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Effect of Regulated Deficit Irrigation on the Quality of 'Arbequina' Extra Virgin Olive Oil Produced on a Super-High-Intensive Orchard

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Abstract: The expansion of the super-high-intensive cultivation of olive groves requires irrigation techniques that are compatible with the increasing scarcity of water due to climate change and olive oil demand. For this, the effect of two regulated deficit irrigation treatments (RDI) and a sustained deficit irrigation (SDI) treatment was studied. The treatments consisted of: (i) control treatment, which supplied 100% of the water lost by evapotranspiration (ET_0); (ii) the "optimal RDI" treatment, which only reduced irrigation water (~37–54% reduction) during the pit hardening stage; (iii) the "confederation RDI" which limited water restriction to the donation of the Guadalquivir hydrographic confederation (~72% reduction); and, (iv) the "confederation SDI", similar water restriction (~72%) but dying the whole tree cycle. In general, the reduction in the irrigation water caused no negative effects on the studied parameters. However, the total phenolic content (TPC) was increased when the deficit irrigation was applied. Fatty acid profile showed changes with respect to the control, increasing oleic acid and the total content of monounsaturated fatty acids (MUFA). For the volatile compound profile, reducing water intake caused changes in mayor volatile compound (*trans*-2-hexenal), related with green flavors. The application of deficit irrigation treatments increased the value obtained in the fruity parameter with respect to the control. On the other hand, irrigation deficit treatments did not generate changes in the olive oil yield.

Keywords: antioxidants; fatty acids; *Olea europea*; sensory profile; volatile composition

1. Introduction

In Spain, one of the most important olive oil producers worldwide [1], olive trees have traditionally been cultivated extensively under rainfed conditions, but in recent decades, there has been a shift towards super-intensive cultivation methods, increasing the number of trees per hectare, mainly due to the increase in consumer demand, promoted by the recommendations of the Food and Agriculture of the United Nations [2] based on its nutritional and sensory profiles [3]. This change in the irrigation system has drastically increased the water needs of olive orchards.

In Spain, the region with the highest cultivation area of olive trees is Andalucía, which is a region with a high level of desertification risk, due to its low rainfall and high temperature. For these reasons, new agricultural strategies are being developed based on reducing irrigation water but without reducing production or quality, such as the use of plant covers [4–6], precision irrigation [7] and/or the application of deficit irrigation [8]. The main two deficit irrigation strategies are: (i) regulated deficit irrigation (RDI), which reduces water during one or more phenological stages of fruit development, and (ii) sustained deficit irrigation (SDI), which reduces water throughout the whole fruit development process.

In the case of olive trees, previous studies have shown that the application of deficient water during pit hardening (phase II) [9,10] did not negatively affect production. In this sense, the Food Quality and Safety research group of the Miguel Hernández University (UMH, Spain) developed a brand, hydroSOStainable products, to recognize farmers and producers that apply and control deficit irrigation techniques and fulfil the brand requirements [11,12]. Thus, the aim of this study was to analyze the effects of two RDI treatments with different intensities and one SDI treatment (adjusted to the reality of the Spanish agriculture and water availability), on the quality of the hydroSOStainable olive oil produced, by analyzing the effects on: (i) the IOC (International Olive Council) quality parameters, (ii) the antioxidant activity ($ABTS^{+\bullet}$ and $DPPH^{\bullet}$ methods), (iii) the total phenolic content (TPC) and (iv) the lipidic, volatile and sensory profiles, along two consecutive seasons (2018 and 2019).

2. Materials and Methods

2.1. Experimental Design and Sample Processing

Olive oil was obtained from 13-year-old 'Arbequina' olive trees cultivated in a super-high density (4.0 m × 1.5 m) orchard located in Carmona (37.49° N, −5.67° W, Seville, Spain). Four irrigation treatments were randomly distributed in blocks (Figure 1) with 60 trees per plot. Olives were mechanically harvested when presented a maturity index of 1.9 [13]; the grape straddle harvester (New Holland Braud Olive) selectively collected samples for each treatment (central line of each plot) and emptied the harvested olives into individual bags; then, the samples were manually cleaned and prepared for the production of the olive oil.

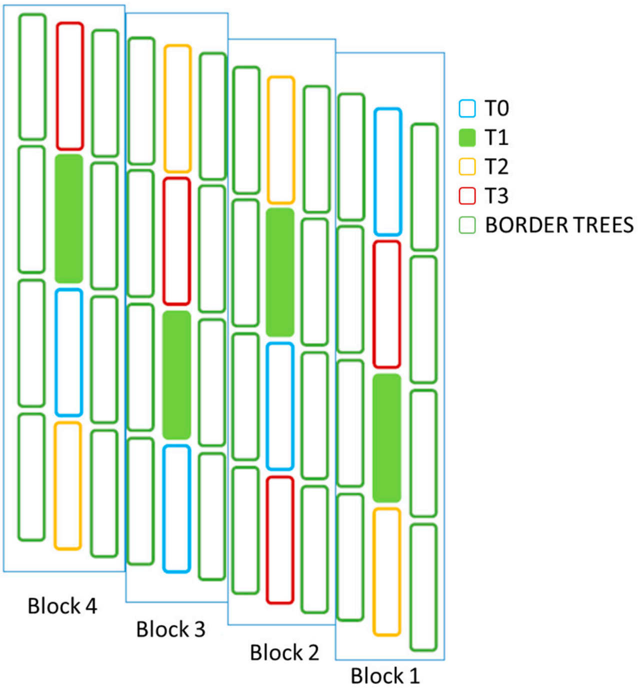

Figure 1. Distribution of deficit irrigation treatments (2018 and 2019 seasons) at a super-high density 'Arbequina' olive trees orchard.

