



Fatty Acid Composition In Olive (*Olea europaea*. L) Oil of Progenies Obtained From Tunisian Cross Breeding Program

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Abstract

Five preselected olive progenies from a Tunisian crossing program between 'Chemlali Sfax' variety and several Mediterranean varieties were evaluated for their fatty acid composition in comparison with the original variety. These progenies were planted in a trial orchard with 4mx6m space and irrigated conditions in the experimental station 'Ettaous' in Sfax (Tunisia). The analysis of variance revealed significant differences among progenies for C16:0, C16:1, C18:1, C18:2 and C18:3. The Hierarchical Cluster Analysis classified the progenies into three main groups. Two groups included four olive progenies characterized mainly by high oleic and low palmitic acid contents. There were significant negative correlations between oleic acid and palmitic, palmitoleic, linoleic and linolenic acids. The principal component analysis showed that two components accounted for 75% of the total variation observed and revealed the importance of the main fatty acids 16:0, 18:1 and 18:2 in the characterization of olive progenies. The fatty acid compositions of the oils from all the progenies comply with international standards and show more beneficial characteristics than the oil obtained from 'Chemlali Sfax' (higher oleic acid content and lower palmitic acid content) except for hybrid 2 oil which showed an unchanged fatty acid composition. From this study, four promising progenies could be candidates for release and further investigation on minor chemical components must be undertaken.

Keywords: Oil characteristics, olive tree, crosses, evaluation, Chemlali Sfax

1. Introduction

The concept of olive oil quality is wide, complex and dynamic (Ozdemir *et al.*, 2018). This has encouraged Mediterranean olive institutions in several producing-countries to perform cross-breeding programs. Most of these programs were interested on crossbreeding among the main outstanding cultivars and selection within the progenies (Fontanazza and Baldoni, 1990). Fatty acid composition, in particular high oleic acid content, has been considered one of the most important breeding objectives for olive oil (Rallo *et al.*, 2008).

In the past few years, olive growing and olive oil production had shown an exponential increase in non-Mediterranean countries (FAOSTAT, 2016). The emergence of the new olive producing areas and the increasing importance of the nutritional features of olive oil for consumers and markets have significantly boosted the development of new and more ambitious olive breeding programs (Lavee, 2013). Thus, the objectives of most recent breeding programs are not only agronomic (Pérez *et al.*, 2018). In fact, as part of an olive genetic improvement program carried out using intervarietal breeding to produce superior progeny, several analytical determinations were carried out in many works based on oil composition (Manai *et al.*, 2018; Mousavi *et al.*, 2018). Evaluation of olive oil composition is considered as a compulsory task in any breeding program aiming at obtaining new olive cultivars (León *et al.*, 2011).

The quality of virgin olive oil is highly determined by its fatty acid composition (high oleic acid content) and minor compounds (León *et al.*, 2018). Several authors have reported advanced selections with enhanced oleic acid, tocopherol, total phenolic contents as well as peroxide and pigments values (De la Rosa *et al.*, 2013; Manai *et al.*, 2018; Pérez *et al.*, 2018).

These benefits of olive oil are associated with their fatty acid contents, mainly monounsaturated fatty acids, and to minor constituents such as tocopherols, phenolic compounds and phytosterols (Al-Bachir and Sahloul, 2017). The acid composition of olive oil varies widely depending on the cultivar, maturity of the fruit, altitude, climate, and several other factors. The major acids in olive oil are (Irmak and Tokuşoğlu, 2017): Oleic acid, a monounsaturated omega-9 fatty acid, Linoleic acid, a polyunsaturated omega-6 fatty acid, Linolenic acid, a polyunsaturated omega-3 fatty acid, Stearic acid, a saturated fatty acid, Palmitic acid, a saturated fatty acid.

Fatty acid composition is important for the commercial properties of oils. It has an influence on the stability of oils due to the contribution of PUFAs to oil rancidity (Tous *et al.*, 1993). In addition to this, several studies have shown that a diet rich in monounsaturated fatty acids may result in a wide range of health benefits such as an improvement in cholesterol levels, and, in turn, prevention of cardiovascular disorders (D'Imperio *et al.*, 2007). In particular, high levels of mainly oleic acid, which have health benefits, are among the

major components of the Mediterranean diet, and they play an important role in the nutritional value of olive oil (Uylaşer and Yildiz, 2014).

