

Impact of olives storage and irrigation with treated wastewater on the oil quality: simulation of mill conditions

Impacto del almacenamiento de aceitunas y el riego con aguas residuales tratadas sobre la calidad del aceite: simulación de las condiciones de almazara

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ABSTRACT

The purpose of this research was to assess the impact of irrigation by treated wastewater on the Tunisian olive oil quality. In this experiment, olives from fields irrigated with treated wastewater were transported to the laboratory and mixed with other batches of olives, at different proportions, of the same variety and coming from not irrigated olive groves. In order to meet the work schedule of Tunisian mills, the mixtures of olives obtained were stored in a laboratory at room temperature for 3, 6 and 9 days before being crushed. The results showed that the free fatty acids and the carotene content of the oils do not depend on the irrigation system, but are dependent on the duration of olive storage before the extraction. On the contrary, the irrigation system affects the oxidation state of the oils and their fatty acid composition.

Key words: treated wastewater, irrigation, mill conditions, olive oil quality

RESUMEN

El propósito de esta investigación fue evaluar el impacto del riego con aguas residuales tratadas sobre la calidad del aceite de oliva de Túnez. En este experimento, las aceitunas de los campos regados con aguas residuales tratadas se transportaron al laboratorio y se mezclaron con otros lotes de aceitunas, en diferentes proporciones, de la misma variedad y procedentes de los olivares no regados. Con el fin de cumplir con el programa de trabajo de los molinos tunecino, las mezclas de aceitunas obtenidas se almacenaron en un laboratorio a temperatura ambiente durante 3, 6 y 9 días, antes de ser prensadas. Los resultados mostraron que los ácidos grasos libres y el contenido de caroteno de los aceites no dependen del sistema de riego, pero dependen de la duración del almacenamiento de oliva antes de la extracción. Por el contrario, el sistema de riego afecta al estado de oxidación de los aceites y su composición en ácidos grasos.

Palabras clave: aguas residuales tratadas, riego, condiciones de almazara, calidad del aceite de oliva

INTRODUCTION

Olive tree (*Olea europaea* L.) is the most ever-green tree grown in arid and semi-arid regions, where plants are frequently subjected to high temperatures and scarcity of water. Moreover, it is demonstrated that the olive trees respond favourably and efficiently to irrigation management (Celano *et al.*, 1999).

Within countries of the southern part of the Mediterranean Sea, natural water resources are limited, whereas their demand is constantly

increasing. Therefore, non-conventional water resources became important to satisfy different agricultural needs. In Tunisia, the re-use of treated wastewater (TWW) has been adopted since the 1960s with the planning of many irrigated perimeters (Klay *et al.*, 2010); about 30–43% of this water was used for agricultural and landscape irrigation. Re-using wastewater for irrigation is viewed as a way to increase water resources, provide supplemental nutrients and protect coastal areas. Reclaimed water is used on 8000 ha to irrigate industrial and fodder crops, cereals, vineyards, citrus and other fruit trees (olives, peaches, pears, apples, pomegranates, etc.).

On the other hand, the use of wastewater in agriculture is often associated with significant health risks because of the presence of high concentrations of micro organisms, enteric in origin, such as bacteria, fungi, viruses, protozoa and helminths (Toze, 2006). These micro organisms secrete substances (enzymes) that can affect the quality of the final product, especially if fruits are stored before processing. During the storage of olives, many mould strains, in particular *Aspergillus* and *Penicillium*, are able to develop and produce ochratoxin A, citrinin and aflatoxins (El Adlouni *et al.*, 2006).

Besides microorganisms, chemical contaminants can be of concern, especially in countries where industrial development has started and industrial effluent enters in contact with domestic wastewater and natural streams (Asehrou *et al.*, 1997).

The quality of the water utilised for irrigation can also affect the trace levels of various metals in vegetable oils (Ansari *et al.*, 2009). Many reports have described the deleterious effects that trace elements have on the flavour of oils and on their oxidative stability (Chen *et al.*, 1999; Murillo *et al.*, 1999). In Tunisia, the production of olives belonging to trees irrigated with TWW is not handled in isolated mills. In fact, batches of these olives can easily be transported to local mills and mixed with fruits from other sources.

To assess the impact of TWW on the quality of olive oil produced, different proportions of olives belonging to trees irrigated with TWW were mixed with olives belonging to trees not irrigated. In order to meet the work schedule of Tunisian mills, the mixtures of olives obtained were stored in a laboratory at room temperature for 3, 6 and 9 days before being crushed. The quality of the oils produced was finally evaluated by performing the following analyses: free fatty acids, specific extinction coefficients K232 and K270, pigments and fatty acid composition. In a previous publication, it has demonstrated that these parameters are affected by the irrigation of the Chemlali olive variety with treated wastewater (Gharsallaoui *et al.*, 2011).

