African Journal of Agricultural Research Vol. 6(29), pp. 4124-4130, 31 July, 2012
Available online at http://www.academicjournals.org/AJAR
DOI: 10.5897/AJAR11.1440
ISSN 1991-637X ©2012 Academic Journals

Full Length Research Paper

Influence of two maturation stages and three irrigation regimes on Chétoui olive oil quality

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Accepted 31 October, 2011

The effects of three-irrigation managements (50% evapotranspiration [ETc], 75% ETc and 100% ETc) and two-maturation degrees (maturation I and maturation II) on the quality on Chétoui olive oil were evaluated. The following parameters were monitored: Pomological parameters, free fatty acids, peroxide value, total phenols, bitterness intensity, oxidative stability, fatty acid composition and aromatic profiles. After the irrigation period, the virgin olive oil exhibited some modifications, mainly in terms of fatty acids composition and volatiles. The amount of oil in the fruit seems not to be influenced by the irrigation management. To obtain the best quality and to use the minimum amount of water, the best irrigation level was 75% ETc.

Key words: Irrigation management, maturation state, Chétoui virgin olive oil, volatiles, oxidative stability.

INTRODUCTION

The olive tree is a crop mainly located in the Mediterranean basin, which cultivation has recently been extended outside of its traditional environment in new producer countries such as Australia, as it is a crop of great economic and social relevance. Almost all these new orchards employ different irrigation techniques in order to accelerate the rate of growth of the olive trees, diminish the characteristic alternate bearing pattern of this crop, and increase fruit yields per hectare and consequently the oil production (Moriana et al., 2003). In Tunisia, the fourth largest producer of olive oil, in which the olive tree represent the most extensive woody crop, *Chétoui* is the second main olive variety, grown mainly in the North. As its name suggests in a traditional Tunisian dialect, Chéta (=like rain), this cultivar requires rainy conditions for optimum growth and fruit production. The olive tree is generally grown under irrigation, especially in the South of the country, a region with limited water resources. Nevertheless, since irrigation increases the yield of the olive orchard, even with a low amount of water, there is a growing interest in irrigated agriculture also in other regions of the country.

In a previous study (Issaoui et al., 2010), we have demonstrated that the *Chétoui* cultivar exhibit a very important plasticity and adaptability in terms of virgin olive oil quality, both in the North and South of Tunisia, in contrast to the cultivar *Chemlali*. In the present study other features of this variety, such as the influence of irrigation regimes and maturity index on the quality of the oil have been investigated. The main goals were therefore to optimize the conditions for sustainable irrigation of the *Chétoui* cultivar grown in the North, a region where aquifers are over-exploited, and to study the effect of different irrigation strategies on the

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composition and quality of the *Chétoui* virgin olive oil.

MATERIALS AND METHODS

Plant material and growing areas

The study was carried out on monovarietal virgin olive oils from the second main Tunisian cultivar, *Chétoui*. Olive oil samples were obtained, in triplicate, from hand-picked good quality fresh and healthy fruits. In the North, the mean precipitation and temperature registered were 600 to 1200 mm year^{-1} and 18°C.

In olive orchard, water was provided three time a week (from June to September) using a localized irrigation system with four drip nozzles of 8 l/h each per tree (two per side), placed in a line along the rows at a distance of 0.5 m from the trunk.

Chétoui cultivars were tested in a factorial combination with three irrigation levels [three plots of 36 m^2 (6 × 6 m) each were designed for each irrigation]: well irrigated (T3 and T2), and stressed (T1), receiving a seasonal water irrigation amount equivalent to 100, 75 and 50% of crop evapotranspiration (ETc) calculated using the Penman–Monteith–FAO method (Allen et al., 1998) with a single estimated crop coefficient (Kc = 0.6) and a coverage coefficient (Kr = 0.5) (D'Andria et al., 2004).

