66 ± 0.00b	ND	ND	ND
M7	11.62 ± 0.07a	1.13 ± 0.01b	0.09 ± 0.01a	4.11 ± 0.03g	76.68 ± 0.17f	4.20 ± 0.05f	0.64 ± 0.01a	0.43 ± 0.02b	0.25 ± 0.02a	0.12 ± 0.03a
M8	11.58 ± 0.06a	1.09 ± 0.01a	ND	4.23 ± 0.04h	76.59 ± 0.07f	4.33 ± 0.05g	0.63 ± 0.01a	0.44 ± 0.01b	0.26 ± 0.01a	0.19 ± 0.01b
Standard Norm**	7.50 - 20.00	0.30 - 3.50	≤ 0.30 (≤ 0.60#)	0.50 - 5.00	55.00 - 83.00	3.50 - 21.00 (2.50 - 21.00#)	no limit (≤ 1.00#)	≤ 0.60	≤ 0.50	≤ 0.20

* Olive oil samples with different fruit maturity.

** Standard Norm refer to COI/T.15/NC No. 3/Rev. 12, 2018 and Codex Stan 33-1981, 2017 (Except for #, the date from COI/T.15/NC No. 3/Rev. 12, 2018).

a - h: different letters in the same column concerning all samples have significantly different values ($p < 0.05$).

ND: not detected.

The ratio of C18:1/C18:2 mainly affects the taste of VOO[33]. The MUFA/PUFA ratio has an important influence on the nutritional properties and OS of VOO[34]. As can be seen in Fig. 2, the general trends decreased during the olive ripening process.

3.4 Total polyphenols (TP) content and total flavonoids (TF) content

Polyphenols that are important to the antioxidant capacity of olive oil are affected by olive varieties, fruit maturity and agronomic conditions[35]. As can be seen from Table 1, the TP content changes approximately roughly in the shape of capital letter "N". Namely, with the increase of fruit ripeness, the TP content in fruit reached the highest level at M5, which was (179.05 ± 2.00) mg GAE / kg, then it dropped rapidly, reached the lowest value at the M6 with (88.77 ± 2.43) mg GAE / kg, and then slowly risen. And there were statistical differences ($p < 0.05$) in TP contents between each fruit maturity.

Flavonoids are also a strong antioxidant, which can reduce the risk of different types of cancers, neurodegenerative, and cardiovascular diseases[36]. The variation trend of TF content was similar to that of TP content, and the highest content, (5.15 ± 0.00) mg RE / kg, also appeared at M5. This was consistent with the change trend of TF content during fruit ripening in the study of Menz *et al.*[37].

3.5 Oxidative stability (OS)

The OS values of different maturation stages were shown in Table 1. In general, the trend of OS values was increasing linearly throughout the maturation process, and the maximum value (15.53 h ± 0.08 h) appeared at M5. In addition, the OS was affected by polyphenols[38]. From Table 1, it can be seen that there was consistency between the changes of OS and the changes of TP content in some periods. In the late stage of fruit ripening, the decrease of fruit moisture content had affected the extraction of some soluble compounds[15], so the OS value had decreased after reaching the maximum at M5.

3.6 Principal component analysis (PCA)

PCA was performed to visually evaluate the changes of some chemical indicators (FFA, PV, K_{232}, K_{270}, TP, TF and OS) and fatty acid content of VOO of '*Cornicabra*' with fruit maturity, respectively[39]. The results were graphically represented by PCA score scatter and loading scatter plots. From Fig. 3a, it can be seen that the distribution distance of each maturity stage is relatively far, indicating that different maturity stages can make a satisfactory distinction between olive oil. The contribution of the first principal

