Changes in the Phenolic Composition of Virgin Olive Oil from Young Trees (*Olea europaea* L. cv. Arbequina) Grown under Linear Irrigation Strategies

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This study reports the HPLC profiles of phenolic compounds of virgin olive oils obtained from young olive trees (*Olea europaea* L. cv. Arbequina) and how the application of a linear irrigation strategy affected these. Hydroxytyrosol, tyrosol, vanillic acid, vanillin, 4-(acetoxyethyl)-1,2-dihydroxybenzene, p-coumaric acid, the dialdehydic form of elenolic acid linked to hydroxytyrosol and to tyrosol, lignans, and the oleuropein aglycon were found in all the oils. Hydroxytyrosol, tyrosol, vanillic acid, and p-coumaric acid contents in the oils were unaffected by linear irrigation. The concentration of lignans was lower in the oils from the least irrigated treatment and the concentration of vanillin increased as the amount of irrigation water applied to olive trees increased. However, 4-(acetoxyethyl)-1,2-dihydroxybenzene, the dialdehydic form of elenolic acid linked to hydroxytyrosol and to tyrosol, and the oleuropein aglycon, all of them hydroxyphenyl derivatives, decreased as the level of irrigation water increased. The latter three compounds represented the most considerable part of the phenolic fraction of the oils and they were shown to be correlated to the oxidative stability, the bitter index (K_{225}), and the bitter, pungent, and sweet sensory attributes. Linear irrigation strategy changed the profile of the oil phenolic compounds and, therefore, changed both the organoleptic properties and the antioxidant capacity of the product.

Keywords: *Arbequina cultivar; linear irrigation; olive oil; phenolics*

INTRODUCTION

Polyphenols are secondary metabolites occurring widely in plants, with the presence of a hydroxy-substituted benzene ring within their structure as a common feature. Over the past decade, phenolic compounds have attracted a great deal of attention in food quality because of their antioxidant property for food stability. They have been shown to play a role in tissue browning, and in the flavor and color characteristics of fruit and derived products (*1*). An understanding of phenolic composition and the factors that affect it is critical for the design of the products and their storage conditions.

Virgin olive oil contains a considerable amount of polyphenols that have a great effect on both the stability and the sensory and nutritional characteristics of the product. The presence of phenolic compounds with antioxidant activity is of particular importance given that it correlates with the resistance of oil to the development of rancidity (*2*). Oil stability has been correlated not only with the total amount of phenolic compounds, but also with the presence of selected phenols (*3*, *4*). The resistance of the oil to self-oxidation processes does not depend on the total amount of phenolic compounds, but is rather a consequence of their composition, and particularly of that of the *o*-diphenolic compounds. The antioxidant properties of phenols are mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donators, and singlet oxygen quenchers (*5*). It is also accepted that the role played by the simple phenols differs from that of the complex phenols (*3*, *4*, *6*). These compounds have been reported to be aglycons derived from oleuropein, demethyloleuropein, and ligustroside, and the dialdehydic form of elenolic acid linked to hydroxytyrosol or tyrosol, which represent the most important part of the total phenolic fraction (*7*−*9*). Aglycons arise from glycosides present in olive fruit that may be hydrolyzed by endogenous β-glucosidases, possibly activated during crushing and malaxation in the oil extraction process.

It has long been known that the level of phenols in olive oils can be influenced by the cultivar, the degree of maturation, and the industrial processes employed for oil extraction, as well as environmental conditions. There are some studies focused on the influence of cultivar (*10*), degree of maturation (*11*), and the industrial processes employed for oil extraction (*12*) on the olive oil phenolic fraction. Environmental factors that are able to influence phenolic metabolism include mineral nutrition, ambient temperature, light, and availability of water (*13*). With regard to water availability, it is generally agreed that the level of phenolic compounds is higher in oils obtained from drought-stressed crops than in those from irrigated crops, and that phenolic compounds in the oil are significantly affected by the irrigation regime (*14*−*16*).

Water stress could influence not only the total amount of phenolic compounds in the oil but also their profile, and therefore both the organoleptic properties and the antioxidant capacity of the product.