Morphological study

The variety characterization was achieved according to the method adopted by the International Olive Oil Council (IOOC), namely 'Methodology for the characterization of olive tree varieties'. Immediately before harvest, 100 fruits were randomly sampled from around the canopy of each tree to determine their maturation index (MI) according to a 0 to 7 scale (Boskou, 1996) and their fresh weight. The mean average weight of 40 fresh fruits was measured for each sample. Then, olives were de-stoned and the flesh (pulp) was separated in order to measure the pulp to stone ratio. The percentage of moisture content was determined using 40 g of olive fruits, which were dried in an oven at 80° till constant weight. Oil content, expressed as a percentage of dry weight, was determined by extracting dried material with 68° hexane in a *Soxhlet* apparatus, according to the procedure described in AOCS Regulation official method. The fruit ripeness index was assigned by qualitative evaluation of the olive skin and flesh colors (Uceda and Frias, 1975). This system is routinely used by the olive oil industry to characterize the degree of ripeness of olives arriving at their facilities. This evaluation was performed in triplicate.

Reagents and standards

p-Hydroxyphenyl-ethanol (*p*-HPEA) was obtained from *Janssen* Chemical Co. (Beerse, Belgium). Pure analytical standards of volatile compounds were purchased from *Fluka* and *Aldrich* (Milan, Italy).

Analytical methods

Quality parameters

Determinations of free acidity and peroxide value were carried out following analytical methods described in the EEC 2568/91 and EEC 1429/92 European Union Regulation.

Fatty acid methyl esters (FAMEs) analysis

The FAMEs were prepared as described by the EU official method. The chromatographic separation was carried out using a Hewlett-Packard (HP 5890) chromatograph, a split/splitless injector, and a flame ionization detector (FID) linked to an HP Chemstation integrator. A fused silica capillary column *HP-Innowax (*30 m × 0.25 mm × 0.25 µm*)* was used with nitrogen as the carrier gas at a flow rate of 1 ml min^{-1}; FID temp. 280°C; injector temperature 250°C and an oven temperature programmed from 180 to 250°C. Results were expressed as relative percent of total area. Iodine values were calculated from fatty acid percentages using the formula reported by Torres and Maestri (2006). The degree of oxidative susceptibility of the oils was estimated according to Cert et al. (1996).

Total phenols were determined colorimetrically as previously reported by Montedoro et al. (1992) and the results are expressed as 3,4-DHPEA equivalents.

Evaluation of the intensity of bitterness was carried according to the procedure described by Beltran et al. (2007).

Rancimat assay

Oxidation stability was evaluated by the Rancimat apparatus (Mod. 743, Metrohm Ω, Switzerland) using an oil sample of 3 g warmed to 120°C and an air flow of 20 L/h. Stability was expressed as induction time (hours).

Volatile compound analyses

Sampling

Solid phase micro extraction was used as a technique for headspace sampling of virgin olive oils. Supelco SPME devices coated with polydimethylsiloxane (PDMS, 100 µm) were used to sample the headspace of 2 ml of virgin olive oil inserted into a 5 ml glass vial and allowed to equilibrate for 30 min. After the equilibration time, the fiber was exposed to the headspace for 50 min at room temperature. Once sampling was finished, the fiber was withdrawn into the needle and transferred to the injection port of the gas chromatography (GC) and GC-MS system.

Identification

GC analyses were accomplished with an HP-5890 series II instrument equipped with a DB-5 capillary column (30 m × 0.25 mm, 0.25 µm film thickness), working with the following temperature programme: 60° for 10 min, ramp of 5° min^{-1} to 220°, injector and detector temperatures, 250°; carrier gas, helium (2 ml min^{-1}); detector FID; splitless injection. The identification of the components was performed by comparison of their retention times with those of pure authentic samples and by means of their linear retention indices (LRI) relative to the series of *n*-hydrocarbons. GC-EIMS analyses were performed with a Varian CP 3800 gas-chromatograph equipped with a DB-5 Capillary column (30 m × 0.25 mm; coating thickness= 0.25 µm) and a Varian Saturn 2000 ion trap mass detector. Analytical conditions were as follows: injector and transfer line temperature at 250 and 240° respectively; oven temperature was programmed from 60 to 240°C at 3° C min^{-1}; carrier gas, helium at 1 ml min^{-1}; splitless injection. Identification of the constituents was based on comparison of the retention times with those of authentic samples, comparing their linear retention indices relative to the series of *n*-hydrocarbons, and on computer matching against commercial (NIST 98 and ADAMS) and home made library mass spectra built from pure substances and components of known oils and MS literature data (Stenhagen and Abrahamsson, 1974; Swigar and Silvestein, 1981; Massada, 1976; Jennings and Shibanoto, 1980; Davies, 1990; Adams, 1995) Moreover, the molecular weights of all the identificated substances

Table 1. Soil characteristics of the experimental site.

