JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Phenolic and Volatile Compounds of Extra Virgin Olive Oil (*Olea europaea* L. Cv. Cornicabra) with Regard to Fruit Ripening and Irrigation Management

AURORA GÓMEZ-RICO,† M. DESAMPARADOS SALVADOR,† MARTA LA GRECA,† AND GIUSEPPE FREGAPANE*,‡

Departamento de Química Analítica y Tecnología de los Alimentos and Instituto Regional de Investigación Científica Aplicada (IRICA), Universidad de Castilla-La Mancha, Ciudad Real, Spain

This study investigated the effect of both the degree of ripening of the olive fruit and irrigation management—rain-fed, two different regulated deficit irrigations (RDI), the method proposed by the Food and Agriculture Organization of the United Nations (known as FAO), and 125 FAO (125% FAO)—on the phenolic and volatile composition of Cornicabra virgin olive oils obtained during two crop seasons. Secoiridoid phenolic derivatives greatly decreased upon increase of both irrigation and ripening, for example, the 3,4-DHPEA-EDA content decreased from 770 to 450 mg/kg through fruit ripening under rain-fed conditions and from 676 to 388 mg/kg from rain-fed conditions to FAO irrigation treatment (at a ripeness index of approximately 4). Moreover, secoiridoid derivatives of hydroxytyrosol decreased more than those of tyrosol. The levels of major volatile components decreased in the course of ripening but were higher in irrigated olive oils: for example, the *E*-2-hexenal content ranged between 4.2 and 2.6 mg/kg (expressed as 4-methyl-2-pentanol) over fruit maturation under rain-fed conditions and between 8.0 and 3.5 mg/kg under FAO scheduling. It is important to note that where water was applied only from the beginning of August (RDI-2), when oil begins to accumulate in the fruit, the resulting virgin olive oil presented a phenol and volatile profile similar to those of the FAO and 125 FAO methods, but with a considerable reduction in the amount of water supplied to the olive orchard.

KEYWORDS: Virgin olive oil; phenols; volatiles; ripening; irrigation; *Olea europaea* L. cv. Cornicabra

INTRODUCTION

Extra virgin olive oil (EVOO) is obtained from healthy olive fruits by mechanical processes only and is the most important vegetable oil ready for direct human consumption. The fine sensory characteristics of this fruit oil, which possesses unique aroma and taste, are mainly due to the presence of minor components, chiefly volatile and phenolic compounds (*1*, *2*). Volatiles are mainly responsible for the aroma of virgin olive oil, especially for the green sensory notes of high-quality virgin olive oils, whereas compounds with a phenolic structure affect both the taste, in particular the positive bitterness organoleptic attribute, and the oxidative stability of the virgin olive oil (*3*, *4*). Phenolics and volatiles are therefore the compounds chiefly responsible for the flavor of EVOOs and to a large extent determine the degree of consumer preference for this highly appreciated product.

It is known that the amount of these minor components in virgin olive oil depends on agronomical and technological factors, for example, the olive cultivar, the degree of ripening of the olive fruit, the irrigation management, and the extraction process, in particular the milling and malaxation conditions and the type of centrifugation system employed. For example, the level of phenolic compounds and volatiles in the olive oil decreases in the course of maturation of the olive fruits (*5*−*8*). In addition, some researchers have reported that of the chemical components of virgin olive oil, phenolic compounds were the most influenced by irrigation, and their concentration is in inverse proportion to the amount of water applied to the olive trees (*9*−*12*). Nevertheless, to date there is no detailed information available on the influence of irrigation and fruit ripening on the volatile composition of virgin olive oil, especially in the case of the Cornicabra variety, which is the second most important variety in Spain (*13*).

The aim of this work was to determine the effect of both (i) the degree of ripening of the olive fruit and (ii) five different types of irrigation management on virgin olive oil volatile and phenolic composition. EVOOs (*Olea europaea* L. cv. Cornicabra) obtained during the 2003/2004 and 2004/2005 crop seasons were used. The ultimate goals are to enhance knowledge with regard to the composition and quality of virgin olive oil

* Corresponding author (e-mail giuseppe.fregapane@uclm.es; fax +34 926 295318).
† Departamento de Química Analítica y Tecnología de los Alimentos.
‡ Instituto Regional de Investigación Científica Aplicada.

10.1021/jf060798r CCC: $33.50
Published on Web 08/25/2006

and to define, if possible, (i) the optimum harvesting period and (ii) sustainable irrigation conditions in the Cornicabra olive cultivar grown in Castilla-La Mancha, a region where aquifers are overexploited.

MATERIALS AND METHODS

Experimental Olive Orchard. The study was conducted during two consecutive crop seasons (2003/2004 and 2004/2005) in an experimental olive (*O. europaea* L.) orchard of cv. Cornicabra maintained by the Consejería de Agricultura (Department of Agriculture) of Castilla-La Mancha, located in Almodóvar del Campo (Ciudad Real, Spain). About 320 50-year-old trees, spaced 12 × 12 m^2, were used in a randomized complete block design with four different treatments and four replications. Each experimental unit consisted of 4 × 3 trees, of which only the central ones were used for sampling. The experimental olive orchard was surrounded by two outer rows of irrigated olives. All of the agronomical treatments applied to the experimental olive orchard were identical, with the exception of irrigation practice.

Irrigation Treatments. Four treatments were applied 2 years before the commencement of this assay: rain-fed conditions (RF), regulated deficit irrigation (RDI), FAO, and 125 FAO. Rain-fed conditions were used as a control to compare the results obtained with the irrigation treatments studied. In the FAO treatment the water requirements were calculated using a methodology based on the crop evapotranspiration (ET_c) proposed by the United Nations Food and Agriculture Organization (*14*). In 125 FAO treatments, a total irrigation dosage 25% higher than the FAO treatment was applied.

For regulated deficit irrigation (RDI), a maximum of 75 mm of water was established because in many Spanish irrigated olive areas there is a legal limitation of 100 mm. Two different strategies were evaluated. In 2003 (RDI-1), water was applied throughout the entire season with different rates of application (10% FAO in May and June, 4% FAO in July and August, and 18% FAO in September), whereas in 2004 (RDI-2), on the basis of the results obtained during the previous crop season, water was applied only from the beginning of August, when the oil starts to form in the fruit, for the purpose of investigating which RDI treatment is more effective in achieving olive production and olive oil quality similar to that obtained by the FAO method while considerably reducing the total amount of water applied. In all irrigation treatments, olive trees were irrigated daily (4 L/h) using eight compensating drippers placed around the trees.

The total water applied in 2003/2004 for the different irrigation treatments was 56 mm for RDI-1, 148 mm for FAO, and 206 mm for 125 FAO; in 2004/2005 these levels were 60 mm for RDI-2, 124 mm for FAO, and 154 mm for 125 FAO. More detailed data on the irrigation management have been previously reported (*12*).

