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Biochemical characterization of olive oil samples obtained from fruit mixtures and from oil blends of four cultivars grown in Central Tunisia

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Abstract – Blends of olive oils obtained from four cultivars (*Olea europaea* L. cv. Chemlali, Chetoui, Oueslati and Koroneiki) were produced by two different methods of blending: processing fruit mixtures or mixing monovarietal oils, using the same proportions of selected cultivars. The obtained blends were biochemically characterized to evaluate quality, and the two methods were compared. The results indicated that the most successful formulations are mainly F8 (60% Chemlali × 20% Oueslati × 20% Koroneiki) characterized by the highest contents of phenols and an elevated oxidative stability, and F5 (50% Chemlali × 50% Koroneiki) containing the highest MUFA level and the highest oxidative stability. The effect of the blending process on pigments and volatiles cannot be easily regulated, unlike phenols, fatty acid composition and OS, all of which positively correlated to the fruit mass ratio in the blend. Results suggest that processing fruit mixtures of different cultivars resulted in a better oil quality than that of oils obtained by the common oil blending method. This blending procedure offers a possibility to modulate the contents of antioxidants, fatty acids and volatile compounds in virgin olive oil, and therefore, its quality and sensorial characteristics.

Keywords: *Olea europaea* L. / blending / antioxidants / fatty acids / volatile compounds

Résumé – Caractérisation biochimique d'échantillons d'huile d'olive obtenus à partir de mélanges de fruits et de mélanges d'huiles de quatre cultivars cultivés dans le centre de la Tunisie. Quatre variétés d'olivier prospectées au centre de la Tunisie (*Olea europaea* L. cv. Chemlali, Chetoui, Oueslati et Koroneiki) ont fait l'objet d'un essai de mélanges variétaux par deux méthodes de coupage, en vert et en huile, tout en utilisant la Chemlali comme variété principale. La méthode classique de coupage à huile consiste à mélanger les huiles monovariétales de deux ou trois variétés à des proportions bien définies. Par contre, La méthode de coupage en vert consiste à mélanger les fruits de ces mêmes variétés avant leur broyage, et en extraire l'huile polyvariétale. Les mélanges obtenus ont été caractérisés biochimiquement pour évaluer la qualité. Les résultats ont indiqué qu'une meilleure qualité d'huile a été obtenue avec la méthode de coupage en vert. Les formulations les plus réussies sont principalement F8 (60 % Chemlali × 20 % Oueslati × 20 % Koroneiki) caractérisée par le contenu le plus élevé de phénols

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(875,32 mg/kg) et une stabilité oxydative élevée (OS = 11,88 h), et F5 (50 % Chemlali × 50 % Koroneiki) contenant le niveau le plus élevé de MUFA (70,69 %) et l'OS la plus élevée (13,83 h). L'effet du coupage sur les pigments et les composés volatiles ne peut pas être facilement régulé, contrairement aux phénols, à la composition acidique et à l'OS, qui sont tous en corrélation positive avec le pourcentage des fruits de chaque variété dans le mélange. Cette procédure offre la possibilité de moduler les teneurs en antioxydants, en acides gras et en composés volatils dans l'huile d'olive, et donc sa qualité et ses caractéristiques sensorielles.

Mots clés : *Olea europaea* L. / coupage / antioxydants / acides gras / composes volatiles

1 Introduction

Olive oil is often the fat preferred by consumers who are conscious of the health benefits of the Mediterranean diet. The health benefits of olive oil are attributed to the high contents of oleic acid and other minor components, especially phenol derivatives. Oils rich in monounsaturated fatty acids (MUFAs) and poor in saturated fatty acids (SFAs) are favored because of the confirmed valuable effect of MUFAs on serum cholesterol levels (Preedy and Watson, 2010). It is well known that the consumption of certain natural antioxidants, in particular polyphenols, has several beneficial effects on health. These bioactive compounds have strong radical scavenging capacity and can play a key role in the protection against oxidative damages and cellular aging (Talhaoui *et al.*, 2015). Besides of their bioactivity, phenols have a major role in the pungent and bitter taste of the oil and greatly improve its oxidative stability (Pedan *et al.*, 2019).

The biological properties of olive oil are also associated to the occurrence of other minor components such as squalene and phytosterols, as well as α-tocopherol and β-carotene which were reported to have an important role in preventing oxidation and could help to prolong the shelf-life of extra virgin olive oil (Jimenez-Lopez *et al.*, 2020). Chlorophylls and carotenoids are the main pigments responsible for the distinctive color of the oil. More importantly, there is a renewed interest in these pigments in relation to the health benefits they may provide (Moyano *et al.*, 2010).

Tunisian orchards are rich in several varieties, but they are dominated by two major ones: Chetoui in the North and Chemlali in the Centre and the South. Thus, it is necessary to conduct research on secondary cultivars in order to diversify Tunisian olive oil resources and enhance the quality.

Chemlali variety, although appreciated by many Tunisian consumers, contains high levels of palmitic and linoleic acids and low levels of monounsaturated fatty acids (especially oleic acid) and phenolic derivatives (Dabbou *et al.*, 2009). These shortcomings could greatly limit Tunisian olive oil exportation, particularly in the existence of a very competitive international market that requires high quality. In addition, taking into consideration the health benefits of MUFA and minor compounds, particularly phenols and their effect on oxidative stability of the oils, there is a need to select an introduced cultivar that contributes to the enhancement of quality and health value of Chemlali oil by blending process. For this reason, Tunisia has encouraged the plantation of several Mediterranean varieties. In their origin areas of cultivation, these cultivars produce oils with different biochemical compositions and sensory profiles. Nevertheless, before using these new cultivars in blending process, their behavior in other sites must be evaluated. Indeed, the biochemical composition of olive oil, besides being strongly dependent on cultivar, is also affected by many other factors, essentially climatic and edaphic conditions (Ben Youssef *et al.*, 2012).

Several studies have investigated the effect of the common method of oil blending on the final quality of Chemlali oil (Issaoui *et al.*, 2009; Youssef *et al.*, 2014). However, very few studies have been conducted on the effect of using the method of processing olive fruits of different cultivars on the final extracted oil. Reboredo-Rodríguez *et al.* (2015) observed that the olive co-crushing of four cultivars in different proportions clearly influenced the sensory property of the resulting oils. Angerosa and Basti (2003) compared only the volatile fraction of oil samples obtained by the two methods of blending using two cultivars.

In the current study, quality indices, pigment and phenolic concentrations, acidic and volatile profiles, as well as oxidative stability, were assessed with the aim: (i) to compare the biochemical compositions of the oils of these cultivars; (ii) to investigate the best combinations of blending that lead to a great improvement of the quality of Chemlali; (iii) to compare between the two methods of blending.

2 Material and methods

2.1 Fruit sampling and blending program

Fruits from 4 different *Olea europaea* L. cultivars, namely Chemlali, Chetoui, Oueslati and Koroneiki, grown in olive groves of central Tunisia (Sidi elHeni, governorate of Sousse), were harvested by hand, in December 2019. The quantity of olive fruits required for the virgin olive oil extraction was calculated previously based on the pre-established blending program. So, about 100 kg of Chemlali fruits and 35 kg of Chetoui, Oueslati and Koreneiki fruits were harvested. The maturity index (MI) of the fruits of each cultivar was determined according to the method described by Gutiérrez *et al.* (1999), which consists of the estimation of olive skin and pulp color. The four studied cultivars didn't have much different dynamics of fruit ripening. At the moment of sampling, Koroneiki, Chetoui, Chemlali and Oueslati had comparable maturity indices (3.5, 3.9, 4.1 and 4.3, respectively). Most of the fruits of Koreneiki and Chetoui had light violet epidermis, and most of the fruits of Chemlali and Oueslati had black epidermis.

The following program was conducted to prepare fruit samples:

a. Mixtures of olive fruits from two or three cultivars were prepared directly after harvest, with pre-established

proportions (20, 40 and 60%). Fruit blends were processed within 24 h of the olive harvest.

b. Monovarietal oils were also extracted within 24 h of the olive harvest.
c. Then, the same proportions of cultivars (20, 40 and 60%) were used to prepare blends from oil mixtures of the same cultivars.

Taking into account variations in conditions in the common oil extraction practice, three independent repetitions were conducted for a single sample (of monovarietal oils and of fruit or oil combinations).

Chemlali is present in all blended oil samples as the main cultivar, at proportions of 50% and 60%, the most used percentages of blending in commercial oils.

2.2 Sample designations

- **From F1 to F8:** oil samples obtained from processing fruit mixtures of two or three cultivars.
- **From O1 to O8:** oil samples obtained from monovarietal oil mixtures.

The following proportions of cultivars were established:

- **bi-varietal blended oils:** F1 and O1: 50% Chemlali × 50% Chetoui; F2 and O2: 60% Chemlali × 40% Chetoui; F3 and O3: 50% Chemlali × 50% Oueslati; F4 and O4: 60% Chemlali × 40% Oueslati; F5 and O5: 50% Chemlali × 50% Koroneiki; F6 and O6: 60% Chemlali × 40% Koroneiki.
- **Multivarietal blended oils:** F7 and O7: 60% Chemlali × 20% Chetoui × 20% Koroneiki; F8 and O8: 60% Chemlali × 20% Oueslati × 20% Koroneiki.

2.3 Oil extraction procedure and blending process

Olive oil was extracted using an Abencor system small-quantity mill, simulating commercial oil-extraction systems (MC2 Ingenieria Sistemas, Seville, Spain). Only healthy fruits were processed after removing leaves. These steps were followed in oil extraction: fruit crushing with a hammer mill (1.5 kg), malaxation of the paste (approximately 700 g) for 30 min at 26 ± 0.5°C, and centrifugation in a two-phase decanter (3500 rpm over 1 min, at room temperature). Malaxers are provided of a top cover to prevent the oxygen exposure and placed in the thermostated water bath. The extracted oil samples were filtered through the filter paper and stored in obscurity in fully filled and amber glass bottles until analyses.

