

# Composition and organoleptic characteristics of oil from *Arbequina* olive (*Olea europaea* L) trees under deficit irrigation

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**Abstract:** This study evaluated the effects of different regulated deficit irrigation (RDI) strategies applied to olive (*Arbequina* cultivar) trees on the qualitative and quantitative parameters of the resulting oil during the maximum evaporative demand period for three consecutive crop seasons. Quality indices, fatty acid composition, pigments, colour,  $\alpha$ -tocopherol and phenolic contents, bitter index, oxidative stability and organoleptic properties of the oil were determined. Irrigation did not affect those parameters used as criteria for classifying olive oil in its commercial grades. Only polyphenol and *o*-diphenol contents and, consequently, the bitter index and oxidative stability were affected by the RDI strategy, with increasing values as the water applied decreased. Regulated deficit irrigation resulted in important savings in irrigation requirements without detriment to oil quality.

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**Keywords:** *Arbequina* cultivar; oil composition; olive oil; regulated deficit irrigation

## INTRODUCTION

The olive tree is one of the oldest and most characteristic crops in the Mediterranean basin, with 95% of the world surface area dedicated to olives being concentrated in this area. Spain is one of the world's leading producing, importing and exporting countries. More than 118000 ha are grown in Catalonia, representing 5.5% of the Spanish olive crop, of which less than 20% are under irrigation.<sup>1</sup> Although Catalonia only occupies fourth place in terms of area of olive groves and oil production, within the quality olive oils produced in Spain it has two of the nine current designations of origin.

Most virgin olive oil produced in the Mediterranean region of Lleida (Catalonia, Spain) is included in the protected designation of origin (PDO) 'Les Garrigues'. The area's production of extra virgin olive oil comes almost exclusively from the *Arbequina* cultivar, and these oils have traditionally been characterised by their excellent sensory quality and distinctive physico-chemical features.<sup>2</sup> This producing area will be partially affected by the new irrigation plans promoted in recent years by the Catalan government. This would represent an amount of irrigation water from 100 to 250 mm year<sup>-1</sup>.<sup>3</sup>

When maximum olive yield is desired, large amounts of water for irrigation are needed, but these amounts are not always available. Until now, most of the research carried out into the effect of irrigation on the olive tree has been based on specific supplies of different amounts of water<sup>4–8</sup> or on constant percentages of crop water demand.<sup>9–13</sup>

The usefulness of regulated deficit irrigation (RDI) strategies has been shown for many fruit trees, such as peaches, pears, almonds and citrus fruits.<sup>14–18</sup> The RDI strategy is based on the effect of water stress on two processes: vegetative growth and photosynthesis. Vegetative growth can be limited by low plant water potentials during specific periods, while fruit growth remains unaffected. This strategy allows a certain degree of water stress during stages of crop development when the tree has a low sensitivity to it, which could coincide with olive pit hardening, with normal irrigation provided during the rest of the season. In the producing area of 'Les Garrigues', fruit pit hardening corresponds to the maximum evaporative demand period of the olive tree (from mid-July to September). Accordingly, reducing the water supply during this period could lead to an increase in water use efficiency. Previous work by Alegre *et al.*<sup>19</sup> showed that

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RDI strategies applied to olive trees negatively affected leaf water potential, stomatal conductance and fruit fresh weight.

On the other hand, it is necessary to study how this irrigation strategy could affect oil yield and oil quality. In previous studies by our research group during the 1996 crop season we found that regulated deficit irrigation applied to *Arbequina* olive during pit hardening accelerated fruit ripening and affected fruit and oil composition during the early stages of ripening.<sup>19,20</sup> However, at harvest, differences in oil content and yield due to irrigation treatment were minimal, with the exceptions of polyphenol content and oil stability, which were marginally affected.

The present study has been carried out to confirm this trend in consecutive crop seasons and to evaluate the long-term effects on oil quality of RDI strategies applied to olive trees during the maximum evaporative demand period, in order to assess their usefulness under limited water irrigation availability in relation to the quality obtained when applying full irrigation.