Olive Oil Samples. Olive fruit samples from rain-fed and irrigated trees were harvested throughout ripening at various ripeness indices (RI), from the immature stage ($1.5 < RI < 2.0$) to the normal harvest period for the Cornicabra variety ($RI \approx 5.5$). The olive ripeness index was determined according to the method proposed by the International Olive Oil Council (IOOC) (*15*), based on the evaluation of the olive skin and pulp colors. RI values range from 0 (100% intense green skin) to 7 (100% purple flesh and black skin). Five and three samplings were gathered in 2003/2004 and 2004/2005, respectively; the samples were collected by hand from the beginning of November to the end of December, whereas the fifth sampling from the 2003/2004 crop was collected by a mechanical shaker at the beginning of January. Four representative subsamples from each treatment (4 subsamples × 4 treatments) were picked at each sampling and brought to the laboratory for oil extraction. Virgin olive oil samples of Cornicabra variety were then obtained using the Abencor analyzer (Abengoa S.A., Sevilla, Spain); this system reproduces at laboratory scale the industrial process through three basic elements: hammer mill, thermomixer, and centrifuge (*16*). The oil obtained was separated by decanting and stored in amber glass bottles at 4 °C in darkness without headspace until analysis.

Methods. *HPLC Analysis of Phenolic Compounds.* A solution of the internal standard (250 μL of 15 mg/kg of syringic acid in methanol) was added to a sample of virgin olive oil (2.5 g), and the solvent was evaporated with a rotary evaporator at 35 °C under vacuum. The oil was then dissolved in 6 mL of hexane, and a diol-bonded phase cartridge (Supelco Co., Bellefonte, PA) was used to extract the phenolic fraction. The cartridge was conditioned first with methanol (6 mL) and then with hexane (6 mL). The oil solution was then applied, and the solid-phase extraction (SPE) column was washed with hexane (2 × 3 mL) and with hexane/ethyl acetate (85:15 v/v; 4 mL). Finally, the phenols were eluted with methanol (15 mL) and the solvent was removed with a rotary evaporator at 35 °C under vacuum until dry. The phenolic residue was dissolved in methanol/water (1:1 v/v; 250 μL).

HPLC analysis was performed using an Agilent Technologies 1100 series system equipped with an automatic injector, a column oven, and a diode array UV detector. A Spherisorb S3 ODS2 column (250 × 4.6 i.d. mm, 5 μm particle size) (Waters Corp., Milford, MA) was used, maintained at 30 °C, with an injection volume of 20 μL and a flow rate of 1.0 mL/min. The mobile phase was a mixture of water/acetic acid (95:5 v/v) (solvent A), methanol (B), and acetonitrile (C): from 95% A−2.5% B−2.5% C to 34% A−33% B−33% C in 50 min. Phenolic compounds were quantified at 280 nm using syringic acid as internal standard and the response factors determined by Mateos et al. (*17*).

GC Analysis of Volatile Compounds [Adapted from Vichi et al. (18)]. Solid-phase microextraction (SPME) followed by GC was used to analyze the volatile compounds in the virgin olive oil samples studied. Olive oil (1.5 g) spiked with 4-methyl-2-pentanol (as internal standard) to a concentration of 1.5 μg/g was placed in a 10 mL vial fitted with a silicone septum. The SPME sampling was performed by exposing the DVB/Carboxen/PDMS fiber (50/30 μm, 2 cm long from Supelco Inc.) for 30 min in the headspace of the sample maintained at 40 °C; it was then retracted into the needle and immediately transferred and desorbed for 1 min in the injection port of an Agilent 6890 series gas chromatoghaph equipped with a flame ionization detector (FID). Compounds were separated on a Supelcowax-10 column (30 m × 0.25 mm × 0.25 μm, Supelco Inc.) under the following conditions: injection port temperature, 260 °C; helium flow, 0.8 mL/min; oven temperature ramp, 35 °C for 10 min, 3 °C/min to 160 °C and then 15 °C/min to 200 °C (maintained for 5 min). Volatile compounds were tentatively identified by comparison with standard substances (Sigma Aldrich) added to the refined oils.

The analytical determinations were carried out at least in duplicate.

Statistical Analysis. Analysis of ANOVA and discriminant analysis were performed using SPSS 13 statistical software (SPSS Inc., Chicago, IL). Duncan's test ($p \leq 0.05$) was used to discriminate among the mean values.

RESULTS AND DISCUSSION

The influence of fruit ripening and irrigation management on (i) the production of the olive grove, which was significantly lower under rain-fed conditions than under irrigation (≈35%), (ii) the characteristics and composition of the olive fruit, and (iii) the quality indices and major components of virgin olive oil has been analyzed and discussed elsewhere (*12*).

Phenolic Compounds. The concentrations of the major phenolic compounds found in virgin olive oil (VOO) obtained throughout ripening from olives subjected to the different irrigation treatments are reported in **Table 1**. As previously reported by our research group, the main phenolic compounds in Cornicabra monovarietal VOO are the dialdehydic form of elenolic acid linked to hydroxytyrosol and tyrosol (3,4-DHPEA-EDA and *p*-HPEA-EDA), oleuropein aglycon (3,4-DHPEA-EA), and ligstroside aglycon (*p*-HPEA-EA). The main simple phenols are tyrosol (*p*-HPEA) and hydroxytyrosol (3,4-DHPEA) (*19*).

The VOO hydroxytyrosol contents (ranging from 1.34 to 3.30 mg/kg, expressed as the 10th and 90th percentiles of the data distribution, respectively) and tyrosol (1.12−4.92 mg/kg) were apparently not affected by the water doses applied or by the degree of ripening of the fruit, because practically no statistically significant differences were observed (**Table 1**). This behavior was similar to that observed for the rest of the minor simple

Table 1. Levels of Major Virgin Olive Oil Phenolic Compounds (Milligrams per Kilogram) with Regard to Fruit Ripening Stage and Irrigation Management[a]

