International Journal of Water Resources and Arid Environments 7(2): 144-152, 2018
ISSN 2079-7079

Using of Treated Wastewater to Irrigate Olive Plants: Some Chemical Constituents of Olive Oils

[1]Nada Zaanouni, [2]Mariem Gharsallaoui and [3]Slimane Gabsi

[1]National School of Engineers Sfax, Sfax University Tunisia
[2]Olive Tree Institute BP1087 Sfax 3000 Tunisia
[3]National School of Engineers Gabès, Gabès University Tunisia

Abstract: This study was carried out to evaluate the effects of irrigation by treated wastewater on the minor element of olive oil. Several parameters were studied: polyphenols, unsaponifiable, terpenes alcohols, tocopherol and sterols. The results showed that the irrigation with treated wastewater didn't have any effects on olive oil quality for both varieties chemlali. positively affected the pigment content and unsaponifiable (vary between 0.98 and 1.34 %) and negatively affected polyphenol content and the values of most of these parameters were within the limits established. Moreover, the olives that fallen and collected from the ground irrigated with wastewater provided oils of poor quality. It is thus recommended to collect olives treated with wastewaters from the plant and to avoid their over ripeness. Moreover, it is to be avoided, during crushing, the mixture of olives collected under different conditions.

Key words: Irrigation · Olive oil · Minor compound · Storage time · Treated wastewater

INTRODUCTION

Agriculture is the major mainstay of the Tunisian economy and the cultivation of olive trees constitutes one of the principal economic sectors of agriculture. In fact, about 65 million olive trees are spread over 1.6 million hectares [1]. The area occupied was 1.7 million hectares (ha), representing more than 30% of the agricultural lands 16.1% of the world olive-growing area (second place after Spain). Due to the scarcity of water resources and the crucial role of the latter in the economic and social development, Tunisia has developed a national strategy for water conservation and the identification of new resources. The use of treated wastewater (TWW) in agriculture is a good way for the water shortage in countries recycle wastewater profitably and thus to more effectively use their water resources [2]. The irrigation of the olive tree is especially important that it has positive effects in increasing the strength and the annual oil yield and weight of the olives. Irrigation has an effect on the composition of the oil [3].

Olive oil is characterized by its unique fatty acid composition in minor compounds belonging to the unsaponifiable fraction of vegetable oils. Its physicochemical characteristics are defined by the trade standard of the International Olive Oil Council [4]. It has beneficial health effects attributed to the presence of a range of biologically active minor components polyphenols, tocopherols and sterols [5]. Determining the profile sterols and terpenes alcohols is used to verify the authenticity of the oil enables the detection of fraud [6].

The Sfax region has an arid climate, where annual rainfall rarely exceeds 200 mm, while it is a significant water deficit. A portion of the treated wastewater is transported to El Hajeb olive field (OTD), as part of a project for the use of wastewater in agriculture. They are used for irrigation of olive trees and intercropping such as cotton, oats and forage sorghum [7].

This study relates to the most spread Tunisian variety chemlali. For the analysis of the qualitative characteristics, the oil sampling was carried out on various trees which were irrigated with both wastewaters and rain; the latter was taken as reference. The natural fall is a consequence of the process of maturation. Indeed, the abscission of the fruits is a phenomenon due to the formation of a layer which appears with the approach of maturation. As maturation advances, resistance to the detachment in a natural way decreases. The olives fall on

Corresponding Author: Nada Zaanouni, National School of Engineers Sfax, Sfax University Tunisia.
E-mail: nadazaanouni@yahoo.fr.

the ground, which implies a fruit loss and a lowering of the quality of oils. Late harvests generate the important fall on the one hand production of the trees. In order to show which effects the wet ground has on the fallen olives, different olive collecting time was considered. Normally, a certain time passes between the arrival of olives to the oil mill and their processing to extract the oil. Ideally, the fruits should be transformed as their arrival but it is not always the case. They are, sometimes, stored for long periods. This time of waiting is particularly critical because the possible deterioration of the fruits between the moment of the harvest and that of the trituration is, without any doubt, the essential cause of the reductions in quality and outputs. The shelf life of olives tends to make increase the extent of the enzymatic systems, either those of the fruit, or those which can come from the proliferation and the development of the colonies of micro-organisms, which attack olives, following their contact with the ground and/or the means of transport and the places and means of storage.

In general, these deteriorations relate on organoleptic qualities and physicochemical characteristics of oils. To study the impact of this practice on the quality of oils coming from both modes of conduits, each category of olive was stored for 3, 6 and 9 days. A control sample was considered at time 0.

