Evolution of Phenotypic Traits and Main Functional Components in the Fruit of ‘Chenggu-32’ Olives (*Olea europaea* L.) Cultivated in Longnan (China)

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Abstract: China has taken olive cultivation as a significant part of its agricultural development. Longnan city of Gansu province was marked into the world olive distribution map by International Olive Oil Council in 1998. However, so far, little research has been done on the growth and development stages of Chinese olives. The objective of this study was to investigate the dynamics changes of several quality characteristics of olive oil at different sampling times. Olive fruit of ‘Chenggu-32’ grown in Longnan were harvested at twenty-four time periods and used for determination of phenotypic traits and oil quality characteristics: total polyphenols and flavonoids contents, as well as fatty acid composition by using Gas Chromatography-Mass Spectrometer (GC-MS) and analysed by using Principal Components Analysis (PCA). Towards maturation, fruit moisture content decreased while oil content increased. Levels of both total flavonoids and total polyphenols contents slightly decreased first then increased. The ratio of unsaturated to saturated fatty acids was close to three. The ratio of monounsaturated fatty acids (MUFA)/ polyunsaturated fatty acids (PUFA) was from 2.28 to 4.05. The oleic acid (C18:1)/linoleic acid (C18:2) ratio was varied between 5.23 and 10.67 according to different sampling dates. The olive oil had lower oleic acid (C18:1) levels, higher linoleic acid (C18:2), linolenic acid (C18:3), and palmitic acid (C16:0) levels compared to Codex values (2017) in some periods, which is the characteristics fatty acid composition of ‘Chenggu-32’ variety in Longnan, China.

Key words: olive oil, Chenggu-32, phenotypic traits, flavonoids, polyphenols, fatty acids

1 Introduction

*Olive* (*Olea europaea* L.) is an important oil tree crop, which is widely distributed in the Mediterranean countries that mainly include Spain, Italy, Tunisia and Greece where have a thousands of years for cultivation and processing. Nowadays, as its beneficial effects on human health were well known all over the world and the increased demand for olive oil market, it has been planted in many different regions, where has diverse environmental conditions¹, such as South America, North America, Oceania and Asia², not just in the Mediterranean countries.

In 1956, olive was imported to China from the Mediterranean³. And based on the complex and multiplex climate, olive was cultivated and processed mainly in the south-western provinces of China⁴,⁵, at present, Yunnan, Gansu and Sichuan⁴,⁵,⁶ are the most suitable regions of China for planting olive. In 1990, the first standard olive groves were established in Wuwu (Longnan, Gansu, China). In 1998, the International Olive Oil Council listed Longnan in the World Olive Oil Distribution Map. After nearly 60 years of introduction, trial and cultivation, China has taken olive cultivation as a significant part of its agricultural development. Nowadays, more and more olive varieties are considered suitable for growth in Longnan city of Gansu province

**Abbreviations:** C16:0, palmitic acid; C16:1, palmitoleic acid; C17:0, margaric acid; C17:1, margaroleic acid; C18:0, stearic acid; C18:1, oleic acid; C18:2, linoleic acid; C18:3, linolenic acid; C20:0, arachidic acid; C20:1, gadoleic acid; C22:0, behenic acid; PCA, principal components analysis; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; GC-MS, Gas Chromatography-Mass Spectrometer
where has become one of the largest domestic olive growing and processing areas in China\(^7\).

The health characteristics of olive fruit and oil have aroused high scientific and commercial interest\(^8\)–\(^9\). Olive oil is directly obtained from the olive fruit by mechanical extraction without other refining process, thus it contains many polar compounds that are usually eliminated from other vegetable oils during the various stages of refining\(^10\). Therefore, compared with the other vegetable oils, olive oil has the unique taste and flavor\(^11\). Its beneficial effects on health associate with their consumption, particularly within the Mediterranean diet. To be specific, it plays a significant role in reducing the risk of chronic diseases such as diabetes, hypertension\(^12\) and cardiovascular disease\(^13\)–\(^14\), as well as preventing colon, breast and ovarian cancer, and inflammatory and autoimmune diseases, such as rheumatoid arthritis\(^15\)–\(^17\). On the one hand, these benefits have been related to the optimal combination of saturated (SFA), monounsaturated (MUFA), and polyunsaturated fatty acids (PUFA), specifically oleic acid (55–83%) in extra-virgin olive oil\(^18\). On the other hand, some minor components such as phyllophanols, pigments and flavonoids also have important impact on nutritional and health-promoting effects, due to their ability of preserving chemical quality of the oil\(^19\), specifically phenolic compounds are attracting considerable attention because they contribute to the olive oil stability of against autooxidation and provide nutritional and sensorial properties\(^20\)–\(^21\).