	ripeness index	3,4-DHPEA	3,4-DHPEA-EDA	3,4-DHPEA-EA	*p*-HPEA	*p*-HPEA-EDA	*p*-HPEA-EA	total phenol
				2003/2004				
rain-fed	1.5 ± 0.5a,w	2.80 ± 0.56b,w	770 ± 49c,y	301 ± 41b,x	1.86 ± 0.59a,w	498 ± 47c,z	138 ± 20b,y	1719 ± 130c,y
RDI-1	1.8 ± 0.6ab,w	1.46 ± 0.25a,w	637 ± 83b,x	180 ± 21a,y	1.89 ± 1.00a,w	440 ± 65bc,y	88 ± 18a,y	1354 ± 42b,y
FAO	2.5 ± 0.4b,w	1.55 ± 0.79a,w	451 ± 31a,y	150 ± 26a,w	2.76 ± 1.22a,w	451 ± 31ab,z	79 ± 23a,x	1076 ± 122a,z
125 FAO	2.0 ± 0.3ab,v	1.46 ± 0.41a,w	433 ± 120a,x	129 ± 33a,w	2.26 ± 0.72a,w	336 ± 81a,y	61 ± 20a,y	968 ± 254a,y
rain-fed	2.7 ± 0.3a,x	2.28 ± 0.42a,w	651 ± 42c,x	245 ± 23c,x	1.62 ± 0.66a,w	378 ± 29c,y	94 ± 12b,x	1380 ± 62c,x
RDI-1	2.8 ± 0.4a,x	2.75 ± 1.99a,w	542 ± 145bc,wx	163 ± 26b,x	2.56 ± 2.52a,w	307 ± 11ab,x	61 ± 10a,x	1084 ± 146b,x
FAO	3.1 ± 0.3a,x	1.91 ± 0.62a,wx	442 ± 13ab,xy	144 ± 22ab,w	3.10 ± 1.10a,w	335 ± 42bc,yz	66 ± 17a,wx	998 ± 85b,yz
125 FAO	2.8 ± 0.2a,w	1.79 ± 0.22a,w	372 ± 85a,wx	113 ± 24a,w	2.41 ± 1.17a,w	264 ± 25a,x	47 ± 7a,xy	805 ± 125a,xy
rain-fed	3.2 ± 0.3a,xy	2.44 ± 0.74a,w	642 ± 48c,x	240 ± 31c,x	1.63 ± 0.73a,w	323 ± 15c,x	77 ± 10b,x	1294 ± 64c,x
RDI-1	3.5 ± 0.4a,xy	1.82 ± 0.51a,w	482 ± 69b,wx	143 ± 16b,x	2.40 ± 1.69ab,w	260 ± 14b,x	49 ± 9a,wx	946 ± 40b,x
FAO	3.6 ± 0.4a,xy	2.13 ± 0.29a,wx	399 ± 24ab,xy	129 ± 13ab,w	3.79 ± 1.35b,w	276 ± 46b,xy	51 ± 10a,w	868 ± 78b,xy
125 FAO	3.4 ± 0.3a,x	1.97 ± 0.12a,w	338 ± 91a,wx	99 ± 27a,w	2.51 ± 0.72ab,w	215 ± 23a,wx	36 ± 8a,wx	699 ± 139a,wx
rain-fed	3.7 ± 0.2a,y	2.14 ± 0.51a,w	675 ± 51c,xy	258 ± 36c,x	1.68 ± 0.85a,w	335 ± 29c,xy	85 ± 15c,x	1364 ± 107c,x
RDI-1	3.8 ± 0.3a,y	2.38 ± 0.71a,w	522 ± 133b,wx	161 ± 25b,x	2.17 ± 1.37ab,w	259 ± 20b,x	53 ± 9b,wx	1004 ± 160b,x
FAO	4.0 ± 0.4a,y	2.22 ± 0.62a,wx	388 ± 39a,x	123 ± 17ab,w	4.05 ± 1.23b,w	255 ± 28b,x	47 ± 8ab,w	824 ± 56ab,x
125 FAO	3.9 ± 0.1a,y	2.87 ± 0.37a,x	310 ± 86a,wx	102 ± 22a,w	3.54 ± 1.47ab,w	192 ± 15a,w	35 ± 4a,wx	651 ± 124a,wx
rain-fed	5.7 ± 0.4b,z	2.44 ± 1.19a,w	446 ± 126b,w	168 ± 53b,w	2.25 ± 1.88a,w	228 ± 21c,w	52 ± 10b,w	905 ± 189b,w
RDI-1	5.4 ± 0.5ab,z	2.53 ± 1.13a,w	384 ± 41ab,w	123 ± 5ab,w	2.34 ± 1.02a,w	200 ± 27bc,w	40 ± 8ab,w	757 ± 12ab,w
FAO	5.5 ± 0.3ab,z	3.22 ± 1.47a,x	302 ± 55a,w	128 ± 41ab,w	4.89 ± 2.51a,w	168 ± 38ab,w	42 ± 13ab,w	654 ± 108a,w
125 FAO	4.9 ± 0.1a,z	3.31 ± 1.05a,x	255 ± 77a,w	90 ± 17a,w	3.87 ± 1.88a,w	151 ± 30a,w	28 ± 4a,w	536 ± 124a,w
				2004/2005				
rain-fed	2.8 ± 0.2b,w	0.99 ± 0.04a,w	519 ± 95b,w	148 ± 46a,w	1.01 ± 0.01a,w	289 ± 58a,w	53 ± 16a,w	1019 ± 216a,w
RDI-2	2.3 ± 0.1a,w	2.09 ± 0.26ab,w	429 ± 11ab,x	142 ± 3a,x	1.88 ± 0.12b,w	266 ± 5a,x	54 ± 3a,x	904 ± 9a,x
FAO	2.5 ± 0.2ab,w	2.71 ± 0.94b,w	401 ± 13ab,x	152 ± 9a,w	2.12 ± 0.09bc,w	253 ± 33a,x	57 ± 1a,y	877 ± 10a,x
125 FAO	2.4 ± 0.1a,w	2.43 ± 0.17b,w	327 ± 14a,x	122 ± 4a,w	2.43 ± 0.16c,w	215 ± 14a,x	44 ± 4a,w	724 ± 38a,x
rain-fed	3.4 ± 0.0a,x	1.09 ± 0.34a,w	478 ± 54c,w	136 ± 50a,w	1.24 ± 0.48a,w	252 ± 54b,w	45 ± 21a,w	921 ± 183b,w
RDI-2	3.4 ± 0.0a,x	8.23 ± 0.99c,x	319 ± 2ab,w	117 ± 2a,w	4.92 ± 1.02c,x	187 ± 8ab,w	45 ± 0a,wx	691 ± 11ab,w
FAO	3.4 ± 0.0a,x	2.97 ± 1.27ab,w	354 ± 42b,wx	125 ± 12a,w	2.43 ± 0.84ab,w	192 ± 3ab,wx	39 ± 1a,w	724 ± 57ab,w
125 FAO	3.5 ± 0.1a,x	3.78 ± 0.38b,w	240 ± 8a,wx	94 ± 15a,w	4.26 ± 0.22bc,w	166 ± 14a,wx	34 ± 4a,w	551 ± 14a,wx
rain-fed	4.1 ± 0.1a,y	1.39 ± 0.15a,w	396 ± 81b,w	145 ± 67a,w	1.79 ± 0.19a,w	229 ± 48b,w	39 ± 25a,w	818 ± 224b,w
RDI-2	4.2 ± 0.0a,y	2.89 ± 1.76a,w	365 ± 23b,w	130 ± 7a,wx	2.97 ± 0.11a,w	186 ± 17ab,w	42 ± 4a,w	738 ± 51b,w
FAO	4.2 ± 0.0a,y	3.84 ± 1.38a,w	307 ± 3b,w	136 ± 14a,w	3.79 ± 0.69a,w	172 ± 5ab,w	46 ± 3a,x	679 ± 18b,w
125 FAO	4.2 ± 0.0a,y	6.97 ± 3.26a,w	160 ± 54a,w	88 ± 15a,w	7.34 ± 3.99a,w	119 ± 36a,w	32 ± 5a,w	422 ± 102a,w

[a] Different letters (a–c) within a column indicate significant differences ($p < 0.05$) with respect to irrigation treatment in each sampling. Different letters (w–y) within a column indicate significant differences ($p < 0.05$) with respect to ripeness index for each irrigation treatment.

phenols identified (data not shown), which were present in very small amounts—vanillin (<0.22 mg/kg), vanillic acid (<0.26 mg/kg), *p*-coumaric acid (<0.25 mg/kg), and ferulic acid (<0.21 mg/kg)—except for pinoresinol (<4.40 mg/kg), the content of which was higher.

