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# Influence of Regulated Deficit Irrigation on Arbequina's Crop Yield and EVOOs Quality and Sensory Profile

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**Abstract:** Regulated deficit irrigation in super-high-density (SHD) olive orchards is a well-known strategy to save water and control plant vigor, without decreasing fruit or oil yield. As there is controversial information about its influence on virgin olive oil quality, a trial was conducted in five SHD olive orchards of Arbequina cultivar in different locations of central, east, north and northeast Spain under full irrigation (FI) and regulated deficit irrigation (RDI) treatments. RDI applied during phase II of fruit growing (40% of total needs) saves more than 20% of water on average, without reductions in olive fruit or extra virgin olive oil (EVOO) yield. No threshold of 3.5 MPa of stem water potential was crossed in any case. RDI modified sterols and the fatty acid profile of EVOOs but not phenols, quality parameters, or the sensory profile. Latitude, altitude, and yearly rainfall have a big impact on some compounds such as campesterol, oleuropein, or margaroleic or linolenic acids.

**Keywords:** olive grove; fatty acids; sterols; phenol profile; climatic conditions



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## 1. Introduction

Nowadays, hedgerow olive orchards (*Olea europaea* L.) have become the most common orchard design in worldwide new plantings [1], mainly due to the need of reducing olive oil production costs. In high-density and super-high-density (SHD) designs , the olive fruits are harvested by overhead harvesters, which are the fastest, most efficient, and cheapest method [2]. In Spain there are more than 89,000 ha with a density above 1000 olive trees per hectare [3], planted over the last two decades [4]. This orchard system requires higher quantities of water, fertilization, and chemicals for pest control.

One of the most important cultivars used in SHD systems is Arbequina because of its low vegetative vigor, compact growth, and early, high, and regular production [1,2]. The production of Arbequina's hedgerow orchard responds positively to irrigation, but Grattan et al. (2006) observed that maximum irrigation did not result in larger production in SHD olive orchards [5]. Furthermore, the irrigation water productivity (production per

unit of total water applied) reaches a maximum and then decreases [6,7]. Oil quality can be affected by different environmental and agronomical factors including climate, growing region, plant nutrition, fruit maturity, crop load, temperature, and available water [1,4,8]. Focusing on irrigation, high doses can negatively affect extra virgin olive oil (EVOO) quality, reducing phenol content and oxidation stability [4,9], but controversial results on fatty acids composition, acidity, and peroxide index have been reported [4].

In arid and semiarid areas, water saving is crucial, so in order to save water with control plant vigor, regulated deficit irrigation (RDI) strategies have been tested [2], evaluating its effect on EVOO quantity and quality [10–12]. These strategies consist of a reduction in the applied water than the theoretically needed amount in a specific phenological stage [13]. There are some phenological periods that have a high sensitivity to water stress [14,15] and full water needs should be applied to avoid negative effects on fruit quantity or quality. However, in other periods, as in phase II of fruit growth, water restrictions can be applied without minimizing these negative effects [6]. There are different methods to assess water stress, with Stem Water Potential (SWP) being the most common [7,16]. Marra et al. (2016) [17] proposed that the optimal SWP values ranged between −2.5 and −3.5 MPa to maximize water productivity and avoid yield reductions. This result is quite similar to the values of Marino et al. (2018) [16], which recommend a moderate stress (−2.0 to −3.5 MPa), or Ahumada-Orellana et al. (2017) [18], which recommend not to cross the −3.5 MPa threshold. However, Gómez del Campo (2013) [19] established the threshold of −2.9 MPa in July and −2.0 MPa in August to avoid oil production decreases, while Hueso et al. (2019) [20] determined −2.21 MPa to maintain oil production and −2.31 MPa to maximize water productivity.

Gucci et al. (2019) [21] reported that RDI strategies did not alter peroxide value, free acidity, or fatty acid composition, but it can modify phenolic composition, as in the results of Ahumada-Orellana et al. (2018) [22] regarding fatty acid composition. On the other hand, Hernández et al. (2018) [23] found changes in fatty acid composition under different deficit irrigation treatments, with a reduction in linoleic acid content under the most restricted irrigation, with an increase in the oleic/linoleic ratio.

The aim of this work is to determine the effect of the Arbequina cultivar on extra virgin olive oil (EVOO) by comparing fully irrigated with regulated-deficit irrigated methods on super-high-density olive orchards. For this purpose, the quantity and quality of the EVOOs of five different olive orchards (central, east, north, and northeast Spain) under both irrigation treatments were analyzed during two consecutive crop seasons.