One of the objectives of this study is to characterize the phenolic fraction of Arbequina virgin olive oil from

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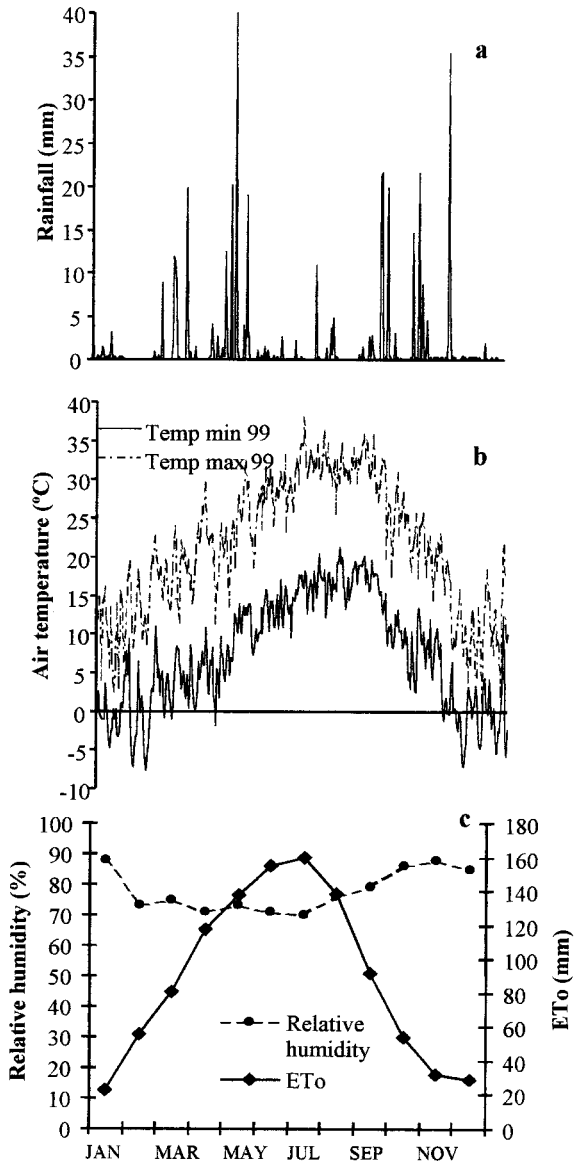

Figure 1. Rainfall (a), air temperature (b), reference crop evapotranspiration (ET_0), and relative humidity (c) for 1999.

young trees. Furthermore, because significant changes in the level of total phenols due to different irrigation regimes have been extensively reported, the present paper also aims to determine the extent to which such changes affect the quantity and nature of the individual phenolic compounds in oils from young olive trees of Arbequina cultivar under a linear irrigation strategy. Also studied was which specific phenolic compounds are more closely related to oil oxidative stability and the bitter taste of oils.

MATERIALS AND METHODS

Plant Material. The trial was carried out in 1999 in a 6-year-old olive orchard (*Olea europea* L. cv. Arbequina) planted on a predominantly clay loam soil located in the Segrià region (Catalonia, Spain) with trees spaced 6 × 4 m (417 trees ha^{-1}). Annual rainfall for 1999 was 427 mm: abundant during the spring and the autumn and almost insignificant during the summer. Annual reference crop evapotranspiration (ET_0) was 1073 mm (Figure 1). In this area, flower bud development started at the beginning of April and full bloom took place at the end of May. Pit hardening began in the second week of July, and olive fruit growth occurred from August to harvest at the end of November.

Table 1. Annual Irrigation Water Applied in 1999 for Every Irrigation Treatment

irrigation treatment (K_c)	water applied (mm/year)
T1 (0.25)	46
T2 (0.38)	84
T3 (0.50)	117
T4 (0.57)	146
T5 (0.64)	171
T6 (0.71)	219
T7 (0.85)	259

The experimental irrigation implementation was based on a linear irrigation design in which the total applied irrigation water changed linearly with the effective crop coefficient (K_c) used when the water budget method proposed by the FAO (*17*) was applied to determine the crop water requirements (ET_c). This used the reference crop evapotranspiration (ET_0) from an agronomic weather station and the effective crop coefficient (K_c) ($ET_c = ET_0 \times K_c$). The water budget method calculates the irrigation requirements by subtracting the effective precipitation (P_{ef}) from the ET_c. Because K_c is almost constant through the year for olive trees, this experimental design allows the relationship between the applied K_c and vegetative growth, olive and oil production, and oil quality to be determined.