Olive oil was obtained using an olive mill model Frantoino Bio (Toscana Enologica Mori, Florence, Italy) at 40–50 kg h^{-1}, with an aqueous two-phase oil extraction system. In total, 100 kg of olive samples was cleaned, grounded (T < 28 °C, 20 min) with 1% (*w*:*w*) talc ore and 2% (*w*:*w*) water.

A pressure chamber (PMS Instrument Company, Albany, OR, USA) was used in 4 trees per treatment to determine the *midday stem water potential* (Ψ_{stem}). The water stress integral (*SI*) was calculated (Equation (1)) to describe the accumulative effect of deficit irrigation strategies, from the beginning of pit hardening to harvest (19 June–14 October in 2018 (117 days) and 14 June–24 October in 2019 (132 days)):

$$SI = \Sigma\,[\Psi - (-0.2)] \times n \qquad (1)$$

where *SI* is the stress integral, Ψ_{stem} is the average midday stem water potential for any interval and *n* is the number of the days in the interval.

Four irrigation treatments were established and conditions were monitored and controlled using the pressure chamber technique and the threshold values of midday stem water potential before and after the pit hardening period:

- Control (T_0): full irrigated conditions [14].
- RDI (T_1): trees had no water restriction along phases I and III (the beginning of olive fruit growth and fruit maturation with oil accumulation, respectively, while regulated irrigation was applied at phase II (pit hardening), with 37% and 54% of water reduction in the 2018 and 2019 seasons, respectively.
- Confederation RDI (T_2): same conditions established in T_1 (deficit irrigation applied at phase II), but with a water restriction set up at donation of Guadalquivir hydrographic confederation (67% of water reduction in 2018 and 72% of water reduction in 2019).
- Confederation SDI (T_3): sustained deficit irrigation with the same water restrictions as T_2 but during the whole cycle of the olive tree.

2.2. Quality Parameters

Chemical quality parameters of olive oil are defined by European Union Regulation [15] and are used to classify olive oils in different commercial categories. Free acidity (% of oleic acid), peroxide value (meq O_2 kg^{-1} oil) and ultraviolet (UV) extinction coefficients (K_{232}, K_{270} and ΔK indexes) were analyzed following the procedure described by European Union Commission Regulation 2568/91 [15] and International Olive Council decisions [16–18]. UV absorption indexes were determined in a UV–visible spectrophotometer (Helios Gamma model, UVG 1002E; Helios, Cambridge, UK), using cyclohexane and a 10 mm quartz cuvette.

2.3. Determination of Antioxidant Activity and Quantification of Total Phenolic Content

The extract used for total phenolic content (TPC) and antioxidant activity (AA) quantification was prepared as previously described by Tuberoso et al. [19] with some modifications. Briefly, 3 g of olive oil was mixed with 5 mL of methanol/water (80/20, *v*/*v*). The mixture was shaken for 2 min and the hydrophilic phase was filtered with a GD/X 0.45 µm cellulose acetate filter (25 mm, Sartorius, Madrid, Spain). This procedure was repeated twice with the lipophilic phases, and all of the hydrophilic extracts were evaporated in a rotary evaporator at 35 °C. Finally, the residue was dissolved in 1.5 mL of methanol.

The antioxidant activity [$ABTS^{+\bullet}$ (azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) and $DPPH^{\bullet}$ (2,2-diphenyl-1-pirylhydrazyl)] were carried out as described by Re et al. [20] and Brand-Williams et al. [21], respectively. The decrease in absorbance was measured at 734 nm ($ABTS^{+\bullet}$) and 515 nm ($DPPH^{\bullet}$) using a UV–visible spectrophotometer (Helios Gamma model, UVG 1002E; Helios, Cambridge, UK). Analyses were run in triplicate and the results were expressed as mmol Trolox eq L^{-1} of olive oil.

Total phenolic content (TPC) was quantified using Folin–Ciocalteu reagent according to Gao et al. [22]. Absorption was measured at 760 nm using a UV–visible spectrophotometer (Helios Gamma model, UVG 1002E; Helios, Cambridge, UK). Analysis was run in triplicate, and the results were expressed as mg gallic acid equivalents (GAE) L^{-1} of olive oil.

2.4. Fatty Acid Profile

The fatty acid profile was determined following ISO-12966-2 [23] and using C13:0 (0.04 mg mL^{-1}) as internal standard for later quantification. After extraction and methylation, for separation and quantification, a gas chromatograph (GC) Shimadzu GC-2030 coupled with a flame ionization detector (FID) an automatic injector AOC-20i was used. Helium was used as carrier gas and nitrogen as a make-up gas (40 mL min^{-1}). FID used hydrogen and air, at rates of 35 mL min^{-1} and 350 mL min^{-1}, respectively. The GC system used a Supelco SP®-2380 capillary column (length: 60 m, internal diameter: 0.25 mm and film thickness: 0.20 µm). The injector and detector temperatures were 250 and 260 °C, respectively and a 1:20 split ratio was used. A helium lineal flow velocity of 28.4 cm s^{-1} was also used. The oven temperature started at 70 °C and increased up to 250 °C at a rate of 3 °C min^{-1}. Methyl fatty acids were identified as compared with retention times with FAME Supelco MIX-37 standards (Supelco Company, Bellefonte, PA, USA). Results were calculated as percentage of each fatty acid in the total fatty acid profile.

Additionally, atherogenic index (*AI*) (Equation (2)) and thrombogenic index (*TI*) (Equation (3)) were calculated according to Sánchez-Rodríguez et al. [24].

$$AI = (4 \times [14:0] + [16:0]) \ / \ \left[\sum\nolimits_{PUFA\ (n-3)} + \sum\nolimits_{PUFA\ (n-6)} + \sum\nolimits_{MUFA}\right] \tag{2}$$

where [14:0] and [16:0] are the contents (%) of myristic and palmitic acids, respectively, and *MUFA* and *PUFA* are the contents of monounsaturated and polyunsaturated fatty acids, respectively.

$$TI = ([14:0] + [16:0] + [18:0]\ ) / \left[0.5 \times \sum\nolimits_{MUFA} + 0.5 \times \sum\nolimits_{PUFA\ (n-6)} + 3 \times \sum\nolimits_{PUFA\ (n-3)} + (n-3)/(n-6)\right] \tag{3}$$

where [18:0] is the content (%) of stearic acid.

