Effects of tree age on the chemical compositions and antioxidant activities of 'Coratina' and 'Koroneiki' olive oils from youth trees in China

Le Yu<sup>1</sup>, Yongjin Wang<sup>2</sup>, Jun Jin<sup>2</sup>, Gangcheng Wu<sup>1</sup>, Qingzhe Jin<sup>1</sup>, and Xingguo Wang<sup>1</sup>

May 8, 2020

### Abstract

China is a new fast-developing olive oil producer country but with non-Mediterranean climate, resulting in the oils from China have been rarely studied. The scale of olive cultivation in China is growing at an amazing rate in recent years. 'Coratina' and 'Koroneiki' are the two major olive cultivated in China. Compared with environment condition, less concern was showed to the influences of olive tree age on the chemical composition of the oils. Thus, it is of great importance to investigate the characterizations of olive oils from trees at different ages to provided references for expanding the olive cultivation in China. Significant differences (p < 0.05) were found in the concentrations of oleic acid, linoleic acid,  $\gamma$ -tocopherol, squalene and phenolic compounds between Coratina and Koroneiki oils from trees at different ages. All kinds of determined antioxidant capacities (DPPH, FRAP, ABTS and ORAC) of olive oils were significantly related with phenolic compounds and tocopherols. The results showed that both cultivar and tree age had influence on the chemical composition and antioxidant capacities of virgin olive oils. The influence of tree age was generally weaker than the cultivar, but tree age had greater effect on the structure of phenolic compounds than cultivar.

#### 1. Introduction

Olive oil is nowadays widely appreciated by consumers all over the world due to its health protection and nutrition. Thus, olive (Olea europaea L.) is now planted in many non-Mediterranean regions across continents under diverse environmental conditions (Vossen, 2007). As a new fast-developing producer country, olive introduction and cultivation in China have been supported by government strongly. Although the scale of olive cultivation in China cannot compare with that in Mediterranean countries, it is growing at an amazing rate over the past few years. There were only 25,300 hectares of olive trees planted around in 2009, and the area rapidly increased to 65,600 hectares in 2015 (Su et al., 2018). Furthermore, the planned area of olive cultivation was about 200,000 hectares, and the main planned area is located in the hot valley zone of the Jinsha River (Su et al., 2018). Therefore, millions of seedlings will be planted in the future.

As a non-Mediterranean country, the climate feature in China is quite different from that in traditionally olive growing regions. The most distinguished feature between China and Mediterranean country is the different timings of rainfall. There is a strong possibility that the trees cultivated in this region encounter rainfall more frequently in anthesis and fruiting periods. It is widely known that environment conditions have great effect on the chemical composition of olive oils (Ceci, Mattar, & Carelli, 2017; Deiana et al., 2019; Fuentes et al., 2018). Though previous studies showed that tree age may affect fatty acid profile, tocopherols, squalene, phytosterol and phenolic compounds in olive oils, much more attention was paid on the influences of environment conditions than tree age (Chtourou et al., 2017a, 2017b). In general, olive has the same growth pattern of the other stone fruits. Several researches have demonstrated that tree age also

<sup>&</sup>lt;sup>1</sup>Affiliation not available

<sup>&</sup>lt;sup>2</sup>Jiangnan University

have effects on the chemical composition of stone fruits, such as Amrapali mango (Meena & Asrey, 2018). Therefore, the influence of tree age on the characterization of olive oils deserves greater attention.

'Coratina' and 'Koroneiki' are two of the main olive cultivars planted in China which is famous on their high oil contents and good contents of phenolic compounds. However, there is a little information available on comprehensive features of olive oils in China. Therefore, the differences between Coratina and Koroneiki oils produced from trees of different ages were evaluated. The evaluation of the characteristics of oils produced from olive trees with different ages has many values for developing Chinese olive industry.

#### 2. Materials and methods

### 2.1 VOO samples

Olives were taken from trees cultivated in the geographical area of Xichang, China (latitude: 27°74′74″N, longitude: 102deg23′22″E, altitude: 953m asl). Previous research had demonstrated that genotype was the main contributor to variance and the influence of crop year was weak (Ceci, Mattar, & Carelli, 2017; Yu et al., 2019), the fruits used in this research were obtained in 2018. Environmental factors, the managements of olive trees, olive fruits collection and VOO extraction were described by our previous study (Yu et al., 2019). The tree ages of 'Coratina', 'Koroneiki' were 2, 7 and 11, respectively. Green fruits were handpicked from all positions of selected trees. The climate conditions of Xichang in 2018 were described in Fig. 1.

# $2.2\ Analytical\ methods$

## 2.2.1 Quality parameters

Fatty acid (ISO 660), peroxide value (ISO 3960), and spectroscopic indices ( $K_{232}$ ,  $K_{270}$  and  $\Delta K$ ) (ISO 3960) were determined according to IOC regulations (IOC, 2013).

### 2.2.2 Chemical compositions

Fatty acid profiles and minor components (tocopherols, total sterols and squalene) were performed as described by our previous reports (Yu et al., 2019). Detailed information associated with the methods used for fatty acid, tocopherols, total sterols and squalene were all presented in the supplementary material.

Phenolic compounds were extracted as described by (Mateos et al., 2001). A 500 mg Sepax Generik Diol tube (Sepax Technologies, Inc., Newark, DE, USA) was used to extract phenolic compounds. The analysis of phenolic compounds was performed by a Waters 1525 HPLC system (Milford, MA, USA) equipped with a Waters 2996 photodiode array detector (Milford, MA, USA) was used. The stationary phase was a Sepax Amethyst C18-H column (250  $\times$  4.6 i.d. mm, 5 $\mu$ m particle size) (Sepax Technologies, Inc., Newark, DE, USA). The mobile phase and solvent gradient conditions used for analysis were according to (IOC, 2009) with some minor modifications. The flow rate was 0.8 mL/min. The quantification of phenolic compounds was carried out at 280 nm.