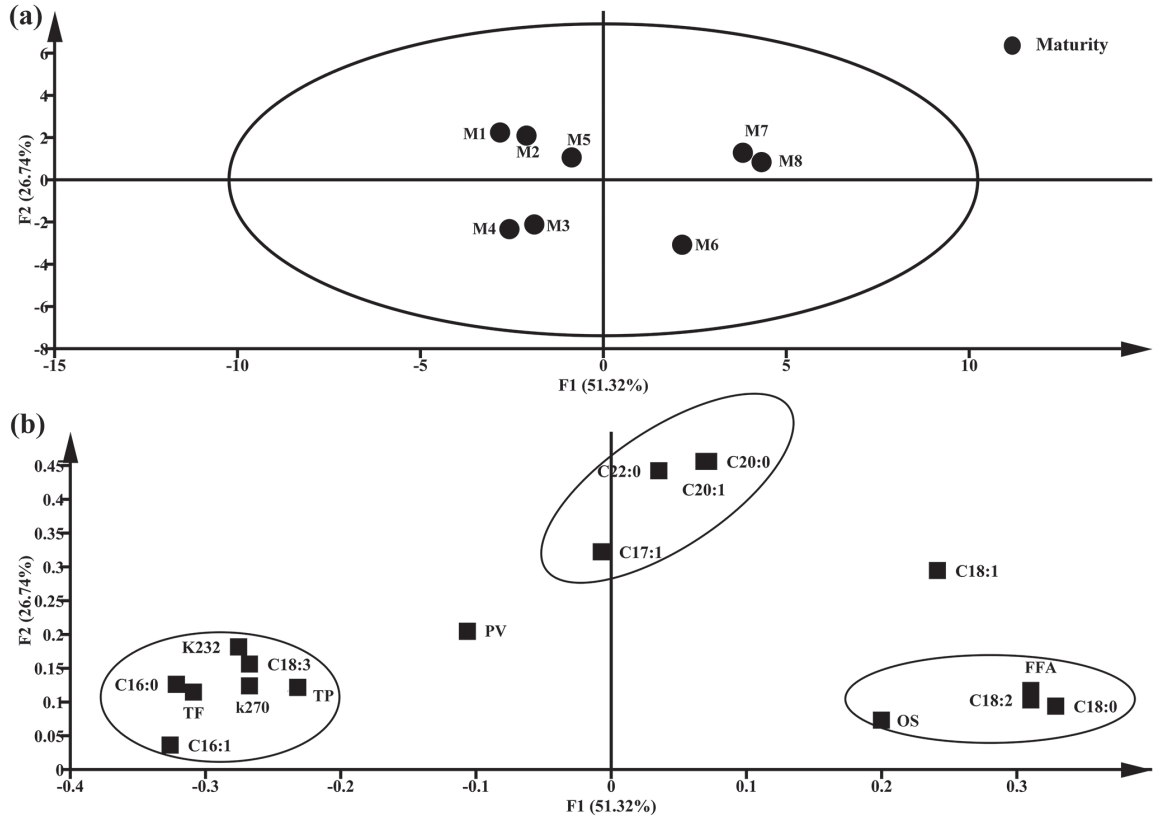

Fig. 3 Scores scatter (a) and loading scatter (b) plots with PCA according to fatty acid profiles and standard chemical indicators of '*Cornicabra*' olive oil.

component (F1) was 51.32%, the contribution of the second principal component (F2) was 26.74%, and the cumulative variance contribution of the two principal components reached 78.06%. As it can be seen from Fig. 3a (score scatter plot) and Fig. 3b (loading scatter plot), the first principal component (F1) is highly correlated with FFA, OS, C18:0 and C18:2 (positive part of F1) and negatively with TP, TF, K_{270}, K_{232}, C16:0, C16:1 and C18:3. It means that the 6th to 8th fruit maturity of *'Cornicabra'* olive oil is higher in 18:0 and 18:2, the OS and FFA are higher, which are related to the positive part of F1, while UFA, TP and TF (the negative part of F1) are more abundant from M1 to M5. The second principal component (F2) is positively correlate with C20:1, C17:1, C20:0 and C22:0. Along this F1, *'Cornicabra'* samples are spread as a function of ripening degree.

According to the eigenvectors and eigenvalues of the principal components, the principal component scores were calculated, respectively. The higher the comprehensive principal component score, the better the traits[40]. As shown in Table 3, 'A' had the highest comprehensive principal component score at M5, indicating that the olive oil at M5 had the best properties from the perspective of 7 indicators including FFA, PV, TP, TF, K_{232}, K_{270} and OS. 'B' had the highest comprehensive principal component score at M7, indicating that the olive oil at M7 had the best fatty acid quality from the perspective of fatty acid content.