MATERIAL AND METHODS

Olives sampling

El Hajeb, located 10 Km south west of Sfax City (34°43'N, 10°41'E) in central eastern Tunisia,

was chosen as experimental site. This site, characterized by sandy soils (84.4% of sand; 9.8% of clay and 5.8% of silt), is affected by the Mediterranean bioclimate. The mean precipitation registered was about 190 mm/year, with a mean temperature of approximately 30 °C (2007 / 2008).

Two experimental plots, consisting of eighteen year old trees with spacing of 24x24 m and divided into six completely randomized blocks, were selected for the experimental research. Each block contained nine "Chemlali" olive trees. On the first experimental plot, TWW was used to irrigate the olive trees at an annual rate of 5,000 m³/ha, by means of a continuous drip irrigation system. This type of irrigation was performed from April to May and from October to December, starting from the crop year 1991/1992. Olive samples were collected in the crop year 2007/2008 in the middle of December; healthy fruit samples (5 Kg) were harvested from each olive tree from each block and immediately brought to the laboratory.

Different mixtures of healthy olives (free from any type of infestation) were prepared by varying the percentage of olives coming from the plot irrigated with treated wastewater (OTWW) and the olives coming from the non-irrigated plot. Ten olive mixtures were finally obtained having the following content of OTWW (%): 5, 10, 20, 25, 30, 35, 40, 45 and 50%. A sample with no OTWW was used as a control. Mixtures with higher percentages of OTWW were not taken into account as TWW is still not widely used to irrigate olive trees and, therefore, it was not very easy to obtain such a mixture. Besides, since in Tunisian mills olives are kept aside for some days at room temperature before being crushed, these mixtures were processed after 3, 6 and 9 days from their harvesting.

Storage condition

Mixtures of healthy fruits were stored in the laboratory at room temperature, in perforated plastic containers for 0, 3, 6 and 9 days. Conditions of storage are detailed in Table 1. Day 0 is taken as reference in determining the effect of olive storage on the quality of olive oil.

Chemical characteristics of TWW

TWW has domestic and industrial origins and is typically treated at secondary level using an aerobic biological process that consists of eliminating the

biodegradable matters by their transformation into microbial residues. The pH of the TWW is 7.60, falling within the 6–9 range, which is regarded as appropriate for irrigation reuse (Rattan *et al.*, 2005). Its electrical conductivity was determined to be 6.30 dS/m, indicating a high level of salinity (Weisman *et al.*, 2004). The monthly average values of the biological oxygen demand of the TWW produced in Sfax varies between 22.1 and 114.5 mg of O₂/L, values which may exceed the limit fixed by the Tunisian standard (30 mg of O₂/L) (Ouali *et al.*, 2008). The chemical oxygen demand varied between 201 and 273 mg of O₂/L, which also exceed the limit fixed by the Tunisian standards (90 mg O₂/L). These high values of chemical oxygen demand can be explained by considering that treated sewage of Sfax city is a mixture of urban and industrial wastewater.

Suspended solid fluctuates between 39 and 60 mg/L, values always greater than the limit required by the Tunisian standard (30 mg/L). These materials may contain pollutants such as heavy metals (Ouali *et al.*, 2008). TWW was also tested for the phyto-toxicity index by means of the germination index of *Lepidium sativum* (a plant very sensitive to organic and mineral pollutants) (Zucconi *et al.*, 1981) and for the micro-toxicity. The latter was accomplished by considering the inhibition of the bioluminescence of *Vibrio fischeri* LCK480 using the LUMISTox system according to ISO/DIS 11348-2 (1998). LUMISTox and germination index of *L. sativum* tests demonstrated that TWW from Sfax exhibited a high micro and phyto-toxicity (Ellouze *et al.*, 2009).

Oil extraction

Olive oil extraction was carried out using an Abencor system. The fresh and stored olives (1.5-2.0 Kg) were crushed by means of a hammer mill and

Table 1. Laboratory conditions during olives storage period.

	Temperature (°C)	Humidity (%)
15/01/2008	12	62
16/01/2008	10	60
17/01/2008	11	60
18/01/2008	10	54
19/01/2008	10	42
20/01/2008	10	72
21/01/2008	12	64
22/01/2008	14	54
23/01/2008	13	67
24/01/2008	14	69

slowly mixed; without adding any water, at 25 °C for 30 min. The resulting paste was centrifuged at 3500 rpm for 3 min, in order to separate the liquid and solid phases. The liquid phase obtained (containing oil and water) was decanted. The volume of olive oil, resulting from decantation, was filtered in order to eliminate any impurity. All samples were subsequently placed in amber glass bottles and stored in the dark at 4 °C.