## 2.2.3 Antioxidant capacity assay

Antioxidant capacity assay (DPPH, FRAP, ABTS and ORAC) was performed according to Shi et al. (Shi et al., 2017) with a few modifications. Polar extract was acquired from 2.0 g of oil. The oil was mixed with 7.5 mL methanol in a brown bottle and vigorously stirred for 20 min. The supernatant was carefully separated as polar extract, and the residue was extracted four times with 7.5mL of methanol each. All polar extracts were transferred into a brown vial and stored at -20 °C in darkness until analysis. Detailed information associated with the methods used for DPPH, FRAP, ABTS and ORAC were all presented in the supplementary material.

#### 2.3 Statistical analysis

All measurements were carried out in triplicate, and results were expressed as means  $\pm$  standard deviations (SD). The data were statistically analyzed by one-way ANOVA. Mean values between different samples were compared by Turkey's multiple tests, and significant differences were established at p < 0.05. Correlation

analysis was performed by Pearson's test. Hierarchical cluster analysis (HCA) was used for classifying the samples. Statistical analyses were carried out with SPSS version 22.0 (IBM Corporation, New York, USA).

#### 3. Results and Discussion

### 3.1 Quality parameters

It is well known that the degree of FA, PV, and the UV absorbencies ( $K_{232}$ ,  $K_{270}$ , and  $\Delta K$ ) are the main indicators to define the quality of olive oil. Table 1 shows the regulated quality criteria of all the analyzed samples. These parameters were all within the limit established for extra virgin olive oil (EVOO) by IOC regulations (IOC, 2013). Although statistically significant differences were observed for the physicochemical quality parameters according to cultivar and tree age in some samples, the numerical difference of these indicators was small and irregularity. Compared with cultivar and tree age, the extraction process may have a more significant effect on the quality of oils.

#### 3.2 Chemical compositions

#### 3.2.1 Fatty acid profiles

The fatty acids of Coratina and Koroneiki in different age are presented in Table 2, and they are all in agreement with the EVOO standard. On the other side, there were significant differences between Coratina and Koroneiki in fatty acids, mainly in oleic acid, linoleic acid and stearic acid. Owing to the differences in the content of oleic acid and stearic acid, there was a gulf existing in the ratio of MUFA to PUFA (MUFA/PUFA) of Coratina and Koroneiki. Contents of other fatty acids were similar, so the ratio of UFA to SFA (UFA/SFA) was little difference in all the samples. Significant differences were also found in the same variety of different ages. As can be seen in Table 2, the percentages of major fatty acids in Coratina and Koroneiki oils varied irregularly according to tree age. The capricious fluctuation in fatty acid resulted in MUFA/PUFA and UFA/SFA in Coratina and Koroneiki oils showed a jumble variation with tree age.

There are plenty literature reports focusing on the effects of varieties on fatty acid profiles. In addition, previous reports have been demonstrated that geographical condition greatly affects the fatty acids of olive oils from the same cultivar. Compared with the Coratina oils around the world, the mean percentage of palmitic acid was in a high level, oleic acid was in a low level, and linoleic acid was in an intermediate level in Coratina oils in the present study (Aparicio & Luna, 2002; Bruscatto et al., 2017; Ceci, Mattar, & Carelli, 2017; Fuentes et al., 2018). Koroneiki was another widely planted olive variety in the world. In comparison with the Koroneiki oils from other regions, the mean percentage of palmitic acid was in a high level, oleic acid was an intermediate level, and linoleic acid was in a low level in the studied Koroneiki oils (Fuentes et al., 2018; Wang et al., 2018). Besides, the effects of tree age on fatty acid profiles were not exactly the same as the existing reports. Chtourou et al. have been concerned about the effects of olive trees age on fatty acid composition and found that the variations of fatty acid composition were not significant with respect to tree age (Chtourou et al., 2017b). However, tree age existed unclear effects on fatty acid compositions in this study.

### 3.2.2 Tocopherols

Tocopherols are recognized as the important antioxidants and nutrient due to their biological activities in olive oil. Four tocopherol isomers were quantified and Table 3 presents the result for all the studied samples. As suggested by previous researches,  $\alpha$ -tocopherol was the major tocopherol in olive oil. Moreover,  $\gamma$ -tocopherol content was greater than  $\beta$ -tocopherol expect 7-year-old Koroneiki. In accordance with previous studies, tocopherol contents were highly cultivar-dependent (Bruscatto et al., 2017). The contents of  $\alpha$ -tocopherol and total tocopherols in Coratina oils were apparently lower than those in Koroneiki oils but the content of  $\gamma$ -tocopherol was higher. Compared with the tocopherols contents in Coratina and Koroneiki produced from origin region, the oils of same cultivar in this study contained fewer contents of tocopherols (Deiana et al., 2019). As reported by Bruscatto et al., the contents of tocopherols in Coratina were evidently higher than those in Koroneiki oils (Bruscatto et al., 2017). In contrast, Koroneiki oils contained higher levels of tocopherols in this study.

From the perspective of tree age, the content of  $\alpha$ - and total tocopherols in VOOs from 7-year-old trees were noticeably higher than those in the samples from 2-year-old and 11-year-old trees of both Coratina and Koroneiki. Meanwhile, the samples from 7-year-old trees contained less  $\gamma$ -tocopherol than those in VOOs from 2-year-old and 11-year-old trees of the two varieties. The increase of  $\alpha$ - and total tocopherols with tree age for the oils from trees of 2-7 years was in consistent with the investigations performed by Chtourou et al., who reported that higher total tocopherol was found in VOO from old trees than young trees (Chtourou et al., 2017b). However, the decrease of  $\alpha$ - and total tocopherols were found in the oils from trees of 7-11 years. This might be related to the state of the trees. Though olive trees can maintain luxuriant for hundreds or even thousands years in Mediterranean region, the natural life span of Chinese olive tree is very short without the professional cultivation and management under the natural conditions (Deng & Yu, 2011). It was much more difficult to maintain the trees in good condition in Xichang, China.

