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Effects of the Planting Density on Virgin Olive Oil Quality of "Chemlali" Olive Trees (*Olea europaea* L.)

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Here, we report the characterization of virgin olive oil samples obtained from fruits of the main Tunisian olive cultivar (Chemlali) grown in four planting densities (156, 100, 69, and 51 trees ha^{-1}). Olive oil samples obtained from fruits of trees grown at 100 trees ha^{-1} had a higher content of oleic acid (65.5%), a higher content of chlorophyll and carotenoids, and a higher content in total phenols (1059.08 mg/kg). Interestingly, olives grown at the two highest planting densities yielded more stable oils than olives grown at the two lowest ones. Thus planting density is found to be a key factor for the quality of olive oils in arid regions.

KEYWORDS: Fatty acids; oxidative stability; phenols; planting density; virgin olive oils

INTRODUCTION

Oil production is influenced by climatic, genetic, and agronomic factors and by their interactions. Many studies have shown that climatic factors such as temperature and precipitation have an effect on plant physiologic behavior and, consequently, on chemical characteristics of its oil (*1*−*4*).

Historically, Tunisian olives were produced under dry-land conditions where trees were spaced widely to take full advantage of the stored soil−water from winter rains for spring and summer growth. More recently, new orchards are irrigated and are planted at higher densities achieving greater yields and resulting in less alternate-bearing behavior (*5*). The increasing demand for olive tree products has led modern plantations to be characterized by a higher number of trees per hectare compared to traditional orchards planted at a density of 50 trees ha^{-1}. However, this can be problematic due to limited water resources available in Mediterranean regions (*6*). This raises many questions as to the effects of the planting density on oil chemical attributes. Hence, we find it judicious to study the relationships between olive oil quality and the planting density of the olive trees to produce more oil and to enhance its quality. For these purposes, in the present work, we evaluate fatty acid contents, oxidative stability, pigments, total phenols, and phenolic compounds of its virgin olive oils in samples obtained from fruits of olive trees cultivated in four planting densities ranging from 51 to 156 tree ha^{-1}.

MATERIALS AND METHODS

Oil Sample Extraction. Olive oil samples were obtained from fruits of the main Tunisian olive cultivar, Chemlali, which were picked by hand at the same stage of maturity during two crop seasons (2007 and 2008) in a 4 ha olive orchard located in Jemmel, center of Tunisia (35°49′ N, 10°30′ E). Olive trees were subjected to identical fertilization regime and to all common olive cultivation practices. The climate of the study area is Mediterranean with an average annual rainfall of 250 mm, mostly distributed outside a 4 month summer drought period. It is characterized by moderate temperatures during spring (25 °C) and high averages temperatures from June to August (37 °C). Olive samples of this cultivar were collected from trees planted in 1988, at four tree spacings, 8×8, 10×10, 12×12, and 14×14 m, corresponding to 156, 100, 69, and 51 trees ha^{-1}, respectively, and were subjected to the same fertilization and common olive cultivation practices.

The soil of the four plant densities has the same textural characteristic, and it was sandy with a water content of 12% at field capacity (−0.02 MPa) and 6.5% at the wilting point (−1.5 MPa) and pH 7.6. Soil−water content was determined monthly by the gravimetric method in the 0−0.8 m layer at 0.75 m from the trunk of three trees from each plant density.

The same laboratory mill was used to prepare the olive oil samples. Only healthy fruits without any visible infection or physical damage were processed. The olives were washed, deleafed, and crushed with a hammer crusher. The paste was then mixed at 25 °C for 30 min, centrifuged without addition of warm water, and transferred into dark glass bottles.

Oil Content. For the determination of oil content, 40 g of olive samples was dried in an oven at 80 °C to constant weight. The dry olives were extracted with petroleum ether using a Soxhlet apparatus. The results were expressed as percentage of dry matter (DM).

Fatty Acid Composition. The fatty acids were converted to fatty acid methyl esters before analysis by shaking a solution of 0.2 g of oil and 3 mL of hexane with 0.4 mL of 2 N methanolic potassium hydroxide and analyzed using a Hewlett-Packard (HP 4890D; Hewlett-Packard Co.,Wilmington, DE) chromatograph equipped with a capillary column (Supelcowax: 30 m × 0.53 mm; 0.25 mm), a split/splitless injector, and an flame ionization detector (FID). The carrier gas was nitrogen, with a flow rate of 1 mL/min. The temperatures of the injector, the detector, and the oven were held at 220, 250, and 210 °C, respectively. The injection volume was 1 μL.