However, the phenolic composition, flavonoids, fatty acid composition and sensory properties of olive oil vary significantly depending on the olive variety, growth condition (altitude, soil composition, latitude, temperature) and ripening stage\(^22\). Due to the diversity and interrelationship of these factors, it is difficult to fully characterize olive oil with their chemical composition or sensory descriptors. Therefore, the dynamic changes of fruit and oil quality characteristics cannot be characterized by a series of compounds or simple data manipulations. Samples should be identified by a large number of variables (compounds and/or sensory descriptors), which should be analyzed by statistical techniques or artificial intelligence algorithms\(^23\)–\(^26\).

Many researches about the factors affecting the quality of olive oil mainly focus on Mediterranean region\(^27\)–\(^28\). In China, there is a lack of in-depth research on variety introduction and cultivation, cultivation environment, fruit and oil quality, chemical composition and so on.

Accordingly, this work records the climate of the Longnan olive planting base, such as temperature, mean precipitation and other factors. At the same time, the dynamic changes of fatty acid, polyphenol, flavonoid and oil content of olive fruits in 24 sampling times (in order to better reflect the overall trend, 24 groups of data were integrated into 8 groups for analysis by month.) of ‘Chenggu-32’ which grows chiefly in Longnan was analyzed, and the fruit characters were evaluated. It is expected that this work might be a source of reference for the olive international researchers that need knowledge on fruits cultivated in Longnan of China.

### 2 Materials and Methods

#### 2.1 Location and plant material

Olive trees of the ‘Chenggu-32’ variety grown in Olive Research Institute Germplasm Resources Gene Bank of Longnan city (33°24'03"N, 104°53'30"E) of China. Five trees were randomly selected from the same variety, and 100 fruits were taken by hand at the position around the middle of each canopy each time. The age of these trees is 11 years. No artificial irrigation. The sampling date is the whole crop seasons from 8 / 10 / 2017 to 3 / 30 / 2018 (sampled olive fruits on the 10\(^{th}\), 20\(^{th}\) and 30\(^{th}\) of each month). After harvesting, the olive fruit samples were immediately sealed in plastic bags and placed in a cryostat and transported to laboratory within 24 hours, and preserved at −20°C for further study. Only healthy fruits, without any kind of infection or physical damage, were processed. The fresh fruits were washed with distilled water and weighed at each sampling time. All experiments were repeated three times. The information of the sampling places in Longnan city (Gansu, China) (Table 1, Fig. 1).

#### 2.2 Reagents and instruments

Standards of a 10-component fatty acid methyl esters (FAMEs) mixture were purchased from Nu - Chek Prep. INC. (USA) Trading Co., LTD. Gallic acid reference substance (BW5007 99.9% pure), rutin reference substance (AB015R ≥ 98% pure), Folin-Ciocalteu, aluminium trichloride, petroleum ether (30 - 60°C), absolute ethanol, methanol, sodium and sodium hydroxide were purchased from local chemical reagent companies in China. All reagents used were of analytical grade; the experimental water is deionized water.

| Soil      | Latitude longitude | Altitude (m) | Temperature (°C) | Precipitation (mm) | Relative Humidity (%) | Sunlight hours (h) |
|-----------|---------------------|--------------|------------------|--------------------|----------------------|-------------------|
| Sandy soil | pH7.9               | 104°53'30"E  | 1036-1048        | 15.3               | 468                  | 56.6              |

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\(^{21}\) Chenggu-32.
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2.3 Analytical methods

2.3.1 Determination of fruit shape index

One hundred olive fruits were randomly selected. The longitudinal and transverse diameters were measured with vernier calipers, and the fruit shape index (fruit shape index = fruit longitudinal diameter / fruit transverse diameter) was calculated and averaged.