4 Discussion

4.1 Oil yield

It can be seen from Fig. 1 that there were significant differences in oil yield of fresh fruits of the same cultivar with different fruit maturity. The oil content kept increasing with the increase of fruit maturity, and after reaching the maximum at M7, the oil content decreased slightly. These behaviors have also been reported by Nergiz *et al.*[41, 42] in two olive cultivars, *'Memecik'* (for both table olive and oil production) and *'Domat'* (for green table olive) in the Aegean region of Turkey. As early as 1994, Sanchez had explained this trend[43]. The profile obtained for the oil accumulation process is a function of the part of the drupe under study. The oil content of the endocarp accumulates slowly and can reach a plateau state at the best mature state, while the oil content of the seeds accumulates quickly, and once the oil content reaches the maximum, it may decrease slightly. The above results indicated that the accumulation trend of olive oil from *'Cornicabra'* cultivar introduced in Longnan, China was similar to that of the Mediterranean region.

4.2 Standard chemical indicators

The UV spectrophotometric characteristics and PV were the important indicators to describe the oxidation state of oil samples[24]. K_{232}, K_{270}, ΔK and PV values were given as ≤ 2.50, ≤ 0.22, ≤ 0.01 and ≤ 20.00 meq O_2 / kg respectively for VOO categorization by Codex Alimentarius (Codex Stan 33-1981, 2017) and IOC (COI/T.15/NC No. 3/Rev. 12, 2018). The values of the above indicators included in this study were all within the limits required by Codex Alimentarius (Codex Stan 33-1981, 2017) and IOC (COI/T.15/NC No. 3/Rev. 12, 2018), which shown that these indicators in the olive oils of various fruit maturities were good. In addition, the FFA first decreased and then increased during the maturation process, the maximum appeared at M7. This result was consistent with the opinions of Baccouri *et al.*[44]. Olives that mature later give the oil a higher FFA level because they experience an increase in enzyme activity, especially by lipolitic enzymes[45], and are more susceptible to pathogenic infections and mechanical damage[44]. Alowaiesh *et al.* has also reported that in two years, regardless of the cultivar, the FFA increased and the PV decreased in the later stage of the olive fruit ripening[46].

4.3 Fatty acid composition

The content of fatty acids in the samples in this study were all within the limits set by the Codex Alimentarius (Codex Stan 33-1981, 2017) and IOC (COI/T.15/NC No. 3/Rev. 12, 2018), but it was not easy to find a unique explanation for the fluctuations in the content of fatty acids in the study samples. The synthesis of certain fatty acids at different stages of fruit maturity, the dilution of fatty acids, the conversion of specific enzymes between different fatty acids, or the antioxidant components of olive drupes may all cause these fluctuations[47]. The concentration of PUFA is one of the key parameters for studying oil quality. Compared with olive oils with low PUFA content, high concentrations of PUFA are more likely to be oxidized, which is conducive to the thermal degradation of olive oils. From

Table 3 The principal components scores.

Comprehensive score*	Olive oil samples with different fruit maturity							
	M1	M2	M3	M4	M5	M6	M7	M8
A	0.38	0.67	0.14	0.82	0.88	−0.96	−0.83	−1.09
B	0.25	0.45	−0.90	−1.02	0.24	−0.66	0.85	0.79

* A: PCA of FFA, PV, TP, TF, K_{232}, K_{270} and OS. B: PCA of fatty acid content.

the linear trend of the ratio of MUFA to PUFA in Fig. 2, it could be found that with the increase of olive fruit maturity, the content of PUFA in olive oil was increasing, which shown that from the perspective of fatty acids, the olive oil of '*Cornicabra*' cultivar was of good quality. In addition, the decrease in C18:1 content and the increase in C18:2 content may be caused by the activity of the oleic acid desaturase that converts oleic acid to linoleic acid[24], and this conversion may also be affected by water stress[48].