Blending process was carried out carefully in a protected environment from oxidative damage. For each blend, a sample of 1.5 L was prepared; the appropriate amounts of monovarietal oils were transferred in a 2 L Erlenmeyer equipped with a magnetic stirrer (to ensure sample homogenization) and a nitrogen bubbling device. Bubbling and stirring were sustained for 15 min. The obtained oil blend was then transferred into three 500 ml fully filled amber glass bottles and saturated with nitrogen before hermetic closure. The blends were stored in obscurity at low room temperature (18 ± 1 °C), until analyses.

2.4 Physico-chemical and biochemical characterization of extracted oil samples

Free fatty acidity and UV absorption coefficients (K_{232} and K_{270}) were determined following the methods described in the European Union Commission Regulation 2568/91.

Chlorophyll and carotenoid concentrations were analyzed following the method of Minguez-Mosquera *et al.* (1991). The chlorophyll content was determined at maximum absorption of 670 nm, while the carotenoid fraction was quantified at 470 nm.

Total phenols were extracted using methanol/Tween 20 mixture (2% v/w), and then colorimetrically quantified, according to the method of Montedoro *et al.* (1992) using the Folin–Ciocalteu reagent. The concentrations of total phenols were measured at 765 nm and values were expressed as mg of gallic acid per kilogram of oil.

Oleuropein aglycon and ligstroside aglycon are the most potent bitter tastants in olive oil (Cui *et al.*, 2021) and have been shown to display antioxidant and free radical-scavenging activities. So, based on the polyphenol content, the bitterness intensity (IBe) can be estimated using the following equation (Beltrán *et al.*, 2007):

$$IBe = -7 \times 10^{-6} \times Phenols^2 + 0.0123 \times Phenols - 0.8722.$$

The fatty acid analyses were carried out by gas chromatography (HP6890, Hewlet Packard Inc., USA) following the procedures described by Tekaya *et al.* (2016) using a method based on gas chromatography of the methyl esters of the fatty acids extracted with hexane. A standard fatty acid methyl ester reference mixture was used to identify the different peaks of fatty acids in each sample.

Oxidative stability was analyzed using a Rancimat apparatus (Mod. 743, Metrohm, Schweiz AG, Zofingen, Switzerland) with the method described by Tura *et al.* (2007). The Oxidation Stability Index (OSI) value is designated as the number of hours required for the rate of change in conductivity to reach a predetermined value.

Finally, the volatile fraction was sampled using Supelco SPME (solid-phase microextraction) and GC–MS analysis was performed following the method described by Ascrizzi *et al.* (2016).

2.5 Statistical analysis

Statistical analyses were conducted using the statistical package for social sciences (SPSS) program, release 11.0 for Windows (SPSS, Chicago, IL, USA). Values of measured parameters are shown as means ± standard deviations (n = 3). Duncan's test was applied to identify significant differences among data. The statistical significance level was fixed at $p < 0.05$.

3 Results and discussion

3.1 Quality parameters

All extracted oil samples are classified as "extra virgin olive oil" samples; values of acidity, K_{232} and K_{270} fell within the range of 0.8%, 2.50 and 0.22, respectively established for the highest quality category "extra virgin olive oil" (Regulation EC/1989/2003) (Tab. 1). As reported by several authors (Borges *et al.*, 2016; Theodosi *et al.*, 2021), these quality indices are basically affected by factors causing damage to the fruits (*e.g.*, olive fly attacks or improper systems of harvesting, inconvenient conditions of transport and storage of olives) or to the oil (the processing technology and conditions of oil storage).

3.2 Effects of the blending process on the content of pigments

ANOVA test shows significant differences between the four cultivars ($P < 0.001$) in pigment concentrations (Tab. 1). Chetoui is the least-pigmented oil (1.01 and 5.38 mg kg^{-1} of chlorophylls and carotenoids, respectively). In contrast, pigments in Chemlali, Oueslati and Koroneiki olive oils were much more abundant; Oueslati is characterized by the highest carotenoid content (18.40 mg/kg) while Koroneiki exhibits the highest content in chlorophylls (5.78 mg/kg). Previous studies reported that the concentration of pigments can vary significantly depending on the variety (or cultivar) (Omri *et al.*, 2020), the fruits ripening degree (Hassine *et al.*, 2021), the climate (Criado *et al.*, 2008) and growing conditions (Bedbabis *et al.*, 2015).

The blending process affected significantly the amounts of pigments according to the proportion of cultivar in the blended oil. Indeed, because of its low contents in pigments, blending with Chetoui oil lead to a sharp decrease of pigment concentration with respect to Chemlali olive oil (F1, O1, F2, O2). However, it seems that blending oils of different cultivars does not necessarily result in a cumulative effect of the constituents of their oils. In fact, blending Chemlali with Oueslati, two oils rich in carotenoids, resulted in a significant decrease of the carotenoid concentration in their final blends (F3, O3, F4, O4). On the other hand, blending with Koroneiki contributed to a significant increase in chlorophyll concentration of Chemlali oil, in F5 and F6 blends. From the above results, it can be concluded that it is rather difficult to produce a blended olive oil with predictable pigment content.

Comparing the two methods of blending, in general, the concentrations of chlorophylls and carotenoids were higher in oil samples obtained by processing blends of fruits than in those obtained by the oil mixing method. Among all tested blends, the combination F8 is the most pigmented oil, followed by F6.

3.3 Variation of total phenol content and bitterness intensity following the proportion of cultivars and the method of blending

As summarized in Table 1, comparing between monovarietal oils, statistical analysis revealed that Koroneiki had the lowest concentration of total phenols (452.39 mg/kg), while the difference between phenol contents in Chemlali, Chetoui and Oueslati oil samples was not significant. These results are in accordance with that reported by some authors about the great influence of olive cultivar on phenolic content in olive oil (Baiano *et al.*, 2013). On the basis of the classification established by Montedoro *et al.* (1992), the three autochthonous cultivars (Chemlali, Chetoui and Oueslati) are characterized by high contents of phenolic compounds (> 500 ppm). The introduced cultivar (Koroneiki) presented a medium concentration of phenols (200–500 ppm).

Regarding blended oil samples, since the contents of polyphenols in Chemlali, Chetoui and Oueslati oils are almost similar, and that of Koroneiki is relatively lower, mixtures between Chemlali and other oils from the three cultivars did not significantly improve the concentration of total phenols in the final blended oil. However, it is clearly observed that milling fruit blends from two or three cultivars contributed to the enhancement of the pool of phenolic compounds in the final blended oil in comparison to the initial amount ($p < 0.001$). In all oil samples obtained by fruit blending process, the amount of total phenols was much higher than in those obtained by the common oil blending method. In addition, the concentrations of total phenols in the final blended oils appeared to change proportionally to the relative amount of each monovarietal fruits in the blends. Phenolic concentration slowly increased with the proportion of Chetoui and Oueslati in the blend. It is also remarkable that by mixing Chemlali fruits with both Oueslati and Koroneiki ones, even at only 20% of each introduced cultivar, total phenol content underwent to about 50% of increase. Indeed, F8 is characterized by the highest phenolic content (875.32 mg/kg), followed by F1 (about 35% of increase) and F2 (about 31.3% of increase).

Bitterness is considered a positive sensory characteristic of olive oil and is often attributed to the presence of phenolic derivatives. By considering the phenol content, the bitterness intensity (IBe) can be predicted and oils can be categorized into different groups as suggested by Beltrán *et al.*, 2007. Thus, in the current work, all the studied olive oil samples (monovarietal and blended oils) should be considered as very bitter oils (IBe > 3).

From a commercial point of view, because of their high levels of phenolic compounds, the obtained blended oils had the disadvantage to be too bitter, characteristics which might be not accepted by some consumers (Beltrán *et al.*, 2007). From a health point of view, these oils are characterized by a high health value due to the bioactive properties of these derivatives.

3.4 Changes in fatty acid composition

Regarding the fatty acid methyl esters (FAMEs) composition, Koroneiki is distinguished by an interesting acidic profile (Tab. 2) with the highest proportion of monounsaturated fatty acids (MUFA; 77.55%) and the lowest level of polyunsaturated fatty acids (PUFA; 7.82%) because of its elevated oleic acid content (C18:1; 76.32%) and low linoleic acid content (C18:2; 6.69%). Consequently, the ratio MUFA/PUFA, which is of great importance for its effects on the health properties of olive

Table 1. Quality parameters and antioxidant profiles of monovarietal and blended oil samples.

