# Summer Deficit-Irrigation Strategies in a Hedgerow Olive cv. Arbequina Orchard: Effect on Oil Quality

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ABSTRACT: Different irrigation treatments were applied to a superintensive orchard of 'Arbequina' olives (Olea europaea L.) during three seasons (2007-2009) to examine the effect of the amount of water and the moment of irrigation in summer on the virgin olive oil (VOO) quality. A control was made (CON) with irrigation to maintain the root zone close to field capacity; two water deficit treatments were employed with irrigation at 30% of CON, either from the end of fruit drop to the end of July (DI-J) or from the end of July until the beginning of oil synthesis (DI-A); and other treatment was tested by irrigating 50% of CON in July and August (DI-JA). DI-J oils exhibited significantly higher oxidative stability, which coincided with significantly higher contents in phenol derivatives. Consequently, the selection of the moment and intensity of summer irrigation played an important role in the nutritional and sensory quality of the VOO.

KEYWORDS: stability, phenol, Olea europaea, virgin oil, water management

# ■ INTRODUCTION

The olive oil industry has been historically conditioned by the high costs of harvesting, which explains why olive oil cannot compete in price with other vegetable oils, the harvest of which is easier, faster, and less expensive. In Spain, in the last decade of the past century, a new planting system was started, which involves densities of above 1000 trees/ha, with the trees planted in rows with a separation of <2 m between them and 4–6 m between rows, for irrigated or nonirrigated orchards, respectively. After 3 years, the trees are trained as a hedge all along the row, and that is why this planting system is called "hedgerow" or "superintensive", due to the high density of trees per hectare.<sup>1,2</sup> This system, with minor modifications, allows for the use of the riding harvester already used for the re-collection of grapes in vineyards, thus reducing the labor costs as well as the period of harvesting. These machines can harvest olives at their early maturity stage even with high fruit retention force.<sup>3,4</sup> This type of harvester can gather as much as 1 ha of such hedgerows in 1-2.5 h, with the participation of only one workman. The cultivar Arbequina is, due to its small size, precocity, oil quality, and branch flexibility, among the traditional Spanish varieties that is best suited to hedgerow orchard and mechanical harvesting.<sup>4-6</sup> Virgin olive oil (VOO) from 'Arbequina' has high acceptance in the international market due to its excellent sensory quality and its characteristic softness, which is highly agreeable to the taste of people accustomed to the tasteless refined oils from rapeseed, sunflower, or soybean, who do not accept excessive attributes, such as bitter and pungent, displayed by other olive cultivars. However, this appreciated quality depends on the maturity of the fruit. Advance maturation results in a clear reduction in sensorial attributes (aroma, taste, and color) due to the decrease in volatile, phenolic, and pigment (chlorophylls and carotenes) contents, which results in the loss of intensity in its characteristic fruity odor, bitter taste, and green-yellow color. Furthermore, the progression of fruit ripening has been related to the increase and decrease in polyunsaturated and monounsaturated fatty acid contents, respectively. These factors, together with the reduction in phenolic compounds, determine a considerable decrease in the oxidative stability of these VOOs.7 These circumstances make it necessary for the 'Arbequina' olive to be harvested at an early stage of maturation and over a short period.<sup>8</sup>

The irrigation of the 'Arbequina' hedgerow orchard results in a yield increase of 3-5 times as compared with the traditional rain-fed cultivation, and this modern practice is spreading.<sup>9,10</sup> Nevertheless, the increase in water supply to 'Arbequina' trees induces a reduction in VOO extractability in the fruit and a significant decrease in the oil pigment and phenol content. This fact determines the obtaining of VOO with low oxidative stability and with poor sensory attributes. Recently, García et al.<sup>11</sup> observed that the application of a sustained deficit irrigation (DI) strategy (65% ETc, 2-3 irrigation events per week) to 'Arbequina' trees (238 trees/ha) induced a significant increase in VOO extraction and the oil content of pigments, phenols, and oleic acid in relation to fully irrigated trees, and also caused a significant decrease in fruit production. In the same way, Gómez del Campo,<sup>12</sup> working with an 'Arbequina' hedgerow orchard, tested four irrigation treatments during the summer, when the olive is most drought resistant. The control (CON) was irrigated to maintain the root zone close to field capacity. Two severe water deficit treatments were applied by irrigating 30% CON from the end of the fruit drop to the end of July (DI-J) or from the end of July until the beginning of oil synthesis (DI-A). Finally, a less severe water deficit treatment

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was applied during July and August (DI-JA) by irrigating 50% CON. CON induced higher fruit and VOO production than DI-A and DI-JA. However, the oil production of DI-J was not significantly different from that obtained by the CON treatment. In a continuation of this work, the aim of this study is to know how these summer deficit irrigation strategies affect the VOO quality parameters and composition and to test if the marketability of the cultivar 'Arbequina' could be improved at the same time that a significant amount of irrigation water could be saved.