### 3.2.3 Squalene and Sterols

The contents of squalene and sterols were illustrated in Table 3. According to the literature, the content of squalene in olive oils ranged from 1100 mg/kg to 8390 mg/kg (Beltrán et al., 2016). The contents were higher than previously reported in both varieties (Salvo et al., 2017). Significant differences were found in the contents of squalene between varieties and tree ages. Koroneiki oils contained notably higher squalene than Coratina oils. The amount of squalene was changed along with the growth of the age depending on variety, first decreased and then increased in Coratina oils while first increased and then decreased in Koroneiki oils. The highest content of squalene was present in 7-year-old Koroneiki oil and 7-year-old Coratina oil was the one with the least squalene. The influences of varieties and tree ages on the content of sterols were generally smaller than on squalene. Squalene is considered as a precursor of sterols and triterpenoids. The less amount of squalene signified that more squalene has been converted to other phytomolecules.

The mean content of sterols in Coratina oils was 1174 mg/kg, a little higher than that in Koroneiki oils. The variation of the content of total sterols with tree age was in the opposite with the change of squalene. The content of sterols first increased and then decreased in Coratina oils while first decreased and then increased in Koroneiki oils. In comparison with the same varieties from other countries, the contents of total sterols were at or just below the average in the present study (Aparicio and Luna, 2002). But the contents of total sterols in all samples still reached the standard requirement (> 1000 mg/kg) for EVOO. The result squared with the theory, as the increased sterols may derive from squalene.

### 3.2.4 Phenolic Compounds

### 3.2.4.1 Visualization of phenolic compounds

Data visualization is an efficient analytical strategy to make it easier for readers to find hidden features in the data. The circos map plays a tremendous role in visualizing the relationships between multidimensional data which is widely used in the genomes. Recently, this method has been successfully applied to characterize the phytochemical profile of edible oils (Zhang et al., 2020). As shown in Table 3 and Fig. 2, the contents of phenolic compounds differed between cultivars and tree ages. It is clear that the presentation of graph is more intuitive compared with traditional tables. The samples and phenolic compounds were automatically divided into two parts (Fig. 2). Different samples were distributed uniformly in upper half circle, while the lower half circle presented the distribution of phenolic compounds at various intervals. The compounds with long intervals indicated high abundance in the samples. The contents of total phenolic compounds were higher in Coratina oils than in Koroneiki oils of all ages. In the other hands, the contents of polyphenols in oils differed greatly of same variety and the variation trend was the same with that of total tocopherols. The oils from 7-year-old trees contained the highest content of phenolic compounds.

In accordance with published data, secoiridoids (including tyrosol derivatives and hydroxytyrosol derivatives) were the major individual phenolic components in olive oil (Deiana et al., 2019). The proportion ranges of the secoiridoids in total polyphenols were from 70% to 94% in this study. However, the structures of secoiridoids in different samples were different. Interestingly, ligstroside aglycone (Try-EA) in both Coratina and Koroneiki oils from 2-year-old trees showed the highest in secoiridoids while decarboxymethyl oleuropein aglycone (Hy-

EDA) was the highest in the remaining samples. Moreover, the diversification of the proportion of Try-EA in samples was opposite to that of the total polyphenol contents. Oils from 2-year-old trees contained the highest proportion of Try-EA, followed by 11-year-old and 7-year-old, but total polyphenol contents in oils from high to low were from 7-year-old, 11-year-old and 2-year-old trees. Owing to the low concentrations, quantity reported of phenolic acids, lignans and flavonoids in different literatures were inconsistent (Deiana et al., 2019; Fuentes et al., 2018). The trend of the amount of secoiridoids was consisting with that of total phenols, and the remaining phenolic components were slightly cultivar-dependent. The proportion of phenolic acids in Koroneiki oils was higher than that in Coratina oils. The contents of phenolic alcohols in Koroneiki and Coratina oils were similar. 11-year-old Coratina oil contained the highest concentration of lignans and the lowest concentration was existed in 7-year-old Coratina oil. The amount of flavonoids was marginally higher in Coratina oils than that in Koroneiki oils.

### 3.2.4.2 Effects of cultivar and tree age on phenolic compounds

The phenolic compounds are a characteristic category substance in olive oils due to its antioxidant activity, nutritive value and sensory characteristics. It is an important index in identifying quality of olive oils. Furthermore, it has been demonstrated that the antioxidant efficiency and the taste threshold of different phenolic fractions were different (Dierkes et al., 2012). 'Coratina' and 'Koroneiki' are widely regarded as the olive cultivars with high content of polyphenol (Vossen, 2007). Though the contents of polyphenols in the studied oils were remarkably lower than those in the same varieties from Mediterranean regions, the contents were similar with those from non-Mediterranean regions (Fuentes et al., 2018; Vossen, 2007). Compared with the other olive cultivars in China, the studied samples contained higher polyphenol contents (Wang et al., 2018).

According to the published reports, endogenous olive polyphenol oxidase and peroxidase played a key role in shaping the phenolic profile of olive oils, particularly for secoiridoid compounds (García-Rodríguez et al., 2011). The increase of total polyphenols in the oils from 2-year-old trees to 7-year-old trees consisted with the findings carried out by Ayton et al., who reported that the polyphenol content rose with tree age (Ayton et al., 2007). The decline of total polyphenols in the oils from 7-year-old trees to 11-year-old trees may also due to the state of the trees.