Analytical indices

Free fatty acids and ultra violet absorbances at 232 and 270 nm were determined according to the European Official Methods of Analysis (EEC, 1991).

Fatty acid composition

Fatty acid methyl esters (FAMES) analysis were carried out after performing alkaline treatment obtained by dissolving the oil (0.05 g) in n-hexane (1 mL) and adding a solution of potassium hydroxide (1 mL; 2N) in methanol (Christie, 1998). FAMES were analyzed by gas chromatography by means of a Shimadzu 17A gas chromatograph equipped with a flame ionization detector (FID) and a capillary column. The operation conditions were as following: the column temperature was set at 180°C; the injector temperature was set at 230°C; the detector temperature was set at 250°C. The carrier gas was nitrogen with a head pressure of 0.6 bar ; for the FID the pressure of air was at 1.5 bar and the hydrogen pressure 0.8 bar. Separation was accomplished by injecting 1 µl of solution onto a capillary column of 15 m length, 0.32 mm of diameter, with a film thickness of 0.25 µm. The polar stationary phase was cyanopropymethyl/phenylmethyl-polysiloxane 1:1. Peaks were identified by comparing their retention times with those of authentic reference compounds. The fatty acid composition was expressed as relative percentages of each fatty acid calculated using internal standard normalization of the chromatographic peak area.

Carotenes and chlorophylls

Chlorophylls and carotenes (mg/Kg of oil) were determined using specific extinction values according to the method of Minguez-Mosquera *et al.* (1991).

Statistical analysis

All analytical determinations were conducted in triplicate. Values of different parameters were

expressed as the mean \pm standard deviation. Significant differences between means ($P < 0.05$) were determined by Fisher's test using SPSS software for windows (SPSS 11, USA).

RESULTS AND DISCUSSION

Free fatty acids

The free fatty acid values of the olive oil mixtures that contain a percentage of OTWW lower than 20% and immediately processed after the harvesting were lower than the upper limit of 0.8% established for "Extra Virgin" olive oil by the EEC Regulation no. 702/2007 (European Union Commission 2007), which amends Commission Regulation (EEC) no. 2568/91. In all the other cases these values were in a range between 0.88 and 3.1 (Table 2). Statistical analysis showed that the free fatty acids do not depend on the percentage of OTWW, but is dependent on the duration of storage of the olives. These results are in good agreement with those found by Al-Absi (2008) and Mousa (2010) who have studied the effect of irrigation with industrial wastewater on the quality of oils extracted from three varieties (Nabli, Improved Nabli and Manzanillo), proving that irrigation by this type of water does not affect the free fatty acids of oils from the three studied varieties. In a previous study (Gharsallaoui *et al.*, 2011), it was found that irrigation of the Chemlali olive variety, by treated wastewater, significantly affects the olive oil free fatty acids when the OTWW proportion is 100%, but Bedbabis *et al.*, (2009, 2010a) who studied the effect of the irrigation with treated wastewater (TWW) on the quality parameters of Chemlali olive oil found that irrigation with TWW did not affect the examined oil quality indices (free fatty acids), the mean values of this parameter was lower than the upper limits established for the best commercial olive oil quality designated as extra virgin. Also, Bedbabis *et al.*, (2010b) in a study to investigate the effects of moderate saline water irrigation in the "Chemlali" olive cultivar grown in Sfax, Tunisia, in comparison to a control plot grown under rain-fed conditions, found that irrigation did not affect free fatty acids.

Oxidative state

The specific extinction coefficient at 232 nm wavelength, K232, is related to the primary oxidation of oil and indicates conjugation of poly-unsaturated fatty acid, whereas K270 is related to the secondary

oxidation products and indicates the presence of carboxylic compounds such as aldehydes and ketones (Garcia *et al.*, 1996). Ultra violet-specific extinction determination permits an approximation of the oxidation process in unsaturated oils (Gutierrez *et al.*, 1992).

The values of the coefficients K270 and K232 were between 0.06 and 0.25 for K270 and between 1.12 and 2.31 for K232. Results showed that during the first 3 days of storage primary oxidation products decreases in the majority of samples (Table 2) but, Bedbabis *et al.*, (2009, 2010a) found that irrigation with treated wastewater did not affect the specific ultraviolet absorbance K232 and K270 of the Chemlali olive oil. This phenomenon can be explained by the fact that during this period the primary products (hydroperoxides) are degraded to a secondary oxidation product, which is confirmed by an increase of the coefficient K270 (Olias-Jiménez and Gutiérrez-González-Quijano, 1970). However, between the 3rd and 6th day of storage, the coefficients K232 and K270 increase in all the samples and in all the mixtures (Table 2). This increase can be explained by the fact that the two oxidation reactions continue simultaneously, with a reaction rate equal to that of primary oxidation, which allows the increase of primary oxidation products, even after the transformation of some of these products into secondary oxidation ones. After 6 days of storage, the primary products of oxidation decrease again which means that the secondary oxidation reaction outweighs the initial reaction of oxidation. In conclusion, during the period of olive storage both oxidation reactions continue, with successive dominance of one over the other in turn.