2.3.2 Determination of moisture content

One hundred fresh fruits were randomly selected and weighed. Then the sample was dried in an oven at 80°C for 2 h, then dried at 120°C to constant weight, and the moisture content was calculated according to the difference in mass before and after fruit drying.

2.3.3 Oil extraction

Cold-press extraction: Oil was extracted using an Abencor laboratory mill, reproduces the industrial process. The extraction process consisted of the following steps: 800 g of fresh olive fruit crushing and malaxation for 30 min at 30°C, two rounds of centrifugation at 25°C, 60 s each at 5000 r/min, with 50 mL water added between rounds. After centrifugation, oils were decanted, filtered, transferred into brown glass bottles and stored at 4°C in the dark until analyses. The above operation was slightly modified with reference to the methods of Dag Arnon et al. and Beltrán, Gabrie et al. The oil was used for the analysis of main functional components.

2.3.4 Determination of oil content

Dried Pulp Soxhlet Extraction Method: The olive fruit was dried to a constant weight in an oven at 50°C and then ground into a fine powder. After accurately weighted, about 1.0 g of fine powder was wrapped with filter paper and put into the extraction bottle of the crude fat analyzer. 50 mL petroleum ether was added and reflux extraction was conducted for 3 hours. Three groups of experiments were conducted in parallel.

The refluxed extraction bottle was placed in a 45°C oven to remove traces of petroleum ether and water. After the flask was cooled, it was weighed and the oil content was calculated based on the quantity of the oil and the quality of the olive powder. Oil content = (m2 - m1)/m × 100%, where “m” is the mass of sample, “m2” is the mass of extraction bottle, “m1” is the mass of oil and extraction bottle. The results were expressed as percentage of dry matter.

2.3.5 Determination of total polyphenols

The total polyphenols was estimated by the Folin–Ciocalteau method. Extraction of the phenols from the olive fruit powder was performed using 1.0 g of the olive fruit powder, which was repeated for 3 times with 10 mL 70% ethanol. The lipid and pigment was removed by petroleum ether. Filtered and evaporated for pre-concentration to a 10 mL volumetric flask. After the test solution was diluted 1 time, took 0.25 mL in a 10 mL volumetric flask. Briefly, 0.25 mL of test solution was, in this order, mixed with 0.5 mL of Folin–Ciocalteu reagent and 2 mL of 10% Na2CO3 solution. The resulting mixture was diluted with deionized water to obtain a 10 mL final volume and then light-proof reaction under 30°C for 2 h. The absorbance was measured at 765 nm. The content of olive polyphenols was calculated according to the below regression equation. The regression equation is: Y = 7.3741X - 0.005 (R = 0.9993), where “X” is the absorbance. The concentration of gallic acid had a good linear relationship between 3.12 and 12.48 mg GAE/mL. The results expressed as gallic acid equivalent (mg GAE / kg).

2.3.6 Determination of total flavonoids

The total flavonoids were estimated by Aluminum Tri-chloride Coloring Method. Extraction of the flavonoids from the olive fruit powder was performed using 1.0 g of the olive fruit powder, which was repeated for 3 times with 10 mL 70% ethanol. The lipid and pigment was removed by petroleum ether. Filtered and evaporated for pre-concentration to a 10 mL volumetric flask. 3.0 mL of test solution was mixed with 4 mL of 0.1 mol/L aluminum trichlo-
ride methanol solution and then shook well. After 5 min, the resulting mixture was diluted with methanol to obtain a 10 mL final volume. The absorbance was measured at 410 nm. The content of olive flavonoids was calculated according to the below regression equation. The regression equation: \[ Y = 31.0967X - 0.3414 \] where \( X \) is the absorbance. The rutin mass concentration had a good linear relationship between 16.8 and 84.0 \( \mu \)g RE/mL. The results expressed as rutin equivalent (mg RE/kg).

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

The fatty acid composition and their relative percentage of extracted olive oils were analysed by GC–MS after methyl esterification by Alkaline Transmethylation.