### 3.3 Antioxidant capacity assay

In terms of antioxidant capacity, there are two approaches for measuring the antioxidant capacities according to the mechanisms of deactivate radicals: single electron transfer (SET) and hydrogen atom transfer (HAT). The DPPH, FRAP and ABTS methods belong to the assays based on the SET. Both SET and HAT happened in the ORAC test (Fernandez et al., 2014). The results of antioxidant capacities of samples evaluated by the four different methods are summarized in Fig.3. Koroneiki oil from 7-year-old trees had the greatest antioxidant capacity evaluated by all three SET methods while 7-year-old Coratina oil showed the strongest antioxidant activity in ORAC test. The differences of the experimental results might be explained by the principles of the methods, as HAT method contained active oxygen whereas SET method did not. On the other hand, 2-year-old Koroneiki oil showed the lowest antioxidant capacity in all experiments.

The correlation between the major chemical compounds and the antioxidant capacity assessed by four methods of all studies oils is presented in Table 4. Correlation analysis revealed that the influences of phenolic compounds to the antioxidant capacity of samples were the most noticeable. The content of total phenolic compounds was extremely significantly positively correlated with all of the four antioxidant capacities. The contents of Hy-EDA and oleuropein aglycone (Hy-EA) were extremely positively correlated with four kinds of free radical scavenging abilities, and the content of tyrosol and p-coumaric acid was extremely negatively correlated with them. In addition, the contents of hydroxytyrosol and decarboxymethyl ligstroside aglycone (Try-EDA) were significantly or extremely significantly correlated with all kinds of antioxidant capacities. Besides, the content of hydroxytyrosol acetate (Hy-AC) was negatively correlated with DPPH and ORAC antioxidant capacity, while the content of o-coumaric acid was positively correlated with ABTS antioxidant capacity. Previous papers have demonstrated that the structure of phenolic content modulates their anti-

oxidant activities, o-diphenols show stronger antioxidant properties due to the formation of intramolecular hydrogen bonds in the detoxification of free radicals (Carrasco-Pancorbo et al., 2005). As a result, the abilities to scavenge free radicals of hydroxytyrosol derivatives are stronger than tyrosol derivatives. Artajo et al. have studied the antioxidant efficiency of the phenolic fraction and found Hy-EDA and Hy-EA emerged the greatest antioxidant ability among VOO phenols (Artajo et al., 2006). This result is suggested to explain the relationship between individual phenolic compounds and the antioxidant capacities of the oils.

Tocopherols are other well-known lipid radical scavengers in VOOs. However, various parameters affect their antioxidant properties. The content of total tocopherols was extremely positively correlated with all antioxidant capacities. Among tocopherol components, the content of  $\alpha$ -tocopherol was positively correlated with DPPH and ORAC antioxidant capacities. The content of  $\beta$ -tocopherol was positively correlated with DPPH, FRAP and ORAC antioxidant capacities. The content of  $\delta$ -tocopherol was positively or extremely positively correlated with DPPH, FRAP and ABTS antioxidant capacities.

Furthermore, the content of squalene was negatively correlated with FRAP and ABTS antioxidant capacities, the percentage of palmitic acid was negatively correlated with the ABTS antioxidant capacity, and UFA/SFA was positively correlated with ABTS antioxidant capacity. Other fatty acid and minor compounds showed no effects on the antioxidant capacity of the oil. Nonetheless, due to insufficient numbers of samples, the conclusions need further verification.

## 3.4 Hierarchical cluster analysis

HCA is an unsupervised classification method, which finishes classification by grouping samples of similar properties. In the present study, two variables were involved into the HCA analysis. One group included major fatty acids with more than 0.5% level together with all minor components; the other one was consist of the structure of phenolic compounds. The dendrograms constructed by standardized data are presented in Fig.4.

As can be seen in Fig.4 (a), Coratina and Koroneiki oils were completely divided into two categories. In addition, the oils from 2-year-old and 11-year-old trees were separated from the oils from 7-year-old trees for both Coratina and Koroneiki. The result demonstrated that the chemical compositions of olive oil were highly cultivar-dependent while tree age had a certain influence on the characteristics of the same varieties. As shown in Fig.4 (b), the oils from 2-year-old Coratina and Koroneiki trees were classified as a group. The structure of phenolic compounds in oil from 7-year-old Koroneiki trees was similar with that from 11-year-old Koroneiki trees. When the 5 distance threshold was chosen, 7-year-old Coratina oil was grouped in the same cluster with 7-year-old and 11-year-old Koroneiki oils. 11-year-old Coratina oil was divided into this cluster at 7 distance threshold. The results implied that both cultivar and tree age have apparent effects the structure of phenolic compounds.

### 4. Conclusions

This work provides information about the quality, chemical composition and antioxidant activity of oils produced from 'Coratina' and 'Koroneiki' cultivars of different ages. Hierarchical cluster analysis traced an overview of the samples and variables, showing evidence of their grouping according to cultivar and tree age based on the composition of oils. Cultivar is the most important factor affecting the characterization of olive oils. VOOs from Coratina showed higher concentrations of linoleic acid, γ-tocopherols and phenolic compounds while those from Koroneiki cultivar were characterized by higher concentrations of oleic acid and squalene. Moreover, the results verified that tree age had effect on chemical composition of oils, but the influence was less than cultivar except for the structure of phenolic compounds. It is very interesting that the phenolic compound structures of oils from 2-year-old trees were unique from other oils which may be related to the enzyme activities of endogenous olive polyphenol oxidase and peroxidase. In general, quality of all samples reached the specifications for EVOO. The contents of total tocopherols and total phenols were extremely positively correlated with antioxidant capacities of DPPH, FRAP, ABTS and ORAC. Among the phenolic compounds, concentrations of Hy-EDA, Hy-EA and tyrosol were extremely correlated with all kinds of antioxidant capacities determined in the study. Data generated in this study provided references

for expanding the cultivation of these cultivars in China.

Declaration of interest: 'Declarations of interest: none'

### References

Aparicio, R., Luna, G., (2002). Characterization of monovarietal virgin olive oils. European Journal of Lipid Science and Technology, 104, 614-627.

