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ARTICLE

Effect of Irrigation on Quality Attributes of Olive Oil

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Two irrigation treatments were applied to olive trees of the major commercial Cretan variety cv. Koroneiki, (a) irrigation with 0.4 evaporation class "A" pan and (b) rain-feed only, in two successive crop years to assess the effect of irrigation on olive oil quality. Olive fruits were harvested at their semiblack maturity stage. Data obtained indicated that irrigation increased fruit weight and oil content, but the standard quality indices (free fatty acids, peroxide value, K_{232}, and K_{270}) of the oil were not affected significantly. However, irrigation affected some aspects of olive oil composition. There were changes in the proportions of polyunsaturated fatty acids (PUFAs), triacylglycerol molecular species, sterols, and aliphatic alcohols. Furthermore, the concentrations of the dialdehydic form of elenolic acid linked to 3,4-DHPEA (3,4-DHPEA-EDA) and the isomer of oleuropeine aglycon (3,4-DHPEA-EA) were higher in oils from non-irrigated trees. Tocopherol and total volatiles were higher in the oil produced from the non-irrigated trees. Such oil was graded more pungent when compared to oils produced from fruits of irrigated trees, although both oils were graded satisfactory by consumers.

KEYWORDS: Volatile compounds; phenolic compounds; quality indices; virgin olive oil; irrigation; cv. Koroneiki

INTRODUCTION

The olive tree (*Olea europaea* L.) is considered to be one of the most drought-resistant crops and has several defense mechanisms in response to water deficit (*1*−*3*). Thus, it is well adapted to the Mediterranean environment where it grows under semi-drought conditions. The most drought-sensitive stage of olive fruit development is the period after the end of pit hardening to the start of maturation. At the same time as stone hardening, oil accumulation starts and achieves a maximum before full maturity, depending on genetic factors and environmental conditions (*4*, *5*).

Although olive trees can grow and produce adequate crops under naturally low annual water supply, irrigation during drought periods is essential to give high yields. Irrigation increases fruit production by increasing both the size and number of fruits and their oil content and reduces fruit drop and alternative bearing (*5*−*10*).

Considerable research has been carried out on the protective physiological mechanisms by which olive trees tolerate drought and grow under unfavorable climatic conditions (*2*, *3*). The effect of water stress on the quality and compositional parameters of olive oil has been less studied, although in recent decades a number of studies have been published related to the above subject. However, results from these studies are not always uniformly consistent and, thus, do not always lead to generally accepted conclusions. Phenolic compounds of olive oil appear to be the most influenced by irrigation, with the effect varying with the amount and time of water applied to the trees (including the type of irrigation management), the climatic conditions, and the variety (*11*−*13*). Moreover, water stress influences the organoleptic properties of olive oil and its oxidative stability. The antioxidant activity of phenolic compounds is well-known (*14*), as is the correlation of some phenolic compounds with sensory attributes of olive oil, especially bitterness and pungency (*15*). More recent research findings (*13*, *16*, *17*) have reported that the volatile profile of olive oil is also influenced by irrigation of olive trees.

On the island of Crete (Greece), olive cultivation occupies 64.2% of the total agricultural land and represents 86% of the crops (35 million trees with average production of 120,000 tonnes of olive oil). Due to the semi-arid climate, irrigation is essential in olive orchards receiving <400 mm of annual rainfall, in new intensive orchards (250−400 trees per hectare), and in soils with low water-holding capacity, which represent $>70\%$ of Cretan olive orchards. At the present time irrigation is becoming more and more common not only in the new intensive olive orchards but in traditional low-density orchards as well. Recent increases in productivity have been mainly due to irrigation of orchards.

Despite its importance for Cretan agriculture, there is very limited information about the Koroneiki cultivar, although some general information concerning growth has been published (*2*). The aim of the current study was to provide a thorough examination of the quality characteristics of olive oils from

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irrigated and non-irrigated trees of this main commercial Cretan variety (Koroneiki) in two successive (and contrasting) seasons.

MATERIALS AND METHODS

Olive Trees and Irrigation. Fifty-year-old olive trees (*O. europaea* L. Koroneiki) in a commercial orchard at Kissamos (Crete) were used during two successive years. The climate at Kissamos is typically Mediterranean with an average rainfall of 560 mm per annum mainly during the winter months. The trees were spaced 5 × 6 m, and the soil was sandy-loam with a pH of 8.2 and an electrical conductivity (EC) of a saturated extract of 0.35 dS/m. The quality of irrigation water was very good with an EC of 0.42 dS/m.

All trees were cared for in the same way (fertilization, pruning, weed control, pest management). Two treatments were applied: (a) deficit irrigation with 0.4 evaporation class A pan and (b) non-irrigated (control). The amount of water applied per week was calculated by the formula Iw = 0.4Ep − 0.8*R*, where Ep is the class A evaporation and *R* is the rainfall. Irrigation water was applied once a week with a drip system, one lateral per row of trees, with 4 L/h using drippers spaced 1 m apart. Irrigation started in mid-May each year until the end of September. Each treatment was applied to 10 trees and replicated three times. The seasonal applications of irrigation water were 275 and 247 mm for years 1 and 2, respectively. In terms of the average temperatures, these were very similar in the two years until August, but in year 1 both the maximum and minimum temperatures decreased more rapidly in the period up to December (**Figure 1**). The two years differed markedly for rainfall in that year 2 saw a significant (50 mm) precipitation in September (**Figure 1**) during the maximum period of fruit growth. Olives were harvested at the end of November.