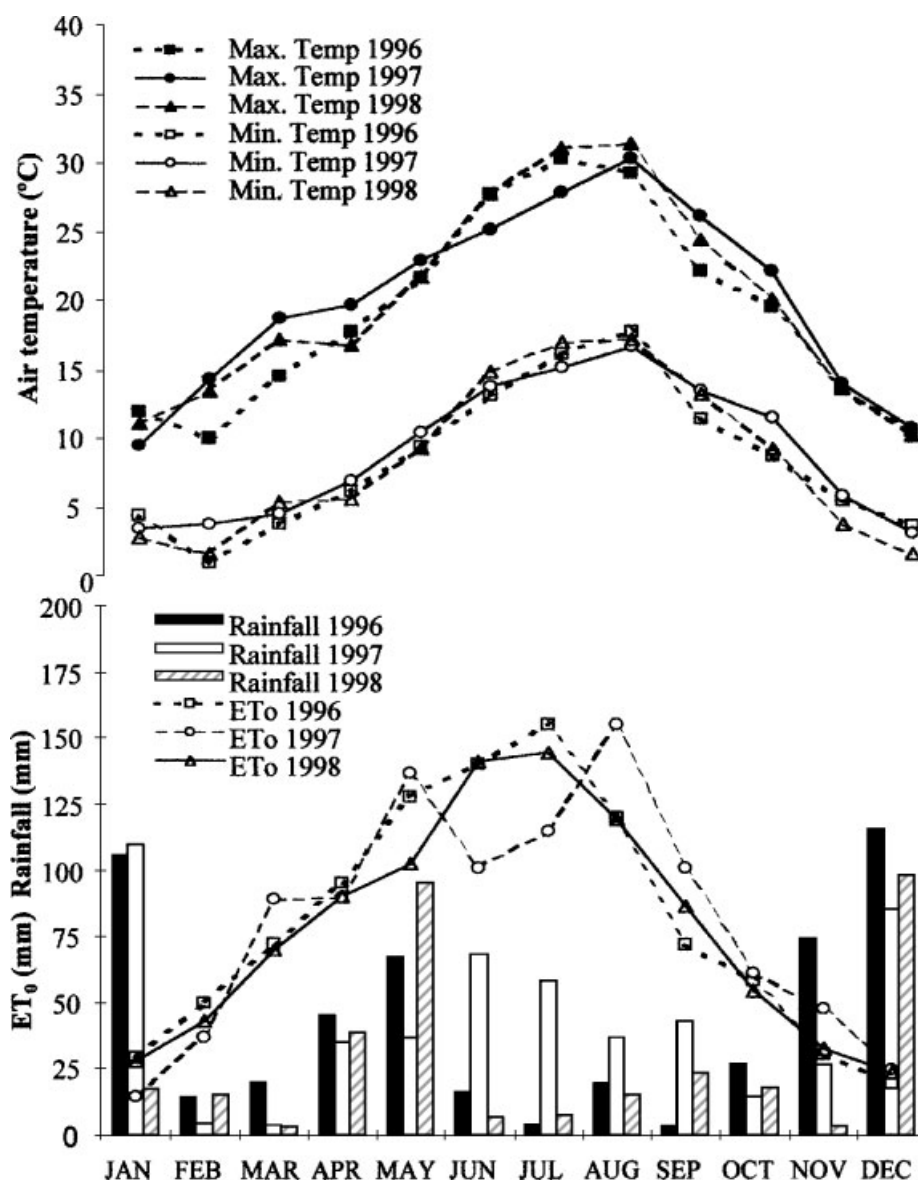
**EXPERIMENTAL**

**Plant material**

The study was carried out during 1996, 1997 and 1998 in a commercial olive grove (*Arbequina* cv) located in the olive-growing region of Les Garrigues (Lleida, Catalonia, Spain; 41°19'21"N, 00°36'43"E). Annual rainfall was 553, 523 and 343 mm for 1996, 1997 and 1998 respectively, with almost no rain from June to September in 1996 and 1998, while it was abundant in that period in 1997. Maximum and minimum monthly temperatures were similar in the three years, apart from June and July in 1997 with lower maximum temperatures and November and December in 1998 with lower minimum temperatures (Fig 1).

The plot was established in terraces, oriented to the East, in a deep (0.8–2.5 m) clay-loam soil. About seventy 100-year-old trees, spaced 9.75 m × 9.75 m, were used in a randomised complete block design with five replications and three or four trees per plot.

Four irrigation treatments were applied, these being



**Figure 1.** Annual patterns of air temperature, rainfall and reference evapotranspiration (ET<sub>0</sub>) for 1996, 1997 and 1998.

the control and three regulated deficit irrigation (RDI) treatments. The control treatment was fully irrigated during the whole season, using crop evapotranspiration ( $ET_c$ ) calculated from FAO-modified Penman-determined reference crop water use ( $ET_0$ ),<sup>21</sup> with estimated crop coefficients  $K_c=0.7$  adapted from Goldhamer *et al.*<sup>10</sup> A reduction of 60% was imposed ( $K_r=0.4$ ) to account for the area shaded by the canopy.<sup>22</sup> Irrigation requirements for each treatment were determined using the water budget approach.<sup>23</sup> Doses were modified *in situ* on the basis of plant water status. Additionally, three RDI treatments were imposed, being irrigated as the control for the whole season, but applying only 75% (T-75), 50% (T-50) and 25% (T-25) of the dose applied to the control between the beginning of massive pit hardening and the end of September (from 5 July to 18 September in 1996, from 17 July to 6 October in 1997 and from 7 July to 25 September in 1998).

The olive trees were irrigated daily with eight compensating drippers ( $6\text{ l h}^{-1}$ ) placed around each tree. Table 1 shows the annual water applied for each irrigation treatment during the three crop seasons.

At harvest period in 1996, which started on 27 November, two representative samples from each irrigation treatment/block were picked and taken to the laboratory for oil extraction and physical and chemical analyses. At harvest period in 1997 and 1998, which started on 15 and 4 December respectively, representative samples were picked from each tree in the experimental plot.

### Oil extraction

An Abencor analyser (MC2, Ingenierias y Sistemas, Seville, Spain) was used to process the olives in a pilot extraction plant. This method determines the industrial yield of olive oil, reproducing the industrial process at laboratory scale and following the same phases: milling, beating, centrifuging and decanting. The unit consists of three essential elements: the mill, the thermobearer and the pulp centrifuge. The oil was separated by decanting, transferred into dark glass bottles and stored in the dark at  $4^\circ\text{C}$ .

### Olive oil analyses

Determination of the free fatty acid content, peroxide value and UV absorption characteristics at 270 nm was carried out following the analytical methods described in the European Union Regulation EEC/2568/91.<sup>24</sup>

**Table 1.** Annual irrigation water applied in 1996, 1997 and 1998 crop seasons for each irrigation treatment

Irrigation treatment	Water applied ( $\text{mm year}^{-1}$ )		
	1996	1997	1998
Control	108	153	151
T-75	85	106	125
T-50	66	90	108
T-25	60	71	90

The results are expressed as percentage of oleic acid (%), milliequivalents of active oxygen  $\text{kg}^{-1}$  oil ( $\text{meq O}_2 \text{ kg}^{-1}$ ) and specific extinction coefficient at 270 nm respectively.