Soil characteristics	Depth (0-40 cm)	Depth (40-80 cm)
pH	8.30	8.10
Clay	14	12
Silt	21	18
Sand	65	70
Organic matter	1.80	1.20
Calcium carbonate	25	33
Total N_2 (%)	0.78	0.65
K_2O (mg/kg)	282	255
P_2O_6	5.4	6
Conductivity PS (mmhos/cm)	0.82	1.03

Table 2. Olive fruit characteristics and composition as affected by different irrigation treatments and maturation state.

Variable	*Chétoui* variety					
Irrigation level	T1	T2	T3	T1	T2	T3
Maturation state		Maturation 1			Maturation 2	
Olives weight (g)	1.6	1.6	1.5	1.6	1.7	1.8
Pulp/ Stone ratio	3.8	4.1	3.5	4.2	4.1	4.2
Fruit damage (%)	10	17	20	12	20	25
Water content (%)	58.2	60.2	58.3	56.5	58.8	57.4
Oil content soxhlet (%)	10.1	9.8	10.3	10.0	9.8	10.1

T1: 50% Etc, flow of 8 l/tree h^{-1} (1540 l tree) being 440 m^3 ha^{-1}, T2: 75% Etc, flow of 12 l/tree h^{-1} (2300 l tree) being 660 m^3 ha^{-1}, T3: 100% Etc, flow of 16 L/tree h^{-1} (3070 l tree) being 880 m^3 ha^{-1}.

were confirmed by GC-CIMS, using MeOH as CI ionizing gas .

Statistical analysis

All the measurements were carried out in triplicate. Significant differences among varieties were determined by analysis of variance, by mean of Student test, using the SPSS program, release 11.0 for Windows.

RESULTS AND DISCUSSION

Pomological parameters

The soil characteristics are described in Table 1, while Table 2 lists the olive fruit characteristics and composition, as affected by the different irrigation treatments, and the ripening index of the fruits of the *Chetoui* cultivar. In the same ripening date, the average level was 1.6 for olives produced from trees submitted to 50% of irrigation volumes. Average values of 1.6 and 1.5 were measured for fruits obtained from trees submitted to a restitution of 75 and 100% respectively. With the progress of ripening, the size of the fruits increased. Consequently, also the pulp to stone ratio increased. This result is in good agreement with previous reports of the positive correlation between the amount of water supplied to the tree and size of the fruits (Gomez-Rico et al., 2007). Unfortunately, also the percentage of damaged fruits increased with the irrigation volumes. Hence, an irrigation volume higher than 75% affected the olive quality more markedly. The irrigation treatment apparently did not affect the oil accumulation in the *Chetoui* fruit since no statistically significant differences in the oil yield were observed during the three different irrigation volumes. Accordingly to this observation, also Lavee and Wodner (1991) and Motilva et al. (1999) reported only a slight delay in oil accumulation in fruits of non-irrigated olive trees because of hydric stress at the end of the summer season (Gomez-Rico et al., 2006).

Analytical parameters

The results of free fatty acid and peroxide value were indicated in the Table 3. The values of free acidity and peroxide value for all types of virgin olive oil (VOO) samples were considerably lower than the maximum limit (0.8% and 20 meqO_2/kg for acidity and peroxide value respectively) established by the EU legislation for the extra virgin olive oil category (EEC, 1991). It can be observed that almost no statistically significant influence of irrigation was visible on the amounts of free fatty acids.

Table 3. Quality index, fatty acid composition (%), iodine value, oxidative susceptibility and oxidative stability of virgin olive oils from *Chétoui* variety growing in the same area under three levels of irrigation.