MATERIALS AND METHODS

Study Site: The experimental site, El Hajeb, is located at 10 km in the south west of Sfax city (34°43'N, 10°41'E) in central eastern Tunisia and belongs to the Mediterranean bioclimatic where the climate is characterized by hot and dry summers and by a relatively cold winter. The annually precipitation is very irregular and varies from year to year. The irrigated area of El Hajeb is part of the large estate of Chaal, managed by the office of the lands. The late harvest generates a falling of an important share of tree production. To determine the quality of oil extracted from fallen fruits, olives were picked up from under the trees from each block.

Olive Sampling and Oil Extraction: Olive oil samples were obtained from Tunisian quality-assured industrial oil mills. The samples stability was analyzed under diffused light and room temperature. The materials used for olive oil packaging was glass. The reference sample was processed immediately after extraction, while the other lots were stored for 3, 6, 9 days at room temperature. Oil was extracted by oleo batcher and was preserved at low temperature until analyses.

Categories of Olives Collected:

WW-Fr: olives picked straight from the tree coming from plot irrigated by wastewater.

TWW-Fr: olives picked straight from the tree coming from plot irrigated by treated wastewater.

Analytical Methods

Content of Unsaponifiable and Extraction of the Total Sterol Fraction and Terpenes Alcohols: The extraction method and metering unsaponifiable used is the one adopted by Stiti *et al* [8]. Unsaponification lipids were determined by saponifying 5 g of lipid extract with 50 mL ethanolic KOH 12% mixed with 200 µL α-cholestanol (0.2% w/v) and 100 µL 1-eicosanol solution (0.1% w/v), as internal standard. The solution was directly injected to gas chromatograph. Sterols and alcoholic fractions were evaluated by CPG-FID. The working temperatures of the chromatograph were 300 °C at the injector, 260 °C at the isothermal column and 300 °C at the detector.

Total Phenols Content: Total polyphenols were determined according to the previously published protocol by using Folin–Ciocalteau methodology described by Zribi [9].

Evaluation of Oxidative Stability: Oxidative stability was measured with the Rancimat 743 apparatus (Metrohm Ω, Basel, Switzerland). Stability was expressed as the oxidation induction time (h), using an oil sample of 3.6 g warmed to 101.6 °C and an air flow of 10 L/h [10].

Tocopherol: For the tocopherol analysis, virgin olive oil samples were diluted in hexane (0.1 g in 10 mL of hexane containing 0.01% BHT), filtered through a 0.22 µm PVDF membrane (Millipore, USA) and then directly injected into the column in the HPLC system. This sample was prepared according to Guinazi [11] and Pinheiro-Sant'ana [12].

Statistical Analysis: All analyzed parameters were carried out in triplicate. The results were reported as mean value of three replicates and standard deviation. Chemical data were analyzed by the SPSS statistics 17.0 for windows and origin 8.The significance of differences at 5% level between averages was determined by one-way ANOVA using Turkey's test. Means were compared using the Duncan test and significance was set at $P < 0.05$.

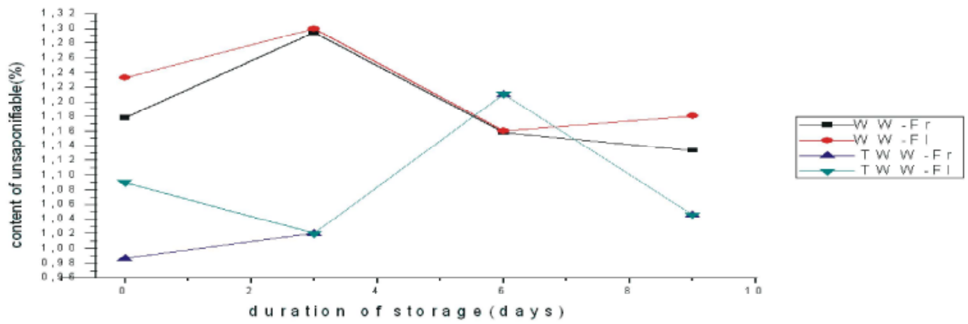

Fig. 1: Evolution of the unsaponifiable fraction (%)

RESULTS

Study of the Unsaponifiable Fraction: The components of the unsaponifiable part and their relative contents are generally regarded as a row property of vegetable oils and represent to some their digital fingerprints. The results are summarized in the Graph 1, showed that irrigation with treated wastewater has, an effect, to reduce this fraction of olive oil. It seems equal that during storage duration, the unsaponifiable oils extracted from olives picked directly from the tree and those that fell below follow the same pattern for each quality of irrigated water and for each storage time as well.