			Free fatty acidity (% oleic acid)	K_{232}	K_{270}	Carotenoids (mg/kg)	Chlorophylls (mg/kg)	Total phenols (mg/kg)	IBe	OS(h)
Chemlali 100%			0.24 ± 0.02 efg	2.38 ± 0.02 bcd	0.18 ± 0.01 g	15.47 ± 0.31 b	4.52 ± 0.04 ef	584.95 ± 36.11 ef	3.92 ± 0.15 def	5.34 ± 0.07 ij
Chetoui 100%			0.20 ± 0.01 ij	2.15 ± 0.01 i	0.14 ± 0.00 j	5.38 ± 0.19 k	1.01 ± 0.02 n	566.16 ± 9.95 ef	3.85 ± 0.04 ef	5.08 ± 0.05 k
Oueslati 100%			0.28 ± 0.02 ab	2.32 ± 0.02 ef	0.19 ± 0.00 efg	18.40 ± 0.42 a	1.04 ± 0.06 n	545.51 ± 39.15 f	3.75 ± 0.18 f	4.25 ± 0.21 m
Koroneiki 100%			0.26 ± 0.01 bcde	2.41 ± 0.02 ab	0.19 ± 0.00 def	12.72 ± 0.55 d	5.78 ± 0.28 c	452.39 ± 25.42 h	3.26 ± 0.15 g	11.84 ± 0.00 b
Chemlali × Chetoui	50% Chetoui	F1	0.22 ± 0.02 ghi	2.25 ± 0.01 g	0.17 ± 0.01 h	5.24 ± 0.11 k	1.99 ± 0.05 k	788.84 ± 19.58 b	4.47 ± 0.02 ab	8.47 ± 0.00 e
		O1	0.19 ± 0.01 j	2.37 ± 0.02 cd	0.17 ± 0.00 h	8.48 ± 0.46 j	1.85 ± 0.00 klm	547.33 ± 23.82 f	3.76 ± 0.11 f	5.50 ± 0.04 hi
	40% Chetoui	F2	0.26 ± 0.01 cdef	2.39 ± 0.01 bc	0.17 ± 0.01 h	8.84 ± 0.30 ij	1.90 ± 0.01 kl	768.00 ± 19.79 b	4.44 ± 0.03 ab	8.34 ± 0.04 e
		O2	0.19 ± 0.01 j	2.44 ± 0.02 a	0.18 ± 0.00 fg	9.27 ± 0.05 hi	2.09 ± 0.21 k	558.18 ± 37.37 ef	3.81 ± 0.17 ef	5.33 ± 0.28 ij
Chemlali × Oueslati	50% Oueslati	F3	0.24 ± 0.02 fgh	2.37 ± 0.02 cd	0.19 ± 0.01 def	10.51 ± 0.38 f	3.05 ± 0.18 i	776.58 ± 8.89 b	4.46 ± 0.01 ab	9.41 ± 0.02 c
		O3	0.25 ± 0.01 def	2.40 ± 0.01 abc	0.20 ± 0.00 bcd	8.76 ± 0.23 j	1.63 ± 0.01 m	541.16 ± 31.45 f	3.73 ± 0.15 f	4.79 ± 0.08 l
	40% Oueslati	F4	0.28 ± 0.02 abc	2.32 ± 0.01 ef	0.20 ± 0.01 a	11.49 ± 0.03 e	2.71 ± 0.20 j	681.63 ± 18.89 c	4.26 ± 0.05 bc	6.83 ± 0.16 f
		O4	0.27 ± 0.01 bcd	2.42 ± 0.01 ab	0.21 ± 0.01 abc	11.45 ± 0.15 e	1.70 ± 0.05 lm	550.28 ± 43.14 f	3.77 ± 0.20 f	4.42 ± 0.14 m
Chemlali × Koroneiki	50% Koroneiki	F5	0.24 ± 0.02 fgh	1.90 ± 0.04 k	0.15 ± 0.01 i	9.38 ± 0.27 h	6.86 ± 0.03 b	610.84 ± 10.32 de	4.03 ± 0.04 cde	13.83 ± 0.08 a
		O5	0.20 ± 0.02 ij	2.32 ± 0.02 ef	0.21 ± 0.01 ab	9.90 ± 0.20 g	4.74 ± 0.11 e	480.46 ± 6.58 gh	3.42 ± 0.04 g	5.85 ± 0.06 g
	40% Koroneiki	F6	0.30 ± 0.02 a	1.97 ± 0.03 j	0.21 ± 0.00 ab	13.65 ± 0.24 c	7.30 ± 0.04 a	658.74 ± 9.58 cd	4.19 ± 0.03 c	8.83 ± 0.16 d
		O6	0.25 ± 0.02 def	2.30 ± 0.02 f	0.19 ± 0.00 cde	10.57 ± 0.30 f	3.71 ± 0.12 g	530.11 ± 18.32 fg	3.68 ± 0.09 f	5.82 ± 0.07 g
Chemlali × Chetoui × Koroneiki	20% Chetoui + 20% Koroneiki	F7	0.21 ± 0.01 hij	2.21 ± 0.03 h	0.20 ± 0.00 cde	9.26 ± 0.16 hi	4.31 ± 0.28 f	649.00 ± 33.00 cd	4.16 ± 0.11 cd	11.91 ± 0.02 b
		O7	0.20 ± 0.01 ij	2.35 ± 0.05 de	0.21 ± 0.00 a	8.50 ± 0.28 j	2.62 ± 0.22 j	556.63 ± 72.87 ef	3.78 ± 0.35 ef	5.20 ± 0.10 jk
Chemlali × Oueslati × Koroneiki	20% Oueslati + 20% Koroneiki	F8	0.28 ± 0.01 abc	1.96 ± 0.02 j	0.20 ± 0.01 cde	13.04 ± 0.19 d	5.45 ± 0.27 d	875.32 ± 35.74 a	4.53 ± 0.01 a	11.88 ± 0.05 b
		O8	0.22 ± 0.02 ghi	2.32 ± 0.01 ef	0.19 ± 0.00 efg	11.15 ± 0.05 e	3.37 ± 0.02 h	563.16 ± 45.50 ef	3.83 ± 0.21 ef	5.71 ± 0.07 gh

IBe: Estimated bitterness intensity; OS: Oxidative Stability; F1...F8: blended oils obtained from fruit mixtures of the four studiedcultivars (Chemlali, Chetoui, Koreneiki, Oueslati) with different proportions; O1...O8: blended oils obtained from oil mixtures. F1 and O1: 50% Chemlali × 50% Chetoui; F2 and O2: 60% Chemlali × 40% Chetoui; F3 and O3: 50% Chemlali × 50% Oueslati; F4 and O4: 60% Chemlali × 40% Oueslati; F5 and O5: 50% Chemlali × 50% Koroneiki; F6 and O6: 60% Chemlali × 40% Koroneiki; F7 and O7: 60% Chemlali × 20% Chetoui × 20% Koroneiki; F8 and O8: 60% Chemlali × 20% Oueslati × 20% Koroneiki.

Each value is an average of three determinations ± S.D. Values in the same column with different letters show statistically significant differences ($P < 0.05$).

Table 2. Fatty acid composition (%) of monovarietal (Chemlali, Chetoui, Oueslati and Koroneiki) and blended olive oil samples obtained from fruit mixtures (F1...F8) or oil mixtures (O1...O8).

	Chemlali 100%	Chetoui 100%	Oueslati 100%	Koroneiki 100%	Chemlali × Chetoui			
					50% Chetoui		40% Chetoui	
					F1	O1	F2	O2
C16:0	16.54 ± 0.04 a	10.92 ± 0.06 i	16.34 ± 0.02 a	10.70 ± 0.03 i	13.71 ± 002 fg	13.64 ± 0.04 fg	14.19 ± 0.09 cde	13.20 ± 0.86 h
C16:1	2.16 ± 0.01 a b	0.43 ± 0.01 p	2.22 ± 0.00 a	0.99 ± 0.01 o	1.21 ± 0.00 n	1.22 ± 0.00 n	1.37 ± 0.01 m	1.36 ± 0.02 m
C17:0	0.01 ± 0.00 d	0.01 ± 0.00 d	0.01 ± 0.00 d	0.04 ± 0.00 bcd	0.08 ± 0.01 bc	0.07 ± 0.01 bc	0.07 ± 0.01 bc	0.19 ± 0.02 a
C17:1	0.10 ± 0.02 b	0.08 ± 0.01 b	0.12 ± 0.00 b	0.11 ± 0.00 b	0.10 ± 0.01 b	0.1 ± 0.00 b	0.1 ± 0.00 b	0.22 ± 0.11 a
C18:0	2.74 ± 0.00 i	3.27 ± 0.00 a	3.00 ± 0.01 c	2.59 ± 0.01 k	3.03 ± 0.01 b	3.02 ± 0.01 b	3.02 ± 0.00 b	3.00 ± 0.02 c
C18:1	60.78 ± 0.01 e	59.33 ± 0.06 e	60.08 ± 0.01 e	76.32 ± 0.11 a	59.58 ± 0.01 e	59.98 ± 0.04 e	59.75 ± 0.05 e	56.34 ± 3.82 f
C18:2	15.47 ± 0.01 ef	23.30 ± 0.05 a	15.76 ± 0.01 e	6.69 ± 0.10 j	19.81 ± 0.00 b	19.48 ± 0.01 b	19.06 ± 0.02 c	17.86 ± 0.98 d
C18:3	1.12 ± 0.01 l	1.40 ± 0.01 a	1.38 ± 0.01 b	1.13 ± 0.01 kl	1.28 ± 0.00 c	1.25 ± 0.00 ef	1.26 ± 0.00 d	1.25 ± 0.00 de
C20:0	0.47 ± 0.01 fgh	0.49 ± 0.01 de	0.55 ± 0.00 a	0.43 ± 0.01 i	0.48 ± 0.01 ef	0.48 ± 0.00 ef	0.49 ± 0.00 ef	0.49 ± 0.23 ef
C20:1	0.14 ± 0.01 d	0.11 ± 0.01 g	0.16 ± 0.00 a	0.13 ± 0.01 e	0.12 ± 0.01 f	0.12 ± 0.01 f	0.13 ± 0.00 f	0.13 ± 0.01 f
C22:0	0.37 ± 0.01 e	0.61 ± 0.02 b	0.28 ± 0.01 h	0.78 ± 0.02 a	0.52 ± 0.01 c	0.54 ± 0.01 c	0.49 ± 0.01 d	0.48 ± 0.01 d
C24:0	0.09 ± 0.00 cdef	0.06 ± 0.00 d	0.10 ± 0.01 bcde	0.07 ± 0.00 defg	0.07 ± 0.00 defg	0.09 ± 0.01 cdef	0.08 ± 0.00 defg	0.08 ± 0.00 defg
SFA	20.22 ± 0.04 a	15.35 ± 0.03 i	20.28 ± 0.01 a	14.62 ± 0.00 j	17.89 ± 0.01 fg	17.84 ± 0.04 fg	18.33 ± 0.08 cd	17.98 ± 0.58 ef
MUFA	63.18 ± 0.04 j	59.95 ± 0.07 o	62.58 ± 0.01 l	77.56 ± 0.10 a	61.02 ± 0.00 n	61.43 ± 0.05 m	61.35 ± 0.05 m	61.29 ± 0.43 m
PUFA	16.59 ± 0.00 i	24.70 ± 0.04 a	17.14 ± 0.00 f	7.82 ± 0.10 q	21.09 ± 0.01 b	20.73 ± 0.01 c	20.32 ± 0.03 d	20.10 ± 0.02 e
MUFA/PUFA	3.81 ± 0.00 j	2.43 ± 0.01 p	3.65 ± 0.00 l	9.92 ± 0.14 a	2.89 ± 0.00 o	2.96 ± 0.00 n	3.02 ± 0.00 mn	3.05 ± 0.02 m