Seven irrigation treatments (T1−T7) were applied from the beginning of April to November, with estimated crop coefficients (K_c) of 0.25, 0.38, 0.50, 0.57, 0.64, 0.71, and 0.85, respectively. The trees were not irrigated in the remaining months because the ($ET_c - P_{ef}$) was negative.

ET_c was calculated from modified Penman-determined reference crop water use (ET_o) for each irrigation treatment (using data from a weather station close to the experimental field) (*18*). To adjust predicted ET_c to the size of the canopy (*19*), a reduction of 65% ($K_r = 0.35$) was imposed on the irrigation treatments T1−T3 and a reduction of 60% ($K_r = 0.40$) was imposed on treatments T4−T7.

The experimental plot consisted of four blocks and seven irrigation levels. Each experimental unit consisted of seven trees with only the five central ones being monitored. Olive trees were irrigated daily with four 8-Lh^{-1} drippers placed around the tree. A water meter was installed at the beginning of each line to verify that the water applied corresponded to each treatment (*20*). Table 1 shows the annual amount of water applied for each irrigation treatment.

At harvest, which started on November 22, representative samples were picked from each tree in the experimental design (7 treatments × 4 blocks) and taken to the laboratory for oil extraction and chemical analyses.

Index of Ripeness. Ripeness was determined according to the proposals of the Spanish National Institute of Agronomic Research (*21*) based on a subjective evaluation of the olive skin and pulp colors.

Oil Extraction. An Abencor analyzer (MC2 Ingenierias y Sistemas, Sevilla, Spain) was used to process the olives in a pilot extraction plant. The unit consists of three essential elements: the mill, the thermobeater, and the pulp centrifuge. After being processed in the mill, the oil was separated by decanting, transferred into dark glass bottles, and stored in the dark at 4 °C.

Olive Oil Analysis. The bitter index (K_{225}) was evaluated by extraction of the bitter components of a sample of 1.0 ± 0.01 g of oil dissolved in 4 mL of hexane passed over a C18 column (Waters Sep-Pack Cartridges), previously activated with methanol (6 mL) and washed with hexane (6 mL). After elution, 10 mL of hexane was passed to eliminate the fat, and then the retained compounds were eluted with methanol/water (1/1) to 25 mL. The absorbance of the extract was measured at 225 nm against methanol/water (1/1) in a 1-cm cuvette (*22*).

Stability is expressed as the oxidation induction time (hours) measured with a Rancimat 679 apparatus (Metrohm Co., Basle, Switzerland) using a 2.5-g oil sample warmed to 120 °C, and 20 Lh^{-1} air flow. The time taken to reach a fixed level of conductivity was measured (*23*).

Table 2. Summary of the Identified Phenolic Components, Retention Time (RT) (min), Mean, and Range Concentration (mg kg^{-1}) in Virgin Olive Oils from Young Olive Trees (Arbequina cv.)

peak	phenolic compound	abbreviation	RT	mean	range
1	hydroxytyrosol	3,4-DHPEA	9.8	0.15	0−0.55
2	tyrosol	p-HPEA	14.1	0.31	0.10−0.69
3	vanillic acid	-	17.5	0.24	0.09−0.55
4	vanillin	-	20.8	0.41	0.20−0.73
5	p-coumaric acid	-	22.9	0.09	0.04−0.15
6	4-(acetoxyethyl)-1,2-dihydrobenzene	3,4-DHPEA-AC	23.5	61.3	21.4−131.0
7	dialdehydic form of elenolic acid linked to hydroxytyrosol	3,4-DHPEA-EDA	35.8	329.2	74.7−780.6
8	dialdehydic form of elenolic acid linked to tyrosol	p-HPEA-EDA	45.2	37.9	13.0−86.4
9	lignans	-	47.3	209.6	112.7−274.8
10	oleuropein aglycon	3,4-DHPEA-EA	53	65.4	25.6−157.7

The organoleptic evaluation of the oils was carried out according to the Official European Methods of Analysis by the Official Test Panel of Virgin Olive Oil of Catalonia. The panel consisted of 10 trained tasters who carried out a description of the oil flavor and quality grading. In this paper only the bitter, pungent, and sweet sensory attributes are reported. The descriptive analysis used a six-point intensity ordinal rating scale from 0 (no perception) to 5 (extreme) to quantify the intensity of sensory attributes.