High variability for most olive oil quality components has been reported in progenies from breeding programs. The significant results come from Israel where several varieties have been characterized and released as ‘Kadesh’, ‘Barnea’, ‘Maalot’, ‘Askal’, ‘Kadeshon’, ‘Sepoka’ and ‘Masepo’ (Lavee, 1978; Lavee *et al.*, 1986; Lavee *et al.*, 1999; Lavee *et al.*, 2003; Lavee *et al.*, 2004). In Spain, a hybridization program has been carried out since 1991 and a new variety ‘Chiquitita’ was released (Rallo *et al.*, 2008). The Moroccan breeding program had released several new cultivars with better fatty acid composition (Charafi *et al.*, 2007).

In Tunisia, most studies were interested in screening progenies mainly for a more interesting chemical composition than that of the cultivar ‘Chemlali Sfax’, which allows the selection of some descendants (Manai *et al.*, 2007; Rjiba *et al.*, 2009; Dabbou *et al.*, 2010). Recently, five new cultivars were released and published in the Official Journal of Republic of Tunisia (JORT, 2017). These new cultivars were mainly characterized by their low palmitic acid levels and high oleic acid levels (Guellaoui *et al.*, 2019; Ben Amar *et al.*, 2019; Ben Amar *et al.*, 2021).

The aim of this study was the oil characterization of five preselected olive progenies. They were selected from a Tunisian controlled crossing program, initiated in 1994 between ‘Chemlali Sfax’ variety and several Mediterranean varieties. Results were compared with the original parent.

2. Material and methods

2.1. Plant material

Five olive progenies were evaluated during their maturity stage for three cropping seasons (2017-2019). Investigated progenies were obtained through controlled crossings between Chemlali Sfax, main Tunisian olive oil variety and foreign varieties (Table 1) which are Coratina (Italy) and Sigoise (Algeria) and through free pollination.

Table 1. Codes and parents of studied progenies

Code	Parents
1	Chemlali Sfax x Coratina
2	Chemlali Sfax x Sigoise
3	Free pollination
4	Chemlali Sfax x Coratina
5	Chemlali Sfax x Coratina
6	Chemlali Sfax (check)

This program was undertaken in order to obtain new oil or table olive varieties meeting the international market requirements. These new cultivars were obtained from crosses made in the period 1993-1996 and planted in 1997

in the Experimental Station ‘Ettaous’ of the Olive Institute at Sfax (Central Tunisia, 34° 44’ Nord, 10° 46’ Est). These progenies were then propagated with semi hardwood cuttings and the obtained plants were planted since 2005 at 4 m × 6 m space in an olive orchard in the ‘Ettaous’ station.

2.2. Oil extraction

Due to alternate bearing of olive tree, one olive sample of 1 kg was harvested each year at a maturity index between 3 and 4 according to the scale of Hermoso *et al.* (1991). The Virgin olive oil was extracted from olive fruit by grinding stoned olives and extracting the oil by mechanical means with small laboratory mill. This equipment consists of a three steps process: a hammer crusher, a thermo beater and a paste centrifuge. After centrifugation, the obtained oil through decantation was transferred into dark glass bottles, and stored at 4°C until further analysis.

2.3. Fatty acid composition

The composition of fatty acids was evaluated after preparation of fatty acid methyl ester using a cold saponification (Stefanouadaki *et al.*, 1999). In brief, 0.2 g of oil were vigorously mixed with 3 mL of hexane and 0.3 ml of a methanolic solution of KOH (2 N), for 1 min. The mixture was allowed to set for 5 min and analyzed by gas chromatography (GC) (Perkin Elmer Gas Chromatograph Clarus 580) equipped with a capillary column (RESTEK Rt-2560) (column temperature 180 °C) coupled to a flame ionization detector. Both the injector and detector were maintained at 250°C. Fatty acids were identified by comparing their retention times with those of standard compounds.