Variable	***Chétoui* variety**					
Irrigation level	T1	T2	T3	T1	T2	T3
Maturation state	Maturation I			Maturation II		
Free acidity (%)	0.2[b]	0.2[b]	0.3[a]	0.4[b]	0.4[b]	0.5[a]
Peroxide index (meqO_2/kg)	8.0[a]	6.7[b]	6.7[b]	7[b]	12.3[a]	12.5[a]
Category	EV	EV	EV	EV	EV	EV
Palmitic acid [&]	11.9[a]	12.5[a]	11.1[a]	9.4[b]	12.9[a]	13.6[a]
Palmitoleic acid	0.5[a]	0.5[a]	0.5[a]	0.4[b]	0.4[b]	0.4[b]
Stearic acid	2.4[a]	2.1[a]	2.5[a]	2.5[a]	2.2[a]	2.4[a]
Oleic acid	70.0[b]	70.0[b]	72.0[a]	74.0[a]	70.0[b]	69.0[b]
Linoleic acid	14.1[a]	14.0[a]	12.9[b]	13.0[b]	14.1[a]	13.8[b]
Linolenic acid	0.9[a]	0.9[a]	0.9[a]	0.7[a]	0.8[a]	0.7[a]
Saturated fatty acid	14.3[a]	14.6[a]	13.6[a]	11.9[b]	15.2[a]	16.0[a]
Monounsaturated fatty acid	70.4[b]	70.4[b]	72.5[a]	74.3[a]	70.0[b]	70.0[b]
Polyunsaturated fatty acid	15.0[a]	14.9[b]	13.9[b]	13.8[a]	14.9[a]	14.5[a]
Oleic/linoleic ratio	5.0[a]	5.0[a]	5.6[a]	5.7[a]	5.0[a]	5.0[a]
Monounsaturated/polyunsatured ratio	4.7[a]	4.7[a]	5.2[a]	5.4[a]	4.7[a]	4.8[a]
Unsaturated fatty acid/ Saturated fatty acid	6.0[a]	5.9[a]	6.3[a]	7.4[a]	5.6[b]	5.2[b]
Oxidative suceptibility	91.5[a]	91.3[a]	91.2[a]	92.5[a]	92.5[a]	89.5[a]
Iodine value	800.0[a]	796.0[a]	749.0[b]	735.0[b]	783.0[a]	765.8[b]
Oxidative stability (hours)	13.4[a]	12.0[b]	14.4[a]	17.0[a]	15.2[b]	15.8[b]
Phenols (mg/kg)	641.1[a]	588[b]	645[a]	871[a]	804.0[b]	862[a]
Bitterness index	4.1[a]	3.9[ab]	4.1[a]	4.5[a]	4.5[a]	4.5[a]
Category	VB	VB	VB	VB	VB	VB

[&](%);[!] hours; VB, very bitter; NB, no bitter; IV, iodine value, OS, oxidative susceptibility; OSI, oxidative stability index; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid. EV, extra Virgin.

On the contrary, a slight increase of acidity was observed between the two-maturation states. The same behaviour was observed for the peroxide value. However, all the samples still remained within the extra virgin olive oil category. It can be concluded that neither the irrigation level nor the maturation index influences the main analytical parameters, and thus the category of olive oil. These results are in good agreements with the findings of Berenguer et al. (2006) for young olive orchards under different irrigation practices in Spain for the Arbequina cultivar and with those of Servili et al. (2007) in Italy for the Leccino cultivar.

Fatty acid composition

The influence of both maturation index and irrigation regimes on fatty acid composition are reported in the Table 3. At the first grade of maturation, the percentage of palmitic, stearic, oleic, linoleic and linolenic acids were not influenced by the kind of irrigation management (Table 3). However, at maturation grade I, at the highest level of water supplied to the olive tree, a statistically significant increase of oleic acid percentage (from 70.0 to 72.0%) and a decrease of linoleic and palmitic acids levels (from 14.0 to 12.9% and from 12.5 to 11.1% respectively) were observed. At the second maturation stage, an inverse trends of the fatty acids composition at the different water managements was noted. Hence, when the percentage of palmitic acid increased (from 9.4 to 13.6%) the percentage of oleic acid decreased (from 74.0 to 69.0%) as the amount of water supplied to the olive tree increased (Table 3). These results could be due the fact that the levels of saturated, polyunsaturated, monounsaturated fatty acids and oleic to linoleic acid ratio may have undergone some changes during ripening and also to the three different amounts of water supplied to the olive tree. However, these changes are very slight and do not have any nutritional relevance. Our results are in good agreement with those of Gomez-Rico et al. (2006, 2007).