Artajo, L.S., Romero, M.P., Morelló, J.R., Motilva, M.J., (2006). Enrichment of refined olive oil with phenolic compounds: Evaluation of their antioxidant activity and their effect on the bitter index. Journal of Agricultural and Food Chemistry, 54, 6079-6088.

Ayton, J., Mailer, R. J., Haigh, A., Tronson, D., & Conlan, D. (2007). Quality and oxidative stability of Australian olive oil according to harvest date and irrigation. Journal of Food Lipids, 14, 138-156.

Beltrán, G., Bucheli, M. E., Aguilera, M. P., Belaj, A., & Jimenez, A. (2016). Squalene in virgin olive oil: screening of variability in olive cultivars. European Journal of Lipid Science and Technology, 118, 1250-1253.

Bruscatto, M. H., Zambiazi, R. C., Crizel-Cardoso, M., Piatnicki, C. M. S., Mendonça, C. R. B., Dutra, F. L. G., & Coutinho, E. F. (2017). Chemical characterization and oxidative stability of olive oils extracted from olive trees of Southern Brazil. Pesquisa Agropecuaria Brasileira, 52(12), 1231–1240.

Carrasco-Pancorbo, A., Cerretani, L., Bendini, A., Segura-Carretero, A., Del Carlo, M., Gallina-Toschi, T., ... & Fernandez-Gutierrez, A. (2005). Evaluation of the antioxidant capacity of individual phenolic compounds in virgin olive oil. Journal of Agricultural and Food Chemistry, 53(23), 8918-8925.

Ceci, L.N., Mattar, S.B., Carelli, A.A., (2017). Chemical quality and oxidative stability of extra virgin olive oils from San Juan province (Argentina). Food Research International, 100, 764-770.

Chtourou, F., Jabeur, H., Lazzez, A., Bouaziz, M., (2017a). Characterization and Discrimination of Oueslati Virgin Olive Oils from Adult and Young Trees in Different Ripening Stages Using Sterols, Pigments, and Alcohols in Tandem with Chemometrics. Journal of Agricultural and Food Chemistry, 65, 3512-3522.

Chtourou, F., Valli, E., Bendini, A., Lazzez, A., Gallina, T., Mohamed, T., (2017b). Effects of Olive Trees Age on the Minor Components of Oueslati Virgin Olive Oils Produced from Olives Harvested at Different Ripening Degrees. JAOCS, Journal of the American Oil Chemists' Society, 94, 435-447.

Deiana, P., Santona, M., Dettori, S., Culeddu, N., Dore, A., Molinu, M.G., (2019). Multivariate approach to assess the chemical composition of Italian virgin olive oils as a function of variety and harvest period. Food Chemistry, 300, 125243.

Deng, M.Q., Yu, N., (2011). Introduction and cultivation techniques of olive (Olea europaea L.)(Chinese). Beijing, China: China Agriculture Press.

Dierkes, G., Krieger, S., Dück, R., Bongartz, A., Schmitz, O.J., Hayen, H., (2012). High-performance liquid chromatography-mass spectrometry profiling of phenolic compounds for evaluation of olive oil bitterness and pungency. Journal of Agricultural and Food Chemistry, 60, 7597–7606.

Fuentes, E., Paucar, F., Tapia, F., Ortiz, J., Jimenez, P., (2018). Effect of the composition of extra virgin olive oils on the differentiation and antioxidant capacities of twelve monovarietals. Food Chemistry, 243, 285–294.

García-Rodríguez, R., Romero-Segura, C., Sanz, C., Sánchez-Ortiz, A., Pérez, A.G., (2011). Role of polyphenol oxidase and peroxidase in shaping the phenolic profile of virgin olive oil. Food Research International, 44, 629-635.

IOC, (2009). Trade Standard Applying to Olive Oils and Olive-Pomace Oils. International Olive Council, Madrid COI/T.20/Doc No 29.

IOC, (2013). Trade standard applying to olive oils and olive pomace oils, COI T.15 NC. no.3-REV.7-2013.

Mateos, R., Espartero, J. L., Trujillo, M., Rios, J. J., León-Camacho, M., Alcudia, F., & Cert, A., (2001). Determination of Phenols, Flavones, and Lignans in Virgin Olive Oils by Solid-Phase Extraction and High-Performance Liquid Chromatography with Diode Array Ultraviolet Detection. Journal of Agricultural and Food Chemistry, 49, 2185-2192.

Meena, N.K., Asrey, R., (2018). Tree age affects physicochemical, functional quality and storability of Amrapali mango (Mangifera indica L.) fruits. Journal of the Science of Food and Agriculture, 98, 3255-3262.

Salvo, A., La Torre, G.L., Rotondo, A., Mangano, V., Casale, K.E., Pellizzeri, V., Clodoveo, M.L., Corbo, F., Cicero, N., Dugo, G., (2017). Determination of Squalene in Organic Extra Virgin Olive Oils (EVOOs) by UPLC/PDA Using a Single-Step SPE Sample Preparation. Food Analytical Methods, 10, 1377-1385.

Shi, L.K., Zheng, L., Jin, Q.Z., Wang, X.G., (2017). Effects of Adsorption on Polycyclic Aromatic Hydrocarbon, Lipid Characteristic, Oxidative Stability, and Free Radical Scavenging Capacity of Sesame Oil. European Journal of Lipid Science and Technology. 119, 1-10.

Su, C.J., Sun, J., Zhu, W., Peng, L., (2018). History, distribution, and potential of the olive industry in China: A review. Sustainability, 10(5), 1-20.

Vossen, P. (2007). Olive oil: history, production, and characteristics of the world's classic oils. HortScience(5), 42, 1093-1100.

Wang, J. W., Ma, L. Y., Gómez-del-Campo, M., Zhang, D. S., Deng, Y., & Jia, Z.K., (2018). Youth tree behavior of olive (Olea europaea L.) cultivars in Wudu, China: Cold and drought resistance, growth, fruit production, and oil quality. Scientia Horticulturae, 236, 106–122.