# MATERIALS AND METHODS

**Site and Orchard.** The experiment was conducted in a 45 ha commercial orchard planted with cv. Arbequina in 1997 in Puebla de Montalbán, Toledo, Spain (latitude, 39° 48' N; longitude, 04° 27'W; altitude, 516 m) at a spacing of  $4 \times 2$  m (1250 olive/ha) with rows oriented 20° N of east–west. At the time of the experiment, hedgerows were 2.3 m high and 1.1 m wide.

Irrigation Treatments. Four irrigation treatments (CON, DI-J, DI-A, and DI-JA) were maintained during the 2007-2009 seasons in an area of 5600  $m^2$  in a completely randomized design of four blocks. Each replicate comprised 36 trees (12 trees in each of 3 adjacent rows). The central 10 trees in the middle row of each replicate were used for measurement. Three of these trees were identified and used only for final harvest. The remaining 26 trees provided a border. Each row of trees was irrigated from a single line with drip emitters of 3 L/h spaced 0.50 m apart. CON trees were irrigated according to continuous readings of six Watermark sensors connected to a data logger (Irrometer, CA, USA) located in pairs at 0.3 m depth and 0.3 m from emitters adjacent to the trunks of three representative trees. Irrigations of 6 h duration were applied from spring until August 15, when sensors indicated a mean soil water potential of -0.03 MPa. Then, to harden the trees for autumn frost, the threshold potential for irrigation was lowered to -0.06 MPa until the end of the irrigation season.

The season was divided into four periods: spring (from March 21 to the beginning of irrigation treatments), first summer irrigation period (July, from the end of fruit drop at June 24, 2007; June 29, 2008; and June 18, 2009; to July 22, 2007; July 24, 2008; and July 21, 2009 respectively); second summer irrigation period (August, from the end of the first period to September 2, 2007, August 31, 2008; and August 24, 2009); and autumn (from the end of the second period to harvest). In the CON, budburst occurred at the beginning of March (March 1, 10, and 10, 2007, 2008, and 2009, respectively), bloom from the end of May to the beginning of June (May 28, 2007; June 1, 2008; and May 24, 2009), and pit hardening in July (July 22, 31, and 12, 2007, 2008, and 2009, respectively).

All treatments were irrigated in the same way as the CON except in summer. DI-J and DI-A were irrigated with 30% of the water applied to the CON during July and August, respectively, whereas DI-JA was irrigated with 50% of CON during both July and August. The amounts of irrigation applied differed from year to year according to climatic conditions. The CON received 221, 284, and 402 mm in 2007, 2008, and 2009 seasons, respectively. Compared to the CON, the reductions in water applied to DI-J, DI-A, and DI-JA were 16, 22, and 27%. CON was irrigated for high water availability, and deficit irrigation treatments significantly modified relative extractable water of the soil and stem water potential in the periods in which they were applied.<sup>13</sup> Agronomic responses of the irrigation treatments were already published in Gómez-del-Campo.<sup>12</sup> In this paper the effect of this irrigation treatment on oil quality is reported.

**Harvesting, Evaluation of Fruit Ripening, and Oil Extraction.** Harvests were made on November 12, 2007, November 5, 2008, and October 30, 2009 when the fruit (4 kg) was removed from the three selected trees in each of four replicates per treatment. The ripening index (RI) of the fruits was evaluated according to the system habitually used in the olive mills, which subjectively measures their skin and flesh color.<sup>14,15</sup> This index was evaluated independently for each of the three irrigation treatments and for control in each of the three years studied.