Statistical analysis showed that the olive storage duration and the percentages of OTWW affect the ultra violet light absorbance of the oils. In fact, the oils with a high percentage of OTWW resulted to be more susceptible to the oxidation, especially if fruits were stored before being processed. These results confirm those of Gharsallaoui *et al.* (2011).

Chlorophylls

Chlorophylls A and B and their oxidation products, pheophytins A and B, are naturally occurring in vegetable oils and are responsible of the greenish colour of the oils. Except for virgin olive oil, where a greenish colour is tolerated, an excessive amount of chlorophyll (> 20 mg/Kg) is considered

Table 2. Evolution of analytical characteristics of oils obtained from different proportion of olives coming from the plot irrigated with treated wastewater (OTWW) olives processed at different times after harvesting.

OTWW percentage	Storage time (days)	Free fatty acids (g of oleic acid/100g of oil)	K232	K270
0	0	0.56 ^{a,x}	1.73 ^{b,x}	0.11 ^{a,x}
	3	0.88 ^{b,x}	1.23 ^{a,x}	0.13 ^{b,x}
	6	2.40 ^{c,x}	2.22 ^{b,x}	0.14 ^{c,x}
	9	3.10 ^{d,x}	2.25 ^{a,x}	0.16 ^{d,x}
5	0	0.60 ^{a,x}	2.25 ^{b,x}	0.11 ^{a,x}
	3	1.30 ^{b,x}	1.21 ^{a,x}	0.13 ^{b,x}
	6	1.98 ^{c,x}	1.93 ^{b,x}	0.15 ^{c,x}
	9	2.90 ^{d,x}	1.85 ^{a,x}	0.16 ^{d,x}
10	0	0.66 ^{a,x}	2.08 ^{b,x}	0.11 ^{a,x}
	3	1.24 ^{b,x}	1.62 ^{a,x}	0.13 ^{b,x}
	6	2.16 ^{c,x}	1.75 ^{b,x}	0.14 ^{c,x}
	9	2.94 ^{d,x}	1.81 ^{a,x}	0.16 ^{d,x}
15	0	0.73 ^{a,x}	1.89 ^{b,y}	0.13 ^{a,y}
	3	1.28 ^{b,x}	1.41 ^{a,y}	0.15 ^{b,y}
	6	2.10 ^{c,x}	2.31 ^{b,y}	0.15 ^{c,y}
	9	2.73 ^{d,x}	1.82 ^{a,y}	0.16 ^{d,y}
20	0	1.06 ^{a,x}	1.91 ^{b,y}	0.13 ^{a,y}
	3	1.26 ^{b,x}	1.99 ^{a,y}	0.15 ^{b,y}
	6	2.00 ^{c,x}	2.18 ^{b,y}	0.15 ^{c,y}
	9	2.72 ^{d,x}	1.41 ^{a,y}	0.16 ^{d,y}
25	0	0.92 ^{a,x}	1.91 ^{b,y}	0.13 ^{a,y}
	3	1.24 ^{b,x}	1.92 ^{a,y}	0.15 ^{b,y}
	6	1.90 ^{c,x}	2.16 ^{b,y}	0.15 ^{c,y}
	9	2.88 ^{d,x}	1.40 ^{a,y}	0.18 ^{d,y}
30	0	0.80 ^{a,x}	2.20 ^{b,y,z}	0.13 ^{a,y}
	3	1.20 ^{b,x}	2.03 ^{a,y,z}	0.16 ^{b,y}
	6	1.92 ^{c,x}	1.64 ^{b,y,z}	0.16 ^{c,y}
	9	2.76 ^{d,x}	1.56 ^{a,y,z}	0.19 ^{d,y}
35	0	0.82 ^{a,x}	2.02 ^{b,z}	0.14 ^{a,z}
	3	1.30 ^{b,x}	2.13 ^{a,z}	0.17 ^{b,z}
	6	2.13 ^{c,x}	2.31 ^{b,z}	0.21 ^{c,z}
	9	2.76 ^{d,x}	1.56 ^{a,z}	0.24 ^{d,z}
40	0	0.92 ^{a,x}	1.68 ^{b,z}	0.14 ^{a,z}
	3	1.40 ^{b,x}	1.52 ^{a,z}	0.17 ^{b,z}
	6	1.90 ^{c,x}	2.13 ^{b,z}	0.20 ^{c,z}
	9	2.74 ^{d,x}	2.24 ^{a,z}	0.25 ^{d,z}
50	0	0.96 ^{a,x}	2.15 ^{b,z}	0.15 ^{a,z}
	3	1.28 ^{b,x}	1.51 ^{a,z}	0.18 ^{b,z}
	6	2.13 ^{c,x}	2.13 ^{b,z}	0.22 ^{c,z}
	9	2.80 ^{d,x}	1.66 ^{a,z}	0.26 ^{d,z}