	Chemlali × Oueslati				Chemlali × Koroneiki				Chemlali × Chetoui × Koroneiki		Chemlali × Oueslati × Koroneiki	
	50% Oueslati		40% Oueslati		50% Koroneiki		40% Koroneiki		20% Chetoui + 20% Koroneiki		20% Oueslati + 20% Koroneiki	
	F3	O3	F4	O4	F5	O5	F6	O6	F7	O7	F8	O8
C16:0	16.36 ± 0.04 a	16.23 ± 0.05 a	16.30 ± 0.04 a	16.47 ± 0.17 a	13.47 ± 0.05 gh	13.85 ± 0.10 ef	14.01 ± 0.03 def	14.34 ± 0.09 cd	14.44 ± 0.00 c	14.54 ± 0.04 c	15.13 ± 0.09 b	15.23 ± 0.11 b
C16:1	2.14 ± 0.02 cd	2.12 ± 0.02 d	2.07 ± 0.00 e	2.18 ± 0.00 b	1.50 ± 0.01 l	1.58 ± 0.01 j	1.63 ± 0.02 i	1.67 ± 0.02 h	1.55 ± 0.00 k	1.62 ± 0.03 i	1.83 ± 0.02 g	1.88 ± 0.01 f
C17:0	0.03 ± 0.02 cd	0.08 ± 0.01 bc	0.08 ± 0.00 bc	0.06 ± 0.01 bcd	0.07 ± 0.00 bc	0.08 ± 0.00 bc	0.05 ± 0.02 bcd	0.08 ± 0.01 bc	0.07 ± 0.01 bc	0.1 ± 0.04 b	0.06 ± 0.00 bcd	0.07 ± 0.02 bc
C17:1	0.10 ± 0.01 b	0.11 ± 0.00 b	0.12 ± 0.00 b	0.12 ± 0.01 b	0.11 ± 0.00 b	0.12 ± 0.00 b	0.11 ± 0.00 b	0.12 ± 0.00 b	0.11 ± 0.00 b	0.11 ± 0.00 b	0.11 ± 0.01 b	0.12 ± 0.00 b
C18:0	2.87 ± 0.01 e	2.90 ± 0.01 d	2.84 ± 0.01 f	2.85 ± 0.01 ef	2.66 ± 0.01 j	2.66 ± 0.01 j	2.66 ± 0.00 j	2.67 ± 0.01 j	2.85 ± 0.01 ef	2.81 ± 0.02 g	2.76 ± 0.01 hi	2.78 ± 0.01 h
C18:1	60.51 ± 0.04 e	60.54 ± 0.01 e	60.64 ± 0.03 e	60.45 ± 0.14 e	68.94 ± 0.09 b	68.32 ± 0.05 bc	67.59 ± 0.04 bc	66.93 ± 0.05 c	63.36 ± 0.02 d	63.03 ± 0.13 d	64.08 ± 0.02 d	63.77 ± 0.10 d
C18:2	15.64 ± 0.01 e	15.60 ± 0.01 ef	15.61 ± 0.00 ef	15.55 ± 0.03 ef	10.82 ± 0.06 i	11.07 ± 0.01 i	11.55 ± 0.05 h	11.88 ± 0.00 h	15.22 ± 0.02 f	15.42 ± 0.01 ef	13.62 ± 0.07 g	13.79 ± 0.00 g
C18:3	1.24 ± 0.00 f	1.26 ± 0.00 d	1.22 ± 0.00 g	1.22 ± 0.00 g	1.14 ± 0.00 j	1.12 ± 0.01 l	1.13 ± 0.01 jk	1.11 ± 0.01 m	1.18 ± 0.00 h	1.18 ± 0.00 h	1.18 ± 0.00 h	1.16 ± 0.01 i
C20:0	0.51 ± 0.00 c	0.53 ± 0.00 b	0.51 ± 0.00 c	0.51 ± 0.01 c	0.46 ± 0.00 fgh	0.45 ± 0.01 gh	0.46 ± 0.01 fgh	0.45 ± 0.01 h	0.47 ± 0.00 efg	0.50 ± 0.03 cd	0.48 ± 0.00 ef	0.49 ± 0.01 ef
C20:1	0.15 ± 0.00 bc	0.15 ± 0.01 bc	0.15 ± 0.00 bc	0.15 ± 0.00 bc	0.13 ± 0.00 e	0.13 ± 0.01 e	0.13 ± 0.01 e	0.13 ± 0.01 e	0.13 ± 0.00 e	0.13 ± 0.00 e	0.15 ± 0.01 bc	0.15 ± 0.00 bc
C22:0	0.35 ± 0.01 f	0.34 ± 0.02 g	0.37 ± 0.00 e	0.33 ± 0.01 fg	0.60 ± 0.01 b	0.54 ± 0.02 c	0.61 ± 0.01 b	0.54 ± 0.01 c	0.53 ± 0.00 c	0.48 ± 0.01 d	0.48 ± 0.01 d	0.47 ± 0.01 d
C24:0	0.10 ± 0.00 bc	0.13 ± 0.03 a	0.10 ± 0.01 bc	0.10 ± 0.00 b	0.10 ± 0.01 b	0.08 ± 0.00 b	0.08 ± 0.00 b	0.08 ± 0.01 b	0.08 ± 0.00 b	0.08 ± 0.01 b	0.11 ± 0.02 b	0.09 ± 0.00 b
SFA	20.21 ± 0.00 a	20.21 ± 0.02 a	20.20 ± 0.03 a	20.32 ± 0.16 a	17.35 ± 0.02 h	17.66 ± 0.06 g	17.85 ± 0.00 fg	18.15 ± 0.05 de	18.44 ± 0.00 c	18.51 ± 0.09 c	19.02 ± 0.03 b	19.12 ± 0.09 b
MUFA	62.91 ± 0.01 k	62.93 ± 0.03 k	62.98 ± 0.03 k	62.90 ± 0.13 k	70.69 ± 0.08 b	70.15 ± 0.04 c	69.46 ± 0.05 d	68.85 ± 0.04 e	65.16 ± 0.02 h	64.90 ± 0.10 i	66.18 ± 0.03 f	65.92 ± 0.09 h
PUFA	16.88 ± 0.01 g	16.86 ± 0.01 g	16.83 ± 0.00 gh	16.78 ± 0.03 h	11.96 ± 0.06 p	12.19 ± 0.02 o	12.69 ± 0.06 n	12.99 ± 0.01 m	16.40 ± 0.03 j	16.6 ± 0.01 i	14.80 ± 0.06 l	14.96 ± 0.01 k
MUFA/PUFA	3.73 ± 0.00 k	3.73 ± 0.01 k	3.74 ± 0.00 k	3.75 ± 0.00 k	5.91 ± 0.04 b	5.76 ± 0.01 c	5.47 ± 0.03 d	5.30 ± 0.00 e	3.97 ± 0.01 h	3.91 ± 0.01 i	4.47 ± 0.02 f	4.41 ± 0.01 g

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

Each value is an average of three determinations ± S.D. Values in the same row with different letters show statistically significant differences ($P < 0.05$). Each value is expressed as wt% total fatty acid methyl esters.

oils (Dag *et al.*, 2011), is high (9.92). In addition, Koroneiki produces an oil with a relatively low level of palmitic acid (C16:0; 10.70%) and saturated fatty acids (SFA; 14.62%).

On the other hand, Chetoui, cultivated in central Tunisia, produced oil with low MUFA and C18:1 levels (59.95% and 59.33%, respectively) and high levels of PUFA, especially C18:2 (24.70% and 23.30% respectively). C18:2 level exceeds the value reported as a maximum limit in Reg. UE 2019/1604 (21.0%). This is may be caused by agronomic and genetic factors. In fact, Chetoui cultivar has been reported to have a low genetic plasticity (Ben Youssef *et al.*, 2012); it showed significant differences in oil quality parameters, including linoleic acid content, when the olive trees were cultivated away from the original plantation site (in the north of the country).