Optimized detecting conditions: **GC conditions:** AE-FFAP elastic quartz capillary column, 30 m length, 0.25 mm i.d., and 0.25 \( \mu \)m films, were used. The carrier gas was helium, and the injection temperature was kept at 250°C; the temperature program was maintained at 160°C for 3 min, held at the rate of 4°C/min to 190°C and held for 2 min, then raised at the rate of 10°C/min to 210°C and held for 5 min, then raised at the rate of 5°C/min to 240°C and held for 5 min. The sample volume was 1 \( \mu \)L; the injection method was split injection with a split ratio of 50:1; the carrier gas velocity was 1.0 mL/min; and the GC-MS interface temperature was 250°C. **MS conditions:** Transmission line temperature was 250°C, and the Ion source temperature was 280°C. The ionization mode was EI. The ionization voltage was 70 eV. The quality Scan mode was Full Scan. The quality scanning range was 50 - 650 amu. The solvent delay time was 3 min. The mass spectrometry database was the NIST 2011 standard mass spectrometry retrieval library.

2.3.8 Statistical analysis

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 PCA using SPSS 22.0.

3 Results and Discussion

3.1 Phenotypic traits

The average fruit weight showed increased first, and then decreased slightly (Fig. 2). When the fruit matures, its weight is relatively high and it is easy to fall off and fall to the ground. The direct collection of olives from trees showed that these fruits were delayed ripening, so the weight of these fruits picked later was lower than that of the best harvesting period. The fruit shape index of the olive fruit was similar to the weight change trend (Fig. 2). When the fruit shape index was gradually increased, it indicated that the fruit was oval. 

Figure 3 was a collection of digital photographs, which visually showed the changes in color of the olives from green to black. The fruit coloring appeared on September 30, 2017, and the color was completely changed between October 20 and 30. And color changes are directly related to agricultural production. The changes can be related to the physical and chemical composition. The fullness or shrinkage of the shape of the fruit is related to the moisture of the fruit. Combined with meteorological conditions, the precipitation is abundant from
August to October, and the precipitation is drastically reduced after November, which can account for the change\textsuperscript{33}. So the fruit is full from August to October, but the fruit begins to shrink from November.

### 3.2 Oil content

The mean values of moisture content ranged from 27.49 to 55.57\%, for February, 2018 to August, 2017 (Fig. 4). In the early stage (August to October) of fruit growth, the moisture content of the fruit was between 54.39 ± 4.37 and 55.57 ± 1.15\%, coherent with the values that the fruit moisture content was often about 55\% or higher on developing fruit\textsuperscript{34}. The striking difference (\(p<0.05\)) in moisture content between the whole sampling period is owing to higher mean precipitation values (8.07 mm) at autumnal period in 2017 (Fig. 1) as compared to other sampling dates, involving an increase of moisture in fruits. The weather relative humidity of air and the oil content of the fruit may also have some effects on the moisture.

As shown in Fig. 4, oil content ranged from 12.49 ± 2.98 to 34.49 ± 0.28\%, which increased towards maturation. Throughout the process, the maximum oil content appeared on December, 2017, and then decreased slightly. The results of\textsuperscript{35} on the oil content of the "Cornicabra" fruit for five consecutive years (1995 - 1999) showed that the trend of change first increased and then decreased slightly with the change of maturity. Although the oil content among the varieties is different, however, the trend of each variety in the maturity period is basically the same\textsuperscript{35}. As the oil content increased, the moisture content decreased (Fig. 4). Correlation between decreases in water and increases in oil content has been confirmed\textsuperscript{35}. Donaire et al.\textsuperscript{37} reported that the moisture content of olives changed with the increase of oil content during fruit ripening. This change may be due to temperature differences in the growing regions of olive fruit during ripening, which may stimulate the biological activity of some enzymes responsible for oil biosynthesis. In addition, a negative relationship between oil synthesis duration and temperature was found\textsuperscript{36}.