Different letters (a-d) within values indicate the significant difference ($P < 0.05$) between olive storage time (0, 3, 6 and 9 days).

Different letters (x,z) within values indicates non significant difference ($P < 0.05$) between different proportion of OTWW olives (0, 5, 10, 15, 20, 25, 30, 35, 40 and 50%).

Each mixture and each storage time was performed in triplicate

K232: Absorbance of the oil at 232 nm and K270: Absorbance of the oil at 270 nm.

undesirable as it is difficult to remove by conventional bleaching processes (Abraham and De Man, 1986). The quantities of chlorophyll found in the oils analysed were low (0.72-1.5 mg/Kg) compared with those found by other authors for the same variety of olives (8.8- 9.67 mg/Kg) (Dabbou *et al.*, 2010; Issaoui *et al.*, 2010) but similar to those of Bedbabis *et al.*, (2010b) who reported chlorophyll contents from 0.46 to 1.28 ppm. These results are not very surprising as the olives were collected in an advanced stage of maturity. Several authors have, in fact, studied the evolution of these pigments with the maturity and have shown that their level is significantly dependent on the harvest period and tend to disappear at the end of maturity (Lazzez *et al.*, 2008; Oueslati *et al.*, 2009). Therefore, olive storage and percentage of OTWW do not affect the oils chlorophyll contents (Table 3).

Results of Gharsallaoui *et al.*, (2011) also showed that irrigation with treated wastewater did not affect the chlorophyll content, whereas in this study, during storage of the fruit, a transfer of chlorophyll

pigments was not observed, as was the case in a previous study (Gharsallaoui *et al.*, 2011). This can be explained by the fact that the content of these pigments is very low, which limits its transfer during storage.

Carotenes

Carotenes are a large group of intense red and yellow pigments found in all plants that photosynthesize. They are vital for the process of photosynthesis and also protect the plant against damage from the free radicals produced during photosynthesis. Virgin olive oil is one of the few vegetable oils consumed in its natural state. Consequently, it is a good source of antioxidants such as polyphenols, α -tocopherol and β -carotene (Rahmani and Saari, 1998). β -carotene is the most important provitamin A source. It is also involved in the oxidative stability of the oil and has a protective role against cancer and cardiovascular diseases (Gimeno *et al.*, 2000). The results obtained (Table 4) have shown that carotene content do not depend on

Table 3. Chlorophyll content (ppm) of the oils obtained from different proportion of olives coming from the plot irrigated with treated wastewater (OTWW) olives processed at different times after harvesting.

Storage time (d)	OTWW percentage									
	0	5	10	15	20	25	30	35	40	50
0	0.72 ^{a,x}	0.95 ^{a,x}	0.97 ^{a,x}	0.93 ^{a,x}	0.93 ^{a,x}	1.18 ^{a,x}	0.77 ^{a,x}	0.78 ^{a,x}	0.76 ^{a,x}	0.78 ^{a,x}
3	1.08 ^{a,x}	1.18 ^{a,x}	0.91 ^{a,x}	1.07 ^{a,x}	1.07 ^{a,x}	1.01 ^{a,x}	1.09 ^{a,x}	0.85 ^{a,x}	0.87 ^{a,x}	0.91 ^{a,x}
6	1.07 ^{a,x}	1.50 ^{a,x}	1.32 ^{a,x}	1.27 ^{a,x}	1.30 ^{a,x}	1.05 ^{a,x}	1.04 ^{a,x}	0.99 ^{a,x}	0.87 ^{a,x}	0.88 ^{a,x}
9	1.09 ^{a,x}	1.31 ^{a,x}	0.97 ^{a,x}	1.11 ^{a,x}	0.96 ^{a,x}	0.95 ^{a,x}	0.96 ^{a,x}	0.97 ^{a,x}	0.90 ^{a,x}	0.92 ^{a,x}

The same letter (a) within values indicates not significant difference ($P \geq 0.05$) between olive storage time (0, 3, 6 and 9 days)

The same letter (x) within values indicates not significant difference ($P \geq 0.05$) between different proportion of OTWW olives (0, 5, 10, 15, 20, 25, 30, 35, 40 and 50%).