Analysis of Phenolic Compounds. *Phenolic Extraction*. Phenols were extracted from virgin olive oil following the procedure of Montedoro et al. (*24*). Methanol/water (80:20 v/v; 2 × 20 mL) was added to 45 g of virgin olive oil and homogenized for 2 min with a Polytron. The two phases were separated by centrifugation at 3000 rpm for 10 min. Hydro alcoholic extracts were then combined and concentrated in a vacuum at <35 °C until a syrup consistency was reached. Acetronitrile (5 mL) was added to the extract and it was washed with 3 × 20 mL of hexane. The apolar phases were also purified with 5 mL of acetonitrile. The resulting acetonitrile solution was evaporated under vacuum and dissolved in 5 mL of acetonitrile. Finally, an aliquot of 2 mL was evaporated under a stream of nitrogen.

HPLC Analysis of Phenolic Compounds. The extracted phenolic fraction was dissolved in 1 mL of methanol and analyzed by HPLC (loop 20 μL). The HPLC system consisted of a Waters 717 plus autosampler, a Waters 600 pump, a Waters column heater module, and a Waters 996 photodiode array detector managed by Millenium 2000 software (Waters Inc., Milford, MA). The column was a Spherisorb ODS-2 (5 μm, 25 cm × 4.6 mm i.d., Technokroma, Barcelona, Spain) maintained at 35 °C and equipped with a Spherisorb S5 ODS-2 (5 μm, 1 cm × 4.6 mm i.d., Technokroma, Barcelona, Spain) precolumn. HPLC analysis was performed following the same procedure as Brenes et al. (*10*) with a slight modification in the elution gradient. The eluents were 0.2% acetic acid (pH 3.1) and methanol, and the flow rate was 1 mL/min. The total running time was 75 min; the initial composition was 90% acetic acid 0.2% and 10% methanol; and the gradient changed as follows: the concentration of methanol was increased to 30% in 10 min and maintained for 15 min, then the methanol percentage was raised to 40% in 10 min and maintained for 5 min. Finally, the methanol percentage was increased to 50, 60, 70, and 100% in 5-min periods. Initial conditions were reached in 15 min. Chromatograms were obtained at 280 nm.

Reference Compounds. Tyrosol and p-coumaric acid were obtained from Extrasynthèse Co. (Genay, France). Vanillic acid and vanillin were obtained from Fluka Co. (Buchs, Switzerland). Hydroxytyrosol was kindly donated by Professor Montedoro (University of Perugia, Italy). The rest of the phenolic compounds were obtained using a semipreparative HPLC column Spherisorb ODS-2 (5 μm, 25 cm × 10 mm i.d., Technokroma, Barcelona, Spain) and a flow rate of 4 mL/min. The mobile phases and the gradient were the same as those described above.

Individual phenols were quantified on a four-point regression curve on the basis of the standards obtained from commercial suppliers or from preparative HPLC as described above.

Mass Spectrometry. The mass spectra of selected (purified) fractions was performed on a micromass ZMD (Waters Inc., Milford, MA). Operational parameters specific to the electrospray mass spectrometry included the following: capillary voltage, 2.5 kV; cone voltage, 10 V; extractor voltage, 5 V; desolvation temperature, 400 °C; source temperature, 120 °C; ion mode, ESI$^-$.

Statistical analysis. Regression analysis was carried out with the 6.12 version SAS System package (SAS Institute Inc., Cary, NC) to evaluate the relationship between irrigation water applied depending on the different irrigation treatments (K_c) and each phenolic compound identified.

RESULTS AND DISCUSSION

Figure 2 shows the HPLC chromatograms of the phenolic extracts from Arbequina virgin olive oils from three of the seven irrigation levels in the trial (T1, T4, and T7 irrigation treatments). Table 2 shows the identified phenolic components, retention times (RT), and average concentrations of the oils examined in this study. In the first part of the chromatogram (Figure 2), a series of simple phenols was found, such as hydroxityrosol (3,4-DHPEA) (peak 1), tyrosol (p-HPEA) (2), vanillic acid (3), and p-coumaric acid (5) already determined by many authors in different cultivars. Vanillin (4) and 4-(acetoxyethyl)-1,2-dihydroxybenzene (3,4-DHPEA-AC) (6) were also found. These were reported in Spanish cultivars for the first time by Brenes et al. (*10*), but other phenolic compounds previously identified in olive oils such as caffeic acid, syringic acid, ferulic acid, and homovanillic acid were not found (*10*, *24*, *25*).