In contrast, there was a considerable difference in the concentrations of secoiridoid derivatives of hydroxytyrosol and tyrosol observed in the VOO in the course of fruit ripening and under the various irrigation treatments studied. In fact, the compounds most affected by irrigation scheduling of the olive grove and by ripening of the fruit were the complex phenol chemical forms, the levels of which decreased significantly in the VOO during ripening and as the water supplied increased. For example, in the 2003/2004 crop the 3,4-DHPEA-EDA content decreased from 770 to 450 mg/kg and the 3,4-DHPEA-EA diminished from 300 to 170 mg/kg in the course of fruit ripening in the rain-fed (RF) VOO samples, whereas from RF conditions to FAO irrigation, at a RI of ≈4.0, the 3,4-DHPEA-EDA content decreased from 676 to 388 mg/kg and the 3,4-DHPEA-EA from 258 to 123 mg/kg (**Table 1**). Tovar et al. (*11*) observed similar behavior for the Arbequina cultivar, for which the levels of secoiridoids diminished as the irrigation dose of olive trees increased.

As far as the 2004/2005 crop season is concerned, although the levels of complex phenols in the VOO samples were lower, the trend was similar to that of the previous crop season (**Table 1**). It is important to note that the differences in phenol contents between the oils from FAO and the second regulated deficit irrigation (RDI-2) strategy were not statistically significant; this indicates that the RDI scheduling employed in the second crop season (RDI-2), when water was applied from the beginning of August only, produced a VOO with a phenolic composition more similar to that of oil from FAO-treated olives than to that from olives grown under RDI-1 water scheduling of the previous year. In this view one of the main goals of the study was attained. Indeed, the phenolic and volatile composition related to the quality of VOO comparable to FAO management was achieved with less demand in water supply.

A high level of bitterness, which as well-known is related to the phenol content (*6*), is a peculiar characteristic of the sensory profile of VOOs of the Cornicabra variety (*8*). Nevertheless, an excessive level of this positive organoleptic attribute could cause consumers to reject the product (*8*). Thus, to meet product quality and marketing needs, the use of irrigation could produce a desirable reduction in the intensity of bitterness and, consequently, improvement in consumer preference may be achieved. A slight but statistically significant decrease in the intensity of bitterness was indeed observed in the organoleptic evaluation of the VOO obtained in this study (*12*), which was carried out by an olive oil taster panel certified by the IOOC.

The observed differences in VOO phenol composition could be a consequence of the different water stress levels of the olive

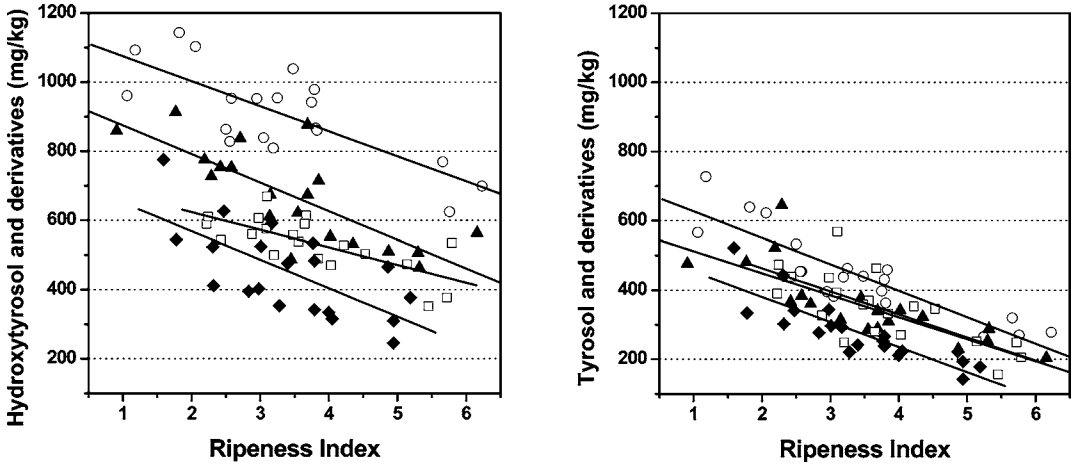

Figure 1. Evolution of hydroxytyrosol and its complex secoiridoid forms and of tyrosol and its derivatives in the course of fruit ripening as affected by irrigation management in crop season 2003/2004: (○) rain-fed; (▲) regulated deficit irrigation, RDI-1; (□) FAO; (◆) 125 FAO.

trees under rain-fed conditions and under the experimental irrigation conditions, which prompts changes in the activity of the enzymes responsible for biosynthesis of the phenolic compounds, such as L-phenylalanine ammonia-lyase, which is more active under higher water stress conditions (*20*, *21*).

The complex phenols were not affected in the same way by irrigation, because the secoiridoid derivatives of hydroxytyrosol decreased more than those of tyrosol, as clearly shown in **Figure 1**. This is very important because hydroxytyrosol and its complex derivative forms are known to possess much greater antioxidant activity and organoleptic influence than the tyrosol group (*22*, *23*). These results were similar to those reported by Tovar et al. (*11*, *24*) for the Arbequina variety.

Volatile Compounds. Table 2 reports the concentrations of C6 volatile compounds from the lipoxygenase (LOX) pathway, expressed as milligrams of internal standard (4-methyl-2-pentanol) per kilogram of oil, in VOO samples taken throughout the course of fruit ripening according to the different irrigation treatments, for the two crop seasons studied.

In all of the Cornicabra VOO samples analyzed, the major volatile component was the C6 aldehyde fraction, the content of which decreased as ripening progressed. For example, the *E*-2-hexenal content ranged between 4.2 and 2.6 mg/kg of IS over fruit maturation for VOOs under RF conditions and between 8.0 and 3.5 mg/kg for those under FAO scheduling; the amount of hexanal was lower and varied between 1.10 and 0.45 mg/kg over fruit ripening under RF conditions and between 0.90 and 0.50 mg/kg under FAO treatment. These compounds, which are responsible for the positive green sensory notes in VOO, are produced through the LOX pathway that takes place during crushing of the olive fruit and olive paste malaxation and are incorporated into the oily phase (*25*).

Figure 2 depicts the evolution of the main volatile compounds found in Cornicabra VOO in the course of fruit ripening as affected by RF and FAO irrigation conditions in crop season 2003/2004. As already mentioned, the decrease of C6 aldehydes was steady from the unripe to over-ripe stages, especially that of *E*-2-hexenal, which is the major volatile compound in various VOO cultivars. Its concentration diminished by about 40% in oils from RF conditions and 60% in oils from FAO treatment throughout fruit maturation (RI between 2.0 and 5.0) (**Figure 2** and **Table 2**). Aparicio et al. (*26*) reported similar behavior, but other researches (*5*, *27*) have shown that during olive ripening the amount of volatile compounds, especially *E*-2-hexenal, increased to a maximum concentration, which occurred when fruit skin color turned from yellow-green to purple; beyond that point the volatile content decreased.

With respect to the evolution of C6 alcohols, there was a significant decrease in *E*-2-hexen-1-ol content, whereas hexan-1-ol and *Z*-3-hexen-1-ol increased slightly during fruit ripening. It is worth noting that this observed increase was statistically significant in VOO from olives under FAO and 125 FAO treatments, which received the highest irrigation doses. A similar trend was observed in the crop season 2004/2005 (**Table 2**). The moderate increase in the hexan-1-ol and *Z*-3-hexen-1-ol content has not been observed by other researchers in other VOO varieties, such as cvs. Picual and Coratina (*5*, *26*), probably due to the different activities of alcohol dehydrogenase (ADH), which is genetically determined in each cultivar (*28*). With regard to C6 esters such as hexyl acetate and *Z*-3-hexyl acetate, these were present in very small amounts in Cornicabra VOO, indicating that there was also little activity of the alcohol acyl transferase (AAT).