2.5. Volatile Compound Profile

Volatile compounds were extracted by headspace solid-phase micro-extraction (HS-SPME). Analysis was carried out according to Sánchez-Rodríguez et al. [25] with some modifications. Briefly, 10 mL of olive oil was added to a 15 mL glass vial with 10 µL of benzyl acetate as internal standard (1000 mg L^{-1}) using 50/30 µm DVB/CAR/PDMS fiber (Supelco Company, Bellefonte, PA, USA). Vials were maintained in a temperature-controlled water bath at 40 °C for 60 min (10 for equilibration and 50 min for extraction) with continuous agitation (500 rpm) in a magnetic stirrer (IKA C-MAG HS 4, IKA-Werke GmbH & Co. KG, Staufen, Germany). After that, fiber was inserted into the injector at 250 °C for 3 min to directly desorb volatile compounds into the GC column.

A gas chromatograph Shimadzu GC-17A coupled to a mass spectrometer (Shimadzu QP-5050A (Shimadzu Corporation, Kyoto, Japan) was used for the isolation and identification of the volatile compounds. To separate volatile compounds, an Rxi-1301Sil MS (Restek Corporation, Bellefonte, PA, USA) column was used (length: 30 m, internal diameter: 0.25 mm and film thickness: 1.0 µm), using helium as a carrier gas at 0.6 mL s^{-1} in a splitless mode. Injector and detector temperatures were 230 and 300 °C, respectively. The oven program was as follows: initial temperature 40 °C for 3 min; rate of 5 °C min^{-1} up to 100 °C; and rate of 3 °C min^{-1} until 300 °C and held for 3 min.

To characterize volatile compounds, chromatogram was analyzed using GCMS Postrun Analysis (Shimadzu Corporation, Kyoto, Japan) software and individual compounds were identified by three methods: (i) mass spectrum (original chemical compound and collection of the Wiley 229 and NIST 14 spectrum libraries); (ii) retention index of standards (RI); and (iii) retention indexes calculated using the C7 to C16 *n*-alkane mix (Sigma-Aldrich, Steinheim, Germany). Experimental retention index was compared with literature index obtained from National Institute of Standards and Technology [26].

2.6. Descriptive Sensory Analysis

Descriptive sensory analysis was carried out using the trained panel of the Food Quality and Safety (CSA) research group of the Miguel Hernández University. This trained panel consisted of 8 panelists (4 males and 4 females, between 25 and 50 years old) with more than 1000 training hours in sensory analysis, specifically in vegetables and fruits. Moreover, panelists had special training in olive oil sensory analysis for 2 days, using standard samples kindly provided by the International Olive Oil Council (IOC).

Four sessions were carried out, one session per each irrigation treatment, following the European regulation n° 2568/91 [15]. The descriptors used were those defined by IOC [27] to classify olive oils into different commercial categories and were classified in two groups: (i) positive attributes (fruity, bitter and pungent) and (ii) negative attributes (musty, fusty and rancid, all scored in the general attribute "defects").

2.7. Statistical Treatment

Two-way analysis of variance, with factors being (i) season (2018 and 2019) and (ii) irrigation treatment (T_0, T_1, T_2 and T_3), and Tukey's multiple range test were carried out. XLSTAT software (Addinsoft, version 2014.1, Paris, France) was used. Statistical significance was stablished at $p < 0.05$, and all analyses were run, at least, in triplicate.

3. Results and Discussion

3.1. Irrigation Stress, Water Used and Production

Figure 2 shows the daily crop reference evapotranspiration (ET_0) and daily rainfall for the 2018 and 2019 seasons. In the 2018 season, last spring rains occurred at the end of May (day of year, DOY, 146) and autumn rains started on DOY 284 (11 October). In the 2019 season, the dry period was longer, from 24 April to 19 October (DOY 116 and 292, respectively). These climatic conditions are typical of Mediterranean areas, with dry and hot periods from late spring until early autumn. Similar reference evapotranspiration (ET_0) behaviors were observed in both seasons, with the highest ET_0 values reached in July and August.

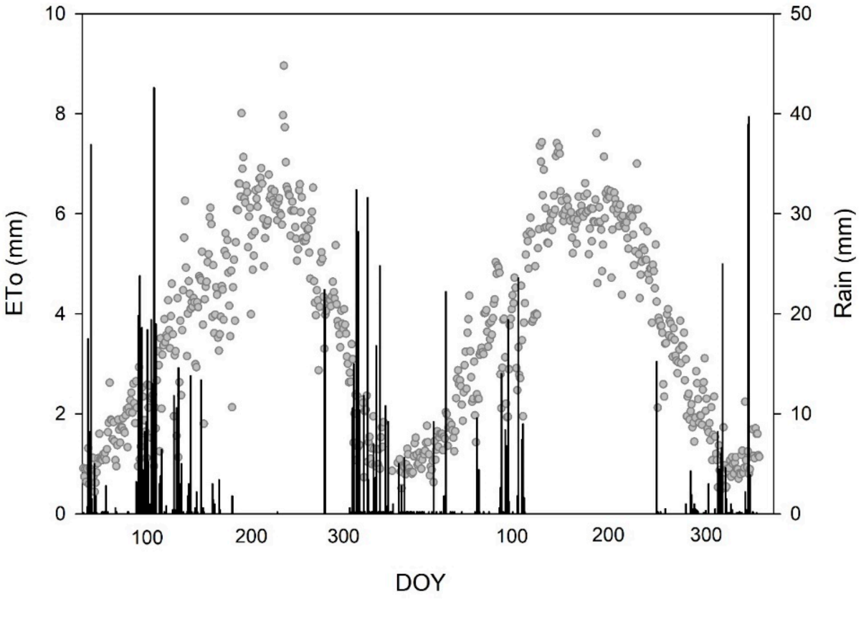

Figure 2. Daily crop reference evapotranspiration (ET_0, dots) and daily rainfall (vertical bars) for the 2018 and 2019 seasons. Data obtained from Villanueva de Rio y Minas (Seville) meteorological station.