The second part of the chromatogram is more complicated because of the presence of a great number of peaks, some of them related to phenols with high molecular weights. Peaks 7, 8, and 10 were confirmed by mass spectrometry as the dialdehydic form of elenolic acid linked to hydroxytyrosol (3,4-DHPEA-EDA), the dialdehydic form of elenolic acid linked to tyrosol (p-HPEA-EDA), and the oleuropein aglycon (3,4-DHPEA-EA), respectively, because they had been described previously (*7*, *8*). Peak 9 corresponds to a mixture of lignans, 1-acetoxypinoresinol, and pinoresinol, identified for the first time in olive oil by Owen et al. (*26*) and reported in Spanish cultivars by Brenes et al. (*27*). Previous studies had suggested that it might correspond to a tyrosol derivative (*24*).

Our results agree with those found by Brenes et al. (*10*) from a qualitative point of view, given that the phenolic compounds identified were practically the same. Nevertheless, the results differ greatly from a quantitative point of view as the main phenolic compound in the oils from our trial was 3,4-DHPEA-EDA,

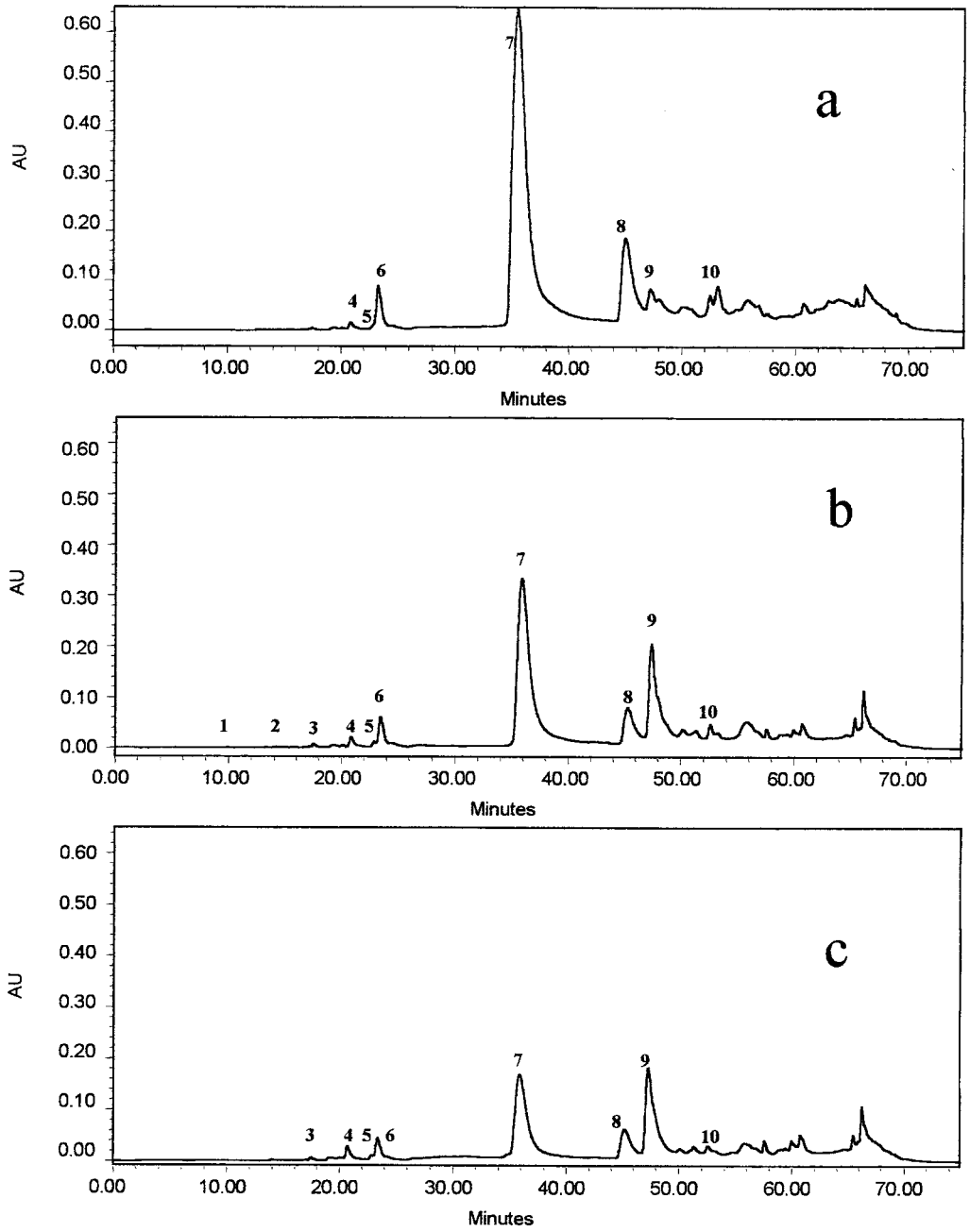

Figure 2. HPLC chromatograms (at 278 nm) of phenolic extracts from virgin olive oil. (a) Tl irrigation treatment ($K_c = 0.25$); (b) T4 irrigation treatment ($K_c = 0.57$); (c) T7 irrigation treatment ($K_c = 0.87$). See Materials and Methods for chromatographic conditions. See Table 2 to identify the peaks.

whereas the main phenolic compound in those from the work of Brenes et al. (*10*) proved to be 1-acetoxypinoresinol.

The concentrations of phenolic compounds in virgin olive oil under different irrigation regimes are reported in Table 3. Among the simple phenols identified, only vanillin and 3,4-DHPEA-AC were affected by the amount of irrigation water applied to the olive tree. The vanillin content in oil increased, whereas 3,4-DHPEA-AC decreased, as the irrigation water applied increased. The responses of these two phenolic compounds to K_c, that defines the amount of irrigation water applied to olive tree, fitted linear regressions (Table 4).