The results presented in Table 1 showed that during the experiments, unsaponifiable contents vary between 0.98 and 1.34 %; values that are in line with standard IOOC [13] for olive oil (< 1.5 %). The analysis of the unsaponifiable fraction by thin layer chromatography on silica gel has identified five groups of chemical substances eluted in the following order: the sterols, aliphatic and terpenes alcohols, tocopherols, hydrocarbons and pigments.

Sterol Composition: The composition of the sterol fraction in olive oil is a very useful parameter to detect fraud or to check its authenticity since they are considered as a footprint of oils. In fact, researchers have shown that each fruit oil seed possesses a specific sterol profile [14]. They are also major compounds for the stability of the oil at high temperature because they act an inhibitor of the polymerization reaction. Table 1.

Total of Sterols Content: Sterols are biologically active compounds of great relevance for olive oil quality**.** The examination of the graphic above shown, first; the samples that were the subject of our study are in line with the IOOC standard that requires concentrations higher than 1000 mg/Kg. In addition to the variety, the contribution out of water can affect the sterol composition of olive oil. Indeed, [15] Fiorino and Nizzi Griffi particularly announce the influence of irrigation on the contents of Δ-5-avenasterol and β-sitosterol in the same way for El Antari and al [16] who noted a variation of the sterol composition between the irrigated and not irrigated areas. In addition, for the irrigation with treated wastewater, oils obtained are less rich in total sterols

The statistical study showed that the state of freshness (gathered directly on a tree or fallen) of olives does not have an effect in the sterol composition of extracted oil.

In order to better illustrate the evolution of six principals sterols of olive oil, we represent the average content of Campesterol, Campestanol, Stigmasterol, Chlerosterol, β-sitosterol, duΔ-5avenasterol and of two terpenes alcohols (erythridion and uvaol). Graph 2.

Content of Campesterol: The results presented in Figure 3 howed that the effect of each studied parameter (quality of water of irrigation, state of freshness of olives and the storage period of olives before their trituration) is lucid on this compound. Indeed, it is noted that oil's coming from the pieces irrigated by treated waste waters are richest in campestral.

Content of Campestanol: Contrary to Campesterol, the irrigation by treated wastewater causes a decrease a campestanol content [17]. found significant negative correlations between the pluviometer and the content of campestanol. The fallen olives give rise to oils richer in campestanol Table 1.

Content of Stigmasterol: The oil containing the lowest content in Stigmasterol is that extracted directly from gathered olives of the tree irrigated by underground water. This result is in agreement with those obtained by Gracia *et al* who found that the content of stigmasterol is connected to various quality parameters.

Table 1: Evolution of sterol composition during storage of olives different samples studied

Storage Olives	Olive categories	Cholesterol	24-Methylene-Compesterol	Campesterol	Campestanol
0 days	WW-Fr	0,71	0,51	76.36	21.56
		0,03	0,02	3,49	0,99
	WWT-Fr	0.99	1.15	95.25	18.24
		0,04	0,04	3,54	0,68
3 days	WW-Fr	2	0.64	61.15	20.05
		0,09	0,03	2,80	0,92
	WWT-Fr	1.23	0.75	88.52	14.97
		0,05	0,03	3,57	0,60
6 days	WW-Fr	1.51	0.54	69.45	22.71
		0,07	0,02	2,99	0,98
	WWT-Fr	0.75	0.37	86.91	16.83
		0,03	0,01	3,46	0,67
9 days	WW-Fr	1.41	0.41	91.58	26.14
		0,06	0,02	3,62	1,03
	WWT-Fr	0.68	1.62	111.25	22.87
		0,03	0,06	4,11	0,85

The black values are the average grades of sterol compounds (mg / kg oil)
Red values correspond to percentages moneys sterols compared to total sterols (%)