The ratio MUFA/PUFA is consequently very low (2.43). Oueslati oil is characterized by relatively low levels of oleic acid (about 60%) and MUFA and high levels of palmitic acid, SFA and PUFA. Regarding Chemlali, this cultivar produces a virgin oil characterized by high levels of palmitic acid (16.54%) and SFA (20.22%), and relatively low levels of oleic acid (60.78%) and MUFA (63.18%). As observed in previous studies (Baccouri *et al.*, 2007), Chemlali olive oil (especially Chemlali from Sfax) is characterized by a low level of oleic acid (53%) and a high level of palmitic acid (20%). Thus, we suggest that, when grown in central Tunisia, Chemlali produces virgin oil with a relatively improved fatty acid profile compared to that produced by the same cultivars grown in the South of Tunisia. Taking into consideration the health benefits of MUFA, especially oleic acid, and their effect on oxidative stability of the oils, there is a need to select an introduced cultivar that contributes to the enhancement of quality and the concentrations of bioactive compounds in Chemlali oil by blending process. As shown in Table 2, blending with Koroneiki oil was the most effective to enhance the levels of oleic acid and MUFA and to reduce the levels of SFA, especially palmitic acid, as well as PUFA, comparing to those in pure Chemlali oil. In fact, at 40% blending with Koroneiki olive oil, oleic acid increased by 10.12% using the method of oil mixtures (O6) and by 11.20% using the method of fruit mixtures (F6). At 50% blending, it increased by 12.40% (in O5, oil mixture method) and by 13.42% (in F5, method of fruit mixtures). In parallel, palmitic acid underwent a significant decrease to 14.34% in O6 and to 14.01% in F6, at 40% blending. When the proportion of Koroneiki increases in the blended oil (at 50%), C16:0 was further reduced to 13.85% in O5 and to 13.47% in F5. Hence, the four bi-varietal blended oils showed improved fatty acid compositions with respect to that of pure Chemlali oil; they are characterized by the highest MUFA content and the lowest SFA and PUFA levels, which lead to a significant amelioration of the ratio MUFA/PUFA, suggesting the enhancement of stability to oxidation of the oil.

Blending with Chetoui oil (which is characterized by reduced palmitic acid and SFA levels along with low oleic acid and MUFA levels and elevated linoleic acid and PUFA levels), caused a significant decrease of C16:0 and SFA of Chemlali oil; however, PUFA and C18:2 exhibit sharp increases compared to the initial concentrations in Chemlali oil, while MUFA and C18:1 levels underwent a significant reduction.

Oueslati oil has an acidic profile quite close to that of Chemlali oil. Thus, blends of Chemlali and Oueslati did not show important modifications in fatty acid levels compared to pure Chemlali oil.

Regarding the multivarietal blends (F7 and F8), when investigating the acidic profiles, it is evident that the concentrations of fatty acids in the final blended oils are proportionally related to the relative percentage of each monovarietal oils or fruits in the mixtures. The presence of Koroneiki, even at only 20% in these oils, contributed to a significant improvement of the acidic profile of Chemlali oil, despite the presence of Chetoui and Oueslati (with relatively modest acidic profiles) in the same proportion (20%).

Comparing between the two methods of blending, in general, the two procedures did not significantly modify the fatty acid composition of the resulting olive oils, except for some changes that essentially affect blends of Chemlali with Koroneiki and Chetoui, which lead to changes in MUFA and PUFA levels. Interestingly, for all blends containing Koroneiki, the method of fruit mixing (F5, F6, F7 and F8) resulted in better quality of the oils in terms of richness in MUFA and an elevated ratio MUFA/PUFA. The assemblage F5 (50% Chemlali × 50% Koroneiki) appeared to be the most successful formulation due to its higher content of MUFA (especially C18:1) and to its lower content of SFA and PUFA (especially C18:2), leading to the enhancement of the ratio MUFA/PUFA. The above results demonstrated that the levels of fatty acids in the oil samples are positively correlated to the fruit mass ratio of the cultivars, which is in agreement with a previous work of Reboredo-Rodríguez *et al.* (2015).

These results suggest that the milling of fruits of different cultivars having different enzymatic activities did not cause any synergetic or antagonistic effect on the endogenous enzymes of each cultivar. Therefore, the fatty acid levels may be easily estimated in the oil obtained by the method of fruit blending.

3.5 Effects of blending on oxidative stability of oil samples

Oxidative stability (OS), measured by the Rancimat test at 120 °C, is a key parameter that contributes to the evaluation of olive oil quality. The results of the current study showed that this parameter depends essentially on the variety ($p < 0.05$). Table 1 shows that Koroneiki oil had the highest induction time, with an average of 11.84 h, followed by Chemlali oil (5.34 h). Oueslati had the lowest OS.

Using the common method of oil blending, the OS of Chemlali did not show a significant modification when blended with Chetoui oil. As expected, it decreased significantly when blended with Oueslati, because of the reduced OS of Oueslati oil. On the other hand, the OS of blends of Chemlali and Koroneiki exhibited a slight increase compared to that of pure Chemlali. Nevertheless, as observed for total phenols, oxidative stability of all oil samples obtained with the fruit blending process was significantly better than stability of samples obtained by the classic oil mixing method. Indeed, the procedure of fruit mixing allowed obtaining different blends with remarkably improved OS. The highest increase of OS (more than two-fold) was observed at 50% of blending with Koroneiki (assemblage F5). A very significant enhancement in Chemlali oil stability was observed using small proportions

Table 3. Changes in the levels of volatile compounds (%) in monovarietal (Chemlali, Chetoui, Oueslati and Koroneiki) and blended olive oil samples obtained from fruit mixtures (F1...F8) or oil mixtures (O1...O8).