Rancimat Assay. Oxidative stability was evaluated by the Rancimat method (*7*). Stability was expressed as the oxidation induction time (h), measured with the Rancimat 743 apparatus (Metrohm, Herisau Switzerland),

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using an oil sample of 3.6 g. The oil temperature was 101.6 °C, and the air flow was 10 L/h.

Extraction of Phenolic Compounds from Olive Oil. The extraction of phenolic compounds was carried out as previously described (*8*). Thirty milliliters of methanol/water (80:20) was added to 30 g of olive oil and mixed with an Ultra-Turrax T25 at 15000*g* for 1 min and then centrifuged at 5000*g* for 10 min. The extraction was repeated twice. The combined methanolic extracts were concentrated in a vacuum rotary evaporator at 35 °C until they reached a syrupy consistency. Ten milliliters of acetonitrile was added to the syrup, which was then washed twice with 20 mL of hexane. The acetonitrile phase was brought to dryness using a rotary evaporator. Finally, the sample was dissolved in gradient grade methanol and analyzed colorimetrically at 725 nm for total phenols and by HPLC for the fractionation of phenolic compounds.

HPLC Analysis of the Phenolic Compounds. The HPLC system consisted of a Hewlett-Packard quaternary pump Series 1100 (Hewelett Packard, Palo Alto, CA) coupled with a UV detector (Jasco UV 970) and HP Chemstation software for processing the acquired data. Injection was by means of a Rheodyne injection valve (Model 7125) with a 10 μL fixed loop (Rheodyne, CA, USA). Analytical separation was achieved on a Lichrosphere 100 RP-18.5 μm column (250 mm × 4 mm i.d.) fitted with a guard column (40 mm) of the same phase (Merck, Darmstadt, Germany). Eluates were detected at 278 nm. The mobile phase consisted of 0.2% acetic acid in water (solvent A) and methanol (solvent B) at a flow rate of 1 mL min $^{-1}$. Phenolic compounds were tentatively identified on the basis of their retention times compared to those of the standard compounds. The quantitative determination was performed using standards. Oleuropein was purchased from Extrasynthese (Geney-France), tyrosol was from Sigma-Aldrich (St. Louis, MO, USA), and hydroxytyrosol was purchased from Cayman Chemical (SPI-BIO, Europe). The gradient changed as follows: 0 min, 95% A (2 min); 10 min, 80% A; 20 min, 70% A; 30 min, 60% A; 50 min, 40% A; 60 min, 0% A.

Determination of Chlorophyll and Carotenoid Compounds. Chlorophyll and carotenoid contents were determined colorimetrically as previously described (*9*). The maximum absorption at 670 nm is related to the chlorophyll fraction, while the maximum absorption at 470 nm is related to the carotenoid fraction. The values of the coefficients of specific extinction applied were $E_0 = 613$ for pheophytin, a major component in the chlorophyll fraction, and $E_0 = 2000$ for lutein, a major component in the carotenoid fraction. Thus, the pigment contents were calculated as follows:

$$\text{chlorophyll (mg/kg)} = (A_{670} \times 10^6)/(613 \times 100 \times d)$$

$$\text{carotenoid (mg/kg)} = (A_{470} \times 10^6)/(2000 \times 100 \times d)$$

where A is the absorbance and d is the spectrophotometer cell thickness (1 cm).

Determination of Oil Quality Parameters. Free acidity, expressed as percent of oleic acid (% 18:1), peroxide value, given as milliequivalents of active oxygen per kilogram of oil (mequiv of O_2/kg), and UV absorption characteristics (K_{232} and K_{270}) were determined according to the analytical methods described in the European Union Commission Regulations EEC/2568/91 and EEC/1429/92.

Statistical Analysis. Significant differences between the planting densities were determined by an analysis of variance, which applied Duncan's test. Differences were considered statistically significant when the probability was greater than 99% ($P < 0.01$). The statistical analysis was performed using SPSS 13.0 for Windows (SPSS Inc., 2004).