In both crop seasons, the volatile compounds most affected by the irrigation were *E*-2-hexenal, *Z*-3-hexen-1-ol, and hexan-1-ol, in the sense that the increase in the water applied to the olive trees produced an increase in these volatiles, mainly in oils from fruits whose ripeness index was >2.5−3.0.

An additional branch of the LOX pathway is active on the linolenic acid substrate, leading to the production of C5 volatile compounds, which are also present in the VOO aroma (*4*, *5*). Reasonable amounts of 1-penten-3-one and 1-penten-3-ol were found in Cornicabra VOO, and their contents ranged between 1.40 and 0.80 mg/kg of IS for 1-penten-3-one and between 0.30 and 0.16 mg/kg for 1-penten-3-ol over the course of fruit maturation (**Table 2**). On the other hand, these volatiles were apparently not affected by the irrigation strategies studied.

RDI-1 resulted in a VOO with a volatile composition similar to those from FAO treatment. On the other hand, the total volatile levels in VOOs produced under RDI-2 conditions were higher than in VOOs produced under FAO conditions and similar to the levels found in VOOs produced under 125 FAO conditions. It is therefore very important to note that the VOOs produced by the second RDI strategy were richer in volatile compounds than those produced under FAO conditions, but with a considerable reduction in the total amount of water used in the olive grove.

Discriminant Analysis. The results of ANOVA and principal component analyses (PCA) were applied to the discriminant analysis to better describe the differences observed in the phenol

Table 2. Levels of Major Virgin Olive Oil Volatile Compounds (Milligrams per Kilogram of IS) with Regard to Fruit Ripening Stage and Irrigation Management[a]

	ripeness index	hexanal	hexan-1-ol	hexyl acetate	*E*-2-hexenal	*Z*-3-hexen-1-ol	*E*-2-hexen-2-ol	*Z*-3-hexenyl acetate	1-penten-3-one	1-penten-3-ol
					2003/2004					
rain-fed	1.5 ± 0.5a,w	1.10 ± 0.16ab,z	0.02 ± 0.00a,w	<0.01	4.18 ± 1.72a,y	0.11 ± 0.03a,w	0.11 ± 0.02a,x	<0.01	1.14 ± 0.17a,z	0.34 ± 0.02a,y
RDI-1	1.8 ± 0.6ab,w	1.38 ± 0.28b,y	0.03 ± 0.02a,w	<0.01	6.46 ± 1.19ab,x	0.15 ± 0.03a,w	0.14 ± 0.00b,y	<0.01	1.51 ± 0.27b,y	0.34 ± 0.08a,y
FAO	2.5 ± 0.4b,w	0.90 ± 0.14a,x	0.04 ± 0.00a,w	<0.01	7.74 ± 2.21ab,x	0.17 ± 0.04a,w	0.12 ± 0.02ab,y	<0.01	1.06 ± 0.16a,y	0.26 ± 0.02a,y
125 FAO	2.0 ± 0.3ab,v	0.91 ± 0.09a,x	0.03 ± 0.01a,w	<0.01	9.74 ± 2.14b,y	0.12 ± 0.04a,w	0.12 ± 0.00ab,y	<0.01	1.07 ± 0.07a,x	0.25 ± 0.05a,w
rain-fed	2.7 ± 0.3a,x	0.68 ± 0.03a,y	0.03 ± 0.01a,wx	<0.01	3.76 ± 0.10a,xy	0.11 ± 0.05a,w	0.06 ± 0.00a,w	<0.01	0.95 ± 0.07a,y	0.29 ± 0.02a,x
RDI-1	2.8 ± 0.4a,x	0.90 ± 0.16b,x	0.06 ± 0.01ab,wx	<0.01	5.69 ± 1.25ab,x	0.18 ± 0.05a,w	0.08 ± 0.00b,x	<0.01	1.18 ± 0.18a,x	0.27 ± 0.02a,xy
FAO	3.1 ± 0.3a,x	0.68 ± 0.06a,w	0.05 ± 0.01ab,w	<0.01	5.82 ± 1.84ab,wx	0.23 ± 0.06ab,w	0.08 ± 0.00b,x	<0.01	0.98 ± 0.07a,xy	0.24 ± 0.01a,xy
125 FAO	2.8 ± 0.2a,w	0.68 ± 0.04a,w	0.08 ± 0.02b,wx	<0.01	7.21 ± 2.27b,xy	0.39 ± 0.12b,y	0.09 ± 0.01b,x	<0.01	0.98 ± 0.11a,wx	0.26 ± 0.03a,w
rain-fed	3.2 ± 0.3a,xy	0.62 ± 0.06a,xy	0.05 ± 0.01a,x	<0.01	3.25 ± 0.35a,wx	0.08 ± 0.01a,w	0.05 ± 0.00a,w	<0.01	0.81 ± 0.07a,xy	0.26 ± 0.03a,x
RDI-1	3.5 ± 0.4a,xy	0.75 ± 0.11a,wx	0.08 ± 0.04ab,wx	<0.01	4.71 ± 0.99ab,wx	0.15 ± 0.03b,w	0.06 ± 0.01a,wx	<0.01	0.97 ± 0.07a,x	0.23 ± 0.04a,wx
FAO	3.6 ± 0.4a,xy	0.66 ± 0.09a,w	0.08 ± 0.03ab,wx	<0.01	4.02 ± 1.19ab,w	0.19 ± 0.05bc,w	0.05 ± 0.01a,wx	<0.01	0.90 ± 0.11a,xy	0.22 ± 0.01a,x
125 FAO	3.4 ± 0.3a,x	0.73 ± 0.03a,w	0.12 ± 0.01b,xy	<0.01	5.48 ± 0.83b,wx	0.23 ± 0.03c,wx	0.06 ± 0.01a,w	<0.01	0.92 ± 0.10a,w	0.23 ± 0.03a,w
rain-fed	3.7 ± 0.2a,y	0.52 ± 0.05a,wx	0.04 ± 0.02a,wx	<0.01	1.90 ± 0.40a,w	0.11 ± 0.09a,w	0.05 ± 0.01a,w	<0.01	0.72 ± 0.08a,x	0.24 ± 0.02a,x
RDI-1	3.8 ± 0.3a,y	0.67 ± 0.09b,wx	0.12 ± 0.07a,x	<0.01	3.40 ± 1.10ab,w	0.27 ± 0.19ab,w	0.06 ± 0.00a,w	<0.01	0.91 ± 0.10b,x	0.26 ± 0.03a,xy
FAO	4.0 ± 0.4a,y	0.58 ± 0.07ab,w	0.11 ± 0.03a,xy	<0.01	3.70 ± 1.35ab,w	0.36 ± 0.19ab,w	0.06 ± 0.01a,wx	<0.01	0.82 ± 0.07ab,x	0.22 ± 0.02a,x
125 FAO	3.9 ± 0.1a,y	0.73 ± 0.08b,w	0.21 ± 0.06b,z	<0.01	5.14 ± 0.74b,wx	0.42 ± 0.08b,y	0.06 ± 0.01a,w	<0.01	0.87 ± 0.07ab,w	0.24 ± 0.03a,w
rain-fed	5.7 ± 0.4b,z	0.44 ± 0.06a,w	0.08 ± 0.03a,x	<0.01	2.46 ± 0.68a,wx	0.23 ± 0.04a,x	0.05 ± 0.02a,w	<0.01	0.48 ± 0.05a,w	0.15 ± 0.02a,w
RDI-1	5.4 ± 0.5ab,z	0.50 ± 0.09a,w	0.12 ± 0.01ab,x	<0.01	3.10 ± 0.77a,w	0.32 ± 0.05ab,w	0.06 ± 0.00a,w	<0.01	0.57 ± 0.12a,w	0.16 ± 0.02a,w
FAO	5.5 ± 0.3ab,z	0.52 ± 0.15a,w	0.14 ± 0.03ab,y	<0.01	3.09 ± 1.79a,w	0.35 ± 0.07b,w	0.05 ± 0.01a,w	<0.01	0.56 ± 0.08a,w	0.16 ± 0.01a,w
125 FAO	4.9 ± 0.1a,z	0.71 ± 0.08b,w	0.17 ± 0.03b,yz	<0.01	3.91 ± 0.76b,w	0.35 ± 0.04b,xy	0.06 ± 0.01a,w	<0.01	0.82 ± 0.04b,w	0.20 ± 0.03b,w
					2004/2005					
rain-fed	2.8 ± 0.2b,w	0.83 ± 0.07a,x	0.05 ± 0.01a,w	<0.01	3.50 ± 0.38a,x	0.08 ± 0.02a,w	0.09 ± 0.00a,x	<0.01	1.10 ± 0.11a,x	0.28 ± 0.01b,w
RDI-2	2.3 ± 0.1a,w	0.74 ± 0.06a,x	0.06 ± 0.00a,w	<0.01	5.45 ± 0.67ab,x	0.14 ± 0.00b,w	0.08 ± 0.01a,w	<0.01	1.17 ± 0.05a,y	0.24 ± 0.01a,w
FAO	2.5 ± 0.2ab,w	0.73 ± 0.12a,x	0.05 ± 0.00a,w	<0.01	4.73 ± 1.47ab,x	0.12 ± 0.00b,w	0.08 ± 0.00a,x	<0.01	1.07 ± 0.02a,y	0.26 ± 0.01ab,w
125 FAO	2.4 ± 0.1a,w	0.83 ± 0.06a,x	0.06 ± 0.01a,w	<0.01	6.38 ± 0.12b,x	0.14 ± 0.04ab,w	0.09 ± 0.00a,x	<0.01	1.21 ± 0.00a,x	0.27 ± 0.00ab,wx
rain-fed	3.4 ± 0.0a,x	0.56 ± 0.11a,wx	0.03 ± 0.01a,w	<0.01	1.92 ± 0.88a,w	0.18 ± 0.07a,wx	0.07 ± 0.01a,wx	<0.01	0.89 ± 0.11a,wx	0.26 ± 0.02a,w
RDI-2	3.4 ± 0.0a,x	0.73 ± 0.08a,x	0.13 ± 0.02b,wx	<0.01	4.80 ± 0.54b,x	0.38 ± 0.13a,w	0.08 ± 0.03a,w	<0.01	0.99 ± 0.03a,x	0.23 ± 0.00a,w
FAO	3.4 ± 0.0a,x	0.58 ± 0.02a,wx	0.07 ± 0.00b,wx	<0.01	3.17 ± 0.30ab,x	0.32 ± 0.02a,x	0.06 ± 0.00a,wx	<0.01	0.86 ± 0.01a,x	0.26 ± 0.00a,w
125 FAO	3.5 ± 0.1a,x	0.72 ± 0.08a,wx	0.19 ± 0.10b,x	<0.01	4.43 ± 0.50b,w	0.32 ± 0.04a,w	0.06 ± 0.00a,w	<0.01	0.87 ± 0.12a,w	0.21 ± 0.03a,w
rain-fed	4.1 ± 0.1a,y	0.36 ± 0.15a,w	0.10 ± 0.00a,x	<0.01	1.40 ± 0.74a,w	0.28 ± 0.02a,x	0.04 ± 0.01a,w	<0.01	0.58 ± 0.20a,w	0.21 ± 0.03a,w
RDI-2	4.2 ± 0.0a,y	0.45 ± 0.02a,w	0.24 ± 0.07b,x	<0.01	2.64 ± 0.00ab,w	0.83 ± 0.17a,x	0.06 ± 0.01b,w	<0.01	0.63 ± 0.02a,w	0.21 ± 0.01a,w
FAO	4.2 ± 0.0a,y	0.43 ± 0.00a,w	0.20 ± 0.07b,x	<0.01	2.09 ± 0.20ab,w	0.64 ± 0.28a,x	0.04 ± 0.00ab,w	<0.01	0.69 ± 0.01a,w	0.27 ± 0.01b,w
125 FAO	4.2 ± 0.0a,y	0.57 ± 0.06a,w	0.23 ± 0.06b,x	<0.01	3.14 ± 0.53b,w	0.55 ± 0.09a,x	0.07 ± 0.00c,w	<0.01	0.86 ± 0.03a,w	0.28 ± 0.01b,x