	Chemlali 100%	Chetoui 100%	Oueslati 100%	Koroneiki 100%	Chemlali × Chetoui			
					50% Chetoui		40% Chetoui	
					F1	O1	F2	O2
Hexanal	2.90 ± 0.28 fghi	6.30 ± 0.28 a	4.40 ± 0.28 bc	3.10 ± 0.28 efgh	2.30 ± 0.14 i	3.30 ± 0.28 efg	3.10 ± 0.28 efgh	5.70 ± 0.28 a
(E)-2-Hexenal	33.80 ± 0.57 i	15.60 ± 0.57 k	50.40 ± 0.57 cd	31.30 ± 0.71 j	45.65 ± 1.20 e	9.70 ± 0.42 l	62.00 ± 1.56 a	29.50 ± 1.98 j
(Z)-2-Hexenal	–	–	–	–	–	–	–	–
(Z)-3-Hexenal	0.80 ± −0.00 fgh	8.10 ± 0.42 a	0.90 ± 0.14 efg	2.10 ± 0.14 b	1.25 ± 0.07 cd	1.00 ± 0.14 def	0.60 ± 0.00 hi	1.40 ± 0.14 c
(Z)-2-Heptenal	0.60 ± 0.00 a	0.55 ± 0.07 b	0.30 ± 0.00 d	0.30 ± 0.00 d	–	–	–	0.55 ± 0.07 b
(E)-4-Oxohex-2-enal	–	2.80 ± 0.28 a	–	1.20 ± 0.14 c	–	0.50 ± 0.00 e	–	2.20 ± 0.28 b
Nonanal	1.40 ± 0.00 efg	1.05 ± 0.07 gh	0.80 ± 0.14 hi	0.45 ± 0.07 i	0.80 ± 0.14 hi	2.95 ± 0.50 ab	1.20 ± 0.14 fgh	3.20 ± 0.28 a
1,3-Butanediol	0.70 ± 0.00 a	0.40 ± 0.00 b	–	–	–	–	–	–
(E)-2-Penten-1-ol	2.40 ± 0.14 bcd	1.70 ± 0.28 fg	0.40 ± 0.00 h	2.10 ± 0.28 bcdef	1.60 ± 0.28 fg	1.95 ± 0.35 cdefg	1.80 ± 0.28 efg	2.60 ± 0.28 b
1-Hexanol	2.60 ± 0.14 fg	3.00 ± 0.14 def	1.60 ± 0.14 h	3.30 ± 0.28 cde	1.40 ± 0.14 h	8.40 ± 0.57 a	2.00 ± 0.28 gh	4.05 ± 0.35 b
(E)-2-Hexen-1-ol	13.90 ± 0.42 b	–	14.10 ± 0.57 b	6.10 ± 0.28 g	17.60 ± 0.71 a	10.75 ± 0.64 d	–	1.90 ± 0.14 h
(Z)-3-Hexen-1-ol	–	7.50 ± 0.42 b	–	1.95 ± 0.07 d	–	14.15 ± 0.92 a	–	6.80 ± 0.71 c
3-Ethyl-1-hexanol	0.30 ± 0.00 bc	0.20 ± 0.00 cd	0.30 ± 0.00 bc	0.20 ± 0.00 cd	–	0.45 ± 0.07 a	–	0.50 ± 0.14 a
Benzyl alcohol	1.70 ± 0.14 a	1.30 ± 0.14 b	0.30 ± 0.00 h	0.50 ± 0.00 gh	0.60 ± 0.00 fg	1.75 ± 0.35 a	0.50 ± 0.00 gh	1.20 ± 0.14 bc
Phenylethyl alcohol	2.40 ± 0.14 b	1.30 ± 0.14 c	0.50 ± 0.00 fg	1.30 ± 0.00 c	0.40 ± 0.00 g	1.25 ± 0.35 cd	0.50 ± 0.00 fg	1.30 ± 0.28 c
(Z)-3-Hexenyl acetate	0.70 ± 0.00 j	12.40 ± 0.42 a	–	8.40 ± 0.57 b	5.25 ± 0.50 g	7.60 ± 0.57 c	4.30 ± 0.42 h	6.90 ± 0.42 cd
1-Hexyl acetate	–	6.80 ± 0.28 a	0.20 ± 0.00 h	3.90 ± 0.28 b	2.95 ± 0.35 cd	3.80 ± 0.42 b	2.40 ± 0.28 de	3.20 ± 0.28 c
Methyl salicylate	3.40 ± 0.42 a	–	–	–	0.75 ± 0.07 f	1.90 ± 0.28 bcd	1.60 ± 0.28 cde	2.15 ± 0.21 b
n-Tetradecane	2.80 ± 0.28 a	1.50 ± 0.14 efg	1.05 ± 0.07 gh	1.50 ± 0.14 efg	2.00 ± 0.14 cd	1.95 ± 0.21 cd	1.20 ± 0.28 fg	1.40 ± 0.28 fg
n-dodecane	0.40 ± 0.00 c	0.40 ± 0.00 c	–	0.30 ± 0.00 d	0.40 ± 0.00 c	0.60 ± 0.14 a	–	0.40 ± 0.00 c
3-Ethyl-1,5-octadiene	4.10 ± 0.14 abc	2.85 ± 0.21 gh	3.20 ± 0.28 efg	2.40 ± 0.14 h	3.00 ± 0.42 fgh	3.50 ± 0.57 cdefg	2.85 ± 0.07 gh	3.50 ± 0.28 cdefg
3,7-Decadiene	7.95 ± 0.35 abc	6.60 ± 0.28 def	6.10 ± 0.14 efg	4.90 ± 0.42 h	4.70 ± 0.28 h	8.05 ± 0.92 abc	5.40 ± 0.28 fgh	7.15 ± 0.64 cde
4,8-Dimethyl-1,3,7-nonatriene	1.50 ± 0.00 def	1.20 ± 0.14 ef	2.60 ± 0.28 a	2.50 ± 0.14 a	0.40 ± 0.00 g	1.10 ± 0.14 f	0.45 ± 0.07 g	0.60 ± 0.00 g
(E)-2-Dodecene	3.20 ± 0.28 j	5.20 ± 0.28 g	1.75 ± 0.21 k	18.55 ± 0.64 a	3.20 ± 0.28 j	5.20 ± 0.57 g	3.60 ± 0.42 i	4.80 ± 0.14 h
Limonene	2.30 ± 0.14 a	1.75 ± 0.07 b	0.80 ± 0.14 fg	0.85 ± 0.07 fg	0.70 ± 0.00 fg	1.20 ± 0.28 cde	0.65 ± 0.07 fg	1.50 ± 0.28 bc
α-Pinene	1.70 ± 0.14 a	0.40 ± 0.00 c	0.20 ± 0.00 d	0.40 ± 0.00 c	–	0.70 ± 0.14 b	–	0.40 ± 0.00 c
(E)-β-Ocimene	3.00 ± 0.28 abc	3.10 ± 0.28 ab	0.25 ± 0.07 j	0.00 j	2.80 ± 0.28 abcd	3.40 ± 0.42 a	2.50 ± 0.28 bcd	3.20 ± 0.14 a
α-copaene	0.5 ± 0.00 b	0.00 f	0.00 f	0.00 f	0.00 f	0.35 ± 0.07 d	0.30 ± 0.00 de	0.60 ± 0.00 a
(E,E)-α-Farnesene	–	5.60 ± 0.28 a	–	–	1.60 ± 0.14 d	3.20 ± 0.42 b	2.00 ± 0.28 c	2.30 ± 0.28 c
hexanal/*(E)*-2-hexenal ratio	0.09 ± 0.01 de	0.40 ± 0.03 a	0.10 ± 0.01 d	0.09 ± 0.01 de	0.05 ± 0.00 h	0.34 ± 0.04 b	0.05 ± 0.00 h	0.19 ± 0.00 c
Total aldehydes	39.50 ± 0.28 jk	31.60 ± 0.71 l	56.80 ± 0.28 de	37.75 ± 0.35 k	50.00 ± 0.85 g	16.95 ± 0.21 m	66.90 ± 1.70 b	40.35 ± 2.33 j
Total alcohols	24.00 ± 0.14 b	15.40 ± 0.28 g	17.20 ± 0.42 f	15.45 ± 0.92 g	21.60 ± 0.85 c	38.70 ± 0.71 a	4.80 ± 0.57 l	18.35 ± 0.07 e
Total esters	4.10 ± 0.42 g	19.20 ± 0.71 a	0.20 ± 0.00 e	12.30 ± 0.28 b	8.95 ± 0.07 de	13.30 ± 1.27 b	8.30 ± 0.42 e	12.25 ± 0.92 b
Total C_6 compounds	54.00 ± 0.85 gh	43.30 ± 1.27 k	71.40 ± 0.00 a	49.55 ± 0.78 ij	68.20 ± 1.56 b	47.80 ± 0.57 j	67.70 ± 1.56 b	51.55 ± 2.05 hi
Total C_5 compounds	2.40 ± 0.14 bcd	1.70 ± 0.28 fg	0.40 ± 0.00 h	2.10 ± 0.28 bcdef	1.60 ± 0.28 fg	1.95 ± 0.35 cdefg	1.80 ± 0.28 efg	2.60 ± 0.28 b
Total identified volatile compounds	95.05 ± 0.50 h	97.60 ± 0.00 fg	90.45 ± 0.35 i	97.80 ± 0.00 f	99.35 ± 0.07 ab	98.70 ± 0.14 cde	98.95 ± 0.21 bcd	99.00 ± 0.14 bc