Olive Samples. Representative fruits (10 kg sample) at a maturity stage at which 50% of the fruit displayed a purple color were hand-picked from trees of each treatment and brought to the laboratory for oil extraction the same day. The same trees were used in each year. Olive oil was extracted by a laboratory scale olive mill using the procedure described previously (*18*). Oil samples were kept in the freezer (−20 °C) under nitrogen prior to analysis. Samples of the same batch of olive fruits were also used for the determination of moisture and oil contents.

Moisture Content. The moisture content of olive fruits was determined by an oven-drying method at 105 ± 1 °C until constant weight was achieved. The moisture content of the fruit was calculated as a percentage loss of the fruit weight.

Oil Content of Fruits. Oil content was determined per gram of dry matter according to the Soxhlet extraction method (*19*) with hexane as extraction solvent.

Analytical Methods. Routine chemical analyses [acidity given as % of oleic acid, peroxide value (PV) expressed as milliequivalents of active oxygen per kg of oil (mequiv O_2/kg), and coefficients of specific extinction K_{232} and K_{270}] were carried out according to standard methods as recommended by the EC (2568/91) with subsequent amendments (*20*).

Oxidative stability was evaluated by the Rancimat method (*21*). Stability was expressed as the induction time (hours) measured with the Rancimat 679 apparatus (Metrohm, Switzerland) at 120 °C with an air flow of 20 L/h.

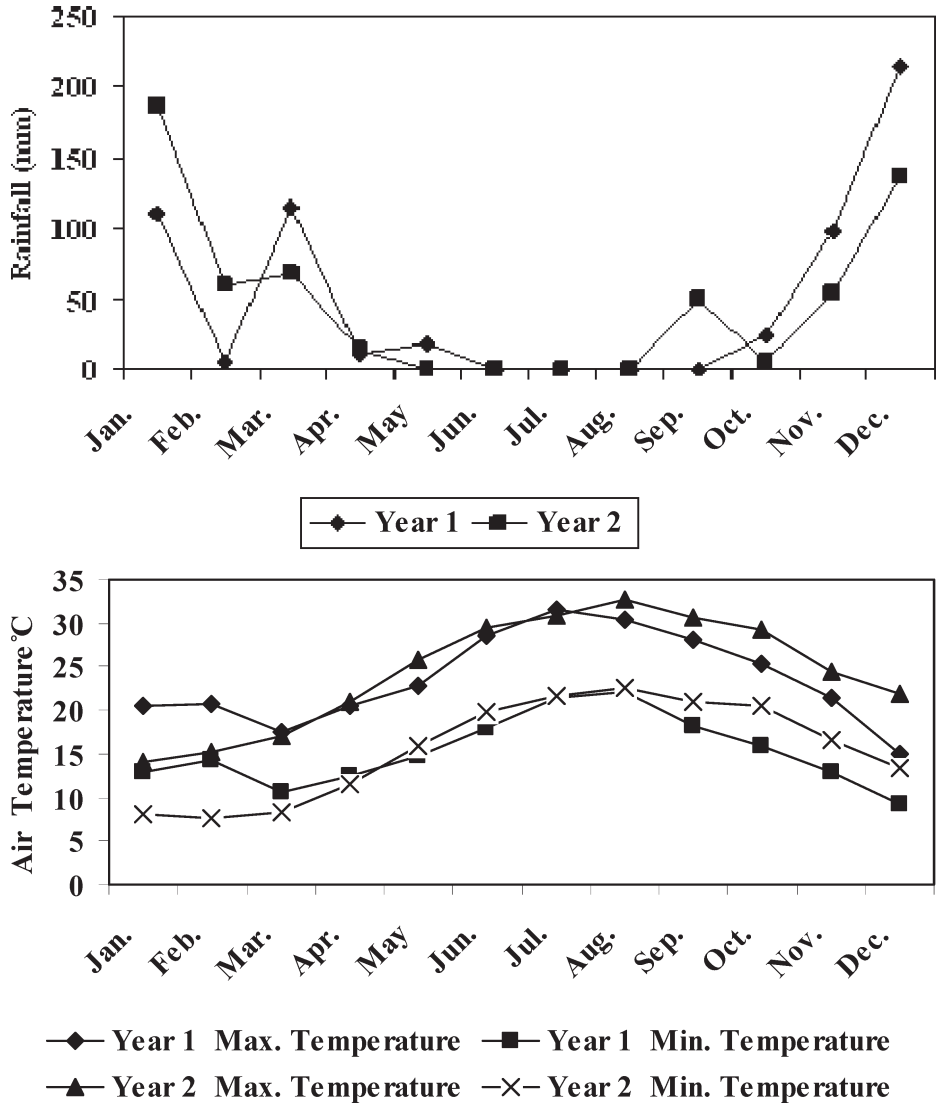

Figure 1. Mean monthly maximum and minimum air temperatures (°C) and total rainfall (mm) recorded during years 1 and 2 in the olive tree orchard.