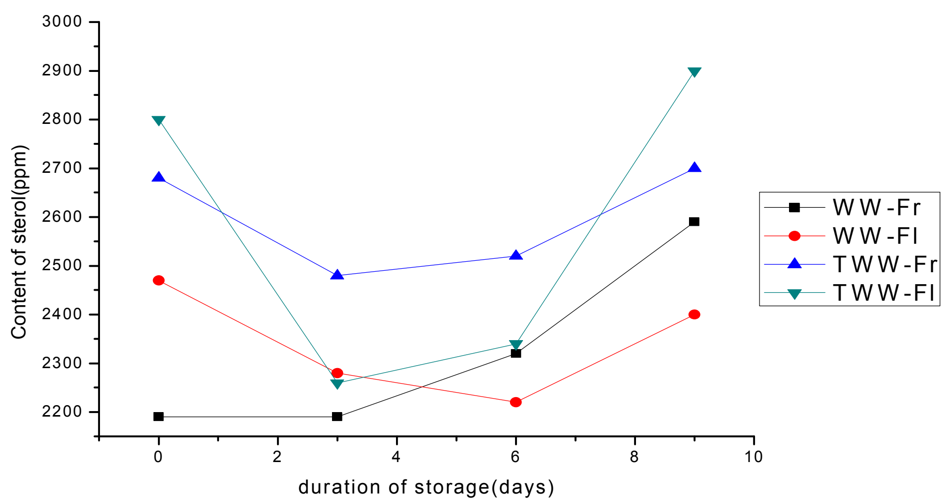

Fig. 2: Evolution of the content of total sterols (ppm)

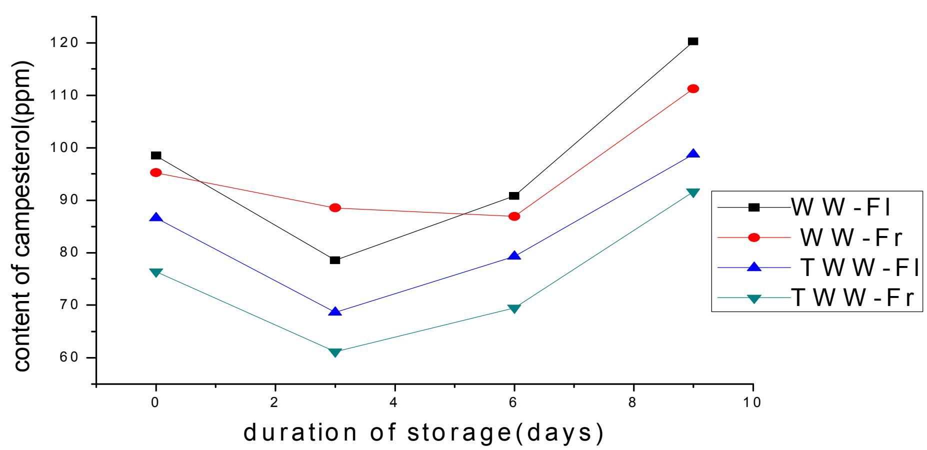

Fig. 3: Evolution of the content of campesterol (ppm) according to the duration of the storage of olives before the triturating.

Table 2: Evolution of sterol composition during storage of olives different samples studied

Storage Olives	Olive categories	Stigmasterol	Chlerosterol	?-Sitosterol
0 days	WW-Fr	2.3	21.36	1826.51
		0,11	0,98	83,4
	TWW-Fr	36.84	17.85	2312.68
		1,37	0,66	86,07
3 days	WW-Fr	6.12	24.09	1815.45
		0,28	1,10	83,26
	TWW-Fr	35.93	19.27	2125.58
		1,45	0,78	85,73
6 days	WW-Fr	6.31	25.84	1960.54
		0,27	1,11	84,42
	TWW-Fr	9.49	17.23	2209.07
		0,38	0,69	88,01
9 days	WW-Fr	7.01	27.39	2110.57
		0,28	1,08	83,36
	TWW-Fr	14.85	17.96	2351.52
		0,55	0,66	86,97

The black values are the average grades of sterol compounds (mg/ kg oil)
Red values correspond to percentages moneys sterols compared to total sterols (%)

Table 3: Sterol compounds (mg/kg oil)

Storage of Olives	Olive categories	Δ5-Avenasterol	Δ5,24-Stigmasta dionol	Δ7-stigmasterol	Δ7-Avenasterol
0 days	WW-Fr	225.88	2.26	4.41	6.66
		10,32	0,10	0,20	0,30
	TWW-Fr	182.14	12.47	3.14	6.14
		6,78	0,46	0,12	0,23
3 days	WW-Fr	239.18	2.98	3.14	5.74
		10,97	0,14	0,14	0,26
	TWW-Fr	174.17	11.97	2.99	4.02
		7,02	0,48	0,12	0,16
6 days	WW-Fr	222.75	3.25	4.61	4.98
		9,59	0,14	0,20	0,21
	TWW-Fr	153.86	5.08	5.2	5.2
		6,13	0,20	0,21	0,21
9 days	WW-Fr	256.49	1.96	2.98	5.84
		10,13	0,08	0,12	0,23
	TWW-Fr	171.84	2.78	3.23	5.28
		6,36	0,10	0,12	0,20