All the complex phenols identified and quantified were affected by the linear irrigation strategy (Table 3). 3,4-DHPEA-EDA, p-HPEA-EDA and 3,4-DHPEA-EA followed the same trend. A linear relationship was found between K_c and the concentration of these compounds in the oil (Table 4). As the irrigation water applied to olive tree increased, the amount of these phenolic substances in the oil decreased.

The relationship between K_c and lignans was quadratic (Table 4). The concentration of these in the oils was lower in the oils from the least irrigated treatment (T1). Pinoresinol and its derivatives are lignans, generally defined as phenylpropane dimers. They have been isolated in the bark of trees, roots, leaves, flowers, stems, fruit, and seeds. Tsukamoto et al. (*28, 29*) isolated them from the bark of olive trees.

Because the degree of fruit maturation has a strong effect on the phenolic composition of the oil, it was decided to determine whether what we observed could be linked to a different fruit ripening phase caused by irrigation. The index of ripeness of the fruit from all

Table 3. Effect of Irrigation Treatment (K_c) on Phenolic Compounds (mg kg^{-1} of Virgin Olive Oil) Content of Arbequina Cultivar Virgin Olive Oil[a]

	irrigation treatment (K_c)[c]							
phenolic compound[b]	T1 (0.25)	T2 (0.38)	T3 (0.50)	T4 (0.57)	T5 (0.64)	T6 (0.71)	T7 (0.85)	
3,4-DHPEA	0.23 (0.11)	0.18 (0.06)	0.17 (0.04)	0.09 (0.02)	0.10 (0.05)	0.19 (0.06)	0.12 (0.02)	NS
p-HPEA	0.33 (0.08)	0.33 (0.06)	0.32 (0.06)	0.28 (0.07)	0.23 (0.03)	0.37 (0.07)	0.28 (0.03)	NS
vanillic acid	0.23 (0.03)	0.25 (0.02)	0.26 (0.02)	0.25 (0.02)	0.23 (0.03)	0.24 (0.05)	0.24 (0.03)	NS
vanillin	0.31 (0.04)	0.34 (0.03)	0.41 (0.02)	0.41 (0.03)	0.42 (0.02)	0.50 (0.05)	0.46 (0.04)	**
p-coumaric acid	0.09 (0.00)	0.10 (0.01)	0.10 (0.01)	0.09 (0.01)	0.09 (0.01)	0.09 (0.01)	0.09 (0.01)	NS
3,4-DHPEA-AC	75.9 (12.3)	69.3 (7.62)	65.2 (10.0)	62.9 (4.78)	53.5 (9.91)	49.3 (5.60)	51.8 (5.72)	**
3,4-DHPEA-EDA	442.8 (73.5)	418.5 (58.8)	398.3 (65.8)	353.6 (46.0)	291.0 (34.1)	212.4 (31.8)	183.0 (12.2)	**
p-HPEA-EDA	50.9 (6.45)	44.4 (4.77)	43.5 (5.00)	38.9 (3.65)	37.1 (3.51)	27.4 (3.73)	23.1 (1.32)	**
lignans	168.5 (10.7)	192.5 (8.24)	198.8 (9.12)	226.5 (6.49)	221.7 (8.61)	232.6 (9.10)	228.1 (8.03)	**
3,4-DHPEA-EA	83.3 (13.2)	77.4 (9.24)	73.3 (10.2)	69.8 (7.77)	66.1 (7.26)	46.1 (5.80)	41.9 (1.82)	**