Fatty Acid Analysis. Fatty acids were extracted and analyzed as described in ref *18*. The fatty acid methyl esters were separated in a 50 m × 0.22 mm (0.25 μm 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 °C/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 ref *20*. Triacylglycerols were separated in a Kromasil 100 C18 column (25 m × 4 mm i.d.; MZ Analysentecknik, 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 comparison to retention times of triacylglycerols from reference chromatograms obtained from standard soybean oil and pure olive oil separated under the same conditions as reported by the official EEC method (*20*). 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 CN−2*n*. where CN is the total acyl carbon number and *n* is the number of double bonds of fatty acids. This gave fractions of ECN42−ECN52 containing the different molecular species calculated according to the EC Regulation 2568/91 method (*20*).

Sterols and Aliphatic Alcohols. Sterols and aliphatic alcohols were determined according to EC Regulation 2568/91 (*20*). The fraction of sterols and aliphatic alcohols was analyzed by a Hewlett-Packard 6890 gas chromatograph with a split injector and FID detector. The analytical column was a 30 m × 0.32 mm with a 0.25 μm film of HP5 (Hewlett-Packard). Sterols were quantified using cholestanol as an internal standard, whereas for the quantitation of aliphatic alcohols arachidyl alcohol was used as internal standard.

Tocopherols. Tocopherols were measured using the IUPAC 2.432 method (*22*). The chromatographic separation of tocopherols was achieved with high-performance liquid chromatography (HPLC; Hewlett-Packard series 1100) with direct injection of the oil dissolved in *n*-hexane using a 250 × 4 mm Lichrosorb Si60 (5 μm film) column MZ Analyzentechnik) coupled with a UV detector (Jasco UV 970). The eluates were monitored at 292 nm. Results were expressed as milligrams per kilogram of oil using a standard curve of α-tocopherol for quantificaiton.

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 by Montedoro et al. (*23*). 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 (*24*). Individual phenolic compounds were separated by a 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. Analytical separation was achieved on a Lichrosphere 100 RP-18, 5 μm column (250 × 4 mm i.d.) equipped with a 5 cm precolumn (Merck, Darmstadt, Germany) with the same packing material as the column. Eluates were monitored at 280 nm for all phenolic compounds. Phenolic compounds were identified on the basis of their retention times compared to those of the standard compounds. Quantitative determination was performed using the calibration curves of authentic standards performed in HP ChemStation software following external standard ESTD procedure. The peak of the dialdehydic form of elenolic acid linked to tyrosol (*p*-HPEA-EDA) was quantified using the standard compound 3,4 DHPEA-EDA.

The following reference compounds were used: gallic acid was purchased from Sigma Chemical Co. (St. Louis, MO) and 2-(*p*-hydroxyphenyl)ethanol (*p*-HPEA) from Aldrich Chemical Co (Milwaukee, WI). The compounds 3,4-dihydroxyphenylethanol (3,4- DHPEA), the dialdehydic form of elenolic acid linked to 3,4-DHPEA (3,4-DHPEA-EDA), and the isomer of oleuropeine aglycon (3,4-DHPEA-EA) were kind gifts from Professor G. F. Montedoro (University of Perugia, Italy). 3,4-DHPEA-EDA and the isomer of oleuropeine aglycon (3,4-DHPEA-EA) were extracted from virgin olive oil and olive fruit, respectively, and their chemical structures were verified by NMR (*25*).

Determination of Volatiles. Volatiles were measured by a dynamic headspace technique as described by Williams et al. (*26*). Half a gram of olive oil was heated at 40 °C and swept with a nitrogen (200 mL/min) stream. Volatiles were trapped in Tenax TA tubes (Perkin-Elmer, Beaconsfield, UK) over a 30 min period. Analysis was carried out using a Perkin-Elmer Autosystem GC with a Supelcowax 10 capillary column (60 m × 0.32 mm i.d., 0.5 μm film thickness). Routine identification of peaks was by reference to authentic standards, but all components had been identified by GC-MS previously (*26*).

Sensory Evaluation. Sensory evaluation of the organoleptic characteristics of oils was carried out according to Annex XII of EC regulation 2568/91 (*20*). The sensory assessments were made up of 16 descriptors (8 positive, 8 negative). The descriptive analysis used a five-point intensity scale, ranging from 0 (no perception) to 5 (extreme). Overall grading used a nine-point scale with 9 for exceptional quality and 1 for the worst. Sample presentation was randomized and performed in triplicate. Ten trained tasters were used.

Statistical Analysis. The significance of differences at a 5% level among means was determined using independent-samples *t* test procedure. The statistical package used was SPSS (version 15.0 for Windows, 2006).

RESULTS AND DISCUSSION

Overall Characteristics of Yields. The mean values for basic yields and characteristics of olive fruits produced from irrigated or non-irrigated trees from the same orchard are shown in **Table 1**. The weight of fruits was significantly higher for irrigated trees in both years but was especially so in year 1 when there was negligible natural rainfall (0.5 mm) in the critical stage (August−September) compared to 50 mm rainfall in September of year 2 (**Figure 1**). In fact, there was a 27% reduction in fruit weight the first year and 17% the second year. These results are in agreement with other studies in which irrigation has been shown to increase fruit size, especially in dry years (*8*, *10*, *27*). Moreover, the oil content (expressed as % dry weight) was also significantly higher (12%) in year 1 for fruits from irrigated trees, in agreement with previous studies for other olive varieties (*5*, *10*), whereas in the second crop year it was only 3% higher and not significantly so. The rainfall of September in the second year probably reduced the difference between the treatments. This shows that Koroneiki, like other cultivars, recovers rapidly from the effect of water stress after autumn rain (*7*). The moisture contents of olive fruits were inconsistent over the two years of the experiment but may be related to the rainfall before harvest (*7*). This study underscores the fact that application of water during the dry season is essential for maximum fruit growth and affects weight as well as oil content of the fruit (**Table 1**).