## 2. Materials and Methods

### *2.1. Plots and Irrigation Description*

A two-year (2015 and 2016) field study was carried out in five field plots (Figure 1) with a super-high-density grove of cv. Arbequina: in Cadreita, Navarra (northern Spain); Torres del Segre, Lleida (northeast Spain); Constantí, Tarragona (northeast Spain); Colmenar de Oreja, Madrid (central Spain); and Villena, Alicante (eastern Spain).

An irrigation trial was performed in the five mature olive groves starting in 2014 and finishing in 2016. This trial consisted of a full irrigation treatment (FI) where 100% of the water needs was applied throughout most of the year following the equations (Equations (1) and (2)) of the water balance method [24,25]. A regulated deficit irrigation (RDI) where 40% of the water needs was applied in mid-July (after massive pit-hardening) and August was combined with 100% application of water during the rest of the year. This method is similar to the moderate RDI strategy described by Martinez-Gimeno et al. (2022) [6]:

$$IN = ETc - Pe \quad (1)$$

where IN: irrigation needs; ETc: crop evapotranspiration under standard conditions (mm); and Pe: effective precipitation

$$ETc = ET_0 \times Kc \times Kr \quad (2)$$

where ETc: crop evapotranspiration under standard conditions (mm); ET0: reference crop evapotranspiration (mm); Kc: crop coefficient; and Kr: reduction coefficient.

Reference crop evapotranspiration ($ET_0$) was determined with the Penman–Monteith method to determine crop evapotranspiration needs.

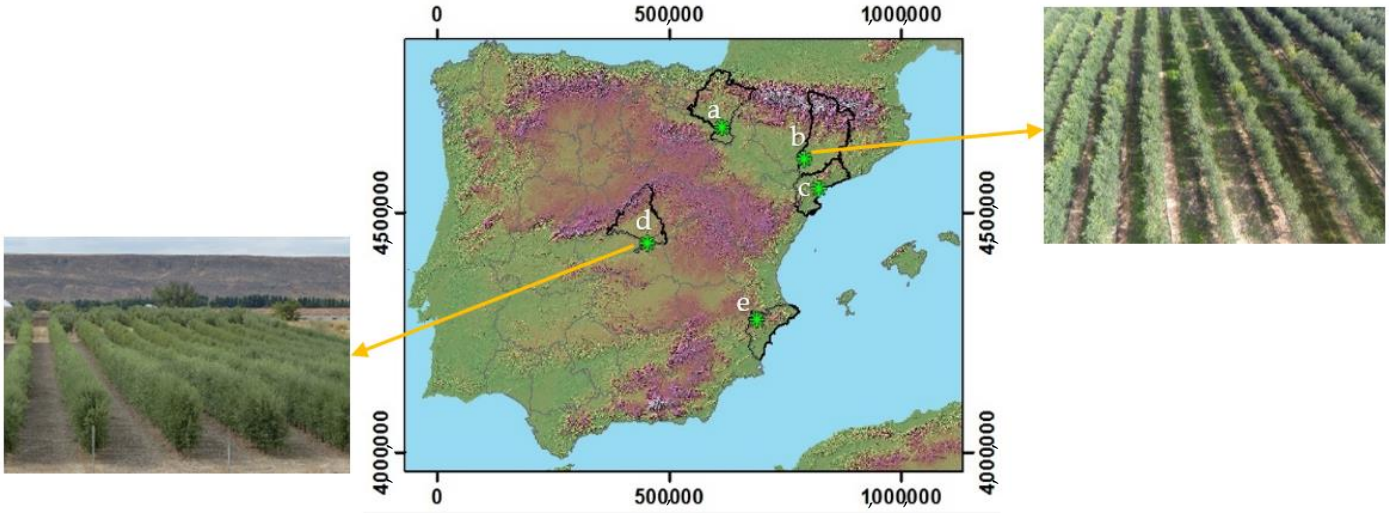



**Figure 1.** Plot location on Iberian Peninsula map: (**a**) Cadreita (Navarra); (**b**) Torres del Segre (Lleida); (**c**) Constantí (Tarragona); (**d**) Colmenar de Oreja (Madrid); and (**e**) Villena (Alicante). Two pictures of the Madrid and Lleida olive orchards are shown.

The main agroclimatic characteristics of the 5 olive groves of the different localities and data of reference crop evapotranspiration and precipitation for 2015 and 2016 are presented in Table 1.