Concentrations were evaluated in this study for the most studied fatty acids (Table 2) and compared with those of the original cultivar and the norms of the International Olive Council (IOC, 2016).

Table 2. Nomenclature, name and norms of the main studied fatty acids of olive oil

Code	Name	Norms* (%)
C16:0	Palmitic acid	7.5-20
C16:1	Palmitoleic acid	0.3 – 3,5
C17:0	Margaric acid	0 - 0,3
C17:1	Margaroleic acid	0 – 0,3
C18:0	Stearic acid	0.5 - 5
C18:1	Oleic acid	55-83
C18:2	Linoleic acid	2.5-21
C18:3	Linolenic acid	≤ 1
C20:0	Arachidic acid	≤ 0.6
C20:1	Gadoleic acid	0 – 0.4

*Norms of the International Olive Council (IOC, 2016)

2.4. Statistical analyzes

Analysis of variance was applied with the Duncan multiple comparison test of the means ($p < 0.01$) to determine the presence of significant differences among the varieties. Results were reported as the mean values of three replications (years) in each analysis. Different letters in the same column of the tables indicate a significant difference.

A tree is then inferred using the unweighted pair group method using an arithmetic average (UPGMA) clustering algorithm. Pearson correlation and principal components analysis (PCA) were performed in order to test the relations among the different fatty acids measured for the different hybrids. Statistical analysis was performed using the SPSS 24.0 program.

3. Results and discussion

3.1. ANOVA analysis

Hybrid effect was significant in five fatty acids measured, C16:0, C16:1, C18:1, C18:2 and C18:3 (Table 3). No significant differences among years were found, with the exception for three fatty acids, C16:0, C18:1 and C18:3.

Table 3. Significance of crop season and genotype factors for all fatty acids

Acids	Hybrid	Year
C 16:0	**	**
C 16:1	**	Ns
C 17:0	Ns	ns
C 17:1	Ns	ns
C 18:0	Ns	ns
C 18:1	**	*
C 18:2	**	ns
C 18:3	*	*
C 20:0	Ns	ns
C 20:1	Ns	ns

***: Significant at 0.01 and 0.05 levels, ns: non significant

The hybrid effect on fatty acid composition was reported by almost all the crossbreeding programs throughout the world, in Spain (Leon *et al.*, 2011; De La Rosa *et al.*, 2013), Tunisia (Guellaoui *et al.*, 2019; Ben Amar *et al.*, 2019; Ben Amar *et al.*, 2021), Israel (Lavee *et al.*, 1986; Lavee *et al.*, 1999; Lavee *et al.*, 2003). The crop season effect on oil fatty acid composition was also reported for olive varieties by many authors (Salvador *et al.*, 2001; Lazzez *et al.*, 2011; El Qarnifa *et al.*, 2019) and precipitation and temperature during olive growth and maturation present the most significant environmental factors that influence the olive oil composition (El Qarnifa *et al.*, 2019).

The variation over years of oleic and palmitic acids must be taken into consideration and then controlled since a healthy diet must contain a limited

amount of saturated acids and high amount of monounsaturated acids, as reported by Zarrouk *et al.* (2009) and El Riachy *et al.* (2019).

3.2. Fatty acid composition

Table 4 gives ranges for each fatty acid in the studied oils. The monounsaturated fatty acids have great importance because of their effect on oil oxidative stability. The main monounsaturated fatty acid, Oleic acid, is present in higher concentrations (69.80–72.82%) for the hybrids 1, 3, 4 and 5 while the hybrid 2 had was with similar oleic acid value (59.35%) than the check (57.23%).