	Chemlali × Oueslati				Chemlali × Koroneiki				Chemlali × Chetoui × Koroneiki		Chemlali × Oueslati × Koroneiki	
	50% Oueslati		40% Oueslati		50% Koroneiki		40% Koroneiki		20% Chetoui + 20% Koroneiki		20% Oueslati + 20% Koroneiki	
	F3	O3	F4	O4	F5	O5	F6	O6	F7	O7	F8	O8
Hexanal	4.95 ± 0.50 b	3.65 ± 0.35 de	3.50 ± 0.28 def	4.10 ± 0.42 cd	2.30 ± 0.28 i	2.70 ± 0.14 ghi	2.50 ± 0.14 hi	2.80 ± 0.28 fghi	3.30 ± 0.14 efg	2.80 ± 0.28 fghi	3.20 ± 0.42 efgh	2.90 ± 0.28 fghi
(E)-2-Hexenal	57.70 ± 0.85 b	62.80 ± 1.13 a	49.50 ± 0.99 d	59.10 ± 1.13 b	41.00 ± 0.57 fg	37.45 ± 0.78 h	48.50 ± 0.71 d	39.60 ± 1.13 g	49.30 ± 0.85 d	36.30 ± 0.57 h	52.20 ± 0.71 c	43.00 ± 0.99 f
(Z)-2-Hexenal	–	–	–	–	–	–	0.30 ± 0.00 c	–	0.60 ± 0.00 a	–	0.20 ± 0.00 d	–
(Z)-3-Hexenal	0.40 ± 0.00 ij	0.65 ± 0.07 ghi	0.40 ± 0.00 ij	0.20 ± 0.00 j	0.60 ± 0.00 hi	1.25 ± 0.07 cd	0.60 ± 0.00 hi	1.10 ± 0.14 de	0.50 ± 0.00 i	1.90 ± 0.14 b	0.40 ± 0.00 ij	1.15 ± 0.07 cde
(Z)-2-Heptenal	–	0.50 ± 0.00 b	0.50 ± 0.00 b	0.50 ± 0.00 b	0.40 ± 0.00 c	–	0.30 ± 0.00 d	0.50 ± 0.00 b	–	0.50 ± 0.00 b	–	0.50 ± 0.00 b
(E)-4-Oxohex-2-enal	–	–	–	–	–	0.80 ± 0.14 d	–	1.10 ± 0.14 c	–	1.20 ± 0.14 c	–	0.40 e
Nonanal	1.70 ± 0.14 def	3.40 ± 0.28 a	3.00 ± 0.28 ab	2.60 ± 0.42 bc	1.50 ± 0.00 efg	1.80 ± 0.28 de	1.55 ± 0.21 efg	2.20 ± 0.14 cd	1.70 ± 0.28 def	1.90 ± 0.14 de	1.80 ± 0.28 de	2.60 ± 0.42 bc
1,3-Butanediol	–	–	–	–	–	–	–	–	–	–	–	–
(E)-2-Penten-1-ol	0.85 ± 0.07 h	1.55 ± 0.21 fg	0.70 ± 0.00 h	1.40 ± 0.14 g	2.30 ± 0.28 bcde	2.45 ± 0.35 bcd	2.10 ± 0.28 bcdef	2.50 ± 0.14 bc	1.90 ± 0.14 defg	3.20 ± 0.42 a	1.80 ± 0.28 efg	2.00 ± 0.14 cdef
1-Hexanol	4.00 ± 0.28 b	3.00 ± 0.14 def	3.80 ± 0.42 bc	3.20 ± 0.28 cdef	1.80 ± 0.28 h	–	2.90 ± 0.28 ef	–	3.60 ± 0.42 bcd	2.00 ± 0.28	2.90 ± 0.42 ef	3.20 ± 0.28 cdef
(E)-2-Hexen-1-ol	2.10 ± 0.28 h	0.80 ± 0.14 i	2.10 ± 0.28 h	0.75 ± 0.07 i	8.80 ± 0.42 e	9.40 ± 0.42 e	1.20 ± 0.00 i	9.60 ± 0.71 e	1.00 ± 0.14 i	12.80 ± 0.57 c	0.90 ± 0.14 i	7.50 ± 0.57 f
(Z)-3-Hexen-1-ol	–	–	–	–	–	–	–	–	–	–	–	–
3-Ethyl-1-hexanol	–	0.10 ± 0.00 de	0.40 ± 0.00 ab	0.30 ± 0.00 bc	0.30 ± 0.00 bc	0.30 ± 0.00 bc	0.20 ± 0.00 cd	–	0.20 ± 0.00 cd	–	0.50 ± 0.14 a	0.50 ± 0.00 a
Benzyl alcohol	0.80 ± 0.14 def	0.60 ± 0.00 fg	1.00 ± 0.14 cd	0.90 ± 0.14 de	0.80 ± 0.14 def	0.70 ± 0.00 efg	0.60 ± 0.00 fg	0.60 ± 0.00 fg	0.75 ± 0.07 defg	0.80 ± 0.14 def	0.90 ± 0.00 de	0.65 ± 0.07 efg
Phenylethyl alcohol	0.55 ± 0.07 efg	0.85 ± 0.07 def	1.20 ± 0.14 cd	0.90 ± 0.00 cde	3.00 ± 0.28 a	1.15 ± 0.07 cd	1.10 ± 0.00 cd	1.30 ± 0.42 c	1.00 ± 0.00 cd	1.15 ± 0.07 cd	1.00 ± 0.00 cd	1.20 ± 0.14 cd
(Z)-3-Hexenyl acetate	–	–	0.50 ± 0.00 j	–	5.90 ± 0.42 efg	6.50 ± 0.42 de	5.40 ± 0.28 g	5.70 ± 0.42 efg	5.60 ± 0.28 fg	6.30 ± 0.57 def	3.20 ± 0.28 i	4.20 ± 0.57 h
1-Hexyl acetate	–	–	–	–	2.20 ± 0.14 ef	2.40 ± 0.42 de	1.80 ± 0.28 f	2.20 ± 0.42 ef	2.10 ± 0.14 ef	2.80 ± 0.42 cd	0.90 ± 0.00 g	1.15 ± 0.07 g
Methyl salicylate	1.50 ± 0.00 de	1.70 ± 0.14 bcd	3.10 ± 0.28 a	2.10 ± 0.14 bc	1.10 ± 0.14 ef	1.55 ± 0.07 de	1.55 ± 0.07 de	1.80 ± 0.28 bcd	2.00 ± 0.14 bcd	1.60 ± 0.28 cde	2.10 ± 0.28 bc	1.80 ± 0.42 bcd
n-Tetradecane	1.80 ± 0.14 de	1.45 ± 0.07 efg	2.00 ± 0.28 cd	2.60 ± 0.28 ab	0.70 ± 0.00 h	1.10 ± 0.14 gh	2.30 ± 0.14 bc	2.30 ± 0.28 bc	1.05 ± 0.07 gh	1.70 ± 0.14 de	1.60 ± 0.14 def	1.20 ± 0.14 fg
n-dodecane	0.40 ± 0.00 c	0.45 ± 0.07 bc	–	0.60 ± 0.00 a	–	0.40 ± 0.00 c	–	0.40 ± 0.00 c	–	–	0.50 ± 0.00 b	0.50 ± 0.00 b
3-Ethyl-1,5-octadiene	4.60 ± 0.28 a	3.25 ± 0.50 defg	4.20 ± 0.14 abc	4.00 ± 0.57 abcd	4.40 ± 0.00 ab	3.10 ± 0.57 fgh	3.60 ± 0.14 cdefg	3.65 ± 0.21 bcdef	3.75 ± 0.21 bcdef	3.50 ± 0.42 cdefg	4.20 ± 0.00 abc	3.90 ± 0.28 abcde
3,7-Decadiene	9.15 ± 0.64 a	7.10 ± 0.28 cde	8.60 ± 0.71 ab	8.00 ± 0.85 abc	7.60 ± 0.00 bcd	6.20 ± 0.85 ef	6.90 ± 0.28 cde	7.25 ± 0.07 cde	7.05 ± 0.78 cde	6.70 ± 0.71 cde	8.00 ± 0.28 abc	8.60 ± 0.85 ab
4,8-Dimethyl-1,3,7-nonatriene	1.55 ± 0.07 de	2.10 ± 0.28 bc	2.40 ± 0.28 ab	1.75 ± 0.21 cd	1.65 ± 0.21 d	1.90 ± 0.28 cd	1.70 ± 0.14 cd	1.80 ± 0.14 cd	1.20 ± 0.14 ef	1.20 ± 0.14 ef	1.50 ± 0.00 def	1.80 ± 0.28 cd
(E)-2-Dodecene	3.10 ± 0.28 j	3.10 ± 0.28 j	6.00 ± 0.42 f	3.10 ± 0.57 j	10.00 ± 0.42 d	15.50 ± 0.71 b	11.10 ± 0.57 c	10.20 ± 0.85 cd	7.50 ± 0.71 e	6.10 ± 0.57 f	7.60 ± 0.71 e	7.20 ± 0.57 e
Limonene	0.60 ± 0.00 g	0.75 ± 0.07 fg	2.20 ± 0.28 a	1.20 ± 0.14 cde	1.00 ± 0.14 def	0.90 ± 0.14 efg	0.75 ± 0.07 fg	1.20 ± 0.14 cde	0.70 ± 0.00 fg	0.90 ± 0.14 efg	1.30 ± 0.14 cd	0.10 ± 0.00 h
α-Pinene	0.40 ± 0.00 c	–	–	–	–	–	0.40 ± 0.00 c	–	0.40 ± 0.00 c	–	0.40 ± 0.00 c	–
(E)-β-Ocimene	2.20 ± 0.28 def	1.45 ± 0.21 ghi	2.20 ± 0.42 def	1.80 ± 0.14 efg	1.00 ± 0.14 i	1.10 ± 0.14 hi	1.40 ± 0.14 ghi	1.50 ± 0.42 ghi	2.45 ± 0.35 cd	2.40 ± 0.28 cde	1.80 ± 0.28 efg	1.70 ± 0.28 fgh
α-copaene	0.30 ± 0.00 de	0.25 ± 0.07 e	0.00 f	0.40 ± 0.00 c	0.00 f	0.30 ± 0.00 de	0.30 ± 0.00 de	0.00 f	0.30 ± 0.00 de	0.00 f	0.40 ± 0.00 c	–
(E,E)-α-Farnesene	–	–	–	–	–	–	–	0.20 ± 0.00 f	1.40 ± 0.14 d	0.80 ± 0.00 e	–	–
hexanal/*(E)*-2-hexenal ratio	0.09 ± 0.01 de	0.06 ± 0.00 g	0.07 ± 0.00 f	0.07 ± 0.01 ef	0.06 ± 0.01 fg	0.07 ± 0.01 ef	0.05 ± 0.00 h	0.07 ± 0.01 ef	0.07 ± 0.00 f	0.08 ± 0.01 e	0.06 ± 0.01 fg	0.07 ± 0.01 ef
Total aldehydes	64.75 ± 1.48 c	71.00 ± 1.13 a	56.90 ± 0.99 de	66.50 ± 0.28 bc	45.80 ± 0.85 h	43.20 ± 0.28 i	53.75 ± 0.64 f	46.20 ± 1.13 h	55.40 ± 0.42 ef	43.40 ± 1.13 i	57.80 ± 0.00 d	50.15 ± 0.92 g
Total alcohols	8.30 ± 0.14 ij	6.90 ± 0.57 k	9.20 ± 0.42 i	7.45 ± 0.35 jk	17.00 ± 0.57 f	14.00 ± 0.71 h	8.10 ± 0.57 ijk	14.00 ± 0.42 h	8.45 ± 0.50 ij	19.95 ± 0.21 d	8.00 ± 0.99 ijk	15.05 ± 0.35 gh
Total esters	1.50 ± 0.00 h	1.70 ± 0.14 h	3.60 ± 0.28 g	2.10 ± 0.14 h	9.20 ± 0.71 de	10.45 ± 0.07 c	8. 75 ± 0.07 de	9.70 ± 0.57 cd	9.70 ± 0.28 cd	10.70 ± 0.71 c	6.20 ± 0.57 f	7.15 ± 0.07 f
Total C_6 compounds	69.15 ± 1.34 ab	70.90 ± 1.13 a	59.30 ± 1.13 cd	67.35 ± 0.35 b	54.50 ± 0.71 fg	51.60 ± 0.85 hi	56.00 ± 0.57 efg	54.20 ± 1.84 gh	58.30 ± 0.99 cde	57.00 ± 1.70 def	59.80 ± 0.28 c	58.15 ± 1.63 cde
Total C_5 compounds	0.85 ± 0.07 h	1.55 ± 0.21 fg	0.70 ± 0.00 h	1.40 ± 0.14 g	2.30 ± 0.28 bcde	2.45 ± 0.35 bcd	2.10 ± 0.28 bcdef	2.50 ± 0.14 bc	1.90 ± 0.14 defg	3.20 ± 0.42 a	1.80 ± 0.28 efg	2.00 ± 0.14 cdef
Total identified volatile compounds	98.65 ± 0.07 cde	99.50 ± 0.14 a	97.30 ± 0.00 g	99.50 ± 0.28 a	98.35 ± 0.07 e	98.95 ± 0.21 bcd	99.05 ± 0.07 bc	99.50 ± 0.00 a	99.35 ± 0.07 ab	98.55 ± 0.21 de	99.30 ± 0.00 ab	97.75 ± 0.07 f

Each value is an average of three determinations ± S.D. Values in the same row with different letters show statistically significant differences ($P < 0.05$).

(20%) of introduced cultivars: OS of Chemlali oil increased from 5.34 h to 11.91 h in F7 and to 11.88 h in F8.

Indeed, Koroneiki oil, despite the lowest phenols content, was characterized by the best OS; this may be due to its interesting fatty acid profile, with high levels of oleic acid and MUFA, and low levels of palmitic acid and SFA. In the same way, for blended oil samples, F5 oil had the highest OS due to its richness in MUFA (especially oleic acid) and its low content in PUFA and SFA. On the other hand, the high OS of F8 may be attributed to its richness in phenolic compounds.

3.6 Changes in the volatile profile

The volatile compounds produced during and after oil extraction contribute to the distinctive flavor of olive oil. The overall GC-MS measurement results are illustrated in Table 3. Twenty-nine compounds were characterized, representing 90.45–99.50% of the total spontaneously emitted volatile compounds (as % of total peak area).