### 3.3 Total polyphenols and total flavonoids content

The content of polyphenols is one of the most important indicators for evaluating the olive oil quality, which mainly affects the flavor, stability and active function of olive oil\textsuperscript{38}. In this study, it can be seen from Fig. 5 that total polyphenols content presented a relatively big variability throughout the sampling period ranging from 4113.33 ± 1053.11 to 9960.00 ± 1053.57 GAE/ kg. Related studies have confirmed that the increase in total polyphenols content is related to the high temperature in autumn\textsuperscript{39}, so in August to December, the total polyphenols content decreases with the decrease of temperature. The increase may be due to the high autumnal temperature stimulating L-phenylalanine aminoase activity. The variation of polyphenols content was generally on the rise, which was consistent with the results of Jiménez et al.\textsuperscript{36}. The results of Franco et al.\textsuperscript{40} showed that the polyphenolic compounds in the seven varieties of olive oil, such as "Arbequina", decreased in the early stage of fruit ripening, the content and change trend of phenolic compounds in different varieties of olive oil were slightly different. Similar results were reported by Sousa et al.\textsuperscript{41} who found that the total polyphenols content of Cv. Cobrançosas varieties also decreased significantly.
from September to November. However, study has shown that when mean precipitation is reduced, it will cause a water stress environment for fruit trees and increase the content of total polyphenols[42]. Higher total polyphenols also was related with low fruit moisture content which during ripening[43]. Differently, the data in this study suggested the opposite of the results of the two studies above. The content of polyphenols is related to many factors such as variety, light, origin, evapotranspiration, crop year, temperature, maturity, storage and processing conditions, so the difference may require more research.

The trend of the total flavonoids content during the whole sampling period was similar with the content of polyphenols. It can be seen from Fig. 5 that total flavonoids content presented a relatively small variability throughout the sampling period ranged from 786.67 ± 184.75 to 2353.33 ± 196.04 mg RE/kg. Therefore, the total flavonoids content decreased with the decrease of temperature from 2353.33 ± 196.04 mg RE/kg. Therefore, the total flavonoids content presented a relatively small variability throughout the whole sampling period. The content of flavonoids varied between 0.71 ± 0.35 and 1.87 ± 1.39. Further -

Table 2 shows mean value and stand deviations of fatty acids at different sampling dates of olives. The most abundant fatty acid was the oleic acid (C18:1) with contents varying from 50.57 ± 1.39% to 59.62 ± 0.35%. Furthermore, the levels of palmitic acid (C16:0) ranged from 14.82 ± 0.85% to 20.42 ± 0.69%. The palmitoleic acid content varied between 0.71 ± 0.00% and 1.87 ± 1.71%; for the arachidic acid, the variety showed values lower than the limit of 0.60% established for the olive oil. Compared with the standard values of each fatty acid (oleic acid: 55.00 - 83.00%, linoleic acid: 3.50 - 21.00%, linolenic acid: ≤ 1.00%, palmitic acid: 7.50 - 20.00%, palmitoleic acid: 0.30 - 3.50%, arachidonic acid: ≤ 0.60%) in the Codex Alimentarius (2017), the contents of palmitic acid, oleic acid, linoleic acid and linolenic acid were not within the standard range. The content of palmitic acid in September was 20.42 ± 0.69%; the contents of oleic acid in September, October and February were 51.17 ± 4.41%, 50.57 ± 1.39% and 53.91 ± 1.73%, respectively, which were all below the minimum limit of 55.00%; the contents of linoleic acid in October, January, February and March were 21.66 ± 2.10%, 22.06 ± 0.51%, 23.06 ± 1.55% and 22.12 ± 4.29%, respectively, which were all higher than the maximum limit of 21.00%; of the eight months, only November and December had linolenic acid content less than 1.00%. According to the report of Zarrouk Wissem et al.[44], after the Verde del l’Hérault variety native to France was cultivated in Tunisia, the changes in oleic acid, linoleic acid, and linolenic acid content were almost the same as those of ‘Chenggu-32’, and its oleic acid content was low to 47.23%; linoleic acid content was up to 27.51%; linolenic acid content was up to 1.43%. Antari, E.I. et al. [45], Ravetti, L. [46] and Meehan, C.K. [47] have all reported that the content of linolenic acid exceeded 1.00%. In addition, the content of major fatty acids in the Leccino olive variety planted in Sfax exceeded the codex values compared to its origin in Italy, but when it was planted in Andalucia (Spain), which has the similar climate conditions as Italy, the content of main fatty acids was within the standard values as in the origin[48]. And Romero, M.P. et al. [49] attributed this change to seasonal differences, especially the availability of water. In view of the fact that the ‘Chenggu-32’ variety originated in China has not undergone geographical changes during the growth process and less precipitation in Longnan (China), so the contents of palmitic acid, oleic acid, linoleic acid and linolenic acid exceeding the standard range is the characteristics fatty acid composition of ‘Chenggu-32’ variety in Longnan, China. Grati Kammoun et al. [50], by studying the pomological and chemical characterization of many varieties, also confirmed that the same name variety would have great genetic differences in different geographical origins, leading to different traits. The oil samples had abundant MUFA content, which was consistent with the observations of oil-producing countries such as Spain, and southern Tunisia[44, 51].

