

Olive Oil Qualitative Parameters after Orchard Irrigation with Saline Water

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The effect of irrigation with saline water on oil quality was studied in the two olive (*Olea europaea* L.) cultivars Koroneiki and Mastoidis, which are the main varieties grown extensively on the island of Crete. Plants (5 years old) were grown outdoors in containers, filled with freely drained light soil. Four treatments were applied, differing in the NaCl added to the irrigation water as follows: 0 (control) 50, 100, and 150 mM NaCl. Drip irrigation was applied regularly, during the dry season (from April to October). Plants in all treatments were irrigated when the soil–water potential reached -40 kPa at a depth of 30 cm. Data showed that increased NaCl levels in irrigation water resulted in a decrease in oil content in the fruits and an increase in total phenols and their secoiridoid derivatives in olive oils from harvested fruits. Furthermore, changes also took place in the composition of fatty acids and triacylglycerol molecular species. The extent of alterations was different for the two varieties and greater in cv. Koroneiki. This fitted with agronomic evidence that cv. Koroneiki is less saline-tolerant than cv. Mastoidis.

KEYWORDS: *Olea europaea*; salinity; salt stress; olive oil; quality indices

INTRODUCTION

Over the past 2 decades, there has been an increasing demand for water, and with climate change, this seems destined to become worse. The problem is more acute in arid or semi-arid regions, such as the Mediterranean, which are characterized by a water imbalance particularly in the summer months. Thus, sources of lower quality water (such as saline groundwater) have become increasingly important for the agricultural industry. The olive tree (*Olea europaea* L.) is one of the species best adapted to the semi-arid Mediterranean environment. It is also considered a moderately salt-tolerant tree crop (1), perhaps because of exclusion of salt by the roots (2, 3) or a capacity to accumulate salt in leaf vacuoles (4). Salinity levels of irrigation water lower than 3.0 dS/m (25 mM NaCl) have no adverse effects on the tree. Levels between 3.0 and 5.5 dS/m (25–50 mM NaCl) generate increasing problems, while salinity levels higher than 5.5 dS/m (50 mM NaCl) can cause severe problems depending upon the cultivar. In agreement with the general theory of crop salt tolerance (1), growth and crop yields of olive are affected by NaCl concentrations above a certain threshold and there are varietal differences in tolerance (2, 3, 5). Most of the experi-

ments with olives have studied vegetative parameters, gas exchange (6), or salt accumulation in young olive trees. In contrast, there has been little research on the effect of saline water irrigation on fruit productivity and olive oil quality. Because of this and with climate change increasing water shortages in the Mediterranean region, we conducted a comprehensive study of olive oil quality for two of the major commercial Greek varieties.

MATERIALS AND METHODS

Plant Material and Salinity Treatment. Olive trees (5 years old, *O. europaea* L.) cv. Koroneiki and cv. Mastoidis were used in this study. Plants were grown outdoors in 200 L containers, filled with freely draining medium-textured soil [sandy/clay/loam (SCL)] with an electrical conductivity (EC) of 0.35 dS/m and pH 7.5. Four different NaCl irrigation treatments containing different concentrations of NaCl were applied: 0 (control), 50, 100, and 150 mM. The corresponding electrical conductivities were 0.32, 4.82, 8.94, and 12.50 dS/m, respectively. Fresh water with an EC of 0.32 dS/m, 0.3 mM Na⁺, and 0.5 mM Cl⁻ was used as a control. Irrigation was applied regularly during the dry season (April–October), when the soil–water potential reached -40 kPa at a depth of 30 cm. During the remainder of the year, trees received natural rainfall. Four trees from each cultivar were used per treatment in a randomized plot design. All trees were cared for in the same way (fertilization, pruning, weed control, and pest management).

Olive Samples and Oil Extraction. Representative fruits were hand-picked in November from trees of each treatment and brought to the laboratory for oil extraction on the same day. The oil content of fruits

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was determined per gram of dry weight by means of the Soxhlet extraction method (7) using hexane as an extraction solvent.