General Quality Characteristics. Overall quality characteristics for virgin olive oils are shown in **Table 2**. Irrigation increased slightly the free fatty acid content of the oil, in agreement with previous data (*11*, *12*), but the increase was only significant in the first year. Similarly, peroxide values were significantly higher in oils from irrigated fruits the first year. For year 2, when 50 mm of

Table 1. Yields and Characteristics of Olive Fruits following Irrigation in Two Successive Years[a]

	sample	irrigated	non-irrigated
total weight of 100 fruits (g)	year 1	93.0 ± 4.0	65.1 ± 1.4*
	year 2	77.1 ± 2.5	59.9 ± 2.5*
moisture (% fresh wt)	year 1	49.2 ± 0.5	54.1 ± 0.7*
	year 2	48.7 ± 0.7	46.5 ± 0.8
oil content (% dry wt)	year 1	51.3 ± 2.1	41.7 ± 0.7*
	year 2	45.5 ± 2.0	43.1 ± 1.5

[a] Data are expressed as means ± standard deviations (n = 6). Significance determined by Student's *t* test with * indicating $p < 0.05$.

Table 2. Standard Quality Characteristics of Oils Obtained from Irrigated or Non-irrigated Olive Trees[a]

	sample	irrigated	non-irrigated
acidity (% oleic acid)	year 1	0.34 ± 0.01	0.22 ± tr. *
	year 2	0.33 ± 0.02	0.30 ± 0.02
peroxide value (mequiv of O_2/kg of oil)	year 1	6.72 ± 0.79	3.54 ± 0.66*
	year 2	5.60 ± 0.12	6.30 ± 0.58
K_{232} (OD at 232 nm)	year 1	1.71 ± 0.06	1.84 ± 0.01*
	year 2	1.44 ± 0.04	1.55 ± 0.04*
K_{270} (OD at 270 nm)	year 1	0.14 ± 0.02	0.16 ± 0.02
	year 2	0.15 ± 0.02	0.17 ± 0.01
total phenol (μg/g of gallic acid)	year 1	201.5 ± 21.0	248.4 ± 12.5*
	year 2	403.6 ± 32.0	479.1 ± 29.9
oxidative stability (h)	year 1	15.3 ± 0.8	14.6 ± 0.5
	year 2	18.4 ± 0.2	15.6 ± 0.3*
total tocopherols (μg/g)	year 1	186.1 ± 15.7	279.3 ± 2.5*
	year 2	228.3 ± 2.4	315.8 ± 37.4*

[a] Data are expressed as means ± standard deviations (n = 6). tr, trace (<0.005). Significance determined by Student's t test with * indicating $p < 0.05$.

rainfall was recorded in September, there was no difference, perhaps reflecting the agronomic conditions. The presence of conjugated triene compounds (as measured at 270 nm) showed no changes on irrigation, whereas the conjugated diene content (as indicated by K_{232}) was higher for the oil from non-irrigated trees. Similar results were obtained for cultivar Picual (*11*). These quality indices of oil depend strongly on fruit quality and soundness, which, in turn, are due to appropriate harvesting, processing, and storage conditions. The values of these quality indices in oils obtained from fruits of both irrigated and non-irrigated trees were well within the limits of extra virgin olive oil established by EC regulations. The total tocopherol content (95−97% of which was α-tocopherol) of olive oil was higher in oils from non-irrigated trees (**Table 2**) as reported by Tovar et al. (*28*).

Oxidation stability was not reflected by the levels of total tocopherols (**Table 2**), in agreement with the data of Baldioli et al. (*14*). Furthermore, there was a higher percentage of polyunsaturated fatty acids in the oil from non-irrigated trees. Moreover, the percentage differences in PUFA for oils from irrigated versus non-irrigated trees were 2.44 and 4.26% in years 1 and 2, respectively (**Table 3**). It is well-known that polyunsaturated fatty acids are more susceptible to oxidation, which, in turn, could shorten the shelf life of the oil. The contribution of different compounds in virgin olive oil to its stability was studied by Aparicio et al. (*29*), who found that the oleic/linoleic acid ratio was one of the main determinants of oxidative stability. Moreover, stability may depend on some synergetic effects (still unknown) between phenolic compounds, fatty acid composition, tocopherols, carotenoids, and chlorophylls (*29*, *30*).