Content of Chlerosterol: While cholesterol is present in animals in relatively high abundance, plants, with very few exceptions, produce negligible amounts of this compound. The content of chlerosterol is dependent on the quality of water for irrigation (Table 2). According to the results found, the irrigation by treated wastewater affects negatively the content of chlerosterol contrary to underground water. For this led mode neither the state for the freshness of olives nor the storage period affects the content of this compound. The olives which fell give oils with the highest content of chlerosterol.

Content of β-Sitosterol: The results relating to the dominating sterol of olive oil (the β-Sitosterol); enable us to conclude that the irrigation by wastewater causes to increase the content of this phytosterol.

Content of Δ5-Avenasterol: Δ5-Avenasterol is among the major sterols in the olive oil and it is classified the second after the β-Sitosterol. The results gathered in the figure above shows that the irrigation of the olive-trees by treated wastewaters affects negatively the concentration of oils in Δ5-Avenasterol (Table 3). It is obvious independently from the control mode; the olives which fell give oils less rich in this phytosterol.

Terpenes Alcohols: The terpenes alcohols are very interesting; mainly because of their activity anti-inflammatory drug [18]. But they are also used because of their cytostatic activity on cancer cells [19], or under the terms of their properties in urology [20], in neurology [21], in cosmetics [22] or for their antibacterial action [23]. The unsaponifiable fraction of the olive oil contains two

Table 4: Sterol compounds (mg/kg oil)

Storage of Olives	Olive categories	Erythridiol	Uvaol	Erythridiol + Uvaol	Total sterols	β-Sitosterol Apparent (%)
0 days	WW-Fr	10.15	0.64	10.79	2188,52	94.86
		0,46	0,03	0.49		
	TWW-Fr	11.45	2.09	12.54	2686,89	93.98
		0,43	0,08	0.51		
3 days	WW-Fr	10.98	0.74	11.72	2180,54	95.47
		0,50	0,03	0.53		
	TWW-Fr	11.97	2.61	14.58	2479,40	94.01
		0,48	0,11	0.59		
	WW-Fr	11.26	0.59	11.85	2322,49	95.26
		0,48	0,03	0.51		
6days	TWW-Fr	12.84	1.36	14.2	2509,99	95.03
		0,51	0,05	0.56		
9 days	WW-Fr	4.25	0.58	4.83	2531,78	94.65
		0,17	0,02	0.19		
	TWW-Fr	6.14	1.41	7.55	2703,88	94.09
		0,23	0,05	0.28		

Table 5: Evolution of the polyphenols contents (ppm)

Time	WW-Fr	TWW-Fr
0 days	88.64±2.36	93.64±1.39
3 days	129±9.61	176.94±4.44
6 days	104.57±1.62	141.59±5.96
9 days	77.26±5.92	96.78±0.1

alcoholic compounds triterpenes pentacyclic: the erythrodiol and the uvaol. The determination of these two compounds can be useful for the detection of the sulfurous oil in the virgin olive oil [24]. According to EC regulation, the rate of the erythrodiol + uvaol should not exceed 4.5% for a virgin olive oil.The results relating to the sum erythridiol+ uvaol, which does not exceed 17 ppm for all the analyzed samples.

Content of Erythridiol: The examination of the results relating to the content of the erythrodiol (Table 4) showed that the irrigation by treated wastewater gives oils rich in this alcohol, whereas the state for the freshness of olives does not affect the content of oils of this compound. The evolution of the concentration of erythrodiol in oils according to the storage of olives tends towards the reduction.

Content of Uvaol: Oils coming from the trees irrigated by underwater are almost deprived of this compound with concentrations that don not exceed 1ppm. For the irrigation by treated wastewater the oils richer in uvaol are obtained after three days of storage of the olives fallen (Table 4).

Content of Polyphenols: The virgin olive oil is the only oil which contains significant amounts of natural phenol substances which confer such a particular taste of bitterness and fruitage of olive oils and at the same time responsible for its stability (oxidation resistance).The results presented in Table 4 showed that the polyphenols contents undergo an increase during the first three days of storage; some is due to the quality of irrigation water and decrease thereafter. This phenomenon can be explained by a transfer of these compounds from the fruit aqueous phase to oily phase, the decrease thereafter is probably due to the decomposition by the polyphenol oxidase (enzymatic reaction). Other authors noted that, the level of polyphenols decreases in the extra virgin olive obtained by the "Coronica" variety, a Spanish-fruit, during 5 and 8 days with 20 and 10°C [25].