Olive oil was extracted using a laboratory-scale olive mill with the procedure as described by Stefanouadaki et al. (8). Oil samples were kept in the freezer at -18°C prior to analysis.

Extraction and Analysis of Phenolics. Phenolic compounds were extracted from virgin olive oil using a methanol/water (80:20, v/v) mixture and purified as described in ref 9. Total phenols were determined colorimetrically using Folin–Ciocalteu reagent. The absorbance was measured at 725 nm and expressed as parts per million (ppm) gallic acid (10). Individual phenolic compounds were separated by a high-performance liquid chromatography (HPLC) system consisting of a Hewlett-Packard quaternary pump series 1100 (Palo Alto, CA) coupled to a UV detector (Jasco UV 970) and with HP Chemstation software. The analytical separation was achieved on a Lichrosphere 100 RP-18, $5\ \mu\text{m}$ column ($250 \times 4\ \text{mm}$ inner diameter) equipped with a 5 cm precolumn (Merck, Darmstadt, Germany) with the same packing material as the column. Eluates were detected at 280 nm as recommended by Ryan et al. (11). The following reference compounds were used: gallic acid was purchased from Sigma Chemical Co. (St Louis, MO), and 2-(*p*-hydroxyphenyl) ethanol (*p*-HPEA) was purchased from Aldrich Chemical Co. (Milwaukee, WI). The compounds 3,4-dihydroxyphenyl ethanol (3,4-DHPEA), the dialdehydic form of elenolic acid linked to 3,4-DHPEA (3,4-DHPEA-EDA), and the isomer of oleuropein aglycon (3,4-DHPEA-EA) were kind gifts from Professor G. F. Montedoro (University of Perugia, Italy).

Fatty Acid Analysis. Fatty acids were extracted and analyzed as described in ref 8. The fatty acid methyl esters were separated in a $50\ \text{m} \times 0.22\ \text{mm}$ ($0.25\ \mu\text{m}$ film thickness) column packed with BP X 70 (SGE Scientific Pty Ltd., Victoria, Australia) using a Hewlett-Packard HP6890 gas chromatograph. The temperature program was 165°C for 5 min, increased to 220°C at $2^{\circ}\text{C}/\text{min}$, and held at 220°C for 15 min. Peak identification was routinely made by reference to authentic fatty acid standards (Polyscience, Niles, IL). Relative percentages were calculated using HP ChemStation software.

Triacylglycerol Molecular Species. Triacylglycerols were analyzed as described in refs (12, 13). Triacylglycerols were separated on a Kromasil 100 C18 column ($25\ \text{m} \times 4\ \text{mm}$ inner diameter; MZ Analysentechnik, Mainz, Germany) using isocratic elution with a mixture of acetone/acetonitrile (60:40, v/v) and a Jasco PU 980 (Tokyo, Japan) liquid chromatograph with a Jasco 830-RI detector. Peak identification was made by a comparison to retention times of triacylglycerols from reference chromatograms obtained from standard soybean oil and pure olive oil (13), separated under the same conditions. The relative percentage composition was calculated using HP ChemStation software. Triacylglycerols in olive oils were separated according to the equivalent carbon number (ECN), defined as $\text{CN}-2n$, where CN is the total acyl carbon number and n is the number of double bonds of fatty acids. This gave fractions of ENC42–ENC52 containing the different molecular species.

RESULTS AND DISCUSSION

General Effects of Salinity. Irrigation of the young olive trees with saline water produced a significant reduction in fruit fresh weight in cv. Koroneiki but not in cv. Mastoidis (Figure 1). This was found even at 50 mM NaCl. The reduction in fruit weight was accompanied by an increase in the percentage content of water and a severe reduction in oil content, which was more intense at higher concentrations of saline water. For cv. Mastoidis, statistically significant changes in moisture and oil content were only found after 150 mM NaCl treatment (Figure 1) and were less marked than for cv. Koroneiki.