Table 4. Mean fatty acid composition (%) of olive oil of five progenies across years compared to the check ‘Chemlali Sfax’

Acids	1	2	3	4	5	6
C 16:0	15.6 b	18.8 a	14.4 bc	13.0 c	14.4 bc	19.8 a
C 16:1	1.0 bc	1.2 b	0.39 d	0.42 d	0.6 c	2.2 a
C 17:0	0.016 a	0.034 a	0.024 a	0.028 a	0.058 a	0.026 a
C 17:1	0.05 a	0.062 a	0.032 a	0.072 a	0.076 a	0.048 a
C 18:0	1.88 a	2.47 a	2.55 a	2.17 a	2.06 a	2.27 a
C 18:1	71.63 ab	59.35 c	69.80 b	72.82 a	71.91	57.23 c
C 18:2	9.18 c	16.93 a	11.81 b	9.71 bc	9.93 bc	17.41 a
C 18:3	0.45 b	0.67 a	0.57 a	0.56 a	0.53 ab	0.64 a
C 20:0	0.19 a	0.31 a	0.22 a	0.42 a	0.27 a	0.27 a
C 20:1	0.18 a	0.14 a	0.18 a	0.55 a	0.21 a	0.14 a

Means in columns followed by the same letter are not statistically significantly different

The level of palmitic acid (C16:0), major saturated fatty acid in olive oil, ranged from 13 for hybrid 4 sample to 19.8% for ‘Chemlali Sfax’ (Table 3). With opposition to oleic acid, palmitic acid level is significantly low and equal for the hybrids 1, 3, 4 and 5 (13 to 15.6%) while the hybrid 2 performed significantly the same amount (18.8%) than the check (19.8%).

Concerning linoleic acid (C18:2), which is much more susceptible to oxidation than monounsaturated fatty acids, the same trend and performance were obtained. Thus, the highest percentage was observed in hybrid 2 (16.93%), whereas the other hybrids shows significantly the lowest ones (9.18-11.81%).

For the other fatty acids, margaric acid (C17:0), margaroleic acid (C17:1), linolenic (18:3), arachidic (C20:0) and gadoleic acid (C20:1) exhibited small amounts (less than 0.67%) and do not varied significantly between all oil samples.

Palmitoleic acid (16:1) content varied significantly between 0.39 (hybrid 3) and 2.2% (Chemlali Sfax) according to progenies, while the level of stearic acid (18:0) was statistically similar for all progenies ranging from 1.88 and 2.55%.

Regarding fatty acid profile, all percentages of fatty acids obtained in the present study fit, more or less, with the requirements established by the IOC (2016) for virgin olive oil, except for C20:1 which slightly exceeded the limit of IOC (< 0.4%) in case of hybrid 5 with 0.55%).

Because of their great importance in human diet, oleic and palmitic acids have wide variability between progenies when compared to the original variety Chemlali Sfax. On the basis of main fatty acid composition, Hybrid 2 is considered similar to Chemlali Sfax with high palmitic acid level and low oleic acid level. On the other hand, hybrids 1, 3, 4 and 5 showed a net genetic improvement regarding to these acids and could be considered for release as new varieties in Tunisia in addition to the released varieties recently (Guellaoui *et al.*, 2019; Ben Amar *et al.*, 2019; Ben Amar *et al.*, 2021; Guellaoui *et al.*, 2021). These results represent a success for the national breeding program of olive tree started in the nineteenth.

The best fatty acid composition with respect mainly to oleic and linoleic acids of the three progenies 1, 4 and 5 is attributed to the genitor 'Coratina'. This variety had been reported with good fatty acid composition in Italy (Muzzalupo, 2012) and outside Italy (Zarrouk *et al.*, 2009; Hashempour *et al.*, 2010). According to Biton *et al.* (2012), phenotypic data of progenies from crosses between different cultivars indicated the potential effects of heterosis as expressed in several olive traits.

3.3. Pearson correlation coefficients

Significant correlations between the fatty acids of olive oil were detected (Table 5). There was a very high negative correlation between oleic acid and palmitic, palmitoleic, linoleic and linolenic acids while linoleic acid was positively correlated with linolenic, palmitic and palmitoleic acids. Palmitic and palmitoleic acids were also positively correlated (0.961). A significant negative correlation between oleic and linoleic acids and significant positive correlation between palmitic and palmitoleic acids were also reported in another study (León *et al.*, 2004).