4.4 Total polyphenols (TP) content and total flavonoids (TF) content

TP content is one of the important factors affecting the quality of olive oil. About changes in TP content, Chimi *et al.* had reported that the content of phenolic compounds progressively increases during the maturation process, until it reached a maximum at the 'spotted' and 'purple' pigmentation stage, after which it decreased[49]. At M5, the color of the peel just turned completely to purple-black. In addition, the polysaccharides on the cell wall affected the release of phenolic compounds during fruit crushing and adverse reactions, resulting in different levels of TP at different fruit maturity[50]. At M6, the sharp drop in TP content may be related to the climate or horticultural conditions at that time[35]. Follow-up studies will continue to monitor the changes in TP content of '*Cornicabra*' cultivar of olive oil.

4.5 Oxidative stability (OS)

OS must be related to the concentration of some olive oil chemical components. In the overall trend of OS and TP content change, it was found that between M2 to M5, with the increase of TP content, the OS continued to increase, and each reached its own maximum. However, after M6 to M8, OS and TP content were not positively correlated. Salvador *et al.* had reported that in addition to the main influence of TP content, OS also received α-tocopherols, UFA and the influence of crop seasons[51]. Therefore, subsequent studies will conduct more studies on a large number of samples from other crop seasons to determine the influence of chemical components in olive oil, such as phenolic compounds on OS, and establish a prediction equation for OS based on several compounds.

4.6 Principal component analysis (PCA)

In the PCA of fatty acids, the fatty acid quality is mainly affected by fatty acid composition, C18:1/C18:2 ratio and UFA content[33]. It can be seen from Table 2 that in the fatty acid composition of '*Cornicabra*', the content of C18:1, C18:2 and C18:3 had always been higher. Jolliffe[52] believes that when calculating the principal component score, the most important and best choice is the front principal component. The main substances on the first principal component were C16:0, C18:1, C16:1, C18:0 and C18:2. The content changes of C18:0, C18:1 and C18:2 was in line with the order of the comprehensive score of fatty acids, which were also the components that had a greater impact on fatty acids. Therefore, the change in fatty acid content of the first principal component can basically explain the proportion of the principal component. In the PCA of chemical indicators (FFA, PV, TP, TF, K_{232}, K_{270} and OS), the content changes of TP, TF, K_{232}, and K_{270} on the first principal component were basically in line with the principal component scores at each maturity level. The OS and FFA values of '*Cornicabra*' olive oil were higher from M6 to M8, which was related to the positive part of F1. The values of PV, TP, TF, K_{232} and K_{270} were relatively high from M1 to M5. PV was correlated with the positive part of F2; TP, TF, K_{232} and K_{270} were all correlated with the negative part of F1.

5 Conclusions

Oils of a pure cultivar or from a specific production area is of great scientific interest. This study was conducted to determine the effect of fruit maturity on the quality parameters and antioxidant activity of VOO of '*Cornicabra*'. According to the results, the contents of all indicators were within the limits set by Codex Alimentarius (Codex Stan 33-1981, 2017) and IOC (COI/T.15/NC No. 3/Rev. 12, 2018). With the increase of fruit maturity, the oil content continued to increase, and the oil content reached the maximum value (20.05%) at M7. With the increase of fruit maturity, K_{232}, K_{270} and PV all decreased. In addition, the maximum values of K_{232} and K_{270} both appeared at M2, which were 1.24 ± 0.04 and 0.13 ± 0.01 respectively, and the maximum of PV appeared at M1, which was (8.87 ± 0.04) meq O_2 / kg. The value of ΔK changed in a small range, always less than 0.01. FFA first decreased and then rose with the increase of olive fruit maturity, and finally reached the maximum value of (0.52 ± 0.03) % (expressed in oleic acid) at M7. TP, TF, and OS which characterizing the antioxidant properties of olive oil increased with the increase of fruit maturity, until the olive peel was purple (at M5), and then decreased with the increase of fruit maturity, in which the oxidation stabilization time and the content of TP increased overall, which indicated that the oxidation stability of VOO of '*Cornicabra*' increased with the increase in fruit maturity. The content of C18:0, C18:1 and C18:2 increased with the increase of fruit maturity, the content of C18:1 remained above 70.00%, and reached the maximum of (76.68 ± 0.17) % at M7. The ratios of MUFA/PUFA and C18:1/ C18:2 decreased during ripening. From the analysis, it can be seen that the fruit maturity is an important parameter to characterize and distinguish the quality of VOO.

Declarations of Interest

None

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