The most important group that contributes to aroma of olive oil was constituted by aldehydes, with 16.95–71% of the total emission, followed by alcohols (4.80–38.70%) and esters (0.20–19.20%). The main volatiles responsible for odour notes of high quality virgin olive oils are the C_6 (43.30–71.40%) and the C_5(0.40–3.20%) volatile compounds produced from primary or secondary lipoxygenase (LOX) pathway, respectively (Angerosa, 2002). Based on the classification of Morales and Aparicio (1999), the major volatile compounds which contribute to the positive attributes of olive oil can be divided into three groups according to their sensory perceptions: hexanal, and hexyl acetate are responsible for the green-sweet aspect of the global green flavor (desirable perceptions). The second group, represented by *(Z)*-3-hexenal, *(Z)*-3-hexenol, and *(Z)*-3-hexenyl acetate, is responsible for the main green perception. Concerning the bitter-astringent aspect, the third group, it is represented by *(E)*-2-hexenal and *(E)*-2-hexen-1-ol.

Comparing the four olive cultivars, harvested at the same date and grown under the same pedoclimatic conditions, it can be observed that the genetic store of each variety, as well as the level and activity of enzymes involved in the LOX pathway, affect considerably the biosynthesis of each volatile compound in monovarietal oils, as also suggested by previous investigations (Angerosa, 2002; Angerosa and Basti, 2003). The total identified volatile compounds in Chemlali oil were 95.05%; this content was significantly improved under the effect of blending process, in all blended oils. It seems that some interactions and/or synergisms could take place among the enzymes involved in the LOX cascade. When more cultivars are mixed, the enzymes involved in the LOX pathway may interact differently (synergic, additive, or antagonistic interactions) in their new environment, the olive paste, depending on the new contents of the fatty acid precursors of volatile compounds.

From a qualitative point of view, the contents of total volatiles differ significantly between monovarietal and multivarietal oils (Tab. 3). It is noticeable that the proportions of C_6 are higher than those of C_5 compounds in all the analyzed oils. Oueslati oil is distinguished by its high level of total C_6 compounds (71.40%) compared to Chemlali (54.00%), Chetoui (43.30%) and Koroneiki (49.55%).

Using the method of oil blending, the concentration of the total C_6 compounds seem to vary in a way proportional to the percentage of each monovarietal oil in the final blend, except for the blend O7, where the final concentration is significantly higher than in Chemlali, Chetoui and Koroneiki oils. Due to its richness in C_6 compounds, the Oueslati oil contributed to a significant increase of the level of these derivatives when added to Chemlali oil (blends O3 and O4). Instead, the changes observed in the total C_6 compounds in oils obtained by processing mixtures of fruits from 2 or 3 corresponding cultivars were not strictly dependent on the percentage of each cultivar in the mixture.

When classifying the volatile compounds on the basis of their chemical classes, it appears that Oueslati oil exhibited high levels of aldehydes (56.80%) and especially *(E)*-2-hexenal (50.40%) which confers "green" aroma or "fresh cut grass" (Kalua *et al.*, 2007). Chetoui oil has the lowest level of *(E)*-2-hexenal (15.60%). On the other hand, the total ester level (sum of *(Z)*-3-hexenyl acetate and 1-hexyl acetate) showed an opposite trend with respect to total aldehydes, and was present in high amount in Chetoui oil (19.20%), but in trace amounts in Oueslati oil. Volatile esters contribute to the good flavor (fruity, sweet, green leaves) appreciated by consumers. Alcohol-acyl-transferases is the key enzyme involved in the biosynthesis of volatile esters; it catalyses esterification of an acyl moiety from an acyl-coenzyme A (acyl-CoA) donor on to an alcohol (Goulet *et al.*, 2015). Thus, we suggest that for Chetoui fruits, alcohol-acyl-transferase activity might be higher than that of other cultivars, which possibly enhanced *(Z)*-3-hexenyl acetate and 1-hexyl acetate biosynthesis, and confer a unique flavor to Chetoui oil. Concerning volatile alcohols, Chemlali oil has the highest content compared to the other studied cultivars. This may be due to the differential activity of the enzymes involved in the biosynthesis of these compounds.

Blending process affected differently the contents of these classes of volatile compounds. Except for O1 and O2, all the blended oils showed a striking accumulation of aldehydes, especially *(E)*-2-hexenal, which reached the maximum concentrations in F2 and O3 blended oils.

In high quality oils, *(E)*-2-hexenal level should be higher than that of hexanal. After induction of oil oxidation, hexanal, an unpleasant-tasting aldehyde, rapidly increases in parallel with the decrease of *(E)*-2-hexenal level, which lead to the development of the "rancid'off-flavour". Thus, as suggested by Jiménez *et al.* (2007), lower hexanal/*(E)*-2-hexenal ratio is a sign of a better quality and lower oxidation degree of the oil. In our study, all blended oils showed very low hexanal/*(E)*-2-hexenal ratio due to the low hexanal content and the high levels of *(E)*-2-hexenal. This indicates that no oxidation occurred after the blending process.

(E)-2-heptenal and nonanal have been associated with the oxidative status of virgin olive oil (Morales *et al.*, 1997). Chemlali oil had the highest levels of these two compounds, while Koroneiki and Oueslati had the lowest.

Total alcohols decreased drastically in all blended oils compared to pure Chemlali (except in O1 oil sample). However, the trend of each alcohol compound is different among samples and there is no strict dependence between the percentage of cultivar in the fruit or oil mixtures and the final concentration of volatile compound in the corresponding

blended oils. This confirms the existence of synergistic or antagonistic phenomena, especially for fruit mixtures, as already observed in previous studies (Angerosa and Basti, 2003; Reboredo-Rodríguez *et al.*, 2015). These interactions and synergisms are probably due to the changed activity of the enzymes involved in the synthesis of volatile compounds in the olive paste, caused by the changed concentrations of each enzyme and of fatty acid precursors (linoleic and linolenic acids) (Reboredo-Rodríguez *et al.*, 2015).

(E)-2-hexen-1-ol, the main alcohol derivative in the oil samples, is characterized by a green odor and by an unpleasant astringent-bitter taste (Aparicio *et al.*, 1996). The level of this volatile was high in Chemlali and Oueslati oils, whereas it was low in Koroneiki and absent in Chetoui oil. The concentration of *(E)*-2-hexen-1-ol decreased significantly in all the blended oils, with the exception of F1.

Total esters appeared to change in a way proportional to the percent of each cultivar in the final blended samples, either produced from fruit mixtures or oil mixtures. The percentage of volatile esters can be simply estimated, since it is proportional to the mass ratio of the components in blended oils. When fruits or oil of the Chetoui cultivar are combined with those of Chemlali, the final blended oil shows a significantly higher ester concentration than that of pure Chemlali.

In this study, 5 terpene hydrocarbons were identified: three monoterpenes, Limonene, α-Pinene, *(E)*-β-Ocimene and two sesquiterpenes, α-Copaene and *(E,E)*-α-Farnesene. Comparing the four studied cultivars, Chemlali has the highest contents in the mentioned terpene compounds, except *(E,E)*-α-Farnesene, which was absent in Chemlali oil but present only in Chetoui oil and all the blended oils that contain Chetoui as introduced cultivar. *(E)*-β-Ocimene is absent in Koroneiki, but present in Tunisian cultivars. Our results are in agreement with those of Cecchi and Alfei (2013), who reported that terpene hydrocarbons are significantly affected by the olive variety.

Taken together, the current study proved that the volatile composition changes qualitatively and quantitatively when fruits or oil of Chemlali cultivar are combined with those of Chetoui, Oueslati and Koroneiki. The blending process could modify the concentrations of some aromatic compounds responsible for the distinctive flavour notes of olive oil, and therefore can change its sensorial quality. However as observed in the present study, the concentrations of volatile compounds in the final blended oil were not strictly dependent on the percentage of each cultivar in the mixture. Thus, the average amount of each compound cannot be easily calculated *a priori*, even if the average volatile composition of monovarietal oils is known.

3.7 Selection of best formulations in terms of oil quality

The results showed that the most successful formulations are mainly F8 and F5 blends. F8 although containing only 20% of the introduced cultivars (Koroneiki and Oueslati) was characterized by a high oxidative stability and the highest contents of phenols, as well as an interesting acidic profile. Blending Chemlali with Koroneiki at 50% (in F5) leads to a significant improvement of MUFA level, especially C18:1, with a significant enhancement of stability to oxidation. The resulted blended oils are characterized by an increased level of total aroma compounds compared to pure Chemlali, due to increased amounts of total aldehydes and esters.

4 Conclusion

All the data obtained in this investigation agree to indicate that oils obtained by processing mixtures of fruits from two or three cultivars at the same time resulted in a better oil quality and elevated bioactive compound contents compared to that of each of the cultivars involved, or to that of oils obtained by the common oil blending method. At the moment of processing, Koroneiki, Chetoui, Chemlali and Oueslati had comparable maturity indices (3.5, 3.9, 4.4 and 4.5, respectively) (Data not shown). Therefore, the studied cultivars didn't have much different dynamics of fruit ripening. Nevertheless, in local production practice, most cultivars are habitually harvested contemporaneously and processed as fruit mixtures, regardless of their different stages of ripeness.

Results suggest that this blending procedure offers a possibility to modulate the contents of antioxidants, fatty acids and volatile compounds in virgin olive oil, and therefore, its quality and sensorial characteristics. The assumption of possible interactions and/or synergisms in the production of volatile compounds from the LOX pathway was suggested. Each cultivar could have provided its particular enzymatic package, and some synergetic or antagonistic interactions have occurred in their new environment, the olive paste. Consequently, these interactions could affect the synthesis and accumulation of biochemical compounds in the resulting oil.

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