The two main olive cultivars grown in Crete, Greece, are cv. Koroneiki (85%) and Mastoidis (14%). The former is cultivated on the plains, lower hillsides, and coastal areas, while cv. Mastoidis is grown at higher altitudes. Although olive is considered a moderately salt-tolerant crop (14), reductions in crop yields have been noted at higher salt concentrations (14, 15) and different cultivars are known to vary in their tolerance (3, 5, 16, 17).

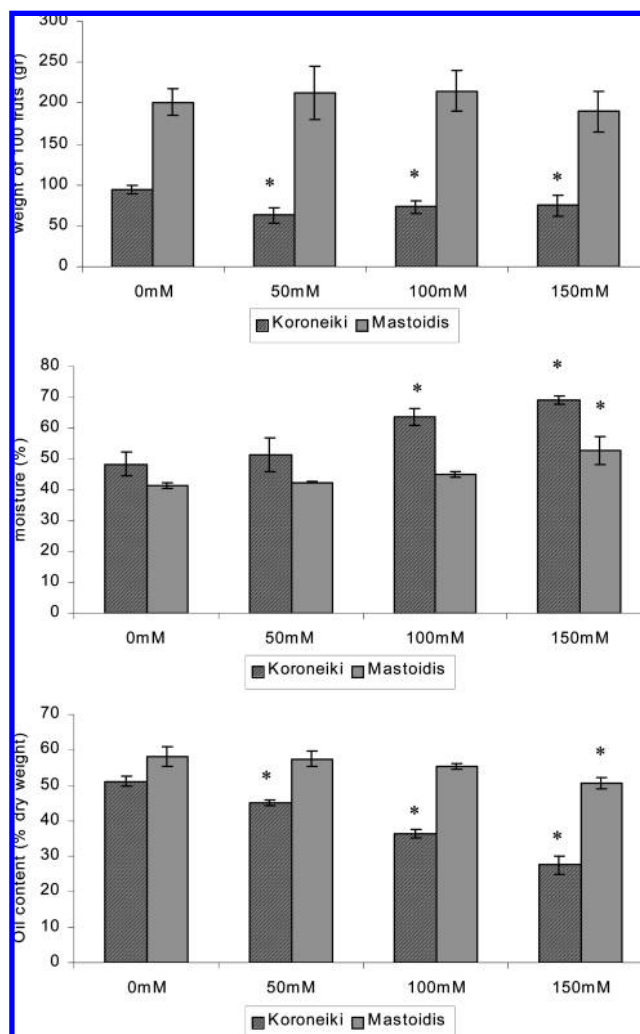


Figure 1. Effect of saline irrigation on fruit weight, moisture, and oil content of olive fruits. Means \pm standard deviation (SD) ($n = 3$) are shown. Significance was determined by Duncan's range test (with * indicating $p < 0.05$) comparing saline irrigation with controls.

For cv. Mastoidis, the decrease in photosynthesis caused by saline irrigation was much less than for cv. Koroneiki (18), perhaps because Na^+ and Cl^- were retained in the root tissue of the former. In fact, it is known that higher leaf concentrations of Na^+ and Cl^- cause reduced CO_2 assimilation rates (3). Thus, cv. Mastoidis appears to be inherently resistant to salt water irrigation. Standard characteristics of the oils produced from fruits of the two varieties after saline irrigation were evaluated according to the European Community guidelines (see the Supporting Information). These tests showed that there were no statistically significant alterations in the concentration of total phenols or in oxidation parameters as measured by K_{232} or K_{270} (data not shown).