The fatty acid composition of the oil was determined by gas chromatography (GC) as fatty acid methyl esters (FAMES). FAMES were prepared by saponification/methylation with sodium methylate according to the modified European Union Regulation EEC/2568/91.<sup>25</sup> Chromatographic analysis was performed in an HP 5890 Series II gas chromatograph equipped with a flame ionisation detector (Hewlett Packard, Palo Alto, CA, USA), using an SP 2330 capillary column (30 m, 0.25 mm id, 0.20  $\mu\text{m}$  film thickness; Supelco, Inc, Bellefonte, PA, USA). The column temperature was isothermal at  $190^\circ\text{C}$  and the injector and detector temperatures were both  $220^\circ\text{C}$ . Fatty acids were identified by comparing retention times with standard compounds. Six fatty acids, palmitic acid (16:0), palmitoleic acid (16:1), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2) and linolenic acid (18:3), expressed as percentages of fatty acids methyl esters, were monitored in this study.

Chlorophyll and carotenoid contents were evaluated at 670 and 470 nm respectively from the absorption spectrum of each oil sample (7.5 g) dissolved in cyclohexane (25 ml),<sup>26</sup> and are expressed as  $\text{mg kg}^{-1}$  oil.

To assess the oil colour, a colorimeter (chroma meter type Color-Eye 3000, Macbeth, Altrincham, UK) with the Optiview 1.1 computer program and the CIELAB colorimetric system was used (chromatic ordinates  $L^*$ ,  $a^*$ ,  $b^*$ ). Oil samples were examined without dilution to avoid colour variation.

$\alpha$ -Tocopherol was evaluated by high-performance liquid chromatography by direct injection of an oil-in-hexane solution. Detection and quantification were carried out in a Waters (Waters Inc, Milford, MA) 600 apparatus with a Waters 996 photodiode array detector at 295 nm. The 25 cm  $\times$  4 mm id column used was filled with Supelcosil LC-NH<sub>2</sub>, 5  $\mu\text{m}$  (Supelco, Inc) and the flow rate was  $1\text{ ml min}^{-1}$ . The injection volume was 20  $\mu\text{l}$ . The mobile phase consisted of hexane/ethyl acetate (70:30) and the total running time was 12 min in isocratic conditions. Quantification was carried out using the external standard method. The linearity of the response was verified by fitting results of the  $\alpha$ -tocopherol analysis in six standard solutions of known concentration to a line. Results are given as  $\text{mg } \alpha\text{-tocopherol kg}^{-1}$  oil.

Phenolic compounds were isolated using the modified method described by Vázquez Roncero *et al.*,<sup>27</sup> by triple extraction of an oil-in-hexane solution with a 60% v/v water/methanol mixture. Folin-Ciocalteu reagent and 5% w/v sodium molybdate in 50% ethanol were added to suitable aliquots of the hydromethanolic extract to measure the absorption of the solution at 725 nm (phenolic compounds) and 370 nm (*o*-diphenolic compounds). Results are given as  $\text{mg caffeic acid kg}^{-1}$  oil.

The bitter index ( $K_{225}$ ) was evaluated by extraction

of the bitter components from a sample of  $1.0 \pm 0.01$  g of oil dissolved in 4 ml of hexane passed over a  $C_{18}$  column (Waters Sep-Pack Cartridges), previously activated with methanol (6 ml) and washed with hexane (6 ml). After elution, 10 ml of hexane was passed to eliminate the fat, and then the retained compounds were eluted with methanol/water (1:1) to 25 ml. The absorbance of the extract was measured at 225 nm against methanol/water (1:1) in a 1 cm cuvette.<sup>28</sup>

The results for the  $\alpha$ -tocopherol and *o*-diphenol contents and the bitter index correspond to the 1997 and 1998 crop seasons.

Stability is expressed as the oxidation induction time (h) measured with a Rancimat 679 apparatus (Metrohm Co, Basle, Switzerland) using an oil sample of 2.5 g warmed to 120 °C, and 20  $l\ h^{-1}$  air flow.<sup>29,30</sup>

The organoleptic evaluation of oils was carried out in accordance with the European Union Regulation EEC/2568/91<sup>24</sup> by the Official Test Panel of Virgin Olive Oil of Catalonia. Only oils from the last crop season of the trial (1998) were evaluated. The panel consisted of 11 tasters who performed the oil flavour description and their quality grading. The descriptive analysis used a six-point intensity ordinal rating scale from 0 (no perception) to 5 (extreme) to quantify the intensity of different sensory attributes (fruity, apple, other ripe fruit, green, bitter, pungent and sweet). Overall grading used a nine-point scale from 1 (lowest quality) to 9 (optimal quality). Depending on the average score of the panel, the oil is classified as extra virgin ( $\geq 6.5$ ), virgin ( $< 6.5$  and  $\geq 5.5$ ), ordinary virgin oil ( $< 5.5$  and  $\geq 3.5$ ) or virgin lampante olive oil.

### Statistical analysis

The data were subjected to analysis of variance using SAS version 6.12 (SAS Institute Inc, Cary, NC,

USA). Separation of the means was obtained using the least square means test, and significant difference was defined at  $P \leq 0.05$ .

### RESULTS AND DISCUSSION

Free fatty acid content, peroxide value and  $K_{270}$  are considered by some authors to be more directly related to the degree of deterioration of an oil than to its quality attributes. The average free fatty acid content values of all the oils in this trial were considerably lower than the limit of 1% oleic acid established by EU legislation<sup>24</sup> for high-quality oils (Table 2). The low free fatty acid content is, to a certain extent, a characteristic of oils extracted from the *Arbequina* cultivar.<sup>31</sup> Free fatty acid content was not influenced by the irrigation regime, as there were no differences between the results from the various irrigation treatments applied. This was also observed by Dettori and Russo<sup>32</sup> in oils from *Leccino*, *Nociara* and *Ogliarola salentina* cultivars and by Patumi *et al*<sup>33</sup> in oils from *Nocellara del Belice* and *Ascolana Tenera* cultivars. Salas *et al*<sup>34</sup> found differences in free fatty acid content between dry-farming and irrigated olive orchards in two out of three years of their trial. However, in all these cases the oil was of the highest quality.