Olive Oil Phenolics. Total phenols were higher in oils from non-irrigated trees but significantly so only in year 1 (**Table 2**). These results are in agreement with previous results (*11*, *12*, *27*, *28*). However, data on phenolic concentrations in olive oils following irrigation in some cases are not in agreement (*31*) and may well reflect varietal differences as well as the exact agronomic conditions. In the latter context, it is noteworthy that for the same trees examined in years 1 and 2 of our study, there were some quantitative and qualitative differences as a result of irrigation (**Tables 2** and **4**). In fact, the lower phenolic concentration that we found for year 1 can be explained by another study which was undertaken using variety Cornicabra, again in two successive years (2003, 2004) (*7*). These authors concluded that the effect of irrigation on phenolic concentration takes place all year round and not just during the oil accumulation phase. According to their data, the total phenol content of the rain-fed trees was sharply reduced in 2004, which had a rainy spring (the last rain falling in June) and a dry autumn (similar to our meteorological data). Therefore, the water status of olive trees throughout the year affects the concentration of phenolics. Another explanation might be the size of fruit. Thus, an inverse relationship between

Table 3. Effect of Irrigation on the Fatty Acid (Percent) Content of Virgin Olive Oils Obtained from Fruits of Cv. Koroneiki[a]

fatty acid	sample	irrigated	non-irrigated
C16:0	year 1	12.75 ± 0.35	12.99 ± 0.19
	year 2	12.46 ± 0.17	12.81 ± 0.03
C16: 1	year 1	0.81 ± 0.02	1.04 ± 0.02*
	year 2	0.73 ± 0.02	0.80 ± 0.01*
C18:0	year 1	2.74 ± 0.06	3.50 ± 0.04*
	year 2	2.45 ± 0.51	3.48 ± 0.02
C18: 1	year 1	75.42 ± 0.33	71.67 ± 0.15*
	year 2	76.51 ± 0.23	71.01 ± 0.16*
C18: 2	year 1	6.50 ± 0.10	8.74 ± 0.15*
	year 2	5.74 ± 0.05	9.80 ± 0.14*
C18: 3	year 1	0.60 ± 0.01	0.82 ± 0.02*
	year 2	0.75 ± 0.03	0.95 ± 0.03*
MUFAs	year 1	76.30 ± 0.34	72.78 ± 0.16*
	year 2	77.29 ± 0.21	71.84 ± 0.18*
PUFAs	year 1	7.12 ± 0.10	9.56 ± 0.08*
	year 2	6.49 ± 0.07	10.75 ± 0.16*
SFA	year 1	16.18 ± 0.39	17.35 ± 0.22*
	year 2	15.66 ± 0.53	17.13 ± 0.04*
MUFAs/PUFAs	year 1	10.72 ± 0.14	7.61 ± 0.06*
	year 2	11.92 ± 0.14	6.68 ± 0.12*

[a] Data are expressed as means ± standard deviation (n = 6) as % total fatty acids. Significance determined by Student's t test with * indicating $p < 0.05$.

Table 4. Effect of Irrigation on the Content (Milligrams per Kilogram) of Phenolic Compounds in Olive Oils[a]

compound	sample	irrigated	non-irrigated
p-HPEA	year 1	1.4 ± 0.3	0.9 ± 0.3
	year 2	2.6 ± 0.15	1.7 ± 0.2*
3,4-DHPEA	year 1	0.4 ± 0.2	0.7 ± 0.4
	year 2	1.6 ± 0.1	2.6 ± 0.4*
3,4-DHPEA-EDA	year 1	159.0 ± 43.7	370.5 ± 48.9*
	year 2	282.2 ± 30.2	468.15 ± 8.90*
p-HPEA-EDA	year 1	219.3 ± 69.3	250.6 ± 42.9
	year 2	337.7 ± 11.2	316.5 ± 53.3
3,4-DHPEA-EA	year 1	162.4 ± 38.6	234.4 ± 34.0
	year 2	267.3 ± 18.2	289.5 ± 28.2

[a] Data are means ± standard deviations (n = 6). For statistical significance see **Table 2**. Response factors: *p*-HPEA = 0.342, 3,4-DHPEA = 0. 252, 3,4-DHPEA-EDA = 1.276, *p*-HPEA-EDA = 1.276, 3,4-DHPEA-EA = 1.718.

oleuropein content and olive fruit size has been reported for different sizes of olive varieties (*32*). Polyphenols are concentrated near the skin of fruits, and the surface area of skin per kilogram of fruit is greater when fruit size is smaller (*33*).

HPLC analysis of individual phenolics from olive oils is shown in **Table 4**. First, in keeping with the general observations on virgin olive oils, the phenolic alcohols *p*-HPEA and 3,4-DHPEA were present at much lower concentrations than the secoiridoid derivatives (*23*) In fact, *p*-HPEA was lower in oils from non-irrigated trees, but this was only statistically significant in the second year. These results are consistent with previous studies (*13*, *16*) reporting that water stress conditions result in a decrease in the activity of the endogenous esterase enzyme in the olive fruit, which hydrolyzes the bond between *p*-HPEA and the elenolic acid of ligstroside (*16*, *27*).

In contrast, 3,4-DHPEA was higher in oils from non-irrigated trees, with these values being again significant in year 2. The secoiridoid derivatives (3,4-DHPEA-EDA and 3,4-DHPEA-EA) were increased under water stress (**Table 4**) as reported by Rico et al. (*13*) and Servili et al. (*16*). The higher concentration of phenolic compounds in oils non-irrigated trees can be attributed to the activity of phenylalanine ammonia-lyase (PAL), a key enzyme in the biosynthetic pathway of phenolic compounds. The activity of this enzyme has been reported to decrease with irrigation. Moreover, the secoiridoid compounds are known to have the highest antioxidant activity of the various constituents of virgin olive oils (*14*, *30*).