Then, oils of each replicate were extracted separately, using an "Abencor" analyzer (Comercial Abengoa S.A., Seville, Spain). The fruits were crushed in a hammer mill (radius = 47.5 mm, with a sieve of 5.0 mm hole diameter) at 3000 rpm. The resulting olive paste was placed in 1 L stainless steel jars and malaxated for 30 min in the thermobeater at 28 °C, using four stainless steel cross blades at 54.5 rpm (radius = 53 mm). Subsequently, the paste was centrifuged in a pulp centrifuge for 1 min at 3500 rpm (radius = 100 mm) to separate the liquid phase (oil and wastewater) from the solid waste. The oil was then decanted into graduated tubes, removed with a pipet, filtered through filter paper, and stored in an N<sub>2</sub> atmosphere at -20 °C until analysis.<sup>16</sup>

**Evaluation of Oil Quality.** Free acidity, peroxide index value, and coefficients of specific extinction at 232 and 270 nm ( $K_{232}$  and  $K_{270}$ ) of the oils were evaluated according to the European Union Standard Methods (EEC, 1991).<sup>17</sup> Oxidative stability was measured by the Rancimat method, which evaluates the time (h) of resistance to oxidation of 3 g of oil sample exposed to a stream of dry air (20 L/h) at a temperature of 100 °C.<sup>18</sup>

The composition of fatty acids was determined by gas chromatographic analysis of the methyl esters. This was performed on an Agilent 6890 equipped with a flame ionization detector, fitted with a fused-silica capillary column (SP-2380, 60 m  $\times$  0.25 mm i.d.) coated with cyanopropylsilicone (0.20 mm film thickness). Hydrogen was employed as carrier gas at flow of 1 mL/min. The oven temperature was maintained at 185 °C, and the injector (split 1:20) and detector were maintained at 225 °C. The data presented here are for the main fatty acids (number of carbons:number of unsaturations): palmitic (16:0), palmitoleic (16:1), stearic (18:0), oleic (18:1), and linoleic (18:2). Other fatty acids including myristic (14:0), palmitoleic (16:1), margaric (17:0), margaroleic (17:1), linolenic (18:3), arachidic (20:0), gadoleic (20:1), or behenic (22:0) were determined but are not shown because values were too small ( $\leq 0.6\%$ ) for any significant role in oil quality. The following formulas using fatty acid content variables were calculated:

> oleic:linoleic ratio (OLR) = |18:1|/|18:2| saturated fatty acid (SAFA) = |16:0| + |17:0| + |18:0| + |20:0| + | 22:0| monounsaturated fatty acid (MUFA) = |16:1| + |17:1| + |18:1| + |20:1| polyunsaturated fatty acid (PUFA) = |18:2| + |18:3| unsaturated fatty acid (UNFA) = |16:1| + |17:1| + |18:1| + |18:2| + |18:3| + |20:1| UNFA/SAFA MUFA/PUFA

Tocopherol content was measured in 2008 and 2009 seasons by HPLC using the IUPAC method.<sup>19</sup> The phenolic fraction of the same samples was isolated by solid-phase extraction and analyzed by reversed-phase HPLC using a diode array UV detector.<sup>20</sup> The quantification of phenolic compounds was carried out at 280 nm using *p*-hydroxyphenylacetic acid as an internal standard, whereas that of flavones was made at 335 nm using *o*-coumaric acid as an internal standard. The content of each phenol compound was calculated by taking into account its individual response factor in relation to the internal standard.

To offer to the readers an idea of the effect of the irrigation treatments on the sensory quality of the oils, an informal sensory evaluation of each oil sample was carried out by two trained tasters only, because we did not have the amount of sample required for an analytical panel of eight tasters. The main positive (olive fruit, bitterness, and pungent) and negative (fusty, musty, winey, rancid, and unspecified others) sensory attributes of the olive oils were evaluated using an unstructured scale, marking the intensity level of each sensory attribute in a 10 cm line, considering the left end of the line the point that indicates the absence of the attribute and the right end its maximum possible intensity. Each determination was evaluated according to the distance (cm) between the left end and the mark carried out by each taster in the intensity scale line. Each presented value corresponds to the median of the distribution of intensities for each sensory attribute in each oil sample. In addition, the tasters described the sensory notes of the oils.

**Statistical Analysis.** Data were subjected to analysis of variance using MSTAT-C (University of Michigan, USA). The effect of year was analyzed in a factorial design. Least significant differences (P < 0.05) were used to separate the means of the parameters evaluated between irrigation treatments using Duncan's multiple-range test.

### RESULTS

**Fruit Ripening.** Harvest date was not determined by maturity index, and large differences between years were observed. The fruits exhibited RI values of 3.0, 0.4, and 1.5 for the first, second, and third seasons, respectively. In this area, harvest should be done before mid-November when fruits can be frozen by low temperature. Not significant differences in this parameter between irrigation treatments were observed in any of the experimental years.