Crop water status (minimum stem water potential, min Ψ_{stem} and water stress integral, *SI*), applied water (*AW*) and olive production are summarized in Table 1. The water used in 2019 to reach the targeted water conditions was higher (484 L m^{-2} or mm) compared to that needed (304 L m^{-2}) in 2018. In both seasons under study, the highest volume of irrigation water was that used in the control treatment, T_0 (mean of the two seasons 753 L m^{-2}) while the lowest volume was applied in T_2 and T_3 treatments, with 221 L m^{-2} and 212 L m^{-2}, respectively. Minimum Ψ_{stem} values were negatively correlated with the applied water volume (R^2 = 0.6689); this way, the lowest values of Ψ_{stem} were observed in T_2 and T_3 olive trees.

Table 1. Effect of the season (2018 and 2019) and irrigation treatment (T_0, T_1, T_2 and T_3) on minimum stem water potential (min Ψ_{stem}), water stress integral (*SI*, MPa day^{-1}), applied water (*AW*, L m^{-2}) and olive oil production in each deficit irrigation treatment during pit hardening in a super-high-density 'Arbequina' olive tree orchard.

Factor	Min Ψ_{stem} (MPa)	SI Total (MPa Day^{-1})	SI Phase II (MPa Day^{-1})	SI Phase III (MPa Day^{-1})	AW (L m^{-2})	Olive Oil Production (kg Oil ha^{-1})
ANOVA †						
Season	n.s.	n.s.	n.s.	n.s.	***	n.s.
Irrigation	**	**	**	**	***	n.s.
Season × Irrigation	**	**	**	**	***	n.s.
Tukey Multiple Range Test ‡						
Season						
2018	−3.35	76.0	54.6	16.9	304 b	2198
2019	−3.47	103	71.9	30.2	484 a	2025
Irrigation						
T_0	−1.88 a	9.53 c	9.25 c	0.17 b	753 a	2192
T_1	−2.94 ab	46.3 bc	43.24 bc	2.09 b	390 b	2162
T_2	−4.96 c	179 a	127 a	48.8 a	221 c	2035
T_3	−3.87 bc	123 ab	73.2 ab	43.9 a	212 c	2056
Season × Irrigation						
2018 × T_0	−2.21 ab	18.4 b	18.2 b	0.00 b	533 b	2348
2018 × T_1	−2.74 abc	40.7 b	38.7 b	0.48 b	334 cd	2169
2018 × T_2	−4.71 bc	140 ab	104 ab	30.0 ab	173 e	2240
2018 × T_3	−3.75 abc	105 ab	57.6 ab	37.1 ab	175 e	2034
2019 × T_0	−1.54 a	0.681 b	0.338 b	0.34 b	972 a	2037
2019 × T_1	−3.15 abc	51.8 b	47.8 ab	3.70 b	446 bc	2154
2019 × T_2	−5.21 c	219 a	151 a	66.0 a	269 de	1831
2019 × T_3	−3.99 abc	141 ab	88.9 ab	50.7 ab	248 de	2077

† n.s. = not significant at $p > 0.05$, ** and *** significant at $p < 0.01$ and $p < 0.005$; ‡ Values followed by the same letter, within the same column and factor, were not significantly different ($p > 0.05$), according to the Tukey's least significant difference test.

Regarding the applied water, the T_2 and T_3 treatments (Guadalquivir hydrographic confederation RDI and confederation SDI) resulted in the highest reductions regarding the volume of applied water, reaching values as high as 69.9 and 70.8%, respectively (mean of seasons 2018 and 2019), of the total water applied in the control trees.

The seasonal stress integral (SI total) was not affected by the factor season, but it was affected by the irrigation treatment, as expected. The highest SI value was achieved in T_2 trees (179 MPa day^{-1}) followed by those included in T_3. Finally, the SI values also depended on the phenological stage, with the highest values being found in the T_2 trees, especially at phase II (pit hardening). Moreover, the minimum Ψ_{stem} and the SI were negatively correlated, with SI total increasing as the Ψ_{stem} decreased (R^2 = 0.9337).

3.2. Analytical Parameters of Olive Oil Quality

In general, no important differences were found for the quality parameters (Table 2); this was expected because all samples were obtained from freshly harvested olives without significant defects. However, in the 2018 season, the peroxide index was higher in the control treatment, T_0 (11.8 meq O_2 kg^{-1}) compared to that observed in the stressed samples.

Similar effects have been previously observed, with lower peroxide index values in samples from water-stressed olive trees [28].

Table 2. Quality parameters of 'Arbequina' olive oil samples, obtained from a super-high-density olive tree orchard, according to the requirements of the International Olive Council [27].