[a] Values are the mean of 8 independent values and the Standard Error. [b] See Table 2 for abbreviations. [c] Significance level of the model by row. NS, not significant ($p > 0.05$); *$p < 0.05$; **$p < 0.01$.

Table 4. Regression Equations Showing the Effect of Irrigation Treatment (K_c) on Some Phenolic Compounds of Arbequina Cultivar Virgin Olive Oil

phenolic compound[a]	model	*a*	*b*	*c*
vanillin	linear	0.238	0.302	-
3,4-DHPEA-AC	linear	86.9	−46.2	-
3,4-DHPEA-EDA	linear	597.1	−482.1	-
p-HPEA-EDA	linear	63.9	−46.8	-
lignans	quadratic	100.6	312.0	−186.8
3,4-DHPEA-EA	linear	105.8	−72.8	-

[a] See Table 2 for abbreviations.

irrigation treatments ranged from 2 (green with red spots epicarp) to 3 (reddish-brown epicarp) at harvest (2.9, 3.0, 2.2, 3.1, 2.0, 1.9, and 2.8, for T1 to T7 irrigation treatments, respectively). Therefore, the difference in the content of phenolic substances cannot be ascribed, under our experimental conditions, to different degrees of fruit ripening.

Although it is widely recognized that phenolic compounds in the oils are significantly affected by water regime, those oils obtained from the most heavily irrigated olive orchards showed a lower phenol total (*14*−*16*). It was observed that irrigation did not affect all the phenolic compounds in the same way. 3,4-DHPEA-EDA, p-HPEA-EDA, and 3,4-DHPEA-EA accounted for the most important part of the phenolic fraction obtained from Arbequina oils in this trial, and they were responsible for most of the decrease in the total phenols in oils from the most irrigated treatments. They are related to a very specific group of coumarin-like compounds called secoiridoids. Secoiridoids are produced from the secondary metabolism of terpenes and are usually derived from the oleoside type of glucosides oleosides, which are characterized by a combination of elenolic acid and a glucosidic residue. It could be stated that these three compounds proceed from the same biogenetic route, the acetate/mevalonate pathway, and consequently respond similarly to water stress: their concentration increases.

Table 5 shows the oxidative stability, the bitter index (K_{225}), and the bitter, pungent, and sweet sensory attributes of Arbequina virgin olive oils in relation to the irrigation treatment applied to olive tree. A good correlation was found between the 3,4-DHPEA-EDA, p-HPEA-EDA, and 3,4-DHPEA-EA concentrations and the oxidative stability of the oils ($r = 0.76$, $p < 0.001$; $r = 0.60$, $p < 0.001$; and $r = 0.73$, $p < 0.001$, respectively). The anti-oxidative activity of polyphenols is generally ascribed to their hydroxyl groups, but this is not the only factor in determining the power of their activities. The most important *o*-diphenol in olive oil is 3,4-DHPEA, which is included in the structure of 3,4-DHPEA-EDA and 3,4-DHPEA-EA. The lack of correlation between 3,4-DHPEA and the oil oxidative stability is probably due to the low concentration of this compound in the oils studied. The antioxidant activity of 3,4-DHPEA-EDA and 3,4-DHPEA-EA has already been evaluated, and they have been shown to extend the shelf life of olive oil, confirming that the *ortho*-diphenolic structure plays an important role in the antioxidant efficiency (*3*, *4*).