The palmitoleic acid (C16:1), margaric acid (C17:0), margaroleic acid (C17:1) and behenic acid (C22:0) contents almost remained unchanged in the whole sampling period. No significant differences were found in ANOVA. Results are in contrast with the findings of previous studies in other varieties, which reported that olive fatty acid composition changed in relation to climatic factors variations[44, 50]. Differences can be explained by cultivar which an essential factor determining olive fatty acid composition[44].

The level of stearic acid (C18:0) was below the upper limit of 5% established for olive oil[54]. In the August - October and December - February periods, the stearic acid content markedly increased, whereas oleic acid content showed an opposite trend (Table 2). The reduction in oleic acid content can also be to the conversion of oleic acid to stearic acid by stearoyl-ACP A9 desaturase activity which is active during triacylglycerol biosynthesis accentuated by more summer precipitation (August)[25].

The percentage of saturated, monounsaturated and polysaturated fatty acids and the ratio of oleic acid / linoleic acid (C18:1 / C18:2) in olive oil were evaluated. It was observed that ‘Chenggu-32’ fruit oil was rich in total saturated fatty acids (SFA), which ranged from 17.51 ± 1.09%.
Table 2  Changes of the fatty acids in olive fruits during ripening (%)\(^a\).

| Items\(^b\) | August         | September       | October        | November\(^c\) | December       | January        | February\(^c\) | March\(^c\)   |
|------------|----------------|-----------------|----------------|----------------|----------------|----------------|----------------|---------------|
| Palmitic (C16:0) | 19.78 ± 0.26ed | 20.42 ± 0.69d   | 18.61 ± 1.19c  | 15.90 ± 0.20ab | 16.63 ± 1.79b  | 16.03 ± 0.21ab | 16.21 ± 0.66ab | 14.82 ± 0.85a |
| Palmitoleic (C16:1) | 0.89 ± 0.11a   | 1.23 ± 0.17a    | 1.08 ± 0.12a   | 0.96 ± 0.09a   | 1.87 ± 1.71a   | 0.83 ± 0.03a   | 0.92 ± 0.08a   | 0.71 ± 0.00a  |
| Margaric (C17:0)  | 0.09 ± 0.01a   | 0.06 ± 0.06a    | 0.03 ± 0.05a   | ND             | 0.04 ± 0.07a   | 0.02 ± 0.04a   | ND             | ND            |
| Margaroleic (C17:1) | 0.11 ± 0.01a   | 0.08 ± 0.07a    | 0.04 ± 0.07a   | 0.09 ± 0.08a   | 0.11 ± 0.19a   | 0.04 ± 0.07a   | ND             | ND            |
| Stearic (C18:0)   | 2.72 ± 0.40ab  | 3.82 ± 0.79c    | 3.32 ± 0.28bc  | 2.40 ± 0.18a   | 2.33 ± 0.41a   | 2.44 ± 0.03a   | 2.51 ± 0.19a   | 2.57 ± 0.05a  |
| Oleic (C18:1)     | 57.62 ± 1.41b  | 51.17 ± 4.41a   | 50.57 ± 1.39a  | 59.62 ± 0.35b  | 58.47 ± 5.39b  | 55.57 ± 0.39ab | 53.91 ± 1.73ab | 55.04 ± 4.46ab|
| Linoleic (C18:2)  | 15.25 ± 0.58a  | 19.47 ± 2.61ab  | 21.66 ± 2.10b  | 18.72 ± 0.50ab | 17.45 ± 7.50ab | 22.06 ± 0.51b  | 23.06 ± 1.55b  | 22.12 ± 4.29b |
| Linolenic (C18:3) | 2.29 ± 0.14c   | 2.06 ± 0.17c    | 1.39 ± 0.41b   | 0.94 ± 0.06a   | 0.93 ± 0.20a   | 1.08 ± 0.06ab  | 1.13 ± 0.04ab  | 1.04 ± 0.17ab |