**Table 1.** Main agroclimatic characteristics of the 5 olive groves and irrigation quantities in each crop season.

| Plot Location | Olive Grove Density (Olive Tree·ha$^{-1}$) | Elevation (MASL) [1] | Year of Planting | 2015 | | | | 2016 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | $ET_0$ [2] (mm) | P [3] (mm) | Irrigation FI (mm) | Irrigation RDI (mm) | $ET_0$ (mm) | P (mm) | Irrigation FI (mm) | Irrigation RDI (mm) |
| Madrid | 1481 | 494 | 2003 | 1312 | 246 | 460 | 335 | 1239 | 450 | 597 | 415 |
| Lleida | 1010 | 119 | 2002 | 1112 | 301 | 372 | 315 | 1107 | 312 | 447 | 327 |
| Tarragona | 1250 | 87 | 2007 | 1065 | 550 | 381 | 259 | 1063 | 500 | 320 | 214 |
| Alicante | 1667 | 505 | 2001 | 1369 | 277 | 449 | 365 | 1346 | 288 | 458 | 383 |
| Navarra | 1667 | 283 | 2005 | 1321 | 320 | 590 | 504 | 1111 | 289 | 345 | 307 |
| Average | | | | 1236 | 339 | 450 | 356 | 1173 | 368 | 433 | 329 |

[1] Meters above sea level; [2] $ET_0$: reference crop evapotranspiration; and [3] P: precipitation.

### 2.2. Field Measurements

In each location (except for Alicante), midday SWP (11:00–13:00 solar time) was used to determine plant water status at 4 different times: (1) at the beginning of the irrigation season (early June); (2) just before pit hardening (mid-July); (3) in phase III of fruit growth (mid-September); and (4) the recovery period when rainfall returns (mid-October). In the Alicante trial the measurements were: (1) before pit hardening (early July); (2) after massive pit hardening (mid-August); and (3) in phase III of fruit growth (mid-September).

Trees were mechanically harvested when the maturity index was around 3 and weighted (4 olive trees per experimental unit). Then, a sample of 5 kg per experimental unit (3 per plot) was picked up in both seasons for olive oil extraction.

### 2.3. Extra Virgin Olive Oil Elaboration and Analysis

For each sample, the maturity index was determined according to Beltran et al. [26] following the 0–7 point scale according to the color of the skin. The Abencor system (MC2

Ingenierias y Sistemas) was employed for extra virgin olive oil (EVOO) elaboration and then the EVOOs were stored at 5 °C for further analysis.

#### 2.3.1. Regulated Physicochemical Parameters

Regulated physicochemical parameters were determined in each EVOO. Free acidity was expressed as a percentage of oleic acid; peroxide value (PV) was expressed as milliequivalents of active oxygen per kilogram of oil (meq $O_2 \cdot kg^{-1}$); and UV spectrophotometric indices ($K232$, $K270$, and $\Delta K$ extinction coefficients) were determined following the analytical methods described in the European Commission Regulation 2568/91 and later amendments [27].

#### 2.3.2. Oil Oxidative Stability

Oil oxidative stability was expressed as the oxidation induction time (h) measured with a Rancimat-679 device (Metrohm Co, Basel, Switzerland) using 2.5 g of oil sample warmed to 120 °C with an air flow of 20 L $h^{-1}$ [28].

#### 2.3.3. Pigment Content and Color

Carotenoids and chlorophylls were determined at a wavelength of 470 nm and 670 nm, respectively, in cyclohexane, according to the method of Minguez-Mosquera et al. (1991) [29]. The concentrations of chlorophyll and carotenoids were expressed as mg of pheophytin and lutein per kg, respectively. Chromatic coordinates were measured by the software CINTRAL v2.2 (GBC Scientific Equipment, Braeside, Victoria, Australia) to obtain the color according to the CIEL $a * b *$ method [30].

#### 2.3.4. Sensory Analysis

Sensory analysis was performed by the "Panel de Catadores de Aceite de Oliva Virgen de la Comunidad de Madrid" according to the method described in the European Commission Regulation (EC) 640/2008 [31]. This method allows for the classification of olive oils based on the detection of negative attributes (fusty/muddy, musty, winey, rancid, wet wood, and others) as well as the measurement of the intensity of three positive attributes (green or ripe fruitiness, bitterness, and pungency). The panel was constituted at least by 8 trained tasters that scored the descriptors on a normalized sheet (from 0 to 10).

#### 2.3.5. Phenolic Profile

Phenolic compounds were isolated by solid phase extraction (SPE) using diol-phase cartridges and the extract was analyzed by HPLC. HPLC analysis was performed using a Perkin Elmer Flexar system (Perkin-Elmer Hispania, S.A., Madrid, Spain) equipped with an automatic injector, a column oven, and a photo diode array UV detector. A Spherisorb ODS-2 column was used (5 µm, 25 cm × 4.6 mm i.d., Technokroma, Barcelona, Spain). Phenolic compounds were quantified at 280 nm using syringic acid as the internal standard and the response factors were determined by the method of Mateos et al. (2001) [32].