**Parameters of Oil Quality.** The values obtained for the extracted oils in the parameters legally established for evaluating the level of commercial quality (free acidity, peroxide value,  $K_{232}$ , and  $K_{270}$ ) were, in all cases, inside the limits established for the commercial quality "extra", the best possible level of quality for virgin olive oils (data not shown). Irrigation treatment and year did not significantly determine changes in those values. However, the oxidative stability, which evaluates the oil's resistance to rancidity, was clearly affected by both factors (Figure 1). DI-J oils exhibited significantly higher mean



**Figure 1.** Oxidative stability (air flow = 20 L/h and temperature = 100 °C) of the oils extracted from olive fruits from a fully irrigated control (100%) and three deficit-irrigation treatments applied in summer: 30% in July, 30% in August, and 50% in July and August. Each value is the mean value of four replicates. For each season, values of different irrigation treatments followed by the same letter are not significantly different by Duncan's test at P < 0.05. Absence of letters means no significant effect due to irrigation detected by ANOVA at P < 0.05.

values for this parameter and during each one of the three tested seasons none of the other irrigation treatment oils showed significantly higher oxidative stability. In contrast, CON oils exhibited an erratic behavior of this variable during these years. Whereas in 2007 the oils of this treatment showed the highest value of oxidative stability (46.0 h), in the following seasons none of the oils of the rest of the treatments showed significantly lower oxidative stability (34.9 and 32.3 h, respectively).

**Fatty Acid Composition.** The presence in the oils of minor fatty acids (<2%) palmitoleic, margaric, margaroleic, estearic, linolenic, araquic, eicosenoic, and behenic was not significantly affected by the irrigation treatment or the season (data not shown). Only the contents of linoleic presented statistically significant changes as a consequence of the different irrigation treatments, whereas the year factor affected both palmitic and oleic contents (Table 1). The greatest differences

Table 1. Fatty Acid Composition of the Oils Extracted from Olive Fruits from a Fully Irrigated Control (CON) and Three Deficit-Irrigation Treatments Applied in Summer: 30% CON in July (DI-J), 30% CON in August (DI-A), and 50% CON in July and August  $(DI-JA)^a$ 

		irrigation treatments			
fatty acid	year	CON	DI-J	DI-A	DI-JA
palmitic	2007	13.4	13.3	13.7	13.6
	2008	12.6	12.8	12.6	12.3
	2009	14.2	14.7	14.0	14.1
	mean	13.4	13.6	13.4	13.3
oleic	2007	73.0	73.6	72.0	72.7
	2008	75.3	75.7	75.1	76.0
	2009	70.1	70.5	70.9	71.1
	mean	72.8	73.3	72.7	73.3
linoleic	2007	9.0 b	8.5 c	9.6 a	8.9 b
	2008	7.5 a	6.9 b	7.4 a	7.0 b
	2009	10.7 a	9.7 b	9.7 b	9.5 b
	mean	9.0 a	8.4 b	8.9 a	8.5 b

"Each value is the mean value of four replicates. For each year, values of the same irrigation treatment followed by the same letter are not significantly different by Duncan's test at P < 0.05. Absence of letters means no significant effect due to irrigation, detected by ANOVA at P < 0.05.

were observed between seasons. The more pronounced differences occurred between the years 2008 and 2009, whereas oils extracted in the 2007 season exhibited intermediate values. Thus, in 2008 the oils exhibited the highest oleic acid content and the lowest palmitic and linoleic contents, regardless of the irrigation treatment from which they were extracted, whereas the opposite situation was observed in 2009. Both CON and DI-A oils, in the average of the three years and during the 2008 season, showed significantly higher linoleic contents than DI-J and DI-JA oils, whereas in 2007 and 2009, respectively, DI-A and CON oils, each individually, showed significantly higher values for this parameter than the oils of the other three treatments. In any case, these differences, although statistically significant, were very slight.

In relation to the fatty acid formulas they basically correlated to the studied factors in the same way as their main components (Table 2). Thus, OLR and MUFA/PUFA exhibited the inverse behavior compared to linoleic content, and SAFA and PUFA followed the same trends as the contents of palmitic and linoleic, respectively. However, the addition of palmitoleic, margaroleic, and gadoleic acids to the predominant content of oleic acid determined that the conjunct of MUFA showed changes due to the different irrigation systems. Therefore, on average, CON and DI-A oils exhibited slightly, but significantly, lower MUFA values than DI-J and DI-JA oils. Finally, UNFA and UNFA/SAFA maintained constant values Table 2. Fatty Acid Formulas of the Oils Extracted from Olive Fruits from a Fully Irrigated Control (CON) and Three Deficit-Irrigation Treatments Applied in Summer: 30% CON in July (DI-J), 30% CON in August (DI-A), and 50% CON in July and August (DI-JA)<sup>*a*</sup>