| Arachidic (C20:0) | 0.46 ± 0.05ab  | 0.57 ± 0.11b    | 0.40 ± 0.16a   | 0.34 ± 0.02a   | 0.35 ± 0.05a   | 0.38 ± 0.00a   | 0.38 ± 0.02a   | ND            |
| Gadoleic (C20:1)  | 0.30 ± 0.03ab  | 0.37 ± 0.06b    | 0.31 ± 0.06ab  | 0.28 ± 0.01ab  | 0.19 ± 0.17a   | 0.30 ± 0.02ab  | 0.28 ± 0.02ab  | ND            |
| Behenic (C22:0)   | 0.13 ± 0.00a   | 0.16 ± 0.03a    | 0.11 ± 0.10a   | ND             | 0.04 ± 0.07a   | 0.13 ± 0.12a   | ND             | ND            |
| \(\Sigma\) SFAs  | 23.18 ± 0.68c  | 25.04 ± 1.48c   | 22.46 ± 1.74b  | 18.64 ± 0.36a  | 19.40 ± 1.79a  | 19.00 ± 0.29ab | 19.11 ± 0.83ab | 17.51 ± 1.09ab|
| \(\Sigma\) MUFAs | 58.86 ± 1.26bc | 52.86 ± 4.26ab  | 52.01 ± 1.19a  | 60.94 ± 0.40c  | 60.64 ± 7.31c  | 56.74 ± 0.36abc | 55.15 ± 1.64abc| 55.75 ± 4.46abc|
| \(\Sigma\) PUFA|s | 17.54 ± 0.52a  | 21.53 ± 2.76a   | 23.06 ± 2.51a  | 19.66 ± 0.51a  | 18.37 ± 7.68a  | 23.14 ± 0.54a  | 24.19 ± 1.59a  | 23.16 ± 4.44a |
| MUFAs/PUFAs       | 3.36 ± 0.17a   | 2.50 ± 0.55a    | 2.28 ± 0.32a   | 3.10 ± 0.10a   | 4.05 ± 2.69a   | 2.45 ± 0.06a   | 2.29 ± 0.21a   | 2.49 ± 0.60a   |
| C18.1/C18.2       | 5.23 ± 0.42a   | 9.00 ± 2.74a    | 10.32 ± 2.36a  | 6.35 ± 0.37a   | 6.60 ± 4.74a   | 9.44 ± 0.47a   | 10.67 ± 1.76a  | 10.11 ± 4.93a  |

\(^a\) Values are means ± sd, n = 3. Means in a row without a common letter differ, \(p < 0.05\).

\(^b\) SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

\(^c\) ND represents not detected.
characteristics which means that the oil theoretically had good stability. The main fatty acids on the first main component are palmitic acid, margaric acid, behenic acid. The main fatty acids on the second main component are palmitoleic acid, margaroleic acid, stearic acid, behenic acid. The main fatty acids on the third main component are linolenic acid, arachidic acid, gadoleic acid.

Using SPSS software for principal component analysis, it is more intuitive to evaluate the effect of fatty acids on olive oil quality at different sampling dates. The number of principal components and the cumulative variance contribution rate are shown in Table 3. The first principal component has the largest variance contribution rate, which is 53.122%. The third principal component has the smallest variance contribution rate, only 9.844%. Because the larger the contribution rate of variance, the greater the impact on the quality of olive oil. Therefore, the fatty acids (include palmitic acid, margaric acid, and behenic acid) on the first principal component have the largest impact on ‘Chenggu-32’ olive oil, and the fatty acids (include palmitoleic acid, margaroleic acid, stearic acid, linoleic acid and oleic acid) on the second principal component have the second largest impact. In addition, the eigenvalues of the three principal components are greater than 1, and the cumulative variance contribution rate reaches 87.881%, which indicates that the three principal components can reflect and explain 87.881% of the effective information of 11 fatty acids in olive oil.