Analysis of Individual Phenolic Compounds. In contrast to the lack of alteration of total phenols, there were changes in the pattern of phenolic compounds, especially for cv. Mastoidis (Table 1). The concentration of phenolics accumulating in olive oil is due to a complex mixture of factors, including cultivar, fruit ripening, pedo-climatic conditions, and some agronomic techniques and agricultural practices as well as technological aspects (19–22). For cv. Mastoidis, there were steady increases in the concentration of the secoiridoid derivatives with increasing salinity treatment. The concentration of these compounds was at least doubled compared to the control treatment. In contrast, for cv. Koroneiki, only 3,4-DHPEA-EDA was raised, although

Table 1. Changes in the Phenolic Compounds (mg/kg) of Olive Oils after Saline Irrigation^a

	cv. Koroneiki			
	NaCl concentration			
	control	50 mM	100 mM	150 mM
3,4-DHPEA	1.1 ± 0.1 a	0.8 ± tr a	0.9 ± tr a	
p-HPEA	1.7 ± 0.1 a	1.3 ± 0.2 b	1.2 ± 0.1 b	
3,4-DHPEA-EDA	237.5 ± 5.4 b	311.8 ± 19.4 a	308.0 ± 28.8 a	
p-HPEA-EDA	208.5 ± 31.7 a	241.2 ± 24.9 a	218.3 ± 24.0 a	
p-HPEA derivative	104.8 ± 17.6 a	108.2 ± 12.9 a	127.7 ± 27.0 a	
3,4-DHPEA-EA	340.3 ± 38.9 a	357.0 ± 36.6 a	345.8 ± 6.6 a	
	cv. Mastoidis			
	NaCl concentration			
	control	50 mM	100 mM	150 mM
3,4-DHPEA	0.6 ± tr a	0.7 ± 0.2 a	0.9 ± tr a	1.0 ± 0.3 a
p-HPEA	2.2 ± 0.3 a	1.8 ± 0.2 a	2.7 ± 0.3 a	1.7 ± 0.3 a
3,4-DHPEA-EDA	165.6 ± 12.5 b	154.9 ± 53.4 b	160.5 ± 52.9 b	451.2 ± 50.6 a
p-HPEA-EDA	146.6 ± 32.7 c	285.8 ± 55.9 b	278.2 ± 41.6 b	392.8 ± 40.6 a
p-HPEA derivative	76.1 ± 14.1 b	106.2 ± 43.7 a	124.9 ± 36.6 a	168.2 ± 44.3 a
3,4-DHPEA-EA	248.9 ± 15.1 b	262.4 ± 18.0 b	270.1 ± 20.8 b	411.9 ± 58.2 a

^a Means ± standard deviations ($n = 3$) are shown. Means within rows followed by the same letter are not significantly different according to Duncan's range test ($p \leq 0.05$). Phenolics are abbreviated as shown in the list of abbreviations. tr = trace ($p < 0.05$).

Table 2. Changes in the Fatty Acid (%) Composition of Olive Oils after Saline Irrigation^a