We found that oils from the 1997 control treatment showed significantly higher peroxide values than those from the RDI treatments. In the previous year, 1996, this difference was not significant, but oils from the control treatment also showed higher peroxide values than the rest (Table 2). This observation agrees with results published by Salas *et al*,<sup>34</sup> who found significantly superior peroxide values in those oils from the most irrigated treatments, except in one year when there was a frost just before harvesting. According to Dettori and Russo<sup>32</sup> and Patumi *et al*,<sup>33</sup> the irrigation

**Table 2.** Quality indices of *Arbequina* cultivar virgin olive oil in relation to crop season and irrigation treatment (mean  $\pm$  standard error)

	Irrigation treatment	Crop season			Mean
		1996 <sup>a</sup>	1997 <sup>b</sup>	1998 <sup>b</sup>	
Free fatty acid content (%)	Control	0.14 $\pm$ 0.03aA	0.081 $\pm$ 0.004aB	0.087 $\pm$ 0.003aB	0.091 $\pm$ 0.008
	T-75	0.15 $\pm$ 0.01aA	0.083 $\pm$ 0.003aB	0.091 $\pm$ 0.004aB	0.098 $\pm$ 0.007
	T-50	0.14 $\pm$ 0.01aA	0.078 $\pm$ 0.003aB	0.085 $\pm$ 0.002aB	0.090 $\pm$ 0.006
	T-25	0.15 $\pm$ 0.01aA	0.083 $\pm$ 0.004aB	0.093 $\pm$ 0.006aB	0.10 $\pm$ 0.01
Peroxide value (meq O <sub>2</sub> kg <sup>-1</sup> )	Control	3.21 $\pm$ 0.27aA	8.92 $\pm$ 0.36aB	6.92 $\pm$ 0.52aB	7.51 $\pm$ 0.74
	T-75	3.15 $\pm$ 0.33aA	7.76 $\pm$ 0.32bB	6.07 $\pm$ 0.24aB	6.65 $\pm$ 0.53
	T-50	3.01 $\pm$ 0.39aA	7.43 $\pm$ 0.32bB	6.94 $\pm$ 0.52aB	6.51 $\pm$ 0.50
	T-25	2.84 $\pm$ 0.24aA	7.04 $\pm$ 0.36bB	6.14 $\pm$ 0.47aB	6.04 $\pm$ 0.62
$K_{270}$	Control	0.15 $\pm$ 0.02aA	0.089 $\pm$ 0.001aB	0.11 $\pm$ 0.00aB	0.11 $\pm$ 0.01
	T-75	0.14 $\pm$ 0.02aA	0.091 $\pm$ 0.001aB	0.10 $\pm$ 0.00aB	0.10 $\pm$ 0.01
	T-50	0.12 $\pm$ 0.01aA	0.088 $\pm$ 0.001aB	0.10 $\pm$ 0.00aB	0.11 $\pm$ 0.01
	T-25	0.15 $\pm$ 0.02aA	0.092 $\pm$ 0.001aB	0.10 $\pm$ 0.00aB	0.11 $\pm$ 0.01

<sup>a</sup>  $n=10$ .

<sup>b</sup>  $n=15$ .

Values within a single column lacking a common lowercase letter are significantly different ( $P < 0.05$ ) with respect to irrigation treatment for each crop season independently.

Values within a single row lacking a common uppercase letter are significantly different ( $P < 0.05$ ) with respect to crop season for each irrigation treatment independently.

regime had no effect on the peroxide value. This is of no great importance, because the average values of this parameter for the oils in our trial fell within the ranges established for the extra virgin olive oil category. There were no differences between irrigation treatments in  $K_{270}$ , and its average value was lower than the limit established by EU legislation<sup>24</sup> (Table 2).

Oil fatty acid composition of the trees under the different irrigation treatments is shown in Table 3. Fatty acid composition was similar in all irrigation treatments in the three crop seasons, with the percentages of palmitic, palmitoleic, stearic, oleic, linoleic and linolenic acids ranging from 12.46 to 15.51%, from 0.86 to 2.12%, from 1.36 to 1.65%, from 69.63 to 74.25%, from 9.17 to 12.31% and from 0.41 to 0.62% respectively. In 1996 there were no differences in oil fatty acid composition between irrigation treatments, maybe because it was the first year in which the RDI strategies were applied. In 1997 and 1998, however, some differences were observed in the percentage of stearic acid and, to a minor extent, in those of oleic and linoleic acids. The crop season had a greater effect on the percentages of fatty acids than the irrigation treatments. The results obtained in this 3 year trial indicate that the regulated deficit irrigation

treatments applied at the beginning of fruit pit hardening, the moment when oil biosynthesis and its accumulation start in an important way, did not significantly affect the fatty acid composition of the oil from the trees under the irrigation treatments. This observation agrees with the results published by Inglese *et al.*<sup>35</sup> and Patumi *et al.*,<sup>33</sup> who found that the fatty acid composition of different Italian cultivars was only affected by varietal factors and not by water regime.

The variability of the fatty acid composition of the oil samples covered the normal range expected for olive oils. Oils from the *Arbequina* cultivar have a high content of palmitic, palmitoleic and linoleic acids, a low stearic acid content and an average content of oleic acid in comparison with Spanish oils from other cultivars.<sup>36</sup> The fatty acid composition of olive oil varies widely, depending primarily on the cultivar but also on the climate and the degree of ripening of the fruit.<sup>37,38</sup> Several researchers have reported the influence of climatic conditions for particular areas and certain production years on the fatty acid composition of olive oil.<sup>39</sup> A study by Tsimidou and Karakostas<sup>40</sup> revealed a greater influence of year of harvest than origin or cultivar.