#### 2.3.6. Sterol and Triterpene Dialcohol Compositions

The analyses of sterol composition and triterpene dialcohols (erythrodiol and uvaol) were performed by elementary plot, with three repetitions for each treatment, according to the official methods described in The European Commission Regulation 2568/91 [33].

#### 2.3.7. Fatty Acid Composition

The fatty acid composition of the oils was determined by gas chromatography as fatty acid methyl esters (FAMEs). FAMEs were prepared by saponification/methylation with sodium methylate according to European regulation (EEC 2568/91) [27]. A chromatographic analysis was performed in an Agilent Technologies 6850 series II Network GC system gas chromatograph equipped with a 60 m × 0.25 mm × 0.20 µm film thickness fused capillary column Supelco 24111 (Agilent Technologies) coupled to a flame ionization

detector. Samples were introduced into the column at 170 °C during a period of 30 min; after this time, the temperature was increased by 5 °C·min$^{-1}$ to 200 °C and maintained for 12 min. The flow rate of He, used as carrier gas, was 0.5 mL/min. Injector and flame ionization detector temperatures were 230 °C and 250 °C, respectively. FAMEs were identified by comparing their retention times with those of standard compounds.

Oxidative susceptibility (OS) was calculated following Equation (3) [34]:

$$OS = m(\%) + 45 \times L(\%) + 100 \times Ln(\%) \quad (3)$$

where $m$ is monounsaturated acids; $L$ is linoleic acid; and $Ln$ is linolenic acid.

### *2.4. Statistical Analysis*

The statistical analysis of the results was carried out using the IBM SPSS Statistics for Windows, Version 29 program (Armonk, NY: IBM Corp) and three-way variance analyses (ANOVA) using the LSD test for the separation of means $p \leq 0.05$ was performed. The 3 independent variables were irrigation (FI or RDI), location (Madrid, Lleida, Tarragona, Alicante, and Navarra), and crop season (2015 and 2016).

Due to the high weight of the olive orchard's locality in VOO composition, a principal component analysis (PCA) was carried out using the SAS (Statistical Analysis Software), Version 9.4 (Cary, NC: SAS Institute Inc.) and including the main meteorological data (yearly rainfall, yearly ETc, and yearly irrigation) of each crop season and some orchard characteristics (latitude, density, and elevation) to establish which of these factors are more related to the different phenolic compounds, sterols, and triterpenes dialcohol, and fatty acids composition.

## 3. Results and Discussion

### *3.1. Irrigation and Plant Water Status*

Reference crop evapotranspiration was similar between crop seasons in each locality except for Navarra, where in 2016 it was 200 mm higher than in 2015 (Table 1). Alicante and Madrid had the highest $ET_0$. Total precipitation was similar between crop seasons in each locality, except for Madrid due to 2016 being a rainy year. Water applied in the FI treatment was 450 mm and 433 mm in 2015 and 2016, respectively, and a RDI of 356 mm and 329 mm in 2015 and 2016, respectively. This represents a water saving of 21 and 25% in each crop season regarding FI treatment.

Midday SWP was measured during the irrigation season in order to determine the water stress on the olive trees. There were statistically significant differences between localities ($p < 0.001$), irrigation treatments ($p = 0.014$), and months ($p < 0.001$), but not between crop seasons ($p = 0.241$). The lowest values were reached after massive pit hardening (after mid-July) (Figure 2).

The limit of high stress (SWP < −3.5 MPa) was not achieved at any time. This value is considered a critical threshold that, if crossed, would cause damage to the photosynthetic apparatus [16]. Only a few measures from mid to the end of the irrigation season in Madrid, Lleida, Tarragona, and Alicante olive groves under RDI treatment dropped below the −2.0 MPa threshold, which is considered the limit of moderate stress. Gómez del Campo (2013) [19] determined that below −2.0 MPa in August reduces oil production and Hueso et al. (2019) [20] fixed on the limit of −2.21 MPa, which was crossed by the Alicante trial in 2015.