	NaCl concentration			
	control	50 mM	100 mM	150 mM
	cv. Koroneiki			
C16:0	13.3 ± 0.2 b	14.0 ± 0.3 b	15.8 ± 0.9 a	15.5 ± 0.5 a
C16:1	1.1 ± tr ab	0.9 ± tr b	1.2 ± 0.1 a	1.1 ± 0.2 ab
C18:0	2.6 ± 0.2 a	3.3 ± 0.02 a	2.8 ± tr a	2.9 ± 0.3 a
C18:1	75.7 ± 1.0 a	74.2 ± 0.9 ab	72.2 ± 2.2 b	70.1 ± 0.6 c
C18:2	5.7 ± 0.7 b	6.5 ± 0.5 ab	7.2 ± 0.8 ab	7.7 ± 1.2 a
C18:3	0.7 ± 0.06 b	0.8 ± tr b	1.3 ± 0.2 a	1.4 ± 0.3 a
total saturated	16.5 ± 0.2 c	18.0 ± 0.3 b	19.6 ± 1.0 a	19.4 ± 0.2 a
total unsaturated	83.4 ± 0.2 a	83.2 ± 0.6 a	80.2 ± 1.3 b	80.4 ± 0.9 b
total PUFAs	6.4 ± 0.8 c	7.3 ± 0.5 bc	8.6 ± 1.0 ab	9.1 ± 1.3 a
C18:1/C18:2	13.6 ± 2.1 a	11.5 ± 0.9 ab	9.8 ± 1.4 b	9.2 ± 1.3 b
	cv. Mastoidis			
C16:0	12.2 ± tr a	12.4 ± 0.6 a	12.7 ± 0.6 a	13.0 ± 0.1 a
C16:1	1.1 ± 0.1 a	0.9 ± 0.2 a	1.0 ± 0.2 a	0.9 ± 0.1 a
C18:0	2.6 ± 0.1 a	2.7 ± 0.2 a	2.8 ± 0.1 a	2.8 ± 0.1 a
C18:1	76.4 ± 0.6 a	74.4 ± 1.6 b	73.4 ± 1.2 b	72.4 ± 0.2 b
C18:2	5.4 ± 0.4 b	7.2 ± 1.1 a	8.4 ± 1.3 a	8.2 ± 1.5 a
C18:3	0.5 ± tr c	0.6 ± tr bc	0.6 ± tr b	0.7 ± tr a
total saturated	15.8 ± 0.1 b	16.0 ± 0.5 b	16.5 ± 0.5 ab	16.9 ± 0.2 a
total unsaturated	84.0 ± 0.1 a	83.7 ± 0.3 a	84.0 ± 0.6 a	82.9 ± 0.6 b
total PUFAs	5.9 ± 0.5 b	7.8 ± 1.2 a	9.0 ± 1.3 a	8.9 ± 1.5 a
C18:1/C18:2	14.2 ± 1.3 a	10.5 ± 1.8 b	8.9 ± 1.6 b	8.8 ± 1.2 b

^a Means ± standard deviations ($n = 3$) are shown. Means within rows followed by the same letter are not significantly different according to Duncan's range test ($p \leq 0.05$). Fatty acids are abbreviated as shown in the list of abbreviations. tr = trace ($p < 0.05$).

the lower crop yield after 150 mM NaCl irrigation prevented a full analysis at this concentration. The increase in secoiridoid derivatives is likely to be a response to salinity because changes in phenolics have been reported for drought-stressed olives (22, 23). Moreover, the phenolic components of olive oil are thought to have a strong effect on sensory perceptions by consumers (22, 23) and can give rise to desirable as well as undesirable (very intense pungent) sensory characteristics. They are also major contributors to oxidative stability, particularly, 3,4-DHPEA and its secoiridoid derivatives (22, 24).

Lipid Composition of Olive Oil. Olive oil is characterized by a very high level of oleate with small but adequate amounts of the essential polyunsaturated acids, linoleate and α -linolenate (8, 25, 26). Oils made from fruits harvested from saline-irrigated trees of both cultivars showed changes in their acyl composition (Table

2). In both cultivars, there was a decrease in the percentage of oleate, whereas the other major monoene, palmitoleate, remained unchanged. In cv. Koroneiki, there was a small but significant increase in palmitate but the main fatty acids, which were increased, were linoleate and α -linolenate. The simplest explanation for the above changes is that saline stress increased the relative activity of the desaturase enzymes responsible for the successive conversion of oleate to linoleate and then to α -linolenate (27). These changes led to a decrease in the proportion of total unsaturated fatty acids after 100 or 150 mM NaCl irrigation, as has been observed in other olive varieties under saline stress (28). These alterations in the overall fatty acid composition of olive oils led to significant increases in total polyunsaturated fatty acid (PUFA) content for both cultivars. At the same time, the ratio of oleate/linoleate fell from about

Table 3. Changes in the Triacylglycerol Molecular Species (%) Composition of Olive Oils Induced by Saline Irrigation^a