Total phenols, bitterness index and intensity of bitterness

Phenols are the main antioxidant components naturally occurring in the olive drupe and in virgin olive oils. The influence of both maturation index and irrigation regimes on total phenols, bitterness and intensity of bitterness were indicated in the Table 3. It can be noted that total phenols were more influenced by the maturation state than the amount of water supplied to the olive tree. Thus, a marked increase of total phenols was observed in parallel to the increase of the maturation index (from 641.1 to 871 mg/kg at the same irrigation regimes).

Table 4. Aromatic composition (%) of virgin olive oils from Chétoui variety growing in the same area under three levels of irrigation.

Variable		***Chétoui* variety**					
Irrigation level		T1	T2	T3	T1	T2	T3
Maturation state			Maturation I			Maturation II	
Aromatic compounds (%)	Sensory notes						
Hexanal	Green, Apple	17.3	16.7	32.9	29.3	29.9	42.4
(E)-2-Hexenal	Green, almonds	20.7	38.1	24.9	17.6	24.3	16.2
Heptanal		3.0	4.0	2.5	2.3	3.5	2.8
(E,E)-2,4-hexadienal		2.1	1.0	trace	0.0	1.4	trace
3-methyl-4-heptanone		9.9	8.2	7.5	6.3	6.6	6.8
5-methyl-3-heptanone		9.3	7.9	7.4	8.3	8.7	9.2
1-Heptanol				5.1			3.7
6-methyl-5-hepten-2-one			1.5				
(E,Z)-2,4-heptadienal		23.6			20.1		
(Z)-3-hexenyl acetate	Fruity, green leaves	0.0	trace	3.1	1.0	4.9	3.4
1-hexyl acetate	Sweet, green, fruity				trace	1.5	trace
Limonene	Greenery, fruity	0.0	3.0	0.9	1.7	7.7	5.7
Phenylacetaldehyde		3.3	4.6	2.6			trace
n-Undecane		trace	trace	trace	trace	0.9	trace
Nonanal		2.3	2.0	1.5	1.0	1.1	1.0
Decanal		1.8	1.8	1.9	0.9	1.6	1.6
α-Copaene		2.9	2.5	2.8	3.2	2.4	2.6
Trans-α-Bergamotene		trace			trace		
(Z)-β-Farnesene		1.6			0.8		
(E,E)-α-farnesene	Soft cooking of vegetale	1.9	2.2	2.2	0.6	2.0	0.8
Total identified %		99.8	93.6	95.4	93.4	96.6	96.3
Number of compounds		17	15	18	16	14	16

Consequently, bitterness index and bitterness intensity of Chétoui virgin olive oil, obtained from trees grown in the north under high precipitation levels, were not influenced by the kind of the irrigation regimes (Table 3). Consumers like less oils with a high level of bitterness and therefore the decrease of this attribute would be desirable in a virgin olive oil rich in phenols, such as the *Chétoui* variety. On the contrary, this could possibly negatively affect the sensory profile of Chemlali VOO (Issaoui et al., 2010). In fact, because of its natural low phenolic content, a decrease in the levels of these compounds could lead to a virgin olive oil with a taste too mild and flat. In addition, it would also cause a decrease of its oxidative stability which would lead to a significant reduction of the shelf life of this product and consequently health properties of the consumer.

Oxidative stability, oxidative susceptibility and iodine value

The influence of both maturation index and irrigation regimes on oxidative stability, oxidative susceptibility and iodine value are presented in Table 3. It can be noted that the irrigation level had no significant influence on oxidative stability, oxidative susceptibility and iodine values of *Chétoui* virgin olive oil. The present results were in accordance with our previous one (Issaoui et al., 2011).