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formula	year	CON	DI-J	DI-A	DI-JA
$OLR^b$	2007	8.2 b	8.7 a	7.5 c	8.2 b
	2008	10.1 b	10.9 a	10.2 b	10.8 a
	2009	6.6 b	7.3 a	7.3 a	7.5 a
	mean	8.3 b	9.0 a	8.3 b	8.9 a
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SAFA	2007	15.8	15.7	16.2	16.0
	2008	15.2	15.3	15.3	14.9
	2009	16.7	17.1	16.7	16.8
	mean	15.9	16.1	16.1	15.9
MITEAd	2007	747 h	75.2 0	737 6	745 h
MOTA	2007	76.8 bc	75.2 a 77.1 ab	76.6 c	7740
	2008	70.8 00	77.1 aU 72.5	73.0	77. T a
	2009	74.5 h	75.0 0	73.0 74.5 h	75.0 0
	mean	/4.5 0	73.0 a	/4.5 0	/ <b>3.</b> 0 a
PUFA <sup>e</sup>	2007	9.4 b	9.0 c	10.0 a	9.4 b
	2008	7.9 a	7.4 b	7.9 a	7.6 b
	2009	11.2 a	10.3 b	10.3 b	10.0 b
	mean	9.5 a	8.9 b	9.4 a	9.0 b
UNFA <sup>f</sup>	2007	84.1	84.2	83.8	83.9
	2008	84.7	84.6	84.6	85.0
	2009	83.2	82.8	83.2	83.2
	mean	84.0	83.9	83.9	84.0
UNFA/SAFA	2007	5.3	5.4	5.2	5.2
	2008	5.6	5.5	5.5	5.7
	2009	5.0	4.8	5.0	5.0
	mean	5.3	5.2	5.2	5.3
MUFA/PUFA	2007	8.0 b	8.4 a	7.4 c	8.0 b
,	2008	9.7 b	10.4 a	9.7 b	10.3 a
	2009	6.5 b	7.1 a	7.1 a	7.3 a
	mean	80 b	86 2	81 b	85 2

<sup>*a*</sup>Each value is the mean value of four replicates. For each year, values of the same irrigation treatment followed by the same letter are not significantly different by Duncan's test at P < 0.05. Absence of letters means no significant effect due to irrigation detected by ANOVA at P < 0.05. <sup>*b*</sup>Oleic:linoleic ratio. <sup>*c*</sup>Saturated fatty acid. <sup>*d*</sup>Monounsaturated fatty acid. <sup>*f*</sup>Disaturated fatty acid.

near 84.0 and 5.2%, respectively, regardless of the year or the treatment of irrigation considered.

**Tocopherol Contents.** No significant differences in the tocopherol content and composition were observed as a consequence of the irrigation treatments (data not shown). In 2008, the mean data were 329.8, 2.6, 2.0, and 334.4 mg/kg for  $\alpha$ ,  $\beta$ ,  $\gamma$ , and total tocopherols, respectively, whereas in 2009 these compounds presented the following mean contents in the oils: 337.4, 3.0, 2.7, and 343.1 mg/kg.

**Phenol Contents.** The concentrations of hydroxtyrosol, tyrosol, vanillic acid, vanillin, *p*-coumaric acid and pinoresinol were  $\leq 2.5$  mg/kg and were not influenced by irrigation treatments or the crop year (data not shown). Meanwhile, irrigation treatment and year significantly determined changes

in the other phenolic compounds, hydroxytyrosol acetate, the dialdehydic form of the decarboxymethyloleuropein aglycone (3,4 DHPEA-EDA), the dialdehydic form of the decarboxymethylligstroside aglycone (p-HPEA-EDA), acetoxypinoresinol, hydroxytyrosylelenolate (3,4 DHPEA-EA), and luteolin (Table 3).

The oils of DI-J presented significantly higher concentrations than the oils from the other irrigation treatments in hydroxytyrosol acetate, 3,4 DHPEA-EDA, *p*-HPEA-EDA, 3,4 DHPEA-EA, total phenols, *o*-diphenols, and total secoiridoids, also showing the highest mean value in tyrosyl elenolate (*p*-