Each mixture and each storage time was performed in triplicate.

Table 4. Carotene content (ppm) in the oils obtained from different proportion of olives coming from the plot irrigated with treated wastewater (OTWW) olives processed at different times after the harvesting.

Storage time (d)	OTWW percentage									
	0	5	10	15	20	25	30	35	40	50
0	12.95 ^{a,x}	11.41 ^{a,x}	12.53 ^{a,x}	11.82 ^{a,x}	12.67 ^{a,x}	12.31 ^{a,x}	14.25 ^{a,x}	9.92 ^{a,x}	11.65 ^{a,x}	13.86 ^{a,x}
3	12.33 ^{c,x}	13.08 ^{c,x}	13.72 ^{c,x}	14.84 ^{c,x}	14.25 ^{c,x}	13.76 ^{c,x}	12.31 ^{c,x}	14.31 ^{c,x}	15.10 ^{c,x}	16.16 ^{c,x}
6	17.54 ^{d,x}	16.74 ^{d,x}	15.82 ^{d,x}	15.53 ^{d,x}	13.01 ^{d,x}	15.34 ^{d,x}	14.23 ^{d,x}	18.24 ^{d,x}	14.75 ^{d,x}	12.08 ^{d,x}
9	14.08 ^{b,x}	16.32 ^{b,x}	13.79 ^{b,x}	13.06 ^{b,x}	12.54 ^{b,x}	13.25 ^{b,x}	12.64 ^{b,x}	13.50 ^{b,x}	12.87 ^{b,x}	11.72 ^{b,x}

Different letters (a-d) within values indicate significant difference ($P < 0.05$) between olive storage time (0, 3, 6 and 9 days)

The same letter (x) within values indicates not significant difference ($P \geq 0.05$) between different proportion of OTWW olives (0, 5, 10, 15, 20, 25, 30, 35, 40 and 50%).

Each mixture and each storage time was performed in triplicate.

the percentage of OTWW. On the contrary, it is significantly dependent on the duration of olive storage before the extraction. Moreover, the carotene content increases during the first 6 days of olives storage and declines thereafter. This can be explained by the fact that during the first 6 days of storage there is a transfer of pigments from the aqueous phase of the fruit to the oily phase followed by a degradation of these pigments, probably due to the action of chemical or enzymatic reactions that occur in the fruit. Other authors have also found that some technological and agronomical factors affect the pigment transfer from the paste to the oil fraction (Criado *et al.*, 2007).

Fatty acid composition

Olive oil has a characteristic fatty acid composition dominated by oleic acid (C18: 1), a mono unsaturated fatty acid present in large quantities, followed by linoleic acid (C18:2), a poly unsaturated fatty acid, and by palmitic acid (C16: 0), a saturated fatty acid. The quality of an olive oil is

even more attractive when the oleic acid content is high and the percentage of palmitic and linoleic acids are low. The fatty acids analysed were as follows: palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2). The results obtained are reported in Table 5. Palmitic acid values range between 17.33 and 18.85%. These values are within the normal range expected for olive oils (Codex Stan 33, 1989) and are similar to those found for Chemlali variety grown in the Sfax region and in the South of Tunisia (Lazzez *et al.*, 2008; Oueslati *et al.*, 2009). Statistical analysis showed that the palmitic acid proportion is independent of the OTWW proportion. This result agrees well with that of (Gharsallaoui *et al.*, 2011) who found that irrigation with treated wastewater has no significant effect on the palmitic acid content. Regarding stearic acid, it shows behaviour similar to that of palmitic acid. The percentages of palmitoleic and linoleic acids cover the normal range expected for olive oils (Rahmani and Saari, 1998) and show significant variance depending on the proportion of OTWW. Bedbabis *et al.*, (2009, 2010a) found a

Table 5. Fatty acid composition (%) of the oils obtained from different proportion of olives coming from the plot irrigated with treated wastewater (OTWW) olives processed at different times after harvesting.

