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# Impact of Olives Storage and Irrigation with Treated Wastewater on the Oil Quality: Simulation of Olive Mill Conditions

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**Abstract**
The purpose of this research was to assess the impact of irrigation bytreated wastewater on the Tunisian olive oil quality. In this experiment, olives from fields irrigated with TWW were transported to the laboratory and mixed with other batches of olives, at different proportions, of the same variety and coming from olive groves 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 results showed that the acidity and the carotene content of the oils does 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.

***Keywords:****treated wastewater, irrigation, mill conditions, olive oil quality*

## 1. Introduction

Olive tree (Oleaeuropaea L.) is mostly 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 OTA, citrinin and aflatoxins (El Adlouni et al., 2006).

Besides micro organisms, 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 (Asehraou 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 el., 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.

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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 workschedule of Tunisianmills, 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: acidity, K232, K270, colorant and fatty acid composition. In a previous publication we have demonstrate that theses parameters are affected by the irrigation of the chemlali olive variety with treated wastewater (Gharsallaoui et al., 2011).

## 2. Material And Methods

### 2.1 Olives sampling

El Hajeb, located 10 Km to the south west of Sfax City (34°43′N, 10°41′E) in central eastern Tunisia, was chosen as the experimental site. This site, characterized by sandy soils (84.4% of sand; 9.8% of clay and 5.8% of silt), and 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.Also, because in Tunisianmills olives are keptaside for somedaysat room temperaturebeforebeingcrushed, these mixtures wereprocessedafter 3, 6 and 9 daysfromtheirharvesting.

### 2.2 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.

**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 |

### 2.3 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 (BOD) of the TWW produced in Sfax varies between 22.1 and 114.5 mg of $O_2$/L, values which may exceed the limit fixed by the Tunisian standard (30 mg of $O_2$/L) (Ouali et al., 2008). The Chemical Oxygen Demand (COD) varied between 201 and 273 mg of $O_2$/L, which also exceed the limit fixed by the Tunisian standards (90mg $O_2$/L). These high values of COD can be explained by considering that treated sewage of Sfax city is a mixture of urban and industrial wastewater.

Suspended Solid (SS) 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 (GI) of L. 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 GI of L. sativum tests demonstrated that TWW from Sfax exhibited a high micro and phyto-toxicity (Ellouze et al., 2009).

### 2.4 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 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.

### 2.5 Analytical indices

Free acidity and UV absorbances at 232 and 270 nm were determined according to the European Official Methods of Analysis (EEC, 1991).

## 2.6 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.

## 2.7 Carotenes and chlorophylls

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

## 2.8 Statistical analysis

All analytical determinations were conducted in triplicate. Values of different parameters were expressed as the mean ± standard deviation. Significant differences between mean ($P < 0.05$) were determined by Fisher's test using SPSS software for windows (SPSS. 11, USA).

# 3. Results

### 3.1 Analytical characteristics

The data represents the mean values (m) of tree replications for each mixture and each storage time.

### 3.2 Chlorophylls content

The data represents the mean values (m) of tree replications for each mixture and each storage time.

### 3.3 Carotenes content

The data represents the mean values (m) of tree replications for each mixture and each storage time.

### 3.4 Fatty acid composition

The data represents the mean values (m) of tree replications for each mixture and each storage time.

# 4. Discussion

### 4.1 Acidity

The free fatty acid values of the olive mixtures that contain a percentage of OTWW lower then 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 acidity does 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 and Mousa (Al-Absi, 2008; 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 acidity of oils from the three studied varieties. In a previous study (Gharsallaoui et al., 2011) it was found that irrigation of the chemléli olive variety, by treated wastewater, significantly affects the olive oil acidity when the OTWW proportion is 100%.

### 4.2 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). Ultraviolet (UV)-specific extinction determination permits an approximation of the oxidation process in unsaturated oils (Guiterrez 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). 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-Gonzàlez-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 UV 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).

**Table 2.** Evolution of analytical characteristics of oils obtained from different proportion of OTWW olives processed at different times after the harvesting

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

Different letters (a-d) within values indicate the significant difference ($P < 0.05$) between olive storage time (0 day, 3 days, 6 days, 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.
K270: Absorbance of the oil at 270 nm.

### 4.3 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 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). These results are not very surprising as the olives were collected in an advanced stage of maturity. Several authors have, infact, 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).

(Gharsallaoui et al., 2011) results 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 our 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.

### 4.4 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 the percentage of OTWW. On the contrary, it is significantly dependent on the duration of olive storage before the extraction. Also, 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).

**Table 3.** Chlorophyll continent (ppm) in the oils obtained from different proportion of OTWW olives processed at different times after the harvesting.

| | | 0 % | 5% | 10% | 15% | 20% | 25% | 30% | 35% | 40% | 50% |
|---|---|---|---|---|---|---|---|---|---|---|---|
| C16 :0 | 0 days | 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 days | 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 days | 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 days | 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 days | 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 days | 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 days | 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 days | 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 days | 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 days | 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 days | 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 days | 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 days | 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 days | 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 days | 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 days | 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 days | 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 days | 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 days | 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 days | 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) within the value indicates non significant difference ($P \geq 0.05$) between olive storage time (0 day, 3 days, 6 days, 9 days)

The same letter (x) within the value indicates non 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 continent (ppm) in the oils obtained from different proportion of OTWW olives processed at different times after the harvesting.

| Storage time | 0% | 5% | 10% | 15% | 20% | 25% | 30% | 35% | 40% | 50% |
|---|---|---|---|---|---|---|---|---|---|---|
| 0 days | 0.72 $^{a,x}$ | 0.95$^{a,z}$ | 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 days | 1.08$^{a,x}$ | 1.18$^{a,z}$ | 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 days | 1.07$^{a,x}$ | 1.50$^{a,z}$ | 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 days | 1.09$^{a,x}$ | 1.31$^{a,z}$ | 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$^{a,x}$ |

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

The same letter (x) within values indicates non-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 5.** Fatty acid composition (%)

| Storage time | 0 % | 5% | 10% | 15% | 20% | 25% | 30% | 35% | 40% | 50% |
|---|---|---|---|---|---|---|---|---|---|---|
| 0 days | 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 days | 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 days | 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 days | 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$^{b,x}$ |

The same letter (a) indicate non-significant difference ($P \geq 0.05$) between olive storage time (0 day, 3 days, 6 days, 9 days).
Different letters (x-z) indicate the 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.

### 4.5 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. 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).
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 content of oleic acid in the oil ($P<0.05$).

Moreover, it is clear from the statistical study that the oil fatty acid composition in the oil does not depend on the duration of olive storage before the extraction.

## 5. 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) we studied the impact of irrigation with treated wastewater on the oilquality. In this study, which is a continuation, we wanted to simulate 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 that 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 us to conclude that the percentage of olives coming from trees irrigated with treated wastewater (OTWW) does not affect the olive oil acidity; 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 stearic and oleic acids. Also, olive storage has a detrimental effect on oil quality.

To answer our main question, we can conclude that 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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## Abbreviation list

TWW: Treated WasteWater
SS: Suspended Solid
ONAS: National Sanitation Office
GI: Germination Index
UV: Ultra violet
BOD: Biochemical Oxygen Demand
EC: Electrical Conductivity
FAMEs: Fatty Acid Methyl Ester
COD: Chemical Oxygen Demand
K232: Absorption characteristic at 232 nm
K270: Absorption characteristic at 270 nm.
OTWW: Olives coming from the plot irrigated with treated wastewater

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