Factor	Peroxide Index (meq O_2 kg^{-1})	Acidity Index (% Oleic Acid)	K_{232}	K_{270}	Δk	Commercial Classification
ANOVA [†]						
Season	***	n.s.	n.s.	***	n.s.	
Irrigation	***	***	**	n.s.	n.s.	
Season × Irrigation	***	***	n.s.	***	n.s.	
Tukey Multiple Range Test [‡]						
Season						
2018	8.21 b	0.240	1.80	0.086 b	0.004	EVOO [¥]
2019	12.0 a	0.214	1.76	0.127 a	0.002	EVOO
Irrigation						
T_0	12.6 a	0.317 a	2.00 a	0.101	0.002	EVOO
T_1	9.73 b	0.207 b	1.78 ab	0.101	0.004	EVOO
T_2	8.74 b	0.192 b	1.66 b	0.111	0.004	EVOO
T_3	9.34 b	0.191 b	1.69 ab	0.113	0.002	EVOO
Season × Irrigation						
2018 × T_0	11.8 ab	0.417 a	1.96	0.082 c	0.004	EVOO
2018 × T_1	8.15 bc	0.206 b	1.96	0.086 bc	0.003	EVOO
2018 × T_2	5.87 c	0.187 b	1.57	0.088 bc	0.004	EVOO
2018 × T_3	7.02 bc	0.150 b	1.72	0.089 bc	0.004	EVOO
2019 × T_0	13.4 a	0.218 b	2.04	0.120 ab	0.001	EVOO
2019 × T_1	11.3 ab	0.208 b	1.61	0.116 abc	0.004	EVOO
2019 × T_2	11.6 ab	0.197 b	1.75	0.134 a	0.004	EVOO
2019 × T_3	11.7 ab	0.232 b	1.66	0.137 a	0.000	EVOO

[†] n.s.= not significant at $p > 0.05$, ** and *** significant at $p < 0.01$ and $p < 0.005$; [‡] Values followed by the same letter, within the same column and factor, were not significantly different ($p > 0.05$), according to Tukey's least significant difference test. [¥] EVOO = Extra Virgin Olive Oil.

A similar behavior to that previously described for the peroxide index was also found for the acidity index in the 2018 season, where the T_0 value (0.42% oleic acid) was higher than those found for the rest of treatments (mean of 0.18%).

All samples under study were classified as extra virgin olive oil according to the Official Commercial Classification stablished by International Olive Oil Council [27]. This way, it can be concluded that deficit irrigation treatments did not reduce the quality of the olive oil; similar results were previously reported by Gómez del Campo and García [29] for the same olive variety, in Toledo (central Spain), despite the water reduction levels.

3.3. Determination of Antioxidant Activity and Quantification of Total Phenolic Content

The irrigation treatment did not have a significant ($p < 0.05$) effect on antioxidant activity (AA) as determined by the two methods used ($ABTS^+$ and $DPPH^\bullet$) (Table 3); however, the antioxidant activity was significantly ($p < 0.05$) higher for the samples from the 2019 season compared to those determined in the samples from the 2018 season. However, the total phenolic content (TPC) was significantly ($p < 0.05$) affected by both factors, the season and the irrigation treatment, with the trees included in the treatments with deficit irrigation (T_1–T_3) showing significantly higher values than those observed in oil samples obtained from the control trees (T_0), and the samples from 2018 had higher values than those from the 2019 season. This experimental positive finding on polyphenol content was possibly due to climatologic conditions.

Table 3. Antioxidant activity (mmol Trolox eq L^{-1}) and total phenolic content (mg GAEL L^{-1}) in 'Arbequina' olive oil samples obtained from a super-high-density olive tree orchard.

Factor	**TPC (mg GAEL L^{-1})**	**ABTS (mmol Trolox eq L^{-1})**	**DPPH (mmol Trolox eq L^{-1})**
ANOVA †			
Season	**	**	*
Irrigation	**	n.s.	n.s.
Season × Irrigation	**	**	*
Tukey Multiple Range Test ‡			
Season			
2018	86.1 a	0.262 b	0.179 b
2019	74.9 b	0.439 a	0.289 a
Irrigation			
T_0	53.7 b	0.325	0.233
T_1	77.0 ab	0.404	0.223
T_2	92.9 a	0.337	0.201
T_3	98.5 a	0.337	0.279
Season × Irrigation			
2018 × T_0	59.0 ab	0.198 b	0.089 b
2018 × T_1	90.4 ab	0.341 ab	0.193 ab
2018 × T_2	91.9 ab	0.220 b	0.196 ab
2018 × T_3	103 a	0.289 ab	0.239 ab
2019 × T_0	48.5 b	0.452 a	0.377 a
2019 × T_1	63.6 ab	0.467 a	0.254 ab
2019 × T_2	93.9 ab	0.454 a	0.206 ab
2019 × T_3	93.7 ab	0.384 ab	0.319 ab

† n.s. = not significant at $p > 0.05$, * and ** significant at $p < 0.05$ and $p < 0.01$; ‡ Values followed by the same letter, within the same column and factor, were not significantly different ($p > 0.05$), according to Tukey's least significant difference test.

Sena-Moreno et al. [30] observed increases in the antioxidant activity of 218.1% and 153.4% when irrigation water was supplied at 20% and 15% of control water dose, respectively. A similar trend to that described for the AA can be observed in the current study but for TPC; this way, the two treatments following the confederation restrictions (T_2 and T_3) reached values of 92.9 and 98.5 mg GAE L^{-1} compared to 53.7 mg GAE L^{-1} found for the control oil (Table 3). Those TPC values were lower than those reported in previous studies [25,31]. On the other hand, Dag et al. [32] concluded that an extremely intense irrigation deficit could negatively affect the TPC.

The effects caused by water stress on the values of the TPC were similar to those previously reported in the literature. For example, Ahumada-Orellana et al. [28] showed that polyphenols increased by 26.8%, 52.2% and 49.3% when minimum Ψ_{stem} was reduced from -1.2 MPA to -3.5 MPA, -5.0 MPA and -6.0 MPA, respectively. In the current study, in the 2018 season, the TPC increased by 55.2%, 56.9% and 77.6% compared to the value of the control treatment when the control min Ψ_{stem} (-2.21 MPa) was reduced to -2.74 MPA, -4.71% and -3.75%, respectively. A similar trend was also observed in the 2019 season, with TPC increasing by 31.3%, 93.8% and 93.8% compared to the control when min Ψ_{stem} decreased from the -1.54 MPa of the control down to -3.15, -5.21 and -3.99 MPa, respectively.