Lipid Composition. The total fatty acid composition of oils from irrigated versus non-irrigated trees showed some differences (**Table 3**). The most notable effects were a significant increase in oleate content and decreases in the polyunsaturated fatty acids linoleate and α-linolenate on irrigation. Thus, the monounsaturated to polyunsaturated fatty acid ratio was significantly influenced by irrigation and, as a result, the intrinsic oxidative stability of the oil was altered. These results are in agreement with previous studies using the cultivars Leccino and Arbequina, respectively (*16*, *28*). In contrast, the opposite changes were found in oils from rain-fed trees of the cultivar Cornicabra (*27*). Furthermore, no differences were found by Patumi et al. (*12*) for oils from the cultivar Kalamata. Taken together, these data indicate that there are strong varietal differences in the response of olive trees to water stress. This may reflect the balance of enzymes used for fatty acid biosynthesis.

The variation in triacylglycerol molecular species composition (**Table 5**) due to irrigation followed trends similar to those of the corresponding fatty acids (**Table 3**). Thus, molecular species containing linoleate or linolenate (LLL, OLL, OOLn, etc.) were decreased in oils from irrigated trees, whereas major species containing oleate but not polyunsaturated fatty acids (e.g., OOO, POO) were significantly increased. Triacylglycerol composition has been established as a measure for olive oil quality control as well as for the determination of their origin and purity. Oils from both irrigation regimes are characterized by four major triacylglycerol fractions, OOO, POO + SOL, OOL, POL. The most abundant triacylglycerol species is OOO, with percentages of 41.8 and 36.2% for oils from irrigated and non-irrigated trees, respectively. Furthermore, the value of (ΔECN42) for both treatments was below the established maximum limit of 0.2 (EC legislation). In addition to changes in fatty acid biosynthesis referred to above, alterations in the balance of triacylglycerol molecular species could also reflect changes in the activity of enzymes used for triacylglycerol assembly (*34*). Thus, irrigation could have resulted in a different balance of isoforms for the lysophosphatidate acyltransferase or diacylglycerol acyltransferase reactions used in triacylglycerol accumulation. Alternatively,

Table 5. Effect of Irrigation on Triacylglycerol Molecular Species (Percent Total Triacylglycerols) in Virgin Olive Oils Obtained from Olive Fruits of Cv. Koroneiki in Year 2[a]

ECN	TAGs	irrigated	non-irrigated	significance
42	LLL + OLLn	0.28 ± tr	0.51 ± 0.02	<0.005
44	OLL	1.43 ± 0.02	2.22 ± 0.05	<0.005
	OOLn	1.24 ± 0.04	1.79 ± 0.14	<0.005
	PLL + PoOL	0.46 ± 0.03	0.70 ± 0.05	<0.005
46	OOL	10.98 ± 0.12	12.74 ± 0.15	<0.005
	POL	5.74 ± 0.18	7.57 ± 0.05	<0.005
	PLL	0.44 ± 0.03	0.71 ± 0.03	<0.005
48	OOO	41.77 ± 0.21	36.18 ± 0.14	<0.005
	POO + SOL	26.15 ± 0.21	24.63 ± 0.09	<0.005
50	POP	3.12 ± 0.09	3.16 ± 0.17	0.73
	GaOO	0.49 ± 0.01	0.36 ± 0.11	0.12
	SOO	5.24 ± 0.06	6.14 ± 0.12	<0.005
	POS	1.24 ± 0.01	1.61 ± 0.06	<0.005
52	AOO	0.91 ± 0.02	0.95 ± 0.01	<0.05
	SOS	0.35 ± 0.03	0.44 ± 0.01	<0.01

[a] Data are expressed as means ± standard deviations ($n = 6$). p values, shown for significance by two-tailed test. tr = trace (<0.005). 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.

the activity of the phospholipid:diacylglycerol acyltransferase could have been altered, which would have allowed preferential incorporation of oleate into OOO or POO + SOL fractions. Finally, membrane species remodeling via a Lands-type mechanism could be involved (*34*−*36*). However, without definite enzymatic evidence on these possibilities it would seem premature to speculate on the mechanism(s) involved. In general, whereas the increase in the amount of overall fatty acid saturation for oils from irrigated fruits might also increase oxidative stability, changes in molecular species could alter consumer perception through mouthfeel (*12*). Recent research findings (*37*) have reported the influence of fatty acid composition on taste receptor cells. These authors proposed that lipid matrices play a key role in the perception of bitterness in virgin olive oil because polyunsaturated matrices produce milder sensations and are less bitter than monounsaturated.

Despite the alterations in fatty acid or triacylglycerol molecular species composition of the olive oil induced by drought or irrigation, all parameters were well within the limits imposed by EC legislation (2568/91).

Sterols and Alcohols. The sterol pattern is also important for the determination of authenticity of virgin olive oil (*20*, *38*). These components contribute to overall quality even though consumers cannot detect them. In addition, phytosterols have structures similar to cholesterol and help to reduce the total plasma and LDL-cholesterol and, as a result, these compounds are being developed as ingredients of functional foods (*39*).