Table 3. Phenolic Compound Contents (Milligrams per Kilogram) of the Oils Extracted from Olive Fruits from a Fully Irrigated Control (CON) and Three Deficit-Irrigation Treatments Applied in Summer: 30% CON in July (DI-J), 30% CON in August (DI-A), and 50% CON in July and August (DI-JA)<sup>*a*</sup>

		irrigation treatments				
phenolic compound	year	CON	DI-J	DI-A	DI-JA	
hydroxytyrosol acetate	2008	12.6 b	22.3 a	10.8 b	12.8 b	
	2009	11.8 b	15.3 a	10.3 b	10.1 b	
	mean	12.2 b	18.8 a	10.5 b	11.5 b	
3,4 DHPEA-EDA <sup>b</sup>	2008	152.2 b	380.3 a	181.9 b	154.8 b	
	2009	285.2 b	401.4 a	322.6 b	283.6 b	
	mean	218.7 b	390.9 a	252.3 b	219.2 b	
tyrosol acetate	2008	4.5 a	4.7 a	0.0 b	0.0 b	
	2009	9.7 b	16.0 a	12.5 ab	9.4 b	
	mean	7.1 b	10.4 a	6.3 b	4.7 b	
<i>p</i> -HPEA-EDA <sup><i>c</i></sup>	2008	54.9 b	137.1 a	61.5 b	65.8 b	
	2009	138.0	175.9	98.9	136.4	
	mean	96.5 b	156.5 a	80.2 b	101.1 b	
pinoresinol	2008	2.3	2.5	2.3	2.5	
	2009	2.8	1.6	1.6	1.6	
	mean	2.5	2.1	2.0	2.0	
acetoxypinoresinol	2008	26.3	23.1	19.7	21.3	
	2009	34.7 a	19.8 b	16.9 b	15.8 b	
	mean	30.5 a	21.5 b	18.3 b	18.6 b	
3,4 DHPEA-EA <sup>d</sup>	2008	15.5 b	46.4 a	23.2 b	19.7 b	
	2009	31.3 b	54.5 a	45.1 a	34.9 b	
	mean	23.4 b	50.5 a	34.1 ab	27.3 b	
<i>p</i> -HPEA-EA <sup><i>e</i></sup>	2008	6.7 b	9.9 a	5.8 b	10.4 a	
	2009	12.9	15.9	14.5	12.4	
	mean	9.8	12.9	10.2	11.4	
luteolin	2008	2.0	1.8	1.8	1.4	
	2009	3.2 a	1.8 b	3.5 a	2.1 b	
	mean	2.6 a	1.8 b	2.7 a	1.8 b	

<sup>*a*</sup>Each value is the mean value of four replicates. For each year, values of the same irrigation treatment followed by the same letter are not significantly different by Duncan's test at P < 0.05. Absence of letters means no significant effect due to irrigation detected by ANOVA at P < 0.05. <sup>*b*</sup>The dialdehydic form of the decarboxymethyl oleuropein aglycone. <sup>*c*</sup>The dialdehydic form of the decarboxymethylligstroside aglycone <sup>*d*</sup>Hydroxytyrosyl elenolate. <sup>*e*</sup>Tyrosyl elenolate.

HPEA-EA), but without achieving statistical significance compared to the other oils. In contrast, CON and DI-A oils exhibited significantly higher luteolin mean contents than the oils from the other tested treatments (Table 3). The oils extracted in 2009 systematically exhibited higher concentrations in the major phenolic compounds than those extracted in 2008 (Figure 2).



**Figure 2.** Contents of the major groups of phenolic compounds (mg/ kg) of the oils extracted from olive fruits from a fully irrigated control (100%) and three deficit-irrigation treatments applied in summer: 30% in July, 30% in August, and 50% in July and August. Each value is the mean value of four replicates. For each season, values of different irrigation treatments followed by the same letter are not significantly different by Duncan's test at P < 0.05. Absence of letters means no significant effect due to irrigation, detected by ANOVA at P < 0.05.

Sensory Evaluation. According to the opinion of two trained tasters, the different summer irrigations determined changes of the bitter and pungent presence in the oils. DI-J oils were bitterer than the other oils and more pungent than CON and DI-A oils. The positive presence of the 'olive fruit' sensory attribute in the oils was affected by irrigation the 2009 season only, when CON oils exhibited a higher intensity of this attribute than the rest of the oils. Oil sensory evaluation was also affected by the season. In 2007 the oils showed the highest presence of 'olive fruit' attribute and the lowest bitter intensity, perhaps due to the higher RI of the fruits in this year. In 2009, in contrast, the oils exhibited the highest presence of bitterness and pungency. The sensory profile of the DI-J oils systematically showed 'green leaf' flavor notes in the three years of the experiment. In contrast, the DI-A oils showed mature fruity notes in 2007 and 2008, whereas CON oils presented "green leaf" notes in 2008 and 2009, but together with 'banana' flavor notes, which indicate an appreciable level of maturity in the original fruit. The best differentiation of the sensory notes occurred in 2007, when DI-A clearly differed from DI-J ('green leaf rather than 'mature fruity'), whereas CON and DI-JA exhibited the same intermediate profile (green and mature fruity).