**Table 3.** Fatty acid composition (%) in virgin olive oil of *Arbequina* cultivar in relation to crop season and irrigation treatment (mean  $\pm$  standard error)

Fatty acid	Irrigation treatment	Crop season			Mean
		1996 <sup>a</sup>	1997 <sup>b</sup>	1998 <sup>b</sup>	
Palmitic (16:0)	Control	15.1 $\pm$ 0.1aA	15.3 $\pm$ 0.2aA	12.6 $\pm$ 0.1aB	14.1 $\pm$ 0.3
	T-75	15.5 $\pm$ 0.2aA	15.0 $\pm$ 0.2aA	12.7 $\pm$ 0.1aB	14.0 $\pm$ 0.2
	T-50	15.2 $\pm$ 0.2aA	15.3 $\pm$ 0.2aA	12.5 $\pm$ 0.1aB	14.1 $\pm$ 0.2
	T-25	15.2 $\pm$ 0.2aA	15.5 $\pm$ 0.2aA	12.5 $\pm$ 0.1aB	14.2 $\pm$ 0.3
Palmitoleic (16:1)	Control	1.59 $\pm$ 0.05aA	2.06 $\pm$ 0.05aB	0.87 $\pm$ 0.03aC	1.48 $\pm$ 0.10
	T-75	1.72 $\pm$ 0.05aA	2.12 $\pm$ 0.05aB	0.86 $\pm$ 0.04aC	1.52 $\pm$ 0.10
	T-50	1.58 $\pm$ 0.05aA	2.05 $\pm$ 0.05aB	0.88 $\pm$ 0.04aC	1.47 $\pm$ 0.09
	T-25	1.52 $\pm$ 0.06aA	1.86 $\pm$ 0.05bB	0.86 $\pm$ 0.04aC	1.37 $\pm$ 0.09
Stearic (18:0)	Control	1.56 $\pm$ 0.11aA	1.36 $\pm$ 0.02aA	1.44 $\pm$ 0.05aA	1.43 $\pm$ 0.03
	T-75	1.37 $\pm$ 0.10aA	1.40 $\pm$ 0.02abA	1.58 $\pm$ 0.04bA	1.48 $\pm$ 0.04
	T-50	1.65 $\pm$ 0.14aA	1.40 $\pm$ 0.02abA	1.64 $\pm$ 0.04bA	1.53 $\pm$ 0.04
	T-25	1.56 $\pm$ 0.14aA	1.45 $\pm$ 0.02bA	1.61 $\pm$ 0.05bA	1.52 $\pm$ 0.05
Oleic (18:1)	Control	72.6 $\pm$ 0.3aA	70.1 $\pm$ 0.4aB	74.3 $\pm$ 0.2aC	72.2 $\pm$ 0.4
	T-75	71.7 $\pm$ 0.3aA	70.4 $\pm$ 0.4aA	73.5 $\pm$ 0.2bB	71.9 $\pm$ 0.3
	T-50	72.1 $\pm$ 0.3aA	69.6 $\pm$ 0.4aB	73.9 $\pm$ 0.2abC	71.8 $\pm$ 0.3
	T-25	72.2 $\pm$ 0.2aA	69.8 $\pm$ 0.4aB	73.7 $\pm$ 0.2abC	71.8 $\pm$ 0.4
Linoleic (18:2)	Control	9.17 $\pm$ 0.29aA	11.94 $\pm$ 0.23aB	10.19 $\pm$ 0.15aC	10.80 $\pm$ 0.21
	T-75	9.68 $\pm$ 0.25aA	12.00 $\pm$ 0.22aB	10.76 $\pm$ 0.14bC	11.17 $\pm$ 0.20
	T-50	9.50 $\pm$ 0.26aA	12.31 $\pm$ 0.22aB	10.51 $\pm$ 0.14abC	11.18 $\pm$ 0.19
	T-25	9.45 $\pm$ 0.12aA	11.96 $\pm$ 0.25aB	10.75 $\pm$ 0.16bC	11.05 $\pm$ 0.19
Linolenic (18:3)	Control	0.41 $\pm$ 0.02aA	0.54 $\pm$ 0.02aA	0.64 $\pm$ 0.02aA	0.58 $\pm$ 0.03
	T-75	0.43 $\pm$ 0.02aA	0.50 $\pm$ 0.02aA	0.60 $\pm$ 0.02aA	0.58 $\pm$ 0.04
	T-50	0.45 $\pm$ 0.02aA	0.52 $\pm$ 0.02aA	0.63 $\pm$ 0.02aA	0.56 $\pm$ 0.03
	T-25	0.45 $\pm$ 0.02aA	0.54 $\pm$ 0.02aA	0.62 $\pm$ 0.02aA	0.57 $\pm$ 0.04

<sup>a</sup>  $n=10$ .

<sup>b</sup>  $n=15$ .

Values within a single column lacking a common lowercase letter are significantly different ( $P < 0.05$ ) with respect to irrigation treatment for each crop season independently.

Values within a single row lacking a common uppercase letter are significantly different ( $P < 0.05$ ) with respect to crop season for each irrigation treatment independently.