Table 5. Correlation coefficients between fatty acids of olive oil

Variables	C 16:0	C 16:1	C 17:0	C 17:1	C 18:0	C 18:1	C 18:2	C 18:3	C 20:0
C 16:1	0.961								
C 17:0	-0.149	-0.176							
C 17:1	-0.232	-0.168	0.676						
C 18:0	0.266	0.103	-0.046	-0.425					
C 18:1	-0.966	-0.914	0.103	0.223	-0.470				
C 18:2	0.918	0.845	-0.065	-0.234	0.594	-0.988			
C 18:3	0.646	0.560	0.113	-0.025	0.770	-0.817	0.887		
C 20:0	-0.160	-0.092	0.199	0.631	0.148	-0.008	0.066	0.427	
C 20:1	-0.638	-0.503	-0.018	0.497	-0.195	0.533	-0.497	-0.158	0.792

In **bold**: Correlation is significant at the 0.05 level

All significant correlations involved five fatty acids, four unsaturated acids (16:1, 18:1, 18:2, 18:3) and one saturated acid (16:0). These fatty acids were considered as the most important acids in olive oil (Zarrouk *et al.*, 2009; El Riachy *et al.*, 2019) and the most correlated each other (Manai *et al.*, 2007; El Riachy *et al.*, 2019). Thus, the variation of the amount of one of the two types during oil storage or during ripening will affect the variation of the other fatty acid type (Manai *et al.*, 2007; El Riachy *et al.*, 2019) and these variations controlled the olive oil stability (Zarrouk *et al.*, 2009).

3.4. Hierarchical analysis

The cluster analysis is conducted on the Euclidean distance matrix. The resulting dendrogram (Figure 1) revealed three major groups. The first group includes two hybrids 4 and 5 showing high oleic (> 72%) and low palmitic (< 14.4%) acid percentages. In contrast to the first group, hybrids in groups 2 (hybrids 1 and 3) have oils with lower oleic (69 to 72%) and higher palmitic (between 14.4 and 15.9 %). The progeny 2 in group 3, to which the Chemlali Sfax belongs, have oils richer in palmitic acid (18.8%) and poorer in oleic acid (59.35%).

Concerning hybrid 2 oil, no significant differences were observed in their fatty acid composition by comparison to those found in the original variety. Thus, the other four hybrids, while clustered in two groups, showed good oil chemical composition. The clustering analysis confirmed the results of one way variance analysis.

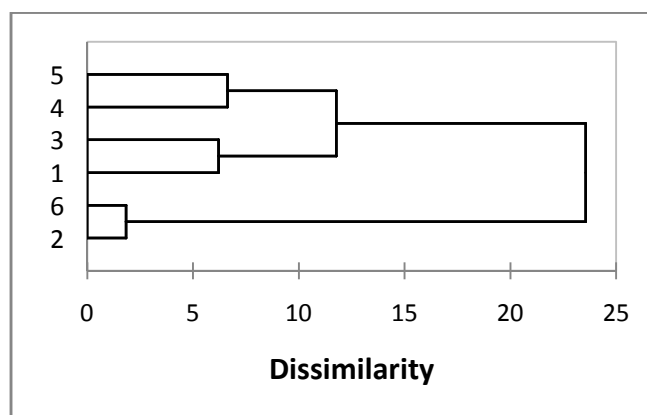


Figure 1. Dendrogram of virgin olive oil data using arithmetic average (UPGMA)

These results are in line with those reported in the bibliography. León *et al.* (2008) and Zarrouk *et al.* (2009) separated 18 varieties growing respectively in the World Olive Germplasm Bank (WOGB) of IFAPA (Cordoba, Spain) and Boughrara collection (Sfax, Tunisia) into groups according to their fatty acid composition. El Riachy *et al.* (2019) clustered 11 olive varieties into groups mainly on the basis of fatty acid composition.

3.5. Principal component analysis

The results of PCA revealed that the first two components (PC1 and PC2) accounted for 75% of the total variation observed. In figure 2, the first component, which accounted for 50.92% of the total variation, revealed that the highest negative contribution to the PC1 was from C16:0 and C18:2 contents while C18:1 had a positive correlation with this component. PC2 accounted for 24.08% of the total variation and had the highest positive correlation with C20:0.