It is also clear that the oil samples coming from the directly gathered fruits of the trees irrigated with treated waste waters (TWW-Fr) are richest in polyphenols, with contents varying from 93.64 mg/kg to 176.94 mg/kg, whereas for the irrigation with underwater (WW-Fr) the concentrations of polyphenol in oils vary from 74.28 to 156 mg/kg. These results are similar to those reported by several authors [26, 27] who found that for Chemlali variety in the area of Sfax, the content of polyphenols in oils are about 100 ppm.

The differences noted according to the quality of irrigation water can be explained by the fact that the treated wastewater irrigated receive more balanced quantity of nutrients (P and K), which improves their nutritional state and avoids the stress. It is well-known that the nutritive disorders can affect the metabolic state of the plant [28, 29]. Former studies showed that the potassium concentration in olives coming from the olive-trees irrigated with treated wastewaters was significantly found higher than that relating to the irrigation by underwater [30].

Table 6: Contents various tocopherols (ppm)

Tocopherol content (ppm)	Standard		Irrigation WWT	
	Fresh	Fallen	Fresh	Fallen
α	58.82	47.11	71.53	54.04
β	5.13	8.41	1.76	3.70
δ	6.82	7.92	0.516	1.69
Δ	ND	ND	0.01	ND
Total	70.77	63.44	73.82	59.43

The wealth of treated wastewater of potassium contribute consequently, in a strong increase in the concentration of K^+ in the fruits, which causes a change of the fruit color of the fruit from the green to the black [31] and an increase as well in the polyphenols content. In addition, Montedoro *et al*. [30] found that the polyphenol content is higher with a degree of earlier maturation. Moreover, the high concentrations of Na and Cl in treated wastewater cause a saline stress of the plants irrigated by this type of water. Studies showed that the increase in the content of polyphenols is associated to the saline stress.

The results obtained in this work are in disagreement with those reported by Bedbabis *et al*. [32] for the variety "Chemlali" irrigated with treated wastewater.

Oxidative Stability: Oxidative stability informs us about the necessary time for the unsaturated fatty acid oxidation. The higher oxidation induction time is, the more stable preserved the oil. Oils relating to pieces irrigated by treated wastewater and extracted from gathered olives of the tree are more stable (most resistant to oxidation). These results confirm those of the polyphenol content where it was found that the irrigation by treated wastewater positively affects the content of polyphenols in oils [33].

The results shown also demonstrate that the oils extracted from fallen olives are the resistant ones to oxidation.

Content of Tocopherol: The tocopherols are hetero acid compounds having high molecular weights. Several isolated forms were identified and indicated by α, β, δ, Δ tocopherols. These compounds play a significant role on in the nutritional level: they have a vitamin activity. The tocopherols are also effective antioxidants. The results illustrated in Table 6 showed that the irrigation by treated waste waters causes to increase the content of α-tocopherol in the oils extracted from fresh olives or dropped olives. The contents of this tocopherol are in general, for our samples, relatively weaker than those found in the literature [34, 35]. Besides α-tocopherols in our samples contain considerable quantities in β and δ tocopherol.Both shapes of tocopherols very interestingly, although the activity of vitamin E is generally allotted to the alpha-tocopherol shape. However, researchers revealed that beta, gamma and delta tocopherols also have a significant biological activity in the organism. In fact, scientists suppose that gamma-tocopherol has an anti-inflammatory and antioxidant properties higher than those of alpha-tocopherol, thus revealing single benefits [36]. The irrigation with treated waste waters affects negatively the contents of beta and gamma tocopherols in oils which reduce their beneficial activities for human health.

CONCLUSION

Although the availability of treated wastewater as a very interesting alternative for urban agriculture, the associated health risks can be a real obstacle to the development of this activity. Moreover, there can be a risk of deterioration of the food quality going down from unconventional water irrigated agriculture. Being based on this study, it can be stressed that:

- Olive trees irrigated with treated wastewater are more sensitive to oxidation in particular when the olives are stored before the extraction.
- The olives fallen and collected from the ground irrigated with treated wastewater provide oils of poor quality.