RESULTS

Fatty Acid Composition. Volumetric soil−water content (θ_v, **Figure 1**) showed similar patterns for the different planting density. It was about 9.5% in April, decreased during June and August, and increased again in September (**Figure 1**). The largest rainfall (146 mm) was recorded in April during the two crop seasons (**Figure 1**).

Our results correspond to the average of the obtained oils from both harvests (2007 and 2008). Olive oil samples obtained from fruits of trees grown at 100 trees ha^{-1} had higher content of oleic

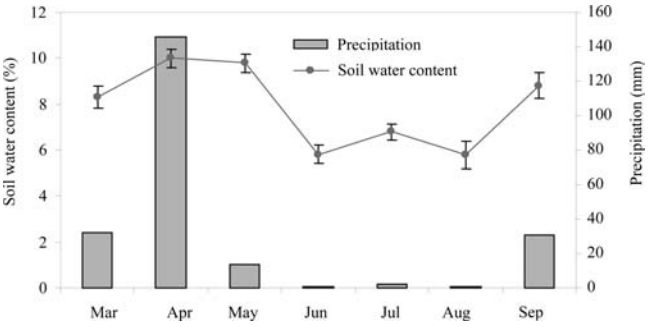

Figure 1. Precipitation (mm) and soil−water content, θ_v (%), in the 0−0.8 m soil layer of the experimental site during the 2007 and 2008 crop seasons.

acid during the two crop seasons (65.5%) (**Table 1**). Palmitic acid content varied between 15.03% and 18.7% according to the planting density. Olive oil samples also contained low amounts of linolenic acid (18:3), arachidic acid (20:0), and palmitoleic acid (16:1) (**Table 1**). Olive oil samples obtained from fruits of trees grown at the lowest planting density (51 trees ha^{-1}) were found to be rich in total saturated fatty acids (more than 20.03%), essentially due to their high content of palmitic acid. Olive oil samples obtained from fruits of trees grown at 100 trees ha^{-1} were found to show the higher content in total monounsaturated fatty acids (about 69%), due to their high percentage in oleic acid. Olive oil samples obtained from fruits of trees grown at 69 trees ha^{-1} were found to have the higher percentage in polyunsaturated fatty acids (16%) due to their high content in linoleic acid.

A great variability in the means of the average fruit fresh weight was observed among the studied olives (**Table 2**); it ranged from a minimum of 0.51 (156 trees ha^{-1}) to 0.71 g (69 trees ha^{-1}). The oil content for all of the olives studied varied slightly among the four densities. So, olive trees grown at the density of 100 trees ha^{-1} had the lowest oil content (48.1%), while olives obtained from fruits of trees grown at 51 trees ha^{-1} recorded the highest content (49.8%), the other densities presented intermediate values (**Table 2**).

Oil Quality Parameters and Pigment Contents. Olives grown at the four planting densities yielded extra virgin olive oils in 2007 and 2008 seasons, but the profile of the analytical parameters (e.g., free fatty acid content, peroxide value, and extinction coefficients at 232 and 270 nm) showed some slight differences (**Table 2**). Oils from the olive trees grown at 100 trees ha^{-1} had a higher content of chlorophyll and carotenoids (**Table 2**).

Changes in the Oxidative Stability. Olive oil samples obtained from fruits of trees grown at the planting density of 100 trees ha^{-1} during the two crop seasons were found to have the higher content in total phenols (1059.08 mg/kg) (**Table 3**).

The oxidative stability of the virgin olive oils was measured with Rancimat equipment, more stable oil being obtained from olives grown at 100 trees ha^{-1} (63.5 h) (**Table 2**). Oxidative stability was positively correlated with total phenols ($r = 0.89$).

Olive oil samples obtained from fruits of trees cultivated at the planting density of 100 trees ha^{-1} were found to contain the dialdehydic form of elenolic acid linked to hydroxytyrosol (3,4-DHPEA-EDA) at a concentration of about 40 mg/kg. Moreover, these olive oil samples were found to have a higher content in oleuropein aglycon (3,4-DHPEA-EA) than olive oil samples obtained from fruits of trees cultivated in the other planting densities (**Table 3**).