Fatty acid	Storage time (d)	OTWW percentage									
		0	5	10	15	20	25	30	35	40	50
C16 :0	0	18.05 ^{a, x}	17.77 ^{a, x}	17.81 ^{a, x}	17.87 ^{a, x}	18.20 ^{a, x}	18.29 ^{a, x}	18.68 ^{a, x}	18.70 ^{a, x}	18.85 ^{a, x}	18.64 ^{a, x}
	3	17.53 ^{a, x}	17.77 ^{a, x}	17.68 ^{a, x}	17.98 ^{a, x}	18.08 ^{a, x}	18.23 ^{a, x}	18.39 ^{a, x}	18.40 ^{a, x}	18.56 ^{a, x}	18.47 ^{a, x}
	6	18.49 ^{a, x}	17.51 ^{a, x}	17.62 ^{a, x}	17.90 ^{a, x}	17.96 ^{a, x}	18.00 ^{a, x}	17.99 ^{a, x}	18.50 ^{a, x}	18.48 ^{a, x}	18.68 ^{a, x}
	9	17.33 ^{a, x}	17.35 ^{a, x}	17.40 ^{a, x}	17.65 ^{a, x}	17.79 ^{a, x}	18.13 ^{a, x}	18.77 ^{a, x}	18.59 ^{a, x}	18.58 ^{a, x}	18.62 ^{a, x}
C16 :1	0	2.01 ^{a, x}	2.00 ^{a, x}	2.02 ^{a, x}	2.02 ^{a, x}	2.05 ^{a, y}	2.07 ^{a, y}	2.13 ^{a, z}	2.11 ^{a, z}	2.13 ^{a, z}	2.08 ^{a, z}
	3	2.01 ^{a, x}	2.05 ^{a, x}	1.98 ^{a, x}	2.06 ^{a, x}	2.06 ^{a, y}	2.07 ^{a, y}	2.09 ^{a, z}	2.11 ^{a, z}	2.12 ^{a, z}	2.09 ^{a, z}
	6	2.00 ^{a, x}	1.99 ^{a, x}	1.99 ^{a, x}	2.02 ^{a, x}	2.05 ^{a, y}	2.03 ^{a, y}	2.05 ^{a, z}	2.10 ^{a, z}	2.07 ^{a, z}	2.09 ^{a, z}
	9	1.98 ^{a, x}	1.92 ^{a, x}	1.98 ^{a, x}	1.98 ^{a, x}	2.01 ^{a, y}	2.02 ^{a, y}	2.05 ^{a, z}	2.05 ^{a, z}	2.05 ^{a, z}	2.05 ^{a, z}
C18 :0	0	1.79 ^{a, x}	1.76 ^{a, x}	1.76 ^{a, x}	1.78 ^{a, x}	1.75 ^{a, x}	1.79 ^{a, x}	1.78 ^{a, x}	1.82 ^{a, x}	1.80 ^{a, x}	1.81 ^{a, x}
	3	1.77 ^{a, x}	1.78 ^{a, x}	1.79 ^{a, x}	1.79 ^{a, x}	1.81 ^{a, x}	1.81 ^{a, x}	1.84 ^{a, x}	1.91 ^{a, x}	1.83 ^{a, x}	1.84 ^{a, x}
	6	1.86 ^{a, x}	1.80 ^{a, x}	1.80 ^{a, x}	1.81 ^{a, x}	1.80 ^{a, x}	1.81 ^{a, x}	1.82 ^{a, x}	1.82 ^{a, x}	1.86 ^{a, x}	1.84 ^{a, x}
	9	1.77 ^{a, x}	1.79 ^{a, x}	1.82 ^{a, x}	1.82 ^{a, x}	1.80 ^{a, x}	1.85 ^{a, x}	1.87 ^{a, x}	1.90 ^{a, x}	1.90 ^{a, x}	1.92 ^{a, x}
C18 :1	0	59.90 ^{a, z}	58.60 ^{a, z}	58.01 ^{a, z}	57.96 ^{a, y}	56.87 ^{a, y}	56.70 ^{a, y}	56.23 ^{a, x}	55.64 ^{a, x}	55.07 ^{a, x}	55.08 ^{a, x}
	3	59.08 ^{a, z}	57.82 ^{a, z}	57.19 ^{a, z}	56.40 ^{a, y}	56.80 ^{a, y}	56.57 ^{a, y}	56.02 ^{a, x}	56.35 ^{a, x}	55.57 ^{a, x}	56.01 ^{a, x}
	6	58.19 ^{a, z}	58.40 ^{a, z}	58.04 ^{a, z}	57.70 ^{a, y}	57.18 ^{a, y}	57.29 ^{a, y}	56.17 ^{a, x}	55.51 ^{a, x}	56.07 ^{a, x}	55.67 ^{a, x}
	9	59.08 ^{a, z}	59.13 ^{a, z}	58.50 ^{a, z}	58.04 ^{a, y}	57.32 ^{a, y}	57.11 ^{a, y}	56.53 ^{a, x}	56.20 ^{a, x}	56.05 ^{a, x}	55.82 ^{a, x}
C18 :2	0	19.34 ^{a, x}	19.59 ^{a, x}	20.21 ^{a, x}	19.45 ^{a, x}	20.45 ^{a, x}	20.16 ^{a, x}	21.16 ^{a, y}	20.91 ^{a, y}	20.13 ^{a, y}	20.65 ^{a, y}
	3	18.71 ^{a, x}	19.60 ^{a, x}	19.67 ^{a, x}	19.71 ^{a, x}	20.23 ^{a, x}	20.32 ^{a, x}	20.67 ^{a, y}	20.72 ^{a, y}	20.86 ^{a, y}	20.53 ^{a, y}
	6	19.34 ^{a, x}	19.27 ^{a, x}	19.47 ^{a, x}	19.54 ^{a, x}	19.57 ^{a, x}	19.83 ^{a, x}	19.96 ^{a, y}	21.05 ^{a, y}	20.48 ^{a, y}	20.63 ^{a, y}
	9	18.83 ^{a, x}	18.78 ^{a, x}	19.18 ^{a, x}	19.47 ^{a, x}	20.03 ^{a, x}	19.87 ^{a, x}	20.23 ^{a, y}	20.19 ^{a, y}	20.21 ^{a, y}	20.61 ^{a, y}