REEFERENCES

1. Hannachi, H., Msallem Mr. M. Ben El Hadj and M. El Gazzah, 2007. Influence of biological site on agronomic and technological potentialities of the olive tree (*Olea europea* L.) in Tunisia.C.R. Biologies, 330: 135-142.
2. Neubert, S.T., 2003. On sprites and their exotic kin, Science, 300, 747- 749,doi:10.1126/ science.1083006.
3. Ouaouich, A. and H. Chimi, 2007. Guide of the producer of olive oil http:// www.unido.org/ fileadmin/ user_media/ Publications/ Pub_free/ Guide_du_producteur_de_huile_olive.pdf.
4. Olive Oil Council International.2015.Trade standard applying to olive oils and olive-pomace oils. COI / T.15 / NC No 3 / Rev. February 8 French 2015.
5. Boskou, D., 2009. Other important minor constituents D.Boskou (Ed.), Olive oil. Minor constituents and health, CRC Press, Boca Raton, FL, pp: 45-54.

6. Reina, R.J. and K.D. White, 1997. EGE Jahngen Validated method for quantification and identification of 4,4-diol and methylsterols triterpene in vegetable oils by gas chromatography mass spectrometry thin layer chromatography high resolution. Journaling of AOAC International, 80 (6) (1997), pp. 1272-1280
7. Charfi, D., A. Trigui and K. Medhioub, 1999. Effects of Irrigation with Treated wastewater is olive trees cv Chemlali of Sfax at the station of el hajeb. Acta Hort. 474: 385-390.
8. Stiti, N., M. Msallem, S. Triki and A. Cherif, 2002. Study of the unsaponifiable fraction of olive oil Tunisian different varieties.Rivista Italiana dell Sostanze Grasse, 9(10): 357-363.
9. Zribi, A., B. Gargouri, H. Jabeur, A. Rebaï, R. Abdelhedi and M. Bouaziz, 2013. Enrichment of pan-frying refined oils with olive leaf phenolic-rich extract to extend the usage life European Journal of Lipid Science and Technology, 115: 1443-1453.
10. Gutiérrez, F., 1989. Determination of the oxidative stability of virgin olive olive oils:comparaison of the active oxygen and the Rancimat methods.Grasas Aceites, 40: 1-5.
11. Guinazi, M., R.C.R.M. Milagres, H.M.P. Pinheiro-Sant'ana and J.B.P. Chaves, 2009. Tocoferois e tocotrienois em óleos vegetais e ovos Quimica Nova, 32: 2098-2103.
12. Pinheiro-Sant'ana, H.M., M. Guinazi, D.S. Oliveira, C.M.D. Lucia, B.L.R. Reis and S.C.C. Brandão, 2011. Method for simultaneous analysis of eight vitamin E isomers in various foods by high performance liquid chromatography and fluorescence detection Journal of Chromatography A, 1218: 8496-8502.
13. Olive Oil Council International 2013 trade standard applying to olive oils and olive-pomace oils COI / T.15 / NC No 3 / Rev. May 7, 2013.
14. Salvador, M.D., F. Aranda and G. Fregapane, 2001. Influence of fruit ripening on the quality of virgin olive oil "Cornicabra". A study of four seasons of successive harvests. Food Chemistry, 73: 45-53.
15. Fiorino, P. and F. Nizzi, 1991. The spread of olive farming.Olivae ,44: 9.
16. AL-Antiri, R., F. Rezgui and B. Aniba, 2002. Wastewater reuse in Tunisia, Waste Water Reuse, toilets in demand management forum.
17. Koutsaftakis, *et al*., 1999. A. Koutsaftakis, F. Kotsifaki, Stefanoudaki E. Effect of the extraction system, the maturation phase and mixing temperature of the sterol composition of virgin olive oils, Journal of the Society of the American Oil Chemists, 76: 1477-148.
18. Vissers, M.N., P.L. Zock, G.W. Meiser and M.B. Katan, 2000. Effect of plant sterols from rice bran oil and triterpenes alcohols from sheanut oil on serum lipoprotein concentrations in humans - Am. J. Clin. Nutr., 72: 1510-1515.
19. Smith-Kielland, I., J.M. Dornish, E. Malterudk, G. Hvistendahl, Romming Chr, O.C. Bockman, P. Kolsakker, Y. Stenstrom and A. Nordal, 1996. Cytotoxic triterpenoids from the leaves of Euphorfia pulcherrima- Planta medica, 62: 322-325.
20. Sunithas , S., M. Nagaraj and P. Varalakshmi, 1999. Effect of lupeol, a pentacyclic triterpene from Cratavea nurvala, on nephrotoxicity induced by cadmium in rats - Medical Science Research, 27: 535-540.
21. Isobe, T., Y. Imai, M. Saito, S. Murata, T. Nagao and C. Tsurumi, XXXX. Preparation of cycloartenols and their use as brain function improvers - Brevet japonais déposé le 17.09.93 (JP 05239088A2).
22. Hayashi, K. and H. Hayashi, XXXX. Hair growth stimulants containing alpha-amyrin triterpenes or extracts of Centella sp. Or Prunelle sp. Brevet japonais enregistred 13.01.95(JP 07010722A2).
23. Ayuso Gonzalez, M.J., Reyes Ruiz and Mr. M. VToro Sainz, 1991. Estudio de la actividad antimicrobiana of compuestos triterpenic aislados Erica andevalensis Cabezudo-Ribera.- An Real Acad. Farm., 57: 419-423.
24. Sanchez Casas, J., E. Osorio Bueno, A.M. Montaño Garcia and M. Martinez Cano, 2004. Sterols and erythrodiol + uvaol content of virgin olive oils from cultivars of Extremadura (Spain). Food Chemistry, 87: 225-230.
25. Inarejos-Garcia, A.M., A. Gomez-Rico, M. Desamparados Salvador and G. Fregapane, 2010. Effect of Preprocessing Olive Storage Conditions on Virgin Olive Oil Quality and Composition. J. Agric. Food Chem., 58: 4858-4865.
26. Dhifi, W., I. Hamrouni, S. Ayachi, T. Chahed, M. Saidani and B. Marzouk, 2004. Biochemical characterization of some Tunisian olive oils. J. Food Lipids, 11: 287-296.
27. Baccouri, O., L. Cerretani, A. Bendini, M.F. Caboni, M., Zarrouk, L. Pirrone and D. Daoud, 2007b. Preliminary chemical characterization of virgin olive oils monovarietal Tunisian and comparison with Sicilian ones. Eur. J. Lipid Sci. Tech., 109: 1208- 1217.
28. Tovar, M., M. Romero, S. Alegre, J. Girona and M. Motilva, 2002. Composition and organoleptic characteristics of oil from Arbequina olive (*Olea europaea* L.) trees under deficit irrigation. J. Sci. Food Agric., 82: 1755-1763.