Volatile compounds

The volatiles of the virgin olive oils obtained from fruits at two different maturation stages and three irrigation managements are presented in Table 4. A considerable variation was noted between these samples. The C6-aldehydes fraction constituted the main volatile chemical class of the virgin olive oil, whatever the maturation stage and the irrigation regime. The principal constituent of this fraction was hexanal (with apple, green and cut grass sensory notes), whose average content ranged from 16.7 to 32.9% during the first maturation stage and from 29.3 to 42.4% during the second one (Table 4). The *(E)*-2-Hexenal percentage varied from 20.7 to 38.1% in the first maturation stage and from 16.2 to 24.3% in the second one. Maturation stage seems to be a strong influent factor on the level of aldehydes. Regarding C6 esters, such as hexyl acetate and *(Z)*-3-hexenylacetate (with sweet, fruity and green leaves notes), these chemicals were detected in very small amounts in *Chétoui* virgin olive oil, indicating a low activity of the alcohol acyl transferase (AAT) in this cultivar.

Although the volatile composition in the same variety may also be influenced by seasonal factors, there were similar and clear changes in the volatiles that can be attributed to the different irrigation practices applied in the orchards. The volatile compounds most affected by the different irrigation regimes were hexanal, *(E)*-2-hexenal, *(Z)*-3-hexenyl acetate and limonene, which have shown an inverse relationship with the water stress suffered by the olive plants (Table 4). These effects were mainly reflected we noticed a marked increase in sensory notes of apple, green and cut grass in *Chétoui* virgin olive oil, due to the increase of hexanal, when the amount of water supplied to the olive tree increase (Table 4). *(E)*-2-Hexenal exhibited a different trend compared to that of hexanal. The highest percentage of *(E)*-2-hexenal occurred, for both the maturation stages, in the *Chétoui* virgin olive oil obtained from plants irrigated at 75% regime. The same was true for limonene. In the case of *(Z)*-3-hexenyl acetate, its percentage increased with the amount of water used (Table 4). Some terpenes were correlated only with an appropriate level of irrigation. The most apparent case regarded (*E,Z)*-2,4-heptadienal (23.6 and 20.1%) that was detected only at the lowest level of irrigation (50%). *(E,E,)*-α-farnesene (1.6 and 0.8%)) behaved the same way. On the contrary, 1-heptanol (5.1 and 3.7%) was detected only when the olive trees were irrigated with the highest percentage of water (100%).

Besides the irrigation regime, also the maturation stage seems to have an important effect on the occurrence and the amounts of some volatiles (Table 4). The level of hexanal increased with the increase of maturation stage passing, at the same irrigation level (T1= 100%ETc), from 32.9 to 42.4%. Conversely, the levels of *(E)*-2-hexenal decreased with ripening passing, at the same irrigation level (T1= 100%ETc), from 24.9 to 16.2% (Table 4). Some compounds such as 1-hexyl acetate, were detected only at the second maturation stage or, as phenyl acetaldehyde, disappeared with ripening.

Conclusion

The selection of an optimal irrigation treatment of the traditional olive orchards in Northern Tunisia, where water resources are sufficiently available, requires the establishment of an appropriate compromise between olive production, quality of virgin olive oil and water consumption. Therefore, based on the results of this study, the best irrigation treatment for this region is apparently 75% ETc. In fact, with 75% Etc, we can obtain in the one hand, an olive oil sample with high level of oleic acid, total phenols, (*E)*-2-Hexenal and a green and fruity taste and in the other hand, olive fruit with acceptable amount of oil.

ACKNOWLEDGEMENTS

The Tunisian Ministry of Enseignement Superieur, Scientific Research and Technology (UR03/ES-08) supported this research. Part of this work was carried out at the Dipartimento di Scienze Farmaceutiche, Sede di Chimica Bioorganica e Biofarmacia, Università di Pisa, Italy. We wish to thank the personnel of the laboratory of ''Human Nutrition and Metabolic Disorder" Faculty of Medicine of Monastir, Institute of Olivier of Sousse''.

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