## DISCUSSION

As in our results, the absence of effect due to different irrigation treatments on the physico-chemical parameters used to evaluate the VOO quality (free acidity, peroxide value,  $K_{232}$ , and  $K_{270}$ ) is the most common trend found in the scientific literature. Thus, Tovar et al.,<sup>21</sup> d'Andria et al.,<sup>22,23</sup> Gómez-Rico et al.,<sup>24</sup> or Garcia et al.<sup>11</sup> did not find any effect on free acidity or on absorbance

in the UV region as a consequence of the different levels of irrigation. However, other authors<sup>9,25,26</sup> observed that the oil free acidity increased when irrigation increased. It seems that irrigation enhances fruit turgidity, making olive fruits more sensitive to mechanical wounding and, subsequently, to fungal infection, resulting in the deterioration of oil quality parameters. Anyway, irrigation treatments did not cause a loss of the commercial quality category of VOO.<sup>26,27</sup>

The general trend observed in our results that DI-J treatment oils presented a higher oxidative stability than those more watered from CON treatment agrees with those obtained by Motilva et al.<sup>28</sup> and Berenguer et al.<sup>9</sup> with 'Arbequina' olives also. In these experiments deficit irrigation was imposed from pit hardening until 2 weeks before the beginning of ripening and throughout the irrigation period, respectively. However, the different response obtained for DI-J, comparing DI-JA and DI-A treatments, is unprecedented and constitutes valuable information for the design of a strategy for deficit irrigation in summer for olive tree cultivation, mainly for this cultivar, the VOO of which is characterized by exhibiting many aromatic components, but low oxidative stability.<sup>29</sup>

The presence of higher linoleic acid content in VOO has been related to a higher level of irrigation of the olive tree.<sup>9,11</sup> In this sense, the results obtained by the CON oils have responded to this guideline. In contrast, the high linoleic values displayed by the DI-A oils contradict this point of view, which has been previously observed by other authors.<sup>27</sup> However, given this contradictory result and the very slight differences found among treatments, it cannot be concluded that a different level of irrigation could affect the content of this fatty acid in the oils, as has been also previously observed.<sup>28,30</sup> The effects on the composition of fatty acids due to the different seasons have been attributed to the alternant crop load typical of the olive tree (bearing cycle). Ben-Gal et al.<sup>31</sup> found higher values for MUFA/PUFA and UNFA/SAFA ratios in high production seasons ("on" years), alternating with lower values of these ratios in low production season ("off" year). In our results a clear alternant behavior is observed in the MUFA/ PUFA ratio during the three tested seasons, but statistical differences were not found in the UNFA/SAFA ratio. Furthermore, SAFA contents, mainly due to the presence of palmitic acid, also exhibited alternant values at this time, conversely coinciding with the MUFA/PUFA ratio values. However, these alternate responses should not be attributed to a different crop load between the seasons tested, because the differences in production were minimal,<sup>12</sup> but to the different degrees of maturity at which the fruit was harvested each year. Thus, the highest and lowest contents in oleic and linoleic acids, respectively, exhibited by the oils extracted from the youngest fruits (0.4 RI) are foreseeable, because the progress of fruit ripening usually coincides with these changes in fatty acid composition.<sup>7</sup> Nevertheless, the fact that the oils extracted from older fruits in 2007 exhibited higher oleic contents than those produced from younger fruits in 2009 is not explained by this argument. Therefore, other season variables, such as the temperature and/or the illumination during fruit growing, could also play an important role in this parameter.

With regard to the tocopherol contents in the oils, our results confirm the findings of Tovar et al.<sup>32</sup> and Palese et al.,<sup>33</sup> who did not find any effect on the presence of these compounds in the oils extracted from 'Arbequina' and 'Coratina' fruits, which had grown under different irrigation strategies. In contrast, Baccouri et al.<sup>34</sup> observed a significant increase in the

tocopherol contents associated with high irrigation in 'Marsalina' olives, and Stefanoudaki et al.<sup>27</sup> found the opposite: higher tocopherol contents in the oil produced from rain-fed 'Koroneiki' olives compared to those obtained from irrigated trees. Possibly, this disparity in results is due to a different varietal response to irrigation treatments.