**Table 4.** Chlorophyll and carotenoid concentrations and colour (expressed as chromatic ordinates  $L^*$ ,  $a^*$  and  $b^*$ ) of *Arbequina* cultivar virgin olive oil in relation to crop season and irrigation treatment (mean  $\pm$  standard error)

	Irrigation treatment	Crop season			Mean
		1996 <sup>a</sup>	1997 <sup>b</sup>	1998 <sup>b</sup>	
Chlorophylls (mg kg <sup>-1</sup> )	Control	8.28 $\pm$ 0.80A	3.84 $\pm$ 0.28B	8.38 $\pm$ 1.19A	5.63 $\pm$ 0.58
	T-75	7.33 $\pm$ 0.54A	4.22 $\pm$ 0.24B	6.08 $\pm$ 0.85A	5.01 $\pm$ 0.32
	T-50	6.62 $\pm$ 0.45A	3.90 $\pm$ 0.24B	7.84 $\pm$ 1.09A	5.13 $\pm$ 0.39
	T-25	6.29 $\pm$ 0.85A	4.45 $\pm$ 0.28B	8.29 $\pm$ 0.45C	5.68 $\pm$ 0.42
Carotenoids (mg kg <sup>-1</sup> )	Control	8.23 $\pm$ 0.74A	5.15 $\pm$ 0.23B	9.75 $\pm$ 1.11A	6.71 $\pm$ 0.52
	T-75	7.20 $\pm$ 0.21A	5.24 $\pm$ 0.21B	9.23 $\pm$ 1.02C	6.25 $\pm$ 0.38
	T-50	7.07 $\pm$ 0.36A	4.94 $\pm$ 0.21B	10.43 $\pm$ 1.01C	6.34 $\pm$ 0.44
	T-25	6.97 $\pm$ 0.55A	5.49 $\pm$ 0.23B	10.88 $\pm$ 0.60C	6.97 $\pm$ 0.51
$L^*$	Control	86.1 $\pm$ 1.7A	90.6 $\pm$ 0.6B	86.8 $\pm$ 1.2A	88.8 $\pm$ 0.6
	T-75	86.3 $\pm$ 2.3A	89.1 $\pm$ 0.5A	88.2 $\pm$ 1.1A	88.5 $\pm$ 0.6
	T-50	88.0 $\pm$ 0.7A	89.6 $\pm$ 0.5A	87.0 $\pm$ 1.0A	88.8 $\pm$ 0.5
	T-25	85.5 $\pm$ 1.6A	89.5 $\pm$ 0.6B	86.0 $\pm$ 0.5A	87.9 $\pm$ 0.6
$a^*$	Control	-6.13 $\pm$ 0.38A	-6.62 $\pm$ 0.24A	-3.70 $\pm$ 0.76B	-5.76 $\pm$ 0.33
	T-75	-5.10 $\pm$ 0.21A	-6.28 $\pm$ 0.21B	-3.40 $\pm$ 0.70C	-5.60 $\pm$ 0.30
	T-50	-5.40 $\pm$ 0.36A	-6.31 $\pm$ 0.21A	-3.48 $\pm$ 0.77B	-5.63 $\pm$ 0.28
	T-25	-4.79 $\pm$ 0.20A	-6.55 $\pm$ 0.24B	-2.61 $\pm$ 0.51C	-5.34 $\pm$ 0.39
$b^*$	Control	92.3 $\pm$ 6.5A	82.0 $\pm$ 2.5A	112.9 $\pm$ 4.3B	90.8 $\pm$ 3.5
	T-75	94.4 $\pm$ 2.4A	84.8 $\pm$ 2.2A	110.0 $\pm$ 5.6B	97.0 $\pm$ 2.6
	T-50	89.5 $\pm$ 6.6A	81.3 $\pm$ 2.2A	114.6 $\pm$ 3.4B	89.0 $\pm$ 2.9
	T-25	91.7 $\pm$ 5.8A	86.7 $\pm$ 2.5A	118.6 $\pm$ 1.5B	95.0 $\pm$ 3.2

<sup>a</sup>  $n=10$ .<sup>b</sup>  $n=15$ .

Values within a single row lacking a common uppercase letter are significantly different ( $P < 0.05$ ) with respect to crop season for each irrigation treatment independently.

The values of the pigment content of the oils from the trees under different RDI treatments are shown in Table 4. Most of the oils obtained were richer in carotenoids than in chlorophylls during the three crop seasons. In 1997 a lower content of these pigments was observed than in other years. A possible explanation could be that in 1997 the summer period was rainy, with 31% of the annual rainfall in that period, implying fewer hours of sun, while in 1996 and 1998 only 8% of the annual rainfall fell during the summer period (Fig 1). There were no differences between irrigation treatments in the chlorophyll and carotenoid contents of the oils in the three crop seasons in this study. It seems that a reduction in the water applied during the summer period did not significantly affect the final contents of these pigments in the fruits at harvest and, subsequently, their contents in the oils obtained. Besides participating in colouring the oil, they play an important role in its oxidative stability owing to their antioxidant nature in the dark and pro-oxidant activity in the light.

The chromatic ordinates  $L^*$ ,  $a^*$  and  $b^*$  of the oils from the RDI treatments are shown in Table 4. There were no differences in any of the chromatic ordinates between irrigation treatments. The oil colour was not affected by water reduction in any of the crop seasons. It was observed that the chromatic ordinate  $a^*$  showed more negative values as the water applied increased, although this trend was not statistically significant. There also seems to be a certain tendency of the

ordinate  $a^*$  towards negative values (green zone) and the ordinate  $b^*$  towards the blue zone in the oils with lower contents of chlorophyll and carotenoid pigments, ie those from 1997. In contrast, in 1998, when oils showed the highest chlorophyll and carotenoid contents, values of  $a^*$  were not so negative and values of  $b^*$  were higher (yellow zone).