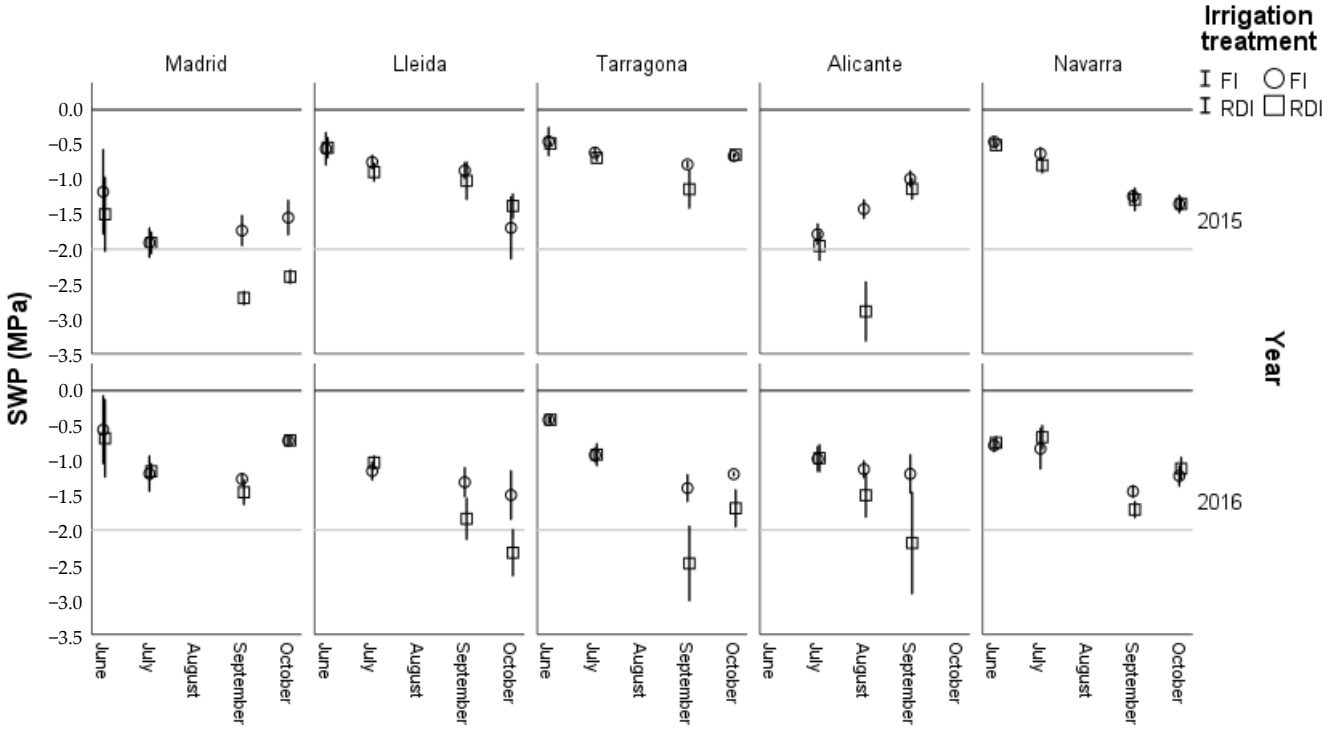


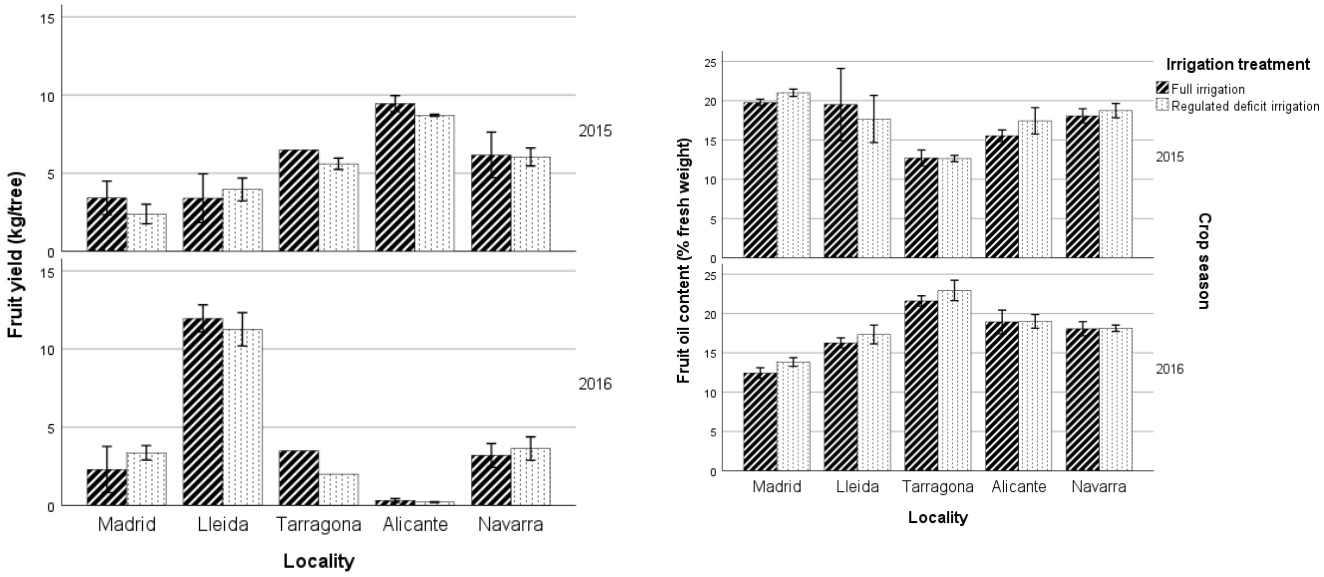

**Figure 2.** Midday stem water potential (SWP) of the irrigation treatments (FI: full irrigation; RDI: regulated deficit irrigation) at different times each year and plot location (n = 4). Vertical error bars represent the mean standard deviation. Grey horizontal line marks the threshold of moderate stress (−2 MPa).

### *3.2. Fruit and Oil Yield*

Olive fruits were harvested when they had a maturity index around 3 (end of October to mid-November, varying between years and localities). Fruit yield was statistically different depending on locality ($p < 0.001$) and crop season ($p < 0.001$), without influence of irrigation treatment ($p = 0.241$). The higher yield was reached in Lleida in 2016 with more than 10 kg·tree$^{-1}$ (Figure 3a). Fruit yield in Alicante and Tarragona was higher in 2015 (ON year) and lower in 2016 (OFF year). On the other hand, the ON year of Lleida was 2016; 2015 was the OFF year. In Madrid and Navarra, slight differences appeared among years, with both being considered medium years.

Fruit oil percentage was not affected by any factor (irrigation treatment $p = 0.141$, locality $p = 0.207$, and year $p = 0.165$), with a global average of 17.6% of fruit oil content in fresh mass (Figure 3b). These results belong to a short-term trial, long-term trials are needed to properly determine the impact on fruit and oil yield.

Similar to our results, no effect of slight deficit irrigation strategies were found by other groups applying the summer deficit irrigation used in this work [10,17,19]. Nevertheless, Hueso et al. (2019) [20] found fruit yield reductions when SWP dropped down to −1.82 MPa during the oil synthesis period (phase III) and oil yield reductions when SWP was lower than −2.21 MPa. No effects on olive oil accumulation were described in several studies with different deficit irrigation treatments as reported in Hernández et al. (2018) [23] and Martínez-Gimeno et al. (2022) [6]; they stated that fruit oil content is less sensitive than olive fruit yield to irrigation shortages. Keeping an adequate stress level with an appropriate RDI strategy is crucial to maintain fruit and olive oil yield [4].