3.4. Fatty Acids Profile

Fifteen fatty acids were identified and quantified in 'Arbequina' extra virgin olive oils (Table 4). In both years, oleic acid (C18:1 *cis* 9) was the major fatty acid in all olive oil samples (49.6% in 2018 and 44.1% in 2019). Regarding the irrigation treatment, higher oleic acid content was reached by sustained deficit irrigation (T_3, 48.9%). These oleic acid contents were lower than that expected for olive oil samples. For instance, Ahumada-Orellana et al. [28] and Gómez del Campo and García [29] observed values close to 70% of total fatty acids for the same variety, although no changes were reported in water-stressed samples. However, Hernández et al. [33] observed similar effects in the oleic acid content of other 'Arbequina' orchards when deficit irrigation was applied. Those differences could be generated by the different locations of the orchards [34] and edaphoclimatic conditions [35]. In this sense, the highest concentration of oleic acid was reached in samples obtained from trees subjected to sustained deficit irrigation (T_3, 48.9%). The second most abundant compound was palmitic acid (C16:0), with 14.6% and 18.4% in the 2018 and 2019 season, respectively. This fatty acid did not present significative differences among irrigation treatments. The third predominant compound was linoleic acid (C18:2 *cis6*), which had a season mean content of 11.6% in 2018 and 17.4 in 2019.

Regarding fatty acid families, monounsaturated fatty acids (MUFAs), with oleic acid as the main compound, were the predominant family (62% in 2018 and 55.6% in 2019), and their content depended on the irrigation treatment, especially higher after T_3 (61.1%). This family is extensively associated with beneficial effects on human health [36]. Saturated fatty acids (SFAs) were the second most concentrated family, with 17% in 2018 and 24.5% in 2019. Finally, polyunsaturated fatty acids (PUFAs) were lower in the 2018 season than in the 2019 season (12.5% and 19.2%, respectively), and control irrigation treatment reached the highest content (16.9%) and lower PUFA content was reached after T_2, 14.9%.

Regarding the health indicators, thrombogenic index (TI) and atherogenic index (AI) values were higher in the 2019 season (0.541 and 0.249, respectively). On the other hand, irrigation treatments only had an effect on the atherogenic index, with lower values reached when sustained deficit irrigation was applied (T_3, 0.221). Both parameters are associated with correct blood circulation [37]. Similar values were obtained by Sánchez-Rodríguez et al. [24] with the same variety and other irrigation treatments.

3.5. Volatile Compound Profile

In general, the concentration of the volatile compounds in olive oil was significantly affected by the irrigation practices, as previously reported by other authors [38,39], but not by the factor season, with 18 compounds being identified and quantified in the volatile profile of the super-high-intensive 'Arbequina' oils under study (Table 5). These compounds provide olive oils with notes of alcohol, apple, sweet, sour, bitter, green, pungent, almond, astringent, leaves, earthy, fruity, floral and herbal, among others, all of them positive characteristics of an extra virgin olive oil, EVOO [25,40,41].

No effect caused by the factor season was observed on the volatile profile of these oils and the mean values of the 2018 and 2019 seasons are summarized in Table 5. The two predominant compounds were *trans*-2-hexenal and *trans*-2-hexen-1-ol; however, the irrigation treatment only caused significant effects on the content of the first compound, with T_1 and T_3 generating equivalent contents (91.67 and 96.28, respectively). The *trans*-2-hexenal compound is one of the main compounds in the volatile fraction of olive oils, especially in extra virgin olive oils [42]. In this sense, the concentration of this compound significantly affected the final concentration of volatile compounds present in the oils, representing ~46.5%. Oils obtained after T_0 and T_3 had the highest values for total volatile content (188 and 186, respectively). These results agreed with those obtained by Gómez-Rico et al. [43], who observed that the volatile compounds most affected by irrigation in 'Cornicabra' oils were *trans*-2-hexenal, *trans*-2-hexen-1-ol and the hexen1-ol.

Olive oils also showed significant differences in the contents of ethanol, benzaldehyde, hexyl acetate, *trans*-ß-ocimene, benzyl alcohol, acetophenone and benzoic acid. Oil samples obtained after T_1 had the highest values of benzaldehyde and *trans*-ß-ocimene (8.14 mg 100 g^{-1} olive oil and 2.19 mg 100 g^{-1} olive oil, respectively). However, some aldehydes present in olive oils can provide undesirable sensory attributes such as sweaty, musty-damp or vinegary [42], although this is not the case. On the other hand, the control and confederation SDI treatments led to olive oil with the highest contents of hexyl acetate (3.54 mg 100 g^{-1} olive oil and 4.77 mg 100 g^{-1} olive oil, respectively). Finally, oil samples from T_0 and T_2 stood out for their contents of benzyl alcohol and benzoic acid.

Table 4. Fatty acid composition (% of total fatty acids) in ‘Arbequina’ olive oil samples obtained from a super-high-density olive tree orchard.