As was foreseeable, the significantly higher values of phenol compound contents exhibited by DI-J oils coincided with significantly higher oxidative stability, because both parameters are strongly related, mainly due to the antioxidant action of odiphenol and secoiridoid molecules.35 As in the case of oxidative stability, the increase in irrigation level has been associated with the decrease in the phenol content of the VOO.<sup>31,36,37</sup> This fact explains the higher values obtained from DI-J oils, but it does not explain the significantly lower values obtained from both DI-JA and DI-A oils, from trees that had received similar and lower amounts of water, respectively, distributed in a different form in summer. It clearly demonstrates that this factor also affects the contents in the oil of these nutritionally important molecules. The phenol compounds of the VOO are mainly formed from the enzymatic hydrolysis of glycosylated phenolic compounds (oleuropein, verbascoside, and/or ligstroside) during the process of oil extraction.<sup>38</sup> Therefore, their presence depends on the interaction of many factors: genetics (variety), environmental (cultivation, harvest, and postharvest conditions), physiological (fruit humidity, age, and health), and the conditions of processing (milling, malaxation, centrifugation, and the water amount used).<sup>39</sup> The different timing of water irrigation in summer would affect the physiological state of the fruit at the time of its processing, favoring the action of these enzymes, slowing its ripening and/or modifying its moisture. The differences found between the two treatments in which irrigation reduction occurred in July suggest that the inductive effect of the increased presence of phenolic derivatives in the oil and/or the delaying of the fruit ripening would be associated with a threshold value of water stress above which this process would trigger. Thus, DI-J treatment, which maintained a reduction of 30% CON during the pit hardening, could induce it, whereas the DI-JA, which reduced irrigation only by 50% CON, was not able to provoke it. In 2009 total phenols were higher than in 2008. This fact may be related to the rainfall (150 mm) that occurred in 2008 before harvesting, which determined a higher fruit water content (57%) compared to 2009 (48%) 12

In a similar way, DI-J oils exhibited the highest scores for bitterness and pungency, probably due to their higher content in secoiridoid derivatives, whereas DI-JA and DI-A oils, on average, showed values similar (even lower) to those of the oils from the most watered treatment (CON). Then, our results only partially agree with those found by Gómez-Rico et al.<sup>40</sup> in 'Cornicabra' olives, who observed lower bitterness in the oils extracted from better irrigated fruit. Taking in account that 'Arbequina' oils habitually exhibited a low level of these sensory attributes, the effect of the moment of irrigation on them would be particularly relevant to the VOO industry to obtain a product that displays a higher presence of positive sensory attributes. The sensory profile of the oils clearly indicated as DI-J oils displayed typical notes of oils extracted from fruits with a lower level of ripening than the other treatment fruits. Curiously, in the 2007 season the sensory notes of these oils especially differed from DI-A oils, which were extracted from fruit irrigated with the same amount of water, but distributed in

other times of application in summer. It seems that DI-J treatment induced a delay in the ripening process of the fruit, which determined the consequent delay in the decrease in the phenol content and in the intensity of sensory attributes, which are typical of the oil extracted from more immature fruits.<sup>41</sup> This fact is especially relevant for 'Arbequina' oils, which lose their original level of sensory quality with the progression of fruit ripening.<sup>8</sup>

In summary, different deficit irrigation strategies in summer to a superintensive olive orchard did not determine significant changes in the physical and chemical parameters legally established for evaluating the level of commercial quality (free acidity, peroxide value,  $K_{232}$ , and  $K_{270}$ ) and the tocopherol contents of the virgin oils subsequently extracted during three consecutive seasons. In addition, the application of severe deficit irrigation exclusively in July (30% of the full irrigated) to the olive trees DI-J did not significantly reduce oil production in any of the experimental years. Sixteen percent of irrigation water was saved,<sup>12</sup> but the quality of VOO was significantly modified. It caused a significant increase in oxidative stability that coincided with a significant increase in hydroxytyrosol acetate, 3,4 DHPEA-EDA, p-HPEA-EDA, 3,4 DHPEA-EA, total phenols, o-diphenols, and total secoiridoids in comparison to the other treatment oils. Furthermore, the taste of these oils showed that they were bitterer than the other oils and more pungent than CON and DI-A oils, exhibiting 'green leaf' flavor notes in the three years of the experiment, whereas the other treatment oils also exhibited typical sensory notes from more mature fruits. When severe deficit irrigation was applied in August, no significant differences to CON were observed in any of the evaluated parameters. In consequence, given the importance of the phenol contents for the nutritional and sensorial quality of VOO, our results demonstrate that the moment and intensity of deficit irrigation in summer are relevant factors to take into account for the VOO industry.

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#### Notes

The authors declare no competing financial interest.

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