ECN	TAGs	NaCl concentration			
		control	50 mM	100 mM	150 mM
cv. Koroneiki					
42	LLL + OLLn	0.2 ± tr b	0.2 ± tr b	0.4 ± 0.1 a	0.5 ± tr a
44	OLL	1.0 ± 0.7 b	1.0 ± tr b	1.2 ± 0.2 b	2.0 ± 0.4 a
	OOLn	1.4 ± tr c	1.4 ± 0.1 c	1.7 ± 0.1 a	2.1 ± 0.4 a
	PLL + PoOL	0.6 ± tr a	0.7 ± 0.1 a	1.0 ± 0.1 a	1.2 ± 0.8 a
46	OOL	9.8 ± 0.9 a	10.2 ± 0.7 a	9.5 ± 0.1 ab	11.8 ± 2.5 a
	POL	5.5 ± 0.6 c	5.8 ± 0.5 bc	6.3 ± 0.2 b	8.4 ± 0.3 a
	PPL	0.5 ± 0.1 b	0.6 ± tr b	0.7 ± 0.1 a	0.8 ± 0.1 a
48	OOO	42.1 ± 1.0 a	38.7 ± 1.8 b	36.8 ± 1.0 b	31.9 ± 1.1 c
	POO + SOL	27.9 ± 0.5 a	28.1 ± 1.8 a	29.2 ± 0.8 a	28.2 ± 1.8 a
	POP	3.4 ± 0.2 a	3.4 ± 0.3 a	4.1 ± 0.2 ab	4.4 ± 1.0 a
50	GaOO	0.4 ± 0.1 a	0.4 ± 0.1 a	0.5 ± 0.1 a	0.3 ± 0.1 a
	SOO	4.7 ± 0.3 b	5.9 ± 0.5 a	5.5 ± 0.6 ab	5.1 ± 0.7 ab
	POS	1.2 ± 0.1 b	1.5 ± 0.1 a	1.5 ± 0.1 a	1.5 ± 0.2 a
52	AOO	0.8 ± tr a	1.0 ± tr a	1.0 ± 0.1 a	0.6 ± 0.5 b
	SOS	0.3 ± tr b	0.4 ± tr a	0.4 ± tr ab	0.4 ± tr a
cv. Mastoidis					
42	LLL + OLLn	0.2 ± tr b	0.2 ± 0.1 ab	0.3 ± 0.1 a	0.3 ± 0.1 a
44	OLL	0.9 ± 0.2 b	1.5 ± 0.3 a	2.0 ± 0.5 a	1.8 ± 0.2 a
	OOLn	1.1 ± 0.1 b	1.1 ± 0.2 b	1.4 ± 0.2 ab	1.5 ± 0.2 a
	PLL + PoOL	0.5 ± 0.1 a	0.6 ± 0.2 a	0.6 ± 0.1 a	0.7 ± 0.1 a
46	OOL	10.1 ± 0.5 b	12.1 ± 1.3 a	13.1 ± 1.2 a	12.7 ± 0.6 a
	POL	5.1 ± 0.3 b	6.0 ± 1.1 ab	7.0 ± 1.0 a	7.0 ± 0.3 a
	PPL	0.9 ± 0.1 b	1.0 ± 0.1 ab	1.1 ± 0.1 ab	1.2 ± 0.1 a
48	OOO	43.7 ± 1.0 a	41.1 ± 3.0 ab	37.9 ± 2.9 b	37.3 ± 0.2 b
	POO + SOL	26.2 ± 0.5 a	24.8 ± 0.3 b	24.8 ± 0.3 b	25.3 ± 0.3 b
	POP	3.0 ± 0.1 a	3.09 ± 0.4 a	3.2 ± 0.3 a	3.5 ± 0.1 a
50	GaOO	0.9 ± 0.1 b	0.9 ± tr ab	0.9 ± 0.1 ab	1.0 ± 0.2 a
	SOO	5.2 ± 0.1 a	5.1 ± 0.5 a	5.1 ± 0.3 a	5.2 ± 0.2
	POS	1.2 ± tr b	1.2 ± tr b	1.3 ± tr b	1.4 ± 0.1 a
52	AOO	0.7 ± 0.1 a	0.7 ± tr a	0.8 ± tr a	0.7 ± 0.1 a
	SOS	0.3 ± 0.1 a	0.3 ± tr a	0.3 ± tr a	0.3 ± 0.1 a

^a Means ± standard deviations ($n = 3$) are shown. Means within rows followed by the same letter are not significantly different according to Duncan's range test ($p < 0.05$). Triacylglycerols were abbreviated as shown in the list of abbreviations. tr = trace ($p < 0.05$).