(**a**) (**b**)

**Figure 3.** Fruit yield (**a**) and fruit oil content (**b**) under irrigation treatments (FI: full irrigation; RDI: regulated deficit irrigation) in both years and plot location (n = 4). Vertical error bars represent the mean standard deviation.

### *3.3. Maturity Index and Olive Oil Quality and Sensory Profile*

The irrigation treatment has no influence on either the quality or the sensory parameters included in Table 2, while location and crop season had a significant effect on most of them. Due to the great influence of the maturity index on the compounds of the EVOO [4], the lack of differences between all the localities, years, and irrigation treatments led us to remove this factor from the explanation of the differences in the composition of the EVOOs.

Similar to other works [9,35], no differences appeared between higher-dose irrigation treatments in extinction coefficients, peroxide value, free acidity, or sensory profile (fruity, pungent, and bitter); however, Sánchez-Rodríguez et al. (2020) [35] found an increase in oxidative stability. When the reduction is important (around 72%), García-Garví et al. (2022) found differences in peroxides, acidity, $K_{232}$, and sensory profile [36] with the most restrictive RDI strategies. Chlorophyll and carotenoid concentrations were not influence by the RDI strategy, similar to results of other groups [37,38], These groups found differences in chlorophylls with severe RDI with 20% and 30% of IN supply.

Alicante's EVOO had high acidity and peroxide value with lower oxidative stability (5.6 h), due to it being the softer EVOO with the lowest values of bitter and pungent attributes. Alicante's and Tarragona's EVOOs had the lowest concentration on total pigments (<5 mg·kg$^{-1}$) and oxidative stability with the less green EVOOs (higher * a and lower * b color parameters) despite their differences in maturity index (Tarragona's EVOO: 2.7 and Alicante's EVOO: 3.4).