[a] Different letters (a–c) within a column indicate significant differences ($p < 0.05$) with respect to irrigation treatment in each sampling. Different letters (w–y) within a column indicate significant differences ($p < 0.05$) with respect to ripeness index for each irrigation treatment.

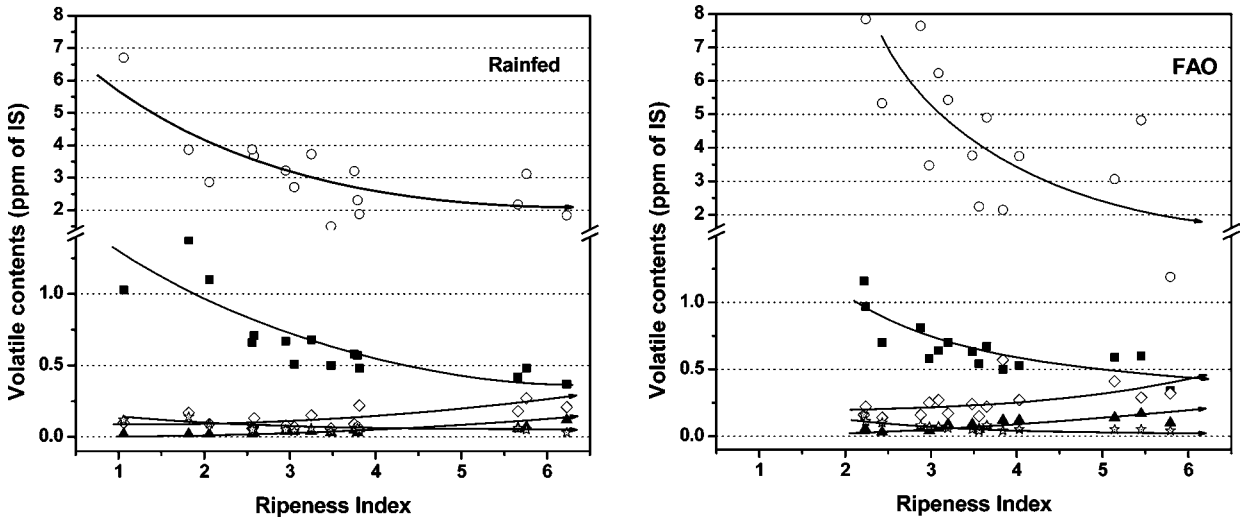

Figure 2. Evolution of main volatile compounds in virgin olive oils under rain-fed conditions and FAO irrigation scheduling in the course of fruit ripening in crop season 2003/2004: (○) *E*-2-hexenal; (■) hexanal; (◇), *Z*-3-hexen-1-ol; (▲) hexan-1-ol; (◆) *E*-2-hexen-1-ol.

and volatile compound profile according to the ripening stage and the different experimental irrigation treatments.