The values of the  $\alpha$ -tocopherol content of the oils from the trees under different irrigation treatments in the 1997 and 1998 crop seasons are shown in Table 5. In 1997, significantly lower  $\alpha$ -tocopherol contents were found in the oils from the most severe treatment (T-25). In the next crop season, although this difference was not statistically significant, a marked tendency was observed. As the water applied increased, so did the tocopherol content.  $\alpha$ -Tocopherol, commonly called vitamin E, is the major tocopherol in olive oil. Besides its nutritional qualities, its antioxidant properties in foods have been known for many years, but little is yet known about its contribution to the stability of olive oil.

As can be observed in Table 5, the total phenol and *o*-diphenol contents in the oils were significantly affected by the irrigation regime in the three crop seasons, with the oils obtained from the treatments with the highest deficits showing higher values. The phenolic content of the oils was more affected by the crop season. In 1997, a year characterised by abundant rainfall during the summer period, the phenolic compound contents were considerably lower than in

other years. The highest values for these compounds were obtained in 1996, when the deficit irrigation treatments were first applied to what had been a dry-farming orchard for many years.

In 1998 the irrigation treatments could be divided into three different groups, with the oils from T-25 and the control treatment respectively showing the highest and lowest polyphenol and *o*-diphenol contents, while T-50 and T-75 occupied an intermediate position. In 1996 and 1997, however, the only differentiation was between T-25 and the other irrigation treatments, although there was a marked trend showing lower polyphenol and *o*-diphenol contents in the oils from the control treatment than in those from the T-50 and T-75 treatments. As the water applied to the olive tree decreased, the polyphenol and *o*-diphenol contents in the oil increased significantly.

The differences in the phenol concentration in the oils could be a consequence of the higher water content of the olives from the less severe deficit irrigation treatments (data not shown). As polyphenols are soluble in both water and oil, significant amounts of such constituents are carried away from the oily phase in the process of oil extraction, because of partitioning between two unmixable liquids.<sup>41</sup> Another supposition that explains the differences found in the polyphenol concentration could be that the water stress implied a greater synthesis of phenolic compounds in the fruits and thus in the oils obtained from them, which would

mean that changes in water status caused a change in the biosynthesis of phenols.<sup>33</sup>

Oil bitterness was characterised by values of the parameter  $K_{225}$  because of the significant correlation between them.<sup>28</sup> The  $K_{225}$  values of the oils under the regulated deficit irrigation treatments are shown in Table 5. A large difference was observed between the average values of  $K_{225}$  in 1997 and 1998. This difference is related to the respective polyphenol and *o*-diphenol contents in those years, as phenolic compounds give the oils their bitter taste.<sup>42,43</sup> In 1997 the irrigation treatments did not influence  $K_{225}$ . It showed very low values corresponding, according to Gutiérrez Rosales *et al.*,<sup>28</sup> to non-bitter or almost imperceptibly bitter oils. We could suppose that in any irrigation treatment the level of phenolic compounds in the oil was high enough to confer a noticeable bitterness. In contrast, in 1998 we found that  $K_{225}$  increased as the water applied decreased, following the same tendency as the polyphenol and *o*-diphenol contents in that year.

Oxidative stability was affected by water regime (Table 5), with the oils from the least irrigated treatment showing significantly higher values. This was as expected, since the oxidative stability of an olive oil is greatly affected by its polyphenol and *o*-diphenol contents.<sup>27,44,45</sup> The average oxidative stability values varied depending on the crop season, being highest in 1996 and lowest in 1997, when the polyphenol content

**Table 5.**  $\alpha$ -Tocopherol, polyphenol and *o*-diphenol concentrations, bitter index and oxidative stability of *Arbequina* cultivar virgin olive oil in relation to crop season and irrigation treatment (mean  $\pm$  standard error)

	Irrigation treatment	Crop season			Mean
		1996 <sup>a</sup>	1997 <sup>b</sup>	1998 <sup>b</sup>	
$\alpha$ -Tocopherol (mg kg <sup>-1</sup> )	Control	—	209.9 $\pm$ 3.9aA	227.3 $\pm$ 2.9aB	217.3 $\pm$ 3.2
	T-75	—	202.4 $\pm$ 3.5aA	220.6 $\pm$ 2.7aB	209.6 $\pm$ 2.2
	T-50	—	201.8 $\pm$ 3.5aA	224.1 $\pm$ 2.7aB	210.7 $\pm$ 3.3
	T-25	—	190.4 $\pm$ 3.9bA	217.7 $\pm$ 3.0aB	206.9 $\pm$ 3.9
Polyphenols (mg kg <sup>-1</sup> )	Control	340.1 $\pm$ 13.7aA	52.7 $\pm$ 4.1aB	175.6 $\pm$ 9.0aC	142.1 $\pm$ 16.9
	T-75	369.6 $\pm$ 10.1aA	59.5 $\pm$ 3.5aB	215.4 $\pm$ 8.3bC	160.8 $\pm$ 16.9
	T-50	366.0 $\pm$ 6.0aA	56.2 $\pm$ 3.7aB	206.2 $\pm$ 8.3bC	152.6 $\pm$ 16.8
	T-25	452.3 $\pm$ 16.9bA	83.4 $\pm$ 4.1bB	255.0 $\pm$ 9.9cC	193.3 $\pm$ 23.5
<i>o</i> -Diphenols (mg kg <sup>-1</sup> )	Control	—	3.8 $\pm$ 0.5aA	20.1 $\pm$ 0.9aB	12.0 $\pm$ 1.7
	T-75	—	4.2 $\pm$ 0.4aA	23.7 $\pm$ 0.9bB	14.0 $\pm$ 1.7
	T-50	—	3.8 $\pm$ 0.4aA	23.5 $\pm$ 0.9bB	13.3 $\pm$ 1.7
	T-25	—	6.7 $\pm$ 0.5bA	28.7 $\pm$ 1.0cB	16.1 $\pm$ 2.2
Bitter index ( $K_{225}$ )	Control	—	0.06 $\pm$ 0.01aA	0.19 $\pm$ 0.01aB	0.14 $\pm$ 0.02
	T-75	—	0.07 $\pm$ 0.01aA	0.21 $\pm$ 0.01abB	0.16 $\pm$ 0.02
	T-50	—	0.06 $\pm$ 0.01aA	0.22 $\pm$ 0.01bB	0.16 $\pm$ 0.02
	T-25	—	0.07 $\pm$ 0.01aA	0.26 $\pm$ 0.01cB	0.17 $\pm$ 0.02
Oxidative stability (h)	Control	22.4 $\pm$ 0.4aA	8.8 $\pm$ 0.3aB	14.7 $\pm$ 0.5aC	12.8 $\pm$ 0.9
	T-75	21.1 $\pm$ 0.4aA	9.0 $\pm$ 0.3aB	15.9 $\pm$ 0.4aC	13.1 $\pm$ 0.7
	T-50	21.7 $\pm$ 0.2aA	8.7 $\pm$ 0.3aB	15.9 $\pm$ 0.4aC	13.1 $\pm$ 0.8
	T-25	24.7 $\pm$ 1.0bA	10.5 $\pm$ 0.3bB	17.6 $\pm$ 0.5bC	14.6 $\pm$ 1.0