The differences among both crop seasons were less than 0.5 points in the maturity index, with slight but statistical significance differences in most of the variables, such as acidity, oxidative stability, pigments, and color parameters.

**Table 2.** Three-way ANOVA (prob > F) and least square means of the maturity index, olive oil quality parameters, and sensory profile for the different factors: irrigation (Irrig: full irrigation: FI; regulated-deficit irrigation: RDI); location (Loc.: Mad: Madrid, Lle: Lleida, Tar: Tarragona, Ali: Alicante, and Nav: Navarra); and crop seasons (CS: 2015 and 2016).

| | Factor (*p*-Value) | | | Factors | | | | | | | | | Standard of Quality for EVOO |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Irrig. | Loc. | CS | Irrig. | | Loc. | | | | | CS | | |
| | | | | FI | RDI | Mad | Lle | Tar | Ali | Nav | 2015 | 2016 | |
| Maturity index | NS | 0.001 | 0.001 | 2.9a | 3.0a | 3.2a | 3.1ab | 2.7b | 3.4a | 2.7b | 3.2a | 2.8b | |
| Indices of quality | | | | | | | | | | | | | |
| Acidity (% oleic acid) | NS | 0.000 | 0.000 | 0.282a | 0.274a | 0.149c | 0.175c | 0.264b | 0.702a | 0.312b | 0.181b | 0.356a | ≤0.8 |
| Peroxide value (meq $O_2 \cdot kg^{-1}$) | NS | 0.000 | NS | 5.157a | 4.816a | 2.775c | 6.717a | 5.130b | 6.073a | 4.782b | 4.906a | 5.051a | ≤20 |
| $K_{232}$ | NS | 0.000 | 0.000 | 1.595a | 1.646a | 1.707a | 1.721a | 1.613b | 1.667ab | 1.418c | 1.733a | 1.531b | ≤2.50 |
| $K_{270}$ | NS | 0.000 | 0.024 | 0.095a | 0.098a | 0.120a | 0.092b | 0.094b | 0.097b | 0.081c | 0.093b | 0.099a | ≤0.22 |
| Oxidative stability (h) | NS | 0.000 | 0.000 | 13.1a | 13.1a | 20.0a | 14.9b | 7.0c | 5.6c | 14.2b | 12.9b | 13.2a | |
| Pigments | | | | | | | | | | | | | |
| Lutein ($mg \cdot kg^{-1}$) | NS | 0.000 | 0.000 | 4.811a | 5.077a | 6.211a | 5.901a | 2.356c | 3.337b | 6.112a | 4.570b | 5.244a | |
| Pheophytin ($mg \cdot kg^{-1}$) | NS | 0.000 | 0.003 | 4.300a | 4.611a | 5.547b | 7.477a | 1.204c | 1.432c | 5.106b | 4.280b | 4.596a | |
| Total pigments ($mg \cdot kg^{-1}$) | NS | 0.000 | 0.000 | 9.111a | 9.689a | 11.758b | 13.378a | 3.560c | 4.768c | 11.218b | 8.850b | 9.839a | |
| Chloro/carot | NS | 0.000 | 0.025 | 0.854a | 0.808a | 0.907b | 1.187a | 0.662c | 0.413d | 0.776c | 0.935a | 0.748b | |
| Color | | | | | | | | | | | | | |
| *L | NS | 0.000 | 0.000 | 87.622a | 88.892a | 87.683b | 84.129c | 91.982a | 92.627a | 87.049b | 88.482a | 87.277b | |
| *a | NS | 0.000 | 0.000 | −8.760a | −9.149a | −10.327b | −9.688b | −6.326a | −8.533a | −9.688b | −8.043a | −9.684b | |
| *b | NS | 0.000 | 0.000 | 66.031a | 69.494a | 80.958ab | 75.804b | 38.542d | 47.100c | 86.078a | 64.833b | 70.106a | |
| Sensory profile | | | | | | | | | | | | | |
| Fruity | NS | 0.000 | NS | 5.0a | 5.0a | 5.2a | 5.3a | 5.0a | 5.2a | 4.5b | 4.9a | 5.1a | Mf > 0 |
| Bitter | NS | 0.000 | NS | 2.3a | 2.5a | 3.5a | 2.8b | 1.9c | 1.6c | 1.8c | 2.3a | 2.4a | |
| Pungent | NS | 0.000 | NS | 2.7a | 3.0a | 4.1a | 3.2b | 2.2cd | 1.8d | 2.5c | 3.0a | 2.8a | |

Irrig.: irrigation; Loc.: location; and CS: crop season. Mf: fruity median. NS = not significant at $p < 0.05$. Different letters mean significant differences between levels of the treatment according to the LSD test $p < 0.05$ (n = 3).