Factor	C14:0	C16:0	C16:1	C16:1	C17:0	C17:1*cis*10	C18:0	C18:1*cis*9	C18:1*cis*11	C18:2*cis*6	C20:0	C20:1n9	C18:3n3	C22:0	C20:4n6	Total	*MUFA* ¥	*PUFA* ¥	*SFA* ¥	*TI* ¥	*AI* ¥
ANOVA †																					
Season	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
Irrigation	n.s.	n.s.	n.s.	***	***	***	n.s.	***	n.s.	***	n.s.	n.s.	n.s.	n.s.	n.s.	***	***	***	***	n.s.	***
Season × irrigation	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
Tukey’s Multiple Range Test ‡																					
Season																					
2018	0.031 b	14.6 b	0.152 b	1.76 b	0.083 b	0.179 b	1.82 b	49.6 a	10.01 a	11.6 b	0.355 b	0.264 b	0.598 b	0.111 b	0.234 a	91.4 b	62.0 a	12.5 b	17.0 b	0.424 b	0.198 b
2019	0.045 a	18.4 a	0.430 a	3.8 a	0.386 a	0.703 a	4.17 a	44.1 b	5.74 b	17.4 a	1.11 a	0.764 a	1.77 a	0.387 a	0.005 b	99.3 a	55.6 b	19.2 a	24.5 a	0.541 a	0.249 a
Irrigation																					
T_0	0.043	16.7	0.281	2.96 a	0.191 b	0.374 c	2.90	45.6 b	8.04	15.5 a	0.722	0.521	1.262	0.253	0.114	95.5 ab	57.8 b	16.9 a	20.8 ab	0.482	0.226 a
T_1	0.037	16.3	0.284	2.72 b	0.222 ab	0.412 bc	2.93	46.0 b	7.88	14.3 bc	0.721	0.516	1.158	0.240	0.113	93.8 b	57.8 b	15.6 bc	20.4 b	0.482	0.224 ab
T_2	0.035	16.3	0.299	2.84 ab	0.265 a	0.502 a	3.07	46.9 b	7.56	13.7 c	0.747	0.484	1.132	0.261	0.118	94.1 b	58.6 b	14.9 c	20.6 ab	0.486	0.223 ab
T_3	0.037	16.8	0.300	2.68 b	0.260 a	0.475 ab	3.10	48.9 a	8.16	14.5 b	0.749	0.534	1.185	0.242	0.133	98.0 a	61.1 a	15.8 b	21.2 a	0.479	0.221 b
Season × irrigation																					
2018 × T_0	0.038 abc	14.6 bc	0.154 b	1.58 c	0.069 c	0.155 d	1.93 c	49.2 b	10.0 a	12.1 c	0.362 b	0.273 b	0.640 b	0.111 b	0.222 a	91.6 b	61.5 b	13.0 cd	17.1 bc	0.426 b	0.198 b
2018 × T_1	0.029 bc	14.1 c	0.145 b	1.65 c	0.083 c	0.168 d	1.70 c	47.7 bc	10.2 a	11.3 cd	0.328 b	0.248 b	0.564 b	0.099 b	0.221 a	88.5 b	60.1 bc	12.1 cd	16.3 c	0.422 b	0.197 b
2018 × T_2	0.028 bc	14.3 c	0.153 b	1.92 c	0.095 c	0.193 d	1.81 c	48.8 b	9.46 a	10.8 d	0.362 b	0.265 b	0.570 b	0.129 b	0.232 a	89.1 b	60.8 b	11.6 d	16.7 bc	0.428 b	0.199 b
2018 × T_3	0.027 c	15.3 b	0.154 b	1.88 c	0.087 c	0.198 d	1.86 c	52.5 a	10.6 a	12.3 c	0.369 b	0.269 b	0.617 b	0.105 b	0.260 a	96.6 a	65.6 a	13.2 c	17.8 b	0.420 b	0.196 b
2019 × T_0	0.048 a	18.8 a	0.407 a	4.34 a	0.314 b	0.592 c	3.86 b	42.0 e	6.05 b	18.9 a	1.08 a	0.768 a	1.88 a	0.395 a	0.005 b	99.4 a	54.1 d	20.8 a	24.5 a	0.538 a	0.254 a
2019 × T_1	0.044 ab	18.5 a	0.424 a	3.79 b	0.362 ab	0.656 bc	4.16 ab	44.3 de	5.56 b	17.4 b	1.12 a	0.785 a	1.75 a	0.382 a	0.005 b	99.2 a	55.5 d	19.2 b	24.5 a	0.543 a	0.250 a
2019 × T_2	0.041 abc	18.2 a	0.445 a	3.76 b	0.436 a	0.811 a	4.33 a	44.9 cde	5.66 b	16.6 b	1.13 a	0.703 a	1.70 a	0.393 a	0.005 b	99.1 a	56.3 d	18.3 b	24.5 a	0.543 a	0.246 a
2019 × T_3	0.046 a	18.2 a	0.445 a	3.49 b	0.434 a	0.752 ab	4.34 a	45.3 cd	5.70 b	16.7 b	1.13 a	0.798 a	1.75 a	0.378 a	0.006 b	99.5 a	56.5 cd	18.4 b	24.6 a	0.539 a	0.246 a

† n.s. = not significant at $p > 0.05$ and *** significant at $p < 0.005$; ‡ Values followed by the same letter, within the same column and factor, were not significantly different ($p > 0.05$), according to the Tukey’s least significant difference test. ¥ *MUFA* = Monounsaturated fatty acid; *PUFA* = Polyunsaturated fatty acid; *SFA* = Saturated fatty acid; *TI* = Thrombogenic index; *AI* = Atherogenic index.

In previous studies, Sánchez-Rodríguez et al. [24] and Sánchez-Rodríguez et al. [25] demonstrated that RDI and SDI led to extra virgin olive oil with similar or even higher amounts of the main volatile. These results agreed well with those obtained in the current study in the sense that both studies demonstrated that the applications of irrigation strategies had no negative effects on the volatile profiles of olive oil.

Table 5. Volatile composition (mg 100 g^{-1} olive oil) of 'Arbequina' olive oil samples obtained from a super-high-density olive tree orchard. Data are the mean of two seasons, 2018 and 2019.