The major sterol components are shown in **Table 6**, and it will be seen that irrigation caused only small changes in the percentage distribution. Irrigation generally caused significant increases in the percentages of individual components with the exception of Δ^5-avenasterol and Δ^5-24-stigmastadienol. The major sterols found were β-sitosterol (67−68%), Δ^5-avenasterol (24−27%),

Table 6. Effect of Irrigation on the Percent of Individual Sterols of Virgin Olive Oils Obtained from Cv. Koroneiki[a]

	irrigated	non-irrigated
cholesterol	0.36 ± 0.06	0.27 ± 0.05
24-methylenecholesterol	0.50 ± 0.01	0.33 ± 0.01*
campesterol	3.29 ± 0.02	2.69 ± 0.02*
campestanol	0.47 ± 0.01	0.32 ± 0.01*
stigmasterol	0.38 ± 0.01	0.24 ± 0.00*
β-sitosterol	67.89 ± 0.37	66.51 ± 0.47*
Δ^5-avenasterol	24.43 ± 0.18	26.94 ± 0.45*
Δ^{5-24}-stigmastadienol	0.86 ± 0.05	0.95 ± 0.03*
Δ^7-stigmastenol	0.25 ± 0.18	0.18 ± 0.02
Δ^7-avenasterol	0.41 ± 0.04	0.34 ± 0.06
total sterols (ppm)	1004.50 ± 11.36	1604.20 ± 36.46*

[a] Data are expressed as means ± standard deviations (n = 6). Significance determined by Student's t test with * indicating $p < 0.05$.

Table 7. Effect of Irrigation on the Aliphatic and Triterpene Alcohol Composition (Milligrams per 100 g of Oil) of Virgin Olive Oil Obtained from Cv. Koroneiki[a]

	irrigated	non-irrigated
docosanol	3.92 ± 0.90	11.15 ± 0.59*
tricosanol	0.35 ± 0.01	0.75 ± 0.03*
tetracosanol	7.83 ± 0.64	18.52 ± 1.14*
pentacosanol	0.51 ± 0.03	0.72 ± 0.06*
hexacosanol	9.00 ± 0.98	12.40 ± 1.34*
heptacosanol	0.47 ± 0.07	0.48 ± 0.02
octacosanol	2.67 ± 0.28	2.90 ± 0.33
total aliphatic alcohols	25.58 ± 2.17	46.91 ± 3.39*
cycloartenol	14.49 ± 0.28	18.23 ± 0.31*
24-methylene cycloartenol	15.30 ± 0.29	9.43 ± 0.65*
citrostadienol	23.02 ± 0.36	12.52 ± 0.30*

[a] Data are expressed as means ± standard deviations (n = 6). Significance determined by Student's t test with * indicating $p < 0.05$.

and campesterol (about 3%). The relative changes in β-sitosterol and Δ^5-avenasterol may be due to an alteration in the activity of the desaturase enzyme that transforms β-sitosterol into Δ^5-avenasterol (*40*). Furthermore, the ratio of campesterol/stigmasterol in oils from Koroneiki was high (8.7 and 11.2, respectively, for oils from both irrigated and non-irrigated trees). A high campesterol/stigmasterol ratio has been reported as an index of high-quality oil (*41*). In contrast to the small changes in sterol quality, irrigation caused a large decrease in the total content of sterols (from 1604 to 1005 ppm).

We also analyzed the aliphatic alcohols in the olive oil samples (**Table 7**). Again, although these components are used to detect adulteration (from solvent-extracted oils), they are not known to be important for oil quality as appreciated by consumers. Almost all of the aliphatic components were reduced significantly on irrigation, with the biggest changes being found for docosanol (from 11.2 to 3.9 mg/100 g of oil) and tetracosanol (from 18.5 to 7.8 mg/100 g of oil). Total aliphatic alcohols were reduced from 46.9 to 25.6 mg/100 g of oil on irrigation. In contrast, for the triterpene alcohols, there were variable effects of irrigation. Whereas cycloartenol was reduced slightly, both 24-methylene-cycloartenol and citrostandienol were increased substantially. The changes in aliphatic alcohols may well reflect gross alterations in the size and morphology of fruits on irrigation.

Volatile Compounds and Panel Tests. The volatile compounds, which are byproducts produced by the lipoxygenase pathway from polyunsaturated fatty acid precursors (*35*), play a vital role in determining the sensory attributes of different olive oils. More than 20 volatile compounds were identified in oils from irrigated or non-irrigated trees (**Table 8**). Major groups of volatiles were hydrocarbons, alcohols, aldehydes, ketones, and esters (*17*, *42*). Aldehydes were the most prevalent volatiles in the extracted oils, accounting for nearly half the total volatiles in oils from both irrigated and non-irrigated trees.

Table 8. Irrigation of Olive Trees Changes the Balance of Volatiles in Virgin Olive Oils

	peak area ratio (×100)[a]	
component	irrigated	non-irrigated
hydrocarbons		
isopentane	4.68 ± 0.36	1.79 ± 0.53*
n-hexane	9.70 ± 0.84	8.55 ± 0.75
alcohols		
1-penten-3-ol	5.94 ± 3.51	14.70 ± 0.28
isoamyl alcohol	3.96 ± 3.57	4.80 ± 0.65
hexan-1-ol	1.67 ± 0.10	1.32 ± 0.24
trans 3-hexen-1-ol	2.53 ± 0.09	4.07 ± 0.40*
cis-3-hexen-1-ol	2.40 ± 1.28	1.83 ± 0.35
trans-2-hexen-1-ol	2.83 ± 0.59	3.54 ± 0.35
aldehydes		
3-methylbutanal	5.77 ± 0.59	1.35 ± 0.09*
acetaldehyde	2.10 ± 0.02	0.72 ± 0.06*
hexanal	8.87 ± 0.77	9.51 ± 0.23
3-hexenal	4.95 ± 0.67	5.21 ± 0.19
trans-2-hexenal	24.03 ± 1.29	26.36 ± 1.22
octanal	1.54 ± 0.11	4.09 ± 0.13*
ketones		
acetone	4.24 ± 1.73	0.87 ± 0.29
3-pentanone	2.73 ± 0.83	6.62 ± 0.43*
esters		
ethyl acetate	4.83 ± 0.22	2.37 ± 0.23*
ethyl propanoate	6.77 ± 1.04	11.49 ± 0.24*
butyl acetate	5.12 ± 0.81	5.60 ± 0.13
hexyl acetate	3.56 ± 2.35	16.66 ± 1.60*
methyl nonanoate	0.73 ± 0.11	0.66 ± 0.10
acids		
acetic acid	0.83 ± 0.15	3.45 ± 2.19
total volatiles	105.26 ± 6.30	136.43 ± 1.14*