The most useful variables for classification of the VOO samples according to the stage of ripening of the fruit in both crop seasons 2003/2004 and 2004/2005 were hexanol, *E*-2-hexen-1-ol, 3,4-DHPEA-EDA, and *p*-HPEA-EDA (**Figure 3**). The first two discriminant functions explained 99.9% of the variance (97.9 and 2.0%, respectively), yielding a good classification (85%) of VOO oil samples obtained from unripe to over-ripe fruits.

On the basis of this result and, in particular, the pattern of evolution of phenolic and volatile compositions throughout fruit

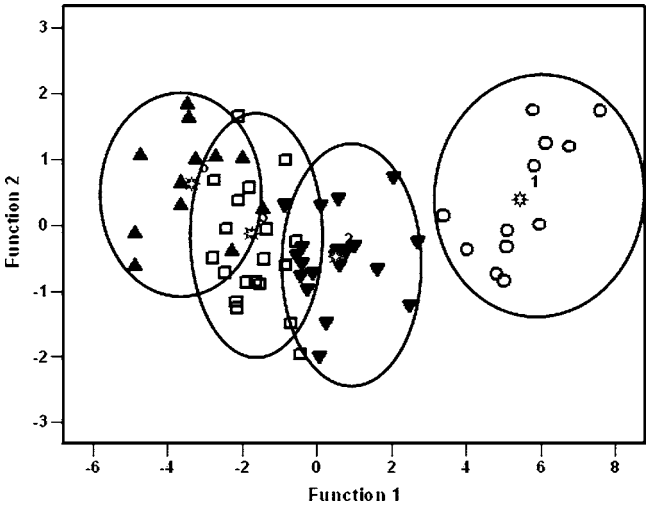

Figure 3. Discriminant functions plot of virgin olive oil composition classified according to the stage of ripening of the fruit in the crop seasons studied: (○) RI 1.1–2.3; (▼) RI 2.5–3.0; (□) RI 3.5–4.3; (▲) RI 5.0–5.9. Variables: hexanol, *E*-2-hexen-1-ol, 3,4-DHPEA-EDA, *p*-HPEA-EDA.

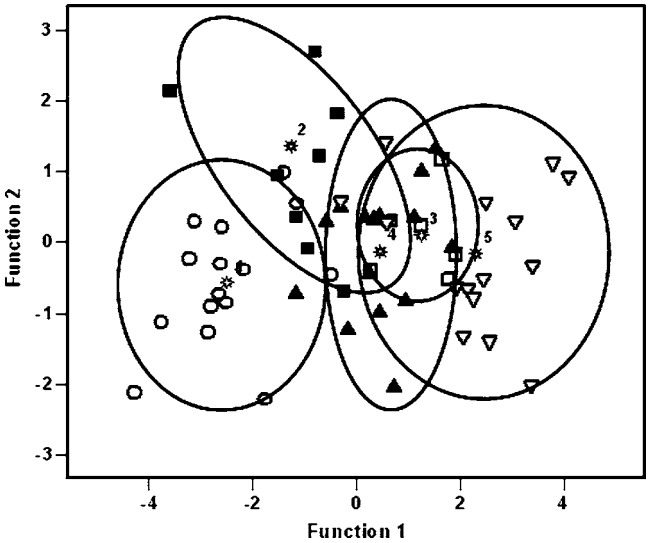

Figure 4. Discriminant functions plot of virgin olive oil composition classified according to the five different types of irrigation management studied in the 2003/2004 and 2004/2005 crop seasons: (○) rain-fed; (■) regulated deficit irrigation (2003/2004), RDI-1; (□) regulated deficit irrigation (2004/2005), RDI-2; (▲) FAO; (▽) 125 FAO. Variables: 3,4-DHPEA-EDA, *E*-2-hexenal, *Z*-3-hexen-1-ol, 3,4-DHPEA-EA.

maturation in this two-year experimental field work and their relationship to VOO quality, the best stage of maturity of Cornicabra olive fruits grown in Castilla-La Mancha for VOO processing would appear to be a RI ranging from 3.0 to 4.0. In fact, at higher RI the concentrations of major volatiles from the LOX pathway in oils from over-ripe fruits (RI > 4.5−5) would be too low, and hence the attractive green odor notes would be less perceptible, which would reduce the level of consumer preference for the product. Moreover, at this RI the decrease in complex phenols would no longer be beneficial in the sense discussed earlier with respect to the Cornicabra olive oil variety, but would produce a significant reduction in the oxidative stability, bitter taste, and nutritional value, especially in irrigated VOO.

With regard to irrigation, 3,4-DHPEA-EDA, *E*-2-hexenal, *Z*-3-hexen-1-ol, and oleuropein aglycon were the most useful variables for classification of the samples obtained under the different types of irrigation scheduling (**Figure 4**). The first two discriminant functions explained 97.3% of the variance (87.5 and 9.7%, respectively), yielding 77% of correctly classified cases.

It is important to note that, as depicted in **Figure 4**, VOO samples obtained using the RDI-1 (no. 2 in the figure) and the RDI-2 (no. 3) strategies were plotted quite far apart, although the total amounts of water applied were similar in both cases (56 and 60 mm, respectively). Moreover, as previously observed, because of their different phenol and volatile profiles, VOO samples from RDI-1 management (2) were situated between samples from RF (1) and FAO (4) treatments, whereas samples from RDI-2 (3) were situated between the VOO samples from FAO (4) and 125 FAO (5) treatments. This clearly shows that the minor compounds in VOO are influenced not only by the total amount of water employed in the olive grove throughout the crop season but in particular by the water irrigation scheduling used.

Finally, the different water stress levels in olive trees affected not only the total amount of phenolic and volatile compounds in the VOO but also their profile. For example, phenolic compounds such as 3,4-DHPEA-EDA and 3,4-DHPEA-EA decreased sharply when the water dosage applied to the olive grove was increased, whereas *E*-2-hexenal, hexan-1-ol, and *Z*-3-hexen-1-ol concentrations were higher in irrigated olive oils. It was further observed that applying water only from the beginning of August (RDI-2), when oil begins to accumulate in the fruit, produced a VOO with similar phenol and volatile profiles to VOOs produced under the FAO and 125 FAO methods, but with a considerable reduction in the amount of water supplied to the olive orchard. This aspect is very important for olive groves in Castilla-La Mancha because it is a region with limited water resources.

ACKNOWLEDGMENT

We express our gratitude to Dr. Alfonso Moriana, Dr. Francisco Ribas, David Pérez, and Nicolás Olmedilla (C.M.A. El Chaparrillo, Servicio de Investigación y Tecnología Agraria, Ciudad Real, Spain) for managing the irrigation scheduling in the experimental olive orchard.