As shown in **Table 3**, olive oil samples obtained from fruits of trees grown at the planting density of 100 trees ha^{-1} were found to contain hydroxytyrosol and tyrosol at concentrations of about

Table 1. Fatty Acid Composition of Virgin Olive Oil Samples from the Four Planting Densities[a]

	156 trees ha^{-1}	100 trees ha^{-1}	69 trees ha^{-1}	51 trees ha^{-1}
saturated fatty acids (SFAs)	18.8 ± 0.30a	19.3 ± 0.23b	19.4 ± 0.41b	20.4 ± 0.34c
monounsaturated fatty acids (MUFAs)	65.8 ± 0.54b	68.6 ± 0.36c	63.6 ± 0.28a	62.9 ± 0.32a
polyunsaturated fatty acids (PUFAs)	14.6 ± 0.13a	14.4 ± 0.32a	16.2 ± 0.20c	15.9 ± 0.12b
palmitic acid C16:0	16.7 ± 028a	17.1 ± 0.25b	16.8 ± 0.38a	17.8 ± 0.28b
palmitoleic acid C16:1	2.6 ± 0.04b	3.1 ± 0.03c	2.2 ± 0.05a	2.8 ± 0.11a
stearic acid C18:0	1.8 ± 0.23a	1.8 ± 0.25a	2.4 ± 0.28b	2.2 ± 0.07b
oleic acid C18:1	63.2 ± 0.58b	65.5 ± 0.21c	61.3 ± 0.47a	60.1 ± 0.40a
linoleic acid C18:2	13.8 ± 0.32a	13.7 ± 0.11a	15.4 ± 1.22b	15.3 ± 0.61b
linolenic acid C18:3	0.7 ± 0.03b	0.7 ± 0.05b	0.8 ± 0.03c	0.6 ± 0.01a
arachidic acid C20:0	0.3 ± 0.02b	0.3 ± 0.06b	0.2 ± 0.02a	0.4 ± 0.02c

[a] Mean ± SD (n = 6). Significant differences within the same row are shown by different letters (P < 0.01).

Table 2. Average Fruit Fresh Weight and Quality Parameters of Chemlali Olive Oil Samples from the Four Planting Densities[a]

	156 trees ha^{-1}	100 trees ha^{-1}	69 trees ha^{-1}	51 trees ha^{-1}
acidity (% C18:1)	0.63 ± 0.06b	0.70 ± 0.10c	0.60 ± 0.03b	0.49 ± 0.12a
PV (mequiv of O_2/K)	5.0 ± 2.00a	6.0 ± 1.00a	7.0 ± 1.15a	9.0 ± 0.58b
K_{232}	2.45 ± 0.18a	2.54 ± 0.09a	2.15 ± 0.07a	2.22 ± 0.08a
K_{270}	0.22 ± 0.01b	0.25 ± 0.09b	0.14 ± 0.08a	0.14 ± 0.01a
chlorophylls (mg/kg)	5.2 ± 0.28b	6.0 ± 0.91b	5.7 ± 0.51b	3.8 ± 0.17a
carotenoids (mg/kg)	3.4 ± 0.22b	3.8 ± 0.55b	3.0 ± 0.43a	2.6 ± 0.58a
oxidative stability (h)	54.3 ± 4.30c	63.5 ± 3.20d	46.6 ± 2.30a	50.2 ± 1.80b
average fruit fresh wt (g)	0.51 ± 0.05a	0.53 ± 0.07a	0.71 ± 0.05b	0.70 ± 0.04b
oil content (fruit dry wt basis) (%)	48.5 ± 0.06a	48.15 ± 0.04a	48.7 ± 0.03a	49.86 ± 0.04b

[a] Mean ± SD. Significant differences within the same row are shown by different letters (P < 0.001). PV, peroxide value; K_{232} and K_{270}, values of specific extinction given as absorbance at 232 and 270 nm, respectively.

Table 3. Phenolic Composition of the Examined Olive Oil Samples (mg/kg)