[a] Data show means ± standard deviations (n = 3) of peak area ratios compared to the internal standard (3.33 ppm isobutyl acetate) peak area × 100. Significance determined by Student's t test with * indicating $p < 0.05$. Samples taken from year 2.

Total volatiles were significantly higher in oils from non-irrigated trees (**Table 8**), which may reflect an activation of the lipoxygenase pathway due to stress (*35*). The same oils also showed significantly more of the 6C "green volatile" compounds, *trans*-3-hexen-1-ol and hexyl acetate. On the other hand, 5C compounds 1-penten-3-ol and 3-pentanone were strongly reduced in oils from irrigated trees. These compounds are products of anaerobic cleavage of the 13-hydroperoxide derivative of α-linolenic acid in the lipoxygenase pathway (*43*). In addition, irrigation changed the levels of some volatiles derived from amino acid metabolism (*42*, *43*). Thus, oils from irrigated trees contained higher levels of 3-methylbutanal, acetaldehyde, acetone, and ethyl acetate but lower amounts of ethyl propanoate and acetic acid.

The lack of change of total 6C volatiles in our work is different from the data of Baccuri et al. (*17*), who reported an increase in irrigation. The lowered levels of hexyl acetate on irrigation (**Table 8**) agreed with a previous study for Leccino (*16*). In contrast, the lowered values of 1-penten-3-ol and 3-pentanone that we found in the oils from irrigated trees agreed only for cv. Leccino (*16*), whereas completely unchanged values were found for oils of cv. Cornicabra (*13*). Again, these data emphasize the different detailed reactions of various olive cultivars to water stress.

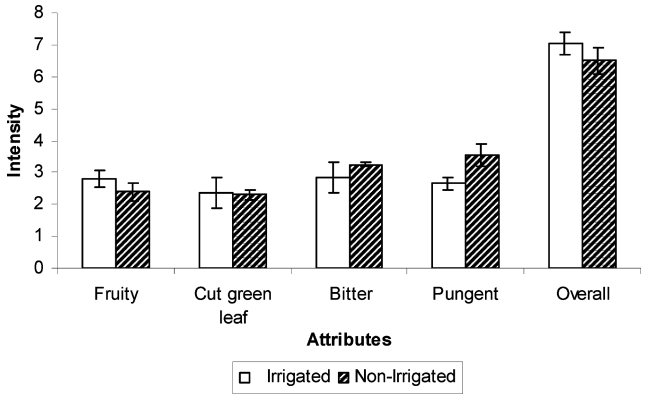

Figure 2. Overall grade and sensory intensities of attributes evaluated by panel tests in olive oils from fruits of irrigated or non-irrigated trees of cv. Koroneiki. Means ± SD (n = 10) shown.

According to the panel test (**Figure 2**) oils from irrigated trees had a slightly better score than those from non-irrigated trees, although both were satisfactory and were in agreement with the volatile contents and phenolic concentrations. Of the volatiles detected (**Table 8**) the lower amounts of 1-penten-3-ol and isoamyl alcohol in oils from irrigated trees would be likely to give a better mouthfeel and flavor (*38*, *43*). The sensory attributes of oils affected by irrigation were fruity, bitter, and pungent, with bitter and pungent having higher values in oils from non-irrigated trees. However, significant differences were observed only in the pungent attribute. The results are in agreement with the fact that phenolic compounds such as 3,4-DHPEA-EDA (which was higher in oils from non-irrigated trees) (**Table 4**) are known to be major contributers to bitterness (*31*)Also noteworthy is the high level of *p*-HPEA-EDA, which is thought to be the phenolic compound mainly responsible for burning, pungent sensory notes in virgin olive oil (*31*). The levels of *p*-HPEA-EDA in oils extracted after the two treatments were comparable.

Furthemore, the organoleptic characteristics of olive oil were also affected possibly due, at least in part, to alterations in the fatty acid composition (*12*, *37*).

Thus, in conclusion, irrigation affected both the yield and quality of olive oil from the Koroneiki cultivar. Although irrigation led to decreased contents of some undesirable sensory qualities, some favorable intense green notes were also reduced. Thus, the effects on quality were complex and may be appreciated more, or less, depending on the consumer's particular needs.

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 (nonesterified) fatty acid; Ga, gadoleic acid, C20:1; *p*-HPEA, (*p*-hydroxyphenyl)ethanol; *p*-HPEA-EDA, dialdehydic form of decarboxymethylelenolic 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.

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