Compound	RT (min) ¥	RI_{Exp} ¥	RI_{Lit} ¥	ANOVA †	Irrigation Treatment			
					T_0	T_1	T_2	T_3
Ethanol	2.263	419	427	***	12.85 a ‡	7.10 b	7.16 b	7.87 b
Acetic acid	3.383	680	660	n.s.	3.14	3.65	3.83	1.96
3-Pentanone	4.381	737	703	n.s.	1.35	1.35	0.77	1.05
Propanoic acid	4.677	750	745	n.s.	0.11	0.33	0.83	1.05
Hexanal	6.688	831	xxx	n.s.	4.45	3.43	3.65	3.33
trans-2-Hexenal	8.903	904	854	***	91.67 a	70.69 b	56.08 c	96.28 a
trans-2-Hexen-1-ol	9.157	910	887	n.s.	47.22	47.46	38.06	43.98
4,8-Dimethyl-1,7-nonadiene	12.998	1000	-	n.s.	1.67	1.72	1.70	2.54
Benzaldehyde	13.893	1017	961	**	2.05 b	8.14 a	1.47 b	1.30 b
3-Hexen-1-ol, acetate	14.579	1031	1009	n.s.	9.47	3.47	11.77	15.53
Hexyl acetate	14.806	1035	997	**	3.54 a	0.35 c	2.33 b	4.77 a
Hexanoic acid	15.571	1050	1010	n.s.	1.03	0.43	0.63	0.38
trans-ß-Ocimene	16.005	1058	1050	***	0.56 b	2.19 a	0.61 b	0.64 b
Benzyl alcohol	18.621	1109	1046	*	2.66 a	0.15 b	2.15 a	0.99 b
1-Octanol	18.967	1115	1068	n.s.	0.47	0.28	0.26	0.06
Acetophenone	19.628	1127	1065	**	0.27 b	4.85 a	0.43 b	0.25 b
Benzoic acid	27.670	1269	1210	**	3.89 a	1.04 b	3.67 a	2.50 b
Nonanoic acid	31.899	1354	1303	n.s.	1.24	1.98	1.98	1.05
Total				***	188 a	159 b	137 b	186 a

† n.s. = not significant at $p > 0.05$, *, ** and *** significant at $p < 0.05$, $p < 0.01$ and $p < 0.005$; ‡ Values followed by the same letter, within the same row, were not significantly different ($p > 0.05$), according to Tukey's least significant difference test. ¥ RT = retention time; RI_{Exp} = experimental retention index; RI_{Lit} = literature retention index.

3.6. Descriptive Analysis

The sensory profiles of the oils under study were established by a trained panel (Table 6). Olive oils from the 2019 season had higher fruity and pungent intensities than those obtain during the 2018 season. In general, water-stressed trees (T_3 and T_2) led to olive oils with a higher intensity of the fruity, bitter and pungent notes compared to those of the control samples, T_0. Previous studies reported that water stress in olive trees negatively affected the attributes of "bitterness" and "pungent" [44]; however, this was not the case in the irrigation treatments applied to the super-high-intensive 'Arbequina' orchard under study, where water stress led to oils of even higher sensory quality than the control samples. A similar trend has also been reported by Sánchez-Rodríguez et al. [25].

An important characteristic for the oils to be classified as "extra virgin" is the absence of defects [27]. Regarding the overall defects presented in the samples under study (rancid, moldy, oxidized, humid and others), none of the studied samples showed significant values of the attribute "defect".

Table 6. Sensory profile (fruity, bitter, pungent and overall defects) by the trained panel of 'Arbequina' olive oil samples obtained from a super-high-density olive tree orchard with deficit irrigation.

Factor	Fruity	Bitter	Pungent	Overall Defects ¥	Commercial Classification
	ANOVA †				
Season	***	n.s.	***	n.s.	
Irrigation	***	**	***	n.s.	
Season × Irrigation	***	**	***	n.s.	

Table 6. *Cont.*

Factor	Fruity	Bitter	Pungent	Overall Defects [¥]	Commercial Classification
	Tukey's Multiple Range Test [‡]				
Season					
2018	4.1 b	1.9	1.1 b	0.0	EVOO [ξ]
2019	5.8 a	2.2	3.1 a	0.0	EVOO
Irrigation					
T_0	4.0 b	1.4 b	1.2 b	0.0	EVOO
T_1	4.6 b	2.1 a	2.0 ab	0.0	EVOO
T_2	5.5 a	2.3 a	2.7 a	0.0	EVOO
T_3	5.7 a	2.5 a	2.5 a	0.0	EVOO
Season × irrigation					
2018 × T_0	3.2 d	1.2 b	0.4 d	0.0	EVOO
2018 × T_1	4.2 cd	2.0 ab	1.4 cd	0.0	EVOO
2018 × T_2	4.1 cd	1.9 ab	1.3 cd	0.0	EVOO
2018 × T_3	5.1 bc	2.7 a	1.4 cd	0.0	EVOO
2019 × T_0	4.9 c	1.5 ab	2.0 c	0.0	EVOO
2019 × T_1	5.1 bc	2.3 ab	2.5 bc	0.0	EVOO
2019 × T_2	7.0 a	2.6 a	4.2 a	0.0	EVOO
2019 × T_3	6.3 ab	2.2 ab	3.5 ab	0.0	EVOO

[†] n.s. = not significant at $p > 0.05$, ** and *** significant at $p < 0.01$ and $p < 0.005$; [‡] Values followed by the same letter, within the same column and factor, were not significantly different ($p > 0.05$) according to Tukey's least significant difference test. [¥] Overall defects included musty, fusty and rancid. [ξ] EVOO = Extra virgin olive oil.

4. Conclusions

This study showed the positive effects of deficit irrigation strategies on the total phenolic content (TPC), variations in the fatty acid profiles, with an increase in the content of oleic acid, as well as an improvement in the sensory profile, especially the fruity attribute. Seasonal effect is another factor that can produce changes in olive oil composition. Moreover, the irrigation treatment did not affect the antioxidant activity of 'Arbequina' oils, but the basic quality parameters were maintained or even improved compared to the control. In addition, the yield of olive oil did not decrease when the applied volume of irrigation water was lower than the control. Therefore, the application of deficit irrigation techniques maintained or improved the quality and composition of olive oil coming from super-high-intensive 'Arbequina' orchards without negatively affecting production.

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