	156 trees ha^{-1}	100 trees ha^{-1}	69 trees ha^{-1}	51 trees ha^{-1}
hydroxytyrosol	9.31 ± 0.08b	13.71 ± 0.04c	5.46 ± 0.03a	4.92 ± 0.04a
tyrosol	12.95 ± 0.06b	20.33 ± 0.04c	9.09 ± 0.03a	8.33 ± 0.02a
vanillic acid	0.22 ± 0.00b	0.11 ± 0.00a	0.31 ± 0.00c	0.52 ± 0.00d
caffeic acid	0.22 ± 0.00a	0.21 ± 0.00a	0.55 ± 0.00b	0.82 ± 0.00c
syringic acid	0.20 ± 0.00a	0.07 ± 0.00a	2.21 ± 0.02b	0.15 ± 0.00a
p-coumaric acid	0.43 ± 0.00c	0.04 ± 0.00a	0.05 ± 0.00a	0.22 ± 0.00b
ferulic acid	0.63 ± 0.00c	0.12 ± 0.00a	0.10 ± 0.00a	0.21 ± 0.00b
3,4-DHPEA-EDA	36.87 ± 1.20b	40.14 ± 2.23c	30.42 ± 1.10a	35.4 ± 1.50b
o-coumaric acid	0.82 ± 0.01b	0.72 ± 0.02b	0.32 ± 0.00a	0.44 ± 0.00a
3,4-DHPEA-EA	754.63 ± 6.50c	865.57 ± 5.70d	513.56 ± 8.50a	573.17 ± 5.30b
total phenols	856.79 ± 12.50c	1059.08 ± 19.50d	613.98 ± 8.50a	706.47 ± 9.53b

13.71 and 20.33 mg/kg, respectively. Other simple phenols such as vanillin, vanillic acid, *o*-coumaric acid, caffeic acid, syringic acid, and ferulic acid were found in very low concentrations.

DISCUSSION

Olive trees planted closer together compete with their neighbors for growing space and resources. Changes in the amount of soil−water available for individual trees could be a key factor for the fruit weight, which is a crucial agronomic parameter for a preliminary comparison between the studied planting densities. Oil content does not constitute a criterion of oil quality determination but is especially a criterion to be envisaged here to study the effects of planting density on the studied olives. As oil content is influenced by olive flesh humidity at the time of olive harvest (*10*), this parameter was expressed as percent of dry matter (**Table 2**). Inglese et al. (*11*) reported lower oil content in the fruits of trees grown under high soil−water deficit, while other authors did not find any difference in oil content between irrigated and nonirrigated trees (*12*−*14*).

Differences in planting densities led to differences in fatty acid composition of virgin olive oil. Morello et al. (*15*) reported that several agronomic parameters could modify the fatty acid composition of olive oil. The most studied aspects include cultivar and origin, fruit ripening, harvest period and climatic conditions, and soil characteristics. However, our results show that the planting density has little influence on the analytical parameters (free fatty acid content, peroxide value, and extinction coefficients at 232 and 270 nm) (**Table 2**), which are affected by factors causing damage to the fruits (e.g., olive fly attacks or improper systems of harvesting, transport, and storage of olives) (*16*). In addition, olive oil samples obtained from fruits of trees grown at the four planting densities were found to have varied chlorophyll and carotenoid contents (**Table 2**). The total pigment content in olive oil is an important quality parameter because it correlates with color, a basic attribute for evaluating olive oil quality. Furthermore, pigments are involved in autoxidation and photooxidation mechanisms (*9*).

The amount of phenolic compounds in EVOO is an important factor when evaluating its quality, given that the natural phenols improve its resistance to oxidation and, to certain extent, are responsible for its sharp bitter taste (*17*). Variations in total phenols observed in the present study are probably related to

the differences in the soil−water availability between the planting densities, because roots of trees explored different volumes of soils. Thus, the soil−water availability in low planting densities was higher although volumetric soil−water contents shown in **Figure 1** were similar to high planting densities. A negative correlation between total phenol content in oil and soil−water availability, depending on irrigation or accumulated rainfall, has been observed by a number of authors for many olive varieties (*18*, *14*, *19*). Moreover, the differences in the concentration of phenolic compounds in olive oil samples which were observed in the present study could be explained by the differences in the water status of the trees grown at different planting density, as reported by other authors (*20*, *21*). With regard to water availability, it is generally agreed that the level of phenolic compounds is higher in oils obtained from drought-stressed crops than in those from irrigated crops (*22*−*24*). Our results also agree with other studies which have established a correlation between phenol content and oxidative stability in virgin olive oil (*25*, *26*).

In summary, olive oil samples were for all planting densities within the limits established in the European Regulation, allowing them to be classified as extra virgin olive oils. However, our results showed that olive oil quality was affected by planting density of the olive orchard. Oils obtained from fruits of trees grown at the two highest planting densities and compared to the lowest ones showed high levels of antioxidants, together with an increased oxidative stability and high level of oleic acid.

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