<sup>a</sup>  $n=10$ .

<sup>b</sup>  $n=15$ .

Values within a single column lacking a common lowercase letter are significantly different ( $P < 0.05$ ) with respect to irrigation treatment for each crop season independently.

Values within a single row lacking a common uppercase letter are significantly different ( $P < 0.05$ ) with respect to crop season for each irrigation treatment independently.

also showed the highest and lowest values respectively. Salas *et al*<sup>34</sup> and Patumi *et al*<sup>33</sup> also found that the polyphenol content and parameters related to it, such as bitter index and oxidative stability, diminished when the amount of water supplied with irrigation increased.

The quality grade of an olive oil is determined not only on the basis of a number of chemical and physical parameters but also on an additional sensory evaluation of it. Sensory appraisal of the oils under the regulated deficit irrigation treatments (Table 6) showed no defects in any of the oils that were classified as extra virgin, independently of the irrigation treatment, the overall grading ranging from 7.8 in oils from the control treatment to 8.2 in those from the T-50 treatment. Aparicio *et al*<sup>46</sup> authenticated *Arbequina* oils by the sweet fruit sensory attributes, whilst, according to McEwan,<sup>47</sup> the sensory characteristics of *Arbequina* oils are sweet and slightly pungent and green. The aroma of the oils from our trial was indeed fruity, reminiscent of both the odour and taste of sound, fresh fruit picked at the optimum stage of ripeness, with the control treatment having a lower mean for this attribute. We observed that the sensory attributes directly related to polyphenol content, such as green, bitter and astringent, were noticeably superior in oils from the T-25 treatment than in those from the control treatment.<sup>48</sup> That is to say, oils from the most severe irrigation treatment, which showed a significantly superior phenol content and bitter index, also scored higher for these sensory attributes. The attributes related to green, such as green banana, apple and artichoke, were higher in the T-25 and T-50 treatments, while the ripe banana attribute, related to mature fruit, was only perceived in the T-75 and control treatments.

**Table 6.** Sensory attributes of *Arbequina* cultivar virgin olive oil (1998 crop season) in relation to irrigation treatment

	Irrigation treatment			
	Control	T-75	T-50	T-25
Overall grading <sup>a</sup>	7.8	8.1	8.2	8.0
Flavour description <sup>b</sup>				
Fruity	2.5	3.0	3.0	2.9
Apple	0.0	0.0	1.2	0.9
Ripe banana	0.8	1.0	0.0	0.0
Green	1.9	2.0	2.5	2.4
Bitter	1.9	2.1	2.1	2.4
Pungent	2.2	2.4	2.6	2.4
Sweet	1.9	1.8	1.8	1.8
Others				
Astringent	0.9	1.3	1.0	1.6
Almond	1.2	1.3	1.1	1.3
Anise–fennel	0.8	0.9	0.5	0.5
Artichoke	0.3	0.3	0.7	0.5
Green banana	0.5	0.3	0.7	0.6
Tomato	0.6	0.9	0.9	0.5

<sup>a</sup> 1 (lowest quality), 9 (optimal quality).

<sup>b</sup> 0 (imperceptible), 1 (barely perceptible), 2 (slight), 3 (average), 4 (great), 5 (extreme).

As a consequence of the results observed in this irrigation experiment, it is possible to conclude that in the Mediterranean area, where summer rainfall is scarce, a regulated deficit irrigation strategy applied to *Arbequina* cultivar did not affect those parameters used as criteria for classifying olive oil in its commercial grades. The fatty acid composition, pigment content and colour of olive oil were not affected by water reduction in the olive tree. As the water applied increased, the  $\alpha$ -tocopherol content also tended to increase. In contrast, the polyphenol and *o*-diphenol contents and, consequently, the bitter index and oxidative stability of the olive oils were affected by the RDI strategy, increasing in value as the water applied decreased. The sensory attributes related to polyphenol content, such as green, bitter and astringent, were superior in oils from the highest deficit irrigation treatment.

All the RDI strategies applied in the current trial, reducing water supply during fruit pit hardening, seemed to be adequate for *Arbequina* olive oil production in this geographical area without negatively affecting oil quality. Only in T-25, the most extreme irrigation treatment, were the parameters related to phenol content significantly affected by water regime. However, the year of harvest was revealed to have a greater influence on olive oil composition. A reduction in water irrigation during fruit pit hardening, which corresponds to the maximum evaporative demand period of the olive tree, could lead to important savings in irrigation requirements and an important increase in water use efficiency.

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