29. Karioti, A., A. Chattzopoulou, A.R. Bili, G. Liakopoulos, S. Stavrianakou and H. Skaltsa, 2006. Novel Secoiridoid glucosides in *Olea europaea* - leaves suffering boron deficiency. Biosci. Biotechnol. Biochem, 70: 1898-1903.
30. Bedbabis, S., AM. Palese, B. Ben Rouina, A. Rhouma, K. Gargouri and M. Boukhris, 2009. The effect of irrigation with wastewater Treated on "Chemlali" olive oil quality. J. Food Qual., 32: 141-157.
31. Chartzoulakis, K.S., 2005. Salinity and olive: Growth, salt tolerance, photosynthesis and yield. Agric.Water Manag., 78: 108-121.
32. Monteleone, E., G. Caporale, L. Lencioni, F. Favati and M. Bertuccioli, 1995. Optimization of virgin olive oil quality in relation to fruit ripening and storage. Dev. Food Sci., 37: 397-418.
33. Owen, RW., A. Giacosa, WE. Hull, R. Haubner, B. Spiegelhalder and H. Bartsch, 2000a. The antioxidant/anticancer potential of phenolic compounds isolated from olive oil. Eur J. Cancer, 36: 1235-47.
34. Dabbou, S., I. Rjiba, A. Nakbi, N. Gazzah, M. Issaoui and M. Hammami, 2010. Compositional quality of virgin olive oils from cultivars introduced in Tunisian arid zones in comparison to Chemlali cultivars. Sci. Hort., 124: 122-127.
35. Lazzez, A., 2009: Study of the phonological characteristics of olives and physio-chemical oil variety Chemlali "Impact of maturity, the site of implantation and olive companion" PhD thesis in chemistry, Faculty of Science Sfax, Sfax, pp: 189.
36. Morris, M.C., D.A. Evans, J.L. Bienias, C.C. Tangney and R.S. Wilson, 2003. Vitamin E and cognitive decline in older persons. Arch Neurol, 60(2): 292-293.ONAS.2003. national Office of Sanitation , Sfax, Tunisia.