Yu, L., Wang, Y. J., Wu, G., Jin, J., Jin, Q. Z., Wang, X. G., (2019). Quality and Composition of Virgin Olive Oils from Indigenous and European Cultivars Grown in China. JAOCS, Journal of the American Oil Chemists' Society.

Zhang, T., Wang, T., Liu, R., Chang, M., Jin, Q., & Wang, X. (2020). Chemical characterization of fourteen kinds of novel edible oils: A comparative study using chemometrics. LWT - Food Science and Technology, 118(October 2019).

Table 1 Regulated physicochemical parameters of Coratina and Koroneiki

Tree age	Coratina	Coratina	Coratina	Koroneiki	Koroneiki	Koroneiki	
	2	7	11	2	7	11	
FA(% oleic acid)	$0.31 {\pm} 0.01 bc$	$0.35 \pm 0.01 b$	$0.31 {\pm} 0.01 bc$	$0.34 \pm 0.01$ b	$0.42{\pm}0.01a$	$0.28{\pm}0.01c$	
PV(Meq O2/kg)	$2.95 \pm 0.03 d$	$2.63 \pm 0.05 e$	$3.59 \pm 0.07 c$	$4.85{\pm}0.03a$	$3.59 \pm 0.07 c$	$3.91 \pm 0.06 b$	
K232	$1.419 \pm 0.034 \mathrm{b}$	$1.486{\pm}0.016{\rm ab}$	$1.188{\pm}0.024c$	$1.269 {\pm} 0.057 \mathrm{c}$	$1.571 \pm 0.053 a$	$1.263{\pm}0.021c$	
K270	$0.131 {\pm} 0.004 a$	$0.131{\pm}0.002a$	$0.105 \pm 0.004 \mathrm{b}$	$0.120 \pm 0.01 \mathrm{ab}$	$0.142{\pm}0.008a$	$0.107 \pm 0.002 \mathrm{b}$	
$\Delta { m K}$	$0.002 \pm 0.000 \mathrm{b}$	$0.001 \pm 0.000 c$	$0.001 \pm 0.000 c$	$0.004{\pm}0.000a$	$0.003 \pm 0.000 a$	$0.001 \pm 0.000 c$	

Table 2 Fatty acid profiles (% weight of methyl esters) of Coratina and Koroneiki

Tree age	Coratina	Coratina	oratina Coratina		Koroneiki	Koroneiki
	2	7	11	2	7	11
C14:0	$0.01 \pm 0.00 a$	$0.01 \pm 0.01a$	$0.01 \pm 0.00 a$	$0.02 \pm 0.00 a$	$0.01 \pm 0.00 a$	$0.01 \pm 0.00 a$
C16:0	$15.76 \pm 0.02 \mathrm{b}$	$16.14 \pm 0.10 a$	$12.93 \pm 0.07 e$	$15.14 \pm 0.07 c$	$13.47 \pm 0.16 d$	$13.74 \pm 0.09 d$
C16:1	$0.74 \pm 0.01 d$	$1.80 \pm 0.01a$	$0.51 {\pm} 0.00 \mathrm{e}$	$1.14 \pm 0.00 \mathrm{b}$	$1.01 \pm 0.04 c$	$0.86{\pm}0.00{ m d}$
C17:0	$0.05 \pm 0.00 a$	$0.08{\pm}0.00a$	$0.05{\pm}0.00a$	$0.05 \pm 0.00 a$	$0.05 \pm 0.01a$	$0.05 \pm 0.00 a$
C17:1	$0.10 \pm 0.00 \mathrm{b}$	$0.27 \pm 0.06 a$	$0.09 \pm 0.00 \mathrm{b}$	$0.08 \pm 0.00 b$	$0.12 \pm 0.02 \mathrm{b}$	$0.09 \pm 0.01 \mathrm{b}$

Tree age	Coratina	Coratina	Coratina	Koroneiki	Koroneiki	Koroneiki
C18:0	$1.76 \pm 0.03 \text{cd}$	$1.58 \pm 0.08 d$	$1.80 \pm 0.03 c$	$2.26 \pm 0.03$ a	$2.03 \pm 0.05$ b	$2.23 \pm 0.03a$
C18:1	$70.03 \pm 0.07 e$	$68.45 \pm 0.18 f$	$73.63 \pm 0.09 d$	$74.83 \pm 0.00c$	$77.67 \pm 0.08a$	$76.71 \pm 0.14$ b
C18:2	$10.15 \pm 0.01$ b	$10.59 \pm 0.12a$	$9.94 \pm 0.03 b$	$5.42 {\pm} 0.01 {\rm c}$	$4.70 \pm 0.02 d$	$5.16 \pm 0.01 cd$
C18:3	$1.00 \pm 0.02a$	$0.76 \pm 0.04 \mathrm{b}$	$0.68 \pm 0.04 \mathrm{b}$	$0.74 \pm 0.01 \mathrm{b}$	$0.65 \pm 0.04 \mathrm{b}$	$0.78 \pm 0.04 \mathrm{b}$
C20:0	$0.01 \pm 0.00a$	$0.01 \pm 0.01a$	$0.01 \pm 0.00a$	$0.01 \pm 0.00 a$	$0.01 \pm 0.00 a$	$0.01 {\pm} 0.01 a$
C20:1	$0.28 \pm 0.01a$	$0.22 \pm 0.01 \mathrm{b}$	$0.27 \pm 0.00a$	$0.19 \pm 0.00 bc$	$0.17{\pm}0.02c$	$0.21 {\pm} 0.01 \mathrm{b}$
C22:0	$0.08 \pm 0a$	$0.11 \pm 0.01a$	$0.07 \pm 0a$	$0.11 \pm 0.01a$	$0.08 \pm 0.03 a$	$0.13{\pm}0.02a$
C24:0	$0\pm0a$	$0\pm0a$	$0\pm0a$	$0\pm0a$	$0\pm0a$	$0\pm0a$
C18:1T	$0\pm0a$	$0\pm0a$	$0\pm0a$	$0\pm0a$	$0\pm0a$	$0\pm0a$
C18:2T+C18:3T	$0.02 \pm 0a$	$0.00 \pm 0b$	$0.02 \pm 0a$	$0.01 \pm 0a$	$0.02 \pm 0a$	$0.01\pm0a$
MUFA/PUFA	$6.38 \pm 0.01 e$	$6.23 \pm 0.03 e$	$7.02 \pm 0 d$	$12.38{\pm}0.04c$	$14.79 \pm 0.17a$	$13.09 \pm 0.09 \mathrm{b}$
UFA/SFA	$4.66 {\pm} 0.02 \mathrm{d}$	$4.58 \pm 0.02 d$	$5.72 \pm 0.04 a$	$4.69 \pm 0.01 d$	$5.37 \pm 0.00 \mathrm{b}$	$5.18{\pm}0.06c$