14 to around 9 (Table 2) (29). On the contrary, Wiesman et al. (30) found an increase, even though not significant, in the percentage of oleic acid with a parallel decrease in linoleic acid percentage in oils extracted from saline-irrigated trees compared to the control (water-irrigated trees).

Although the oleate percentage in both olive oils was significantly reduced by saline treatment, this reduction was not reflected in all of the triacylglycerol molecular species that contained it (Table 3). Thus, the major molecular species, triolein, was reduced significantly in both cultivars. In marked contrast, species such as OLL (oleate/linoleate/linoleate) or POL (palmitate/oleate/linoleate) were both increased significantly. Presumably, in these cases, the increased linoleate content (Table 2) dominated the alterations. The increase in species with an ECN of 42 is especially important because this is used to detect adulteration of virgin olive oils (31, 32). There were also increases in the amounts of some of the more saturated molecular species [e.g., palmitate/oleate/stearate (POS)], although because these are minor components, the changes are unlikely to alter the perception of the olive oil quality by as much as mouth-feel, by consumers. Irrigation with saline water has been shown to be a realistic option for olive oil varieties (such as cv. Mastoidis), which maintain good crop production under these conditions. The changes that we found in the quality of the olive oil product are not sufficient to reduce its value. For cultivars such as Koroneiki, the alterations in olive oil composition were similar to cv. Mastoidis, indicating that salt stress has some consistent effect on the lipid and phenolic components of olive oil. However, the acute affect of saline irrigation on vegetative growth, fruit yield, and oil content mean

that only low levels of salt can be tolerated by trees of the Koroneiki variety and, hence, be considered for general agricultural practice.

ABBREVIATIONS USED

A, arachidic acid, C20:0; 3,4-DHPEA, 3,4-dihydroxyphenyl)ethanol; 3,4-DHPEA-EA, isomer oleuropein aglycon; 3,4-DHPEA-EDA, dialdehydic form of decarboxymethyl elenoic acid linked to 3,4-dihydroxyphenyl)ethanol; ECN, equivalent carbon number; FFA, free (non-esterified) fatty acid; Ga, gadoleic acid, C20:1; *p*-HPEA, (*p*-hydroxyphenyl)ethanol; *p*-HPEA-EDA, dialdehydic form of decarboxymethyl elenolic acid linked to (*p*-hydroxyphenyl)ethanol; HPLC, high-performance liquid chromatography; L, linoleic acid, C18:2; Ln, α -linolenic acid, C18:3; O, oleic acid, C18:1; P, palmitic acid, C16:0; Po, palmitoleic acid, C16:1; PUFA, polyunsaturated fatty acid; S, stearic acid, C18:0; TAG, triacylglycerol; UV, ultraviolet; LLL, C18:2-C18:2-C18:2; OLLn, C18:1-C18:2-C18:3; PLLn, C16:0-C18:2-C18:3; OLL, C18:1-C18:2-C18:2; OOLn, C18:1-C18:1-C18:3; PLL, C16:0-C18:2-C18:2; PoOL, C16:1-C18:1-C18:2; OOL, C18:1-C18:1-C18:2; POL, C16:0-C18:1-C18:2; PPL, C16:0-C16:0-C18:2; OOO, C18:1-C18:1-C18:1; POO + SOL, C16:0-C18:1-C18:1 + C18:0-C18:1-C18:2; POP, C16:0-C18:1-C16:0; GaOO, C20:1-C18:1-C18:1; SOO, C18:0-C18:1-C18:1; POS, C16:0-C18:1-C18:0; AOO, C20:0-C18:1-C18:1; SOS, C18:0-C18:1-C18:0.

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Supporting Information Available: Effects of saline irrigation on standard quality characteristics of oils from fruits of two olive varieties. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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