According to the eigenvectors and eigenvalues of the principal components, the principal component scores are calculated. From the perspective of determining the best time to obtain high-quality fatty acids, three principal component scores were ranked according to different sampling dates, and the higher the principal component scores, the better the quality of the oil fatty acids. The results are shown in Table 4. The top three scores of the main component score are August, September and December. Fatty acid quality of oil was usually mainly affected by fatty acid composition, C18:1/ C18:2 ratio, and UFA content. As shown in Table 2, in the fatty acid composition of ‘Chenggu-32’, the levels of oleic acid, linoleic acid, and linolenic acid were always high. Therefore, Saporta M. considered that when calculating the principal component score, the most important and best choice was the first principal component. The main substances on the first main component are palmitic acid, margaric acid, behenic acid. The content of palmitic acid was the largest 20.42 ± 0.06% in September, followed by 19.78 ± 0.26% in August; the content of margaric acid was 0.09 ± 0.01% in August, 0.06 ± 0.06% in September, and 0.04 ± 0.07% in December.

Table 3  Principal component eigenvalues and contribution rates.

| Main          | Eigenvalues | Variance contribution rate % | Cumulative variance contribution rate % |
|---------------|-------------|------------------------------|----------------------------------------|
| 1             | 5.843       | 53.122                       | 53.122                                 |
| 2             | 2.741       | 24.916                       | 78.037                                 |
| 3             | 1.083       | 9.844                        | 87.881                                 |

Fig. 6  Fatty acid principal component analysis load map.
and they were also the top three in all sampling dates; for the content of behenic acid, the maximum was 0.16 ± 0.03% in September, and it was 0.13% in August. The change in fatty acid content of the first principal component can basically explain the fraction of the principal component. Therefore, the main component scores in August, September, and December were high, which also means that the quality of olive oil fatty acids harvested during these three periods was better.

4 Conclusions

In the present study, we sampled the olive fruits for 24 times in 8 months (August, 2017 to March, 2018), processed the sample by GC-MS, PCA of the fatty acids, and finally summarized the phenotypic traits and the change rule of main functional components of ‘Chenggu-32’ variety fruits in Longnan, China. The fruit veraison discoloration time was earlier, appeared in late September. Due to the different natural and meteorological conditions in the area, the dried fruit weight was generally increased. Oil content was closely related to moisture content, temperature and precipitation, and reached the maximum in December. Polyphenols content and flavonoids content fluctuate or elevate. The ratio of SFA: MUFA: PUFA was 1:3:1, the most abundant fatty acid was oleic acid, which was close to 60%. The content of oleic acid, linoleic acid, linolenic acid, and palmitic acid in the fatty acid composition is out of the Codex Alimentarius values, which is the characteristic of ‘Chenggu-32’ variety in Longnan, China. In future research, in order to analyze the unique fatty acid composition and content at the molecular level and explore the expression patterns and pathways of key regulatory genes in the process of substance transformation, modern methods of lipid metabolomics and transcriptomics will be used. In addition, oil stability against oxidation, related anti-oxidant levels and more quality parameters will also be measured to provide a theoretical basis for the directional improvement of olive oil quality and the guidance of harvesting. This work might be a source of reference for the olive international researchers that need knowledge on fruits of cultivated in Longnan of China.

Declarations of Interest

None

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Table 4  Score of main components of fruit fatty acids in different sampling dates.

| Sampling date | Fatty acid principal component score |
|---------------|-------------------------------------|
| Aug.          | 2.03                                |
| Sep.          | 1.81                                |
| Oct.          | 0.11                                |
| Nov.          | -0.32                               |
| Dec.          | 0.75                                |
| Jan.          | -0.52                               |
| Feb.          | -1.29                               |
| Mar.          | -2.56                               |
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