This PCA allowed the classification of the samples according to the progeny with progenies 2 and 6 samples having higher percentages of C16:0 and C18:2 and clustering together closely in the negative side of PC1. On the other hand, progenies 4 and 5 were located in the positive side of PC2 and characterized with highest percentages of oleic acid. While progenies 1 and 3 were clustered together in the negative side of PC2 and positive side of PC1 having lower oleic acid levels than the group of progenies 4 and 5.

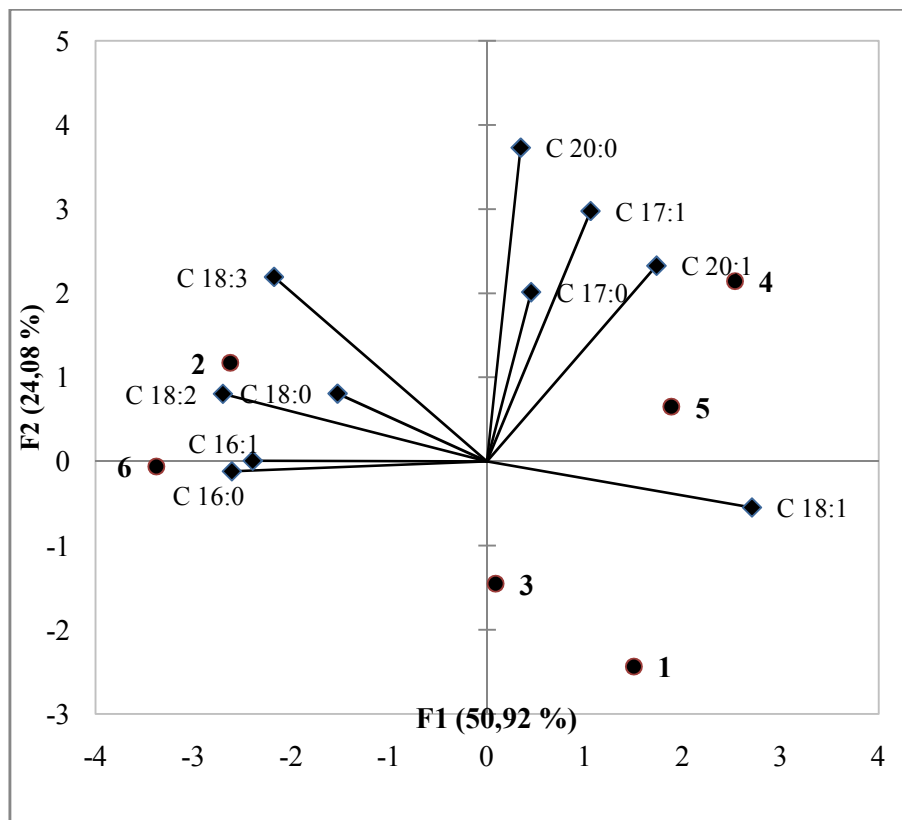


Figure 2. Biplot of principal components 1 and 2 based on fatty acid profile components recorded for each progeny

The PCA analysis showed the importance of the main fatty acids 16:0, 18:1 and 18:2 in the characterization of olive progenies. Previous studies approved this conclusion. Zarrouk *et al.* (2009) reported that C18:1, C18:2 are relevant in describing the olive oil samples. According to Leon *et al.* (2008), the percentages of C18:1, C18:2 and saturated fatty acids were the main contributors of variation in the World Olive Germplasm Bank of Cordoba.

The principal component analysis corroborates with the above clustering analysis and approved the good fatty acid composition of the four hybrids 1, 3, 4 and 5. It revealed that the progeny 2 is with similar fatty acid composition as the original variety 'Chemlali Sfax'.

4. Conclusion

The controlled crossings on Chemlali Sfax variety provides four new progenies having a better oil fatty acid composition and within the range expected for olive oil. These progenies have the potential for registration as new cultivars. In a future work, these promising progenies will be explored mainly for oil stability against oxidation and related anti-oxidant levels and sterolic composition before recommendation for release. Nevertheless, one progeny did not provide a significant gain in comparison with the original variety regarding mainly to oleic and palmitic acids. This progeny must be discarded from the actual breeding program and could be interesting in another breeding program based on other traits than the fatty acid composition.

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