Table 3 Tocopherols, total polyphenols, squalene and phenolic compounds of Coratina and Koroneiki

Tree age	Coratina	Coratina	Coratina	Koroneiki	Koroneiki	Koroneiki	
	2	7	11	2	7	11	
$\alpha$ -T (mg/kg)	$163.9 \pm 0.8c$	$178.6 \pm 1.7 \mathrm{b}$	$166.9 \pm 0.6 c$	$178.1 \pm 0.1$ b	$187.8 {\pm} 0.6 a$	$178.4 \pm 2.0 \mathrm{b}$	
$\beta$ -T (mg/kg)	$5.3 \pm 0.2 b$	$3.4 {\pm} 0.1 c$	$2.5 \pm 0.0 de$	$2.2 \pm 0.1e$	$6.4 {\pm} 0.1 a$	$2.6 \pm 0.0 d$	
$\gamma$ -T (mg/kg)	$13.7 \pm 1.0 b$	$8.8 {\pm} 0.2 c$	$15.6 \pm 0.2a$	$6.3 \pm 0.5 d$	$2.6 {\pm} 0.3 \mathrm{e}$	$5.7 \pm 0.4 d$	
$\delta$ -T (mg/kg)	$0.7 {\pm} 0.1 c$	$0.0 \pm 0.0 d$	$1.4 \pm 0.0 b$	$0.0 \pm 0.0 d$	$1.8 \pm 0.1a$	$0.0 \pm 0.0 d$	
TTs (mg/kg)	$183.5 \pm 0.3c$	$190.8 \pm 1.6 \mathrm{b}$	$186.4 {\pm} 0.7 {\rm c}$	$186.5 {\pm} 0.4 {\rm c}$	$198.5 {\pm} 0.3 a$	$186.6 \pm 2.15 c$	
TSs(mg/kg)	$1190\pm37ab$	$1254 \pm 48a$	$1096\pm22 bc$	$1204\pm12a$	$1028 \pm 54c$	$1183 \pm 39a$	
Squalene(mg/kg)	$8102 \pm 77 \mathrm{d}$	$7179 \pm 305 e$	$8400 \pm 74 d$	$11072 \pm 341 b$	$12971 \pm 283a$	$10030 \pm 147 c$	
Ну	$4.6 {\pm} 0.2 a$	$5.0 \pm 0.2a$	$4.4 {\pm} 0.2 a$	$3.2 \pm 0.1 b$	$4.5 \pm 0.3a$	$4.6 \pm 0.1a$	
Try	$4.8 \pm 0.1 b$	$1.7 \pm 0.1 d$	$4.0 \pm 0.2 c$	$7.2 \pm 0.0 a$	$1.9 \pm 0.2 d$	$4.2 \pm 0.0 c$	
Vanillic acid	$2.6 \pm 0.0 b$	$1.4 \pm 0.1 c$	$1.5 \pm 0.0 c$	$3.0\pm0.0\mathrm{ab}$	$3.2 {\pm} 0.3 a$	$1.5 \pm 0.0 c$	
Vanillin	$0.0 {\pm} 0.0 c$	$0.5 \pm 0.2 b$	$0.5 \pm 0.0 b$	$0.9 \pm 0.0 a$	$0.0\pm0.0c$	$0.4 \pm 0.0 b$	
p-coumaric acid	$1.2 \pm 0.0 ab$	$0.4 {\pm} 0.1 c$	$0.8 \pm 0.0 $ b	$1.3 \pm 0.2a$	$0.0 \pm 0.0 d$	$0.4 {\pm} 0.1 c$	
Hy-AC	$0.5 \pm 0.0 b$	$0.3 \pm 0.0 c$	$0.7 \pm 0.0 a$	$0.5 \pm 0.1 b$	$0.0 \pm 0.0 d$	$0.2 \pm 0.0 c$	
Ferulic acid	$0.8 \pm 0.0 d$	$0.2 \pm 0.0 e$	$0.3 \pm 0.0 e$	$9.8 \pm 0.0 b$	$13.3 \pm 0.1a$	$5.8 {\pm} 0.2 c$	
o-coumaric acid	$0.0 \pm 0.0 a$	$0.2 \pm 0.1a$	$0.2 \pm 0.0 a$	$0.0 \pm 0.0 a$	$0.0 \pm 0.0 a$	$0.1 \pm 0.2a$	
Hy-EDA	$12.1 \pm 0.4 e$	$98.0 \pm 1.1a$	$51.0 \pm 0.3 d$	$5.7 \pm 0.2 f$	$84.2 \pm 2.1 b$	$61.9 \pm 0.3 c$	
Try-AC	$0.6 {\pm} 0.0 a$	$0.4 \pm 0.1 b$	$0.7 \pm 0.0 a$	$0.0 \pm 0.0 c$	$0.0\pm0.0c$	$0.0 \pm 0.0 c$	
Try-EDA	$6.9 \pm 0.2 b$	$39.1 {\pm} 0.5 a$	$8.6 \pm 0.2 b$	$3.3 \pm 0.0c$	$8.5 \pm 0.9 b$	$1.9 \pm 0.0c$	
Pinoresinol	$5.5 \pm 0.1 d$	$0.0 \pm 0.0 f$	$20.6 {\pm} 0.1 a$	$2.0 \pm 0.3 e$	$12.6 \pm 0.9 b$	$7.6 {\pm} 0.2 c$	
Hy-EA	$13.7 {\pm} 0.2 c$	$30.9 \pm 1.5a$	$14.3 {\pm} 0.2 c$	$6.2 \pm 0.8 d$	$28.4 \pm 2.0a$	$19.7 \pm 0.3 b$	
Luteolin	$3.4 \pm 0.1a$	$2.3 \pm 0.0 b$	$2.6 {\pm} 0.2 \mathrm{b}$	$1.0 \pm 0.0 c$	$1.1{\pm}0.2\mathrm{c}$	$0.4 {\pm} 0.0 {\rm d}$	
Apigenin	$3.4 \pm 0.1a$	$1.4 \pm 0.3 bc$	$1.3 \pm 0.0 bc$	$0.4 {\pm} 0.1 c$	$2.7 \pm 0.9 ab$	$0.3 \pm 0.0 c$	
Methy-luteolin	$2.6 {\pm} 0.1 \mathrm{b}$	$1.4 \pm 0.1 d$	$3.1 \pm 0.1a$	$1.9\pm0.1c$	$2.6 \pm 0.0 b$	$0.4 {\pm} 0.0 \mathrm{e}$	
Try-EA	$48.4{\pm}0.2c$	$58.3 \pm 0.3 a$	$48.9 {\pm} 0.1 c$	$55.6 \pm 0.5 b$	$26.9 \pm 0.9 d$	$25.5 {\pm} 0.1 {\rm d}$	
TPC	$111.1 \pm 0.2 e$	$241.4 \pm 0.1a$	$163.5 {\pm} 0.4 {\rm c}$	$101.9 {\pm} 1.0 \mathrm{e}$	$189.8 \pm 0.4 \mathrm{b}$	$134.7 \pm 0.3 d$	