The same letter (a) indicate not significant difference ($P \geq 0.05$) between olive storage time (0, 3, 6 and 9 days) Different letters (x-z) indicate significant difference ($P < 0.05$) between different proportion of olives OTWW (0, 5, 10, 15, 20, 25, 30, 35, 40 and 50%).

Each mixture and each storage time was performed in triplicate.

significant increase of palmitic, palmitoleic, linoleic, linolenic and stearic acid contents in plots irrigated with treated wastewater compared to the one treated with well water and a decrease of oleic acid was observed at the end of experimental period. The results obtained lead to the conclusion that irrigation by TWW has the effect of increasing the production of these acids, a finding which led to the work of (Gharsallaoui *et al.*, 2011). Also, Bedbabis *et al.*, (2010b) found that rain-fed virgin olive oils showed a statistically significant higher content of oleic and linoleic acids and virgin olive oils extracted from moderate saline water irrigated trees had higher contents of palmitic, palmitoleic, stearic and linolenic acids.

Finally, considering the results of oleic acid, mixtures of olives with a high percentage of OTWW gave the lowest amounts of this fatty acid. In fact, the values obtained were very close to the lower limit (55%) established by the Commission Regulation (EEC) no. 2568/91. The value seems to increase slightly in the oils obtained from mixtures stored for longer periods. The statistical study shows that the percentage of OTWW in the studied mixture of olives has a significant effect on content of oleic acid in the oil ($P < 0.05$). Moreover, it is clear from the statistical study that the fatty acid composition in the oil does not depend on the duration of olive storage before the extraction

CONCLUSION

In Tunisia, the use of wastewater is an integral part of the national water resources strategy. This kind of water is used to irrigate industrial and fodder crops, cereals, vineyards, citrus and many olive tree plots in different localities. The production of olive trees irrigated by treated wastewater will not be handled alone in isolated mills, on the contrary these olives will be transported to local mills where they will be mixed with fruits from other sources and stored under ambient conditions before the crushing.

In a previous study, Gharsallaoui *et al.*, (2011) studied the impact of irrigation with treated wastewater on the oil quality and it was simulated the conditions of pressing in an oil mill where the fruits from plots irrigated with TWW will be mixed with olives from other plots that are not normally irrigated, as is the case for most of the olive tree plots in Tunisia. With the view that the practice of irrigation by TWW is not yet widely used in Tunisia; it is

assumed that the percentage of olives from plots irrigated by OTWW does not, in practice, exceed 50%. The principal objective of this study was to determine at which percentage of OTWW the quality of the oil begins to be affected.

The results obtained in this work, where normal conditions of olive oil mills were simulated, allowed to conclude that the percentage of olives coming from trees irrigated with treated wastewater (OTWW) does not affect the olive oil free fatty acids; that the percentage of OTWW affects slightly the oil oxidation state; that the percentage of OTWW does not affect the content of pigments (carotene and chlorophylls); that the increase of OTWW percentage has the effect of increasing the percentage of palmitoleic, linoleic and linolenic acid, whereas it has the effect of decreasing the percentage of oleic acid. Also, olive storage has a detrimental effect on oil quality.

The effect of irrigation with treated wastewater begins to appear from 20% of OTWW for the C16: 1 content and a percentage of 15% of OTWW for the C18: 1 content. It begins to affect the oil content of C18: 2 from an OTWW proportion of 30%.

With respect to the oxidation state of oils, the effect of irrigation by TWW begins to appear when the proportion of OTWW exceeds 15%. The mixture of olives from plots irrigated by treated wastewater with olives from other sources can be done without harming the quality of oil provided it does not exceed 20% of OTWW.

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