It was also observed that, as well as derivatives containing *o*-diphenols, the ester containing p-HPEA (p-HPEA-EDA) also played a role as an antagonist to the oxidation reaction, although to a lesser extent. This observation agrees with the work by Angerosa and di Giovacchino (*30*).

In the current study, lignans did not correlate with oil stability. This agrees with the work by Brenes et al. (*27*), who observed that they were not easily oxidized under air, and with that by Montedoro et al. (*24*), who indicated that a peak named 10, which must correspond to lignans, was very stable during oxidation.

The bitter index (K_{225}) evaluates the intensity of the bitter taste in virgin olive oil. A work by Gutiérrez et al. (*22*) showed a significant correlation with the intensity of bitterness evaluated in a sensorial manner by a panel. We found a strong positive correlation between

Table 5. Stability, Bitter Index (K_{225}), and Bitter, Pungent, and Sweet Sensory Attributes of Arbequina Cultivar Virgin Olive Oil[a]

irrigation treatment (K_c)	stability (hours)	bitter index (K_{225})	sensory attributes		
			bitter	pungent	sweet
T1 (0.25)	20.3 (0.9)	0.369 (0.016)	2.6 (0.08)	2.5 (0.08)	1.6 (0.05)
T2 (0.38)	18.8 (1.1)	0.305 (0.021)	2.1 (0.09)	2.4 (0.07)	1.6 (0.09)
T3 (0.50)	19.6 (1.2)	0.334 (0.027)	2.3 (0.07)	2.5 (0.09)	1.6 (0.07)
T4 (0.57)	18.0 (1.1)	0.297 (0.022)	1.7 (0.07)	2.4 (0.08)	1.7 (0.07)
T5 (0.64)	17.9 (1.0)	0.294 (0.019)	2.2 (0.08)	2.4 (0.06)	1.7 (0.04)
T6 (0.71)	16.4 (0.7)	0.241 (0.017)	1.6 (0.07)	2.2 (0.04)	1.8 (0.07)
T7 (0.85)	16.5 (0.7)	0.235 (0.012)	1.6 (0.08)	2.0 (0.13)	2.0 (0.03)

[a] Values are the mean and the Standard Error.

the 3,4-DHPEA-EDA, p-HPEA-EDA, and 3,4-DHPEA-EA concentrations in the oil and the bitter index ($r = 0.78$, $p < 0.001$; $r = 0.81$, $p < 0.001$; and $r = 0.83$, $p < 0.001$, respectively). They are all related to the major bitter principle of olive fruit, the secoiridoid oleuropein. We also found good positive correlations between these three compounds and the bitter ($r = 0.79$, $p < 0.01$; $r = 0.87$, $p < 0.01$; $r = 0.83$, $p < 0.01$) and pungent sensory attributes ($r = 0.90$, $p < 0.01$; $r = 0.93$, $p < 0.01$; $r = 0.93$, $p < 0.01$), whereas the correlation between them and the sweet attribute was negative ($r = -0.92$, $p < 0.01$; $r = -0.93$, $p < 0.01$; $r = -0.93$, $p < 0.01$).

Vanillin present in the oils studied seemed to have an effect on the sensory characteristics of the oils of the trial since a negative correlation was found ($r = -0.41$, $p < 0.01$) between the vanillin content of the oils and their bitter index (K_{225}) and also between vanillin and the bitter sensory attribute ($r = -0.79$, $p < 0.01$). Vanilla is one of the most widely used flavoring ingredients in food.

The different HPLC profiles found for the oils from different irrigation regimes emphasize the complexity of the biochemical processes that control the formation of olive phenolic compounds. In conclusion, we could say that the content of oleuropein related compounds (3,4-DHPEA-EDA, p-HPEA-EDA, and 3,4-DHPEA-EA) increased under water stress conditions, whereas vanillin content increased as irrigation water applied to olive trees increased, and lignans content was lower in the oils from the least irrigated treatment. Given that the linear irrigation strategy changed the profile of oil phenolic compounds, both the organoleptic properties and the antioxidant capacity of the product were affected, as these are correlated to the contents of some phenolic compounds found in the oil.

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