LITERATURE CITED

(1) Angerosa, F. Influence of volatile compounds on virgin olive oil quality evaluated by analytical approaches and sensor panels. *J. Lipid Sci. Technol.* **2002**, *104* (9−10), 639−660.

(2) Aparicio, R.; Luna, G. Characterisation of monovarietal virgin olive oils. *J. Lipid Sci. Technol.* **2002**, *104* (9−10), 614−627.

(3) Morales, M. T.; Tsimidoou, M. The role of volatile compounds and polyphenols in olive oil sensory quality. In *Handbook of Olive Oil: Analysis and Properties*; Harwood, J., Aparicio, R., Eds.; Aspen Publishing: Gaithersburg, MD, 2000; pp 393−458.

(4) Angerosa, F.; Mostallino, R.; Basti, C.; Vito, R. Virgin olive oil odour notes: their relationship with volatile compounds from the lipoxygenase pathway and secoiridoid compounds. *Food Chem.* **2000**, *68*, 283−287.

(5) Angerosa, F.; Mostallino, R.; Basti, C.; Vito, R. Influence of malaxation temperature and time on the quality of virgin olive oils. *Food Chem.* **2001**, *72*, 19−28.

(6) Gutiérrez-Rosales, F.; Perdiguero, S.; Gutiérrez, R.; Olías, J. M. Evaluation of bitter taste in virgin olive oil. *J. Am. Oil Chem. Soc.* **1992**, *69*, 394−395.

(7) Morales, M. T.; Aparicio, R.; Calvente, J. J. Influence of olive ripeness on the concentration of green aroma compounds in virgin olive oil. *Flavour Fragrance J.* **1996**, *11*, 171−178.

(8) Salvador, M. D.; Aranda, F.; Fregapane, G. Influence of fruit ripening on Cornicabra virgin olive oil. A study of four crop seasons. *Food Chem.* **2001**, *73*, 45−53.
(9) Motilva, M. J.; Romero, M. P.; Alegre, S.; Girona, J. Effect of regulated deficit irrigation in olive oil production and quality. *Acta Hortic.* **1999**, *474*, 377−380.
(10) Motilva, M. J.; Tovar, M. J.; Romero, M. P.; Alegre, A.; Girona, J. Influence of regulated deficit irrigation strategies applied to olive trees (Arbequina cultivar) on oil yield and oil composition during the fruit ripening. *J. Sci. Food Agric.* **2000**, *80*, 2037−2043.
(11) Tovar, M. J.; Romero, M. P.; Motilva, M. J. Changes in the phenolic composition of olive oil from young trees (*Olea europaea* L. cv. Arbequina) grown under linear irrigation strategies. *J. Agric. Food Chem.* **2001**, *49*, 5502−5508.
(12) Gómez-Rico, A.; Salvador, M. D.; Soriana, A.; Pérez, D.; Olmedilla, N.; Ribas, F.; Fregapane, G. Influence of different irrigation strategies in a traditional Cornicabra cv. olive orchard on virgin olive oil composition and quality. *Food Chem.* **2006**, doi: 10.1016/j.foodchem. 2005.09.075.
(13) M.A.P.A. *Anuario de Estadística Agroalimentariaqq*; Ministerio de Agricultura, Pesca y Alimentación: Spain, 2004.
(14) Doorenbos, J.; Pruitt, W. O. *Crop Water Requirements*; FAO Irrigation and Drainage Paper 24; Food and Agriculture Organization of the United Nations: Rome, Italy, 1977.
(15) IOOC. Document 6; Internacional Olive Oil Council: Madrid, Spain, 1984.
(16) Martínez, J. M.; Muñoz, E.; Alba, J.; Lanzón, A. Informe sobre utilización del analizador de rendimiento Abencor. *Grasas Aceites* **1975**, *26*, 379−385.
(17) Mateos, R.; Espartero, J. L.; Trujillo, M.; Ríos, J. J.; León-Camacho, M.; Alcudia, F.; Cert, A. Determination of phenols, flavones and lignans in virgin olive oil by solid-phase extraction and high-performance liquid chromatography with diode array ultraviolet detection. *J. Agric. Food Chem.* **2001**, *49*, 2185−2192.
(18) Vichi, S.; Castellote, A. I.; Pizzale, L.; Conte, L. S.; Buxaderas, S.; Lopez-Tamames, E. Analysis of virgin olive oil volatile compounds by headspace solid-phase microextraction coupled to gas chromatography with mass spectrometric and flame ionisation detection. *J. Chromatogr. A* **2003**, *983*, 19−33.
(19) Gómez-Alonso, S.; Salvador, M. D.; Fregapane, G. Phenolic compounds profile of Cornicabra virgin olive oil. *J. Agric. Food Chem.* **2002**, *50*, 6812−6817.
(20) Patumi, M.; d'Andria, R.; Fontanazza, G.; Morelli, G.; Giori, P.; Sorrentino, G. Yield and oil quality of intensively trained trees of three cultivars of olive under different irrigation regimes. *J. Hortic. Sci. Biotechnol.* **1999**, *74*, 729−737.
(21) Tovar, M. J.; Romero, M. P.; Girona, J.; Motilva, M. J. L-Phenylalanine ammonia-lyase activity and concentration of phenolics in developing fruit of olive tree (*Olea europaea* L. cv. Arbequina) grown under different irrigation regimes. *J. Sci. Food Agric.* **2002**, *82*, 892−898.
(22) Baldioli, M.; Servili, M.; Peretti, G.; Montedoro, G. F. Antioxidant activity of tocopherols and phenolic compounds of virgin olive oil. *J. Am. Oil Chem. Soc.* **1996**, *73*, 1589−1593.
(23) Gennaro, L.; Piciola Bocca, A.; Modesti, D.; Masella, R.; Coni, E. Effect of biophenols on olive oil stability evaluated by thermogravimetric analysis. *J. Agric. Food Chem.* **1998**, *46*, 4465−4469.
(24) Tovar, M. J.; Romero, M. P.; Alegre, S.; Girona, J.; Motilva, M. J. Composition and organoleptic characteristics of oil from Arbequina olive (*Olea europaea* L) trees under deficit irrigation. *J. Sci. Food Agric.* **2002**, *82*, 1755−1763.
(25) Sanchez, J.; Harwood, J. L. Byosinthesis of triacylglycerols and volatiles in olives. *Eur. J. Lipid Sci. Technol.* **2002**, *104*, 564−573.
(26) Aparicio, R.; Morales, M. T. Characterization of olive ripeness by green aroma compounds of virgin olive oil. *J. Agric. Food Chem.* **1998**, *46*, 1116−1122.
(27) Solinas, M.; Maesilio, V.; Angerosa, F. Evoluzione di alcuni componenti dell'aroma degli oli vergini di oliva in relazione del grado de maturazione delle olive. *Riv. Ital. Sostanze Grasse* **1987**, *64*, 475.
(28) Angerosa, F.; Basti, C.; Vito, R. Virgin olive oil volatile compounds from lipoxigenase pathway and characterization of some Italian cultivars. *J. Agric. Food Chem.* **1999**, *47*, 836−839.

Received for review March 22, 2006. Revised manuscript received July 5, 2006. Accepted July 20, 2006. This research project was supported by the Conserjería de Ciencia y Tecnología de la Junta de Comunidades de Castilla-La Mancha (Project PBI-03−015).

JF060798R