Hy: Hydroxytyrosol; Try: Tyrosol; Hy-AC: Hydroxytyrosol acetate; Hy-EDA: Decarboxymethyl oleuropein aglycone; Try-AC: Tyrosol acetate; Try-EDA: Decarboxymethyl ligstroside aglycone; Hy-EA: Oleuropein aglycon; Try-EA: Ligstroside aglycone; TPC: total phenolic compounds.

Table 4 Correlation coefficient between antioxidant capacities and chemical compounds of Coratina and

#### Koroneiki oils

	C16:0	C18:1	C18:2	MUFA/PUFA	UFA/SFA	α-Τ	β-Т	γ-Τ
DPPH	-0.260	-0.015	0.083	0.020	0.271	0.566*	0.527*	-0.320
FRAP	-0.253	-0.101	0.217	-0.114	0.297	0.429	0.536*	-0.171
ABTS	-0.469*	-0.030	0.280	-0.178	0.538*	0.273	0.311	0.021
ORAC	0.018	-0.211	0.164	-0.073	-0.014	0.536*	0.564*	-0.343
	$\delta$ - $\mathrm{T}$	TTs	TSs	Squalene	Ну	Try	Vanillic acid	Vanillin
DPPH	0.524*	0.875**	0.180	-0.464	0.484*	-0.867**	0.070	-0.372
FRAP	0.577*	0.803**	0.069	-0.490*	0.544*	-0.882**	0.012	-0.431
ABTS	0.630**	0.662**	-0.045	-0.510*	0.605**	-0.864**	-0.266	-0.353
ORAC	0.297	0.786**	0.037	-0.234	0.574*	-0.885**	0.027	-0.413
	p-coumaric acid	Hy-AC	Ferulic acid	o-coumaric acid	Hy-EDA	Try-AC	Try-EDA	Pinoresinol
DPPH	-0.755**	-0.516*	0.196	0.205	0.814**	-0.057	0.596**	0.154
FRAP	-0.704**	-0.407	0.067	0.267	0.788**	0.102	0.613**	0.225
ABTS	-0.710**	-0.244	-0.097	0.491*	0.818**	0.243	0.525*	0.495*
ORAC	-0.718**	-0.586*	0.099	0.163	0.808**	-0.074	0.727**	-0.131
	Hy-EA	Luteolin	Apigenin	Methy-luteolin	Try-EA	TPs		
DPPH	0.841**	0.007	0.348	0.234	-0.118	0.870**		
FRAP	0.818**	0.152	0.427	0.329	-0.066	0.871**		
ABTS	0.739**	0.151	0.265	0.316	-0.126	0.856**		
ORAC	0.909**	0.070	0.415	0.061	-0.055	0.877**		

<sup>\*</sup> Significant correlation (p < 0.05)

Figure legends

- Fig. 1 The climate conditions of Xichang, Sichuan Province
- Fig. 2 Circos map displaying the relationships between Coratina and Koroneiki oils and phenolic compounds.
- Fig. 3 Antioxidant capacities of Coratina and Koroneiki oils

Fig. 4 Hierarchical cluster dendrogram of studied samples according to (a) major fatty acids (>0.5%, C16:0, C16:1, C18:0, C18:1, C18:2, C18:3), and minor components ( $\alpha$ -tocopherol,  $\beta$ -tocopherol,  $\gamma$ -tocopherol,  $\delta$ -tocopherol, phenolic compounds, total sterols, and squalene); (b) the structure of phenolic compounds

#### Hosted file

Figure.docx available at https://authorea.com/users/319780/articles/449487-effects-of-tree-age-on-the-chemical-compositions-and-antioxidant-activities-of-coratina-and-koroneiki-olive-oils-from-youth-trees-in-china

<sup>\*\*</sup> Significant correlation (p < 0.01)