### *3.4. Phenolic Profile*

There were no effects on any of the different phenolic compounds (Table 3) nor in the total phenolic concentration (TPC). These results are similar to those of Gómez-Rico et al. (2006) [39] who found differences in TPC between rainfed and well-irrigated olive trees, but not in the intermediate cases. Sanchez-Rodriguez et al. (2020) [35] found statistically significant differences with all the deficit treatments regarding FI. This lack of differences in phenols between irrigation treatments also showed no differences in the pungent or bitter properties of the EVOOs (Table 2). Phenol compounds are the main compounds responsible for these sensory attributes [40].

There was an important effect of the location on almost all the phenolic compounds. The EVOOs from Madrid showed a higher content of TPC (497 $mg \cdot kg^{-1}$) followed by Lleida in TPC (377 $mg \cdot kg^{-1}$), which was opposite to the EVOOs from Alicante with a lower TPC (169 $mg \cdot kg^{-1}$). Navarra and Tarragona's EVOOs had an intermediate concentration (274 $mg \cdot kg^{-1}$ and 202 $mg \cdot kg^{-1}$, respectively). Regarding oleuropein, Madrid's EVOOs had 6.89 $mg \cdot kg^{-1}$, around 4-fold more than the EVOOs from Tarragona and Navarra (1.85 $mg \cdot kg^{-1}$ and 1.52 $mg \cdot kg^{-1}$, respectively), with an intermediate position of Alicante and Lleida (3.38 $mg \cdot kg^{-1}$ and 3.00 $mg \cdot kg^{-1}$, respectively).

Statistical differences were also observed in different phenolic compounds regarding crop season. EVOOs of the 2016 crop season had around 25 $mg \cdot kg^{-1}$ of TPC higher than EVOOs of 2015, although the oleuropein content was lower (2.821 $mg \cdot kg^{-1}$ and 3.832 $mg \cdot kg^{-1}$ in 2016 and 2015 EVOOs, respectively).

Principal component analysis (PCA) was carried out to determine the relationships between main meteorological data (yearly rainfall, yearly ETc, and yearly irrigation) of each crop season, orchard characteristics (latitude, density, and elevation), and the phenolic compounds (Figure 4). Axis 1 explained 30.15% of the total variance and axis 2 explained 20.25%. Plots at

higher altitudes are linked to higher contents of luteolin, apigenin, oleuropein, total secoiridoids, and total phenols, and lower contents of vanillic acid and hydroxytyrosol acetate.

**Table 3.** Three-way ANOVA (prob > F) and least square means of the phenolic profiles of the different factors: Irrig: irrigation (full irrigation: FI; regulated deficit irrigation: RDI); Loc: locality (Mad: Madrid; Lle: Lleida; Tar: Tarragona; and Ali: Alicante; Nav: Navarra); and CS: crop season (2015 and 2016).

| Phenolic Compounds (mg kg$^{-1}$) | Factor (*p*-Value) | | | Factors | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Irrig. | Loc. | CS | Irrig. | | Loc. | | | | | CS | |
| | | | | FI | RDI | Mad | Lle | Tar | Ali | Nav | 2015 | 2016 |
| Hydroxytyrosol | NS | 0.000 | 0.000 | 6.800a | 6.301a | 2.038b | 2.551b | 1.555b | 1.698b | 24.912a | 9.869a | 3.232b |
| Tyrosol | NS | 0.000 | 0.000 | 3.702a | 3.604a | 1.440c | 1.696bc | 2.102bc | 2.588b | 10.438a | 4.395a | 2.911b |
| Vanillic acid | NS | 0.000 | 0.000 | 2.079a | 2.093a | 0.710d | 2.029c | 3.269a | 2.496b | 1.926c | 2.424a | 1.748b |
| Caffeic acid | NS | 0.000 | 0.000 | 0.028a | 0.032a | 0.000b | 0.000b | 0.000b | 0.000b | 0.151a | 0.000b | 0.060a |
| Vanillin | NS | 0.000 | NS | 2.558a | 2.394a | 1.841c | 3.121a | 2.543b | 2.008c | 2.868ab | 2.506a | 2.447a |
| p-Coumaric acid | NS | 0.000 | NS | 2.867a | 3.199a | 2.621b | 2.388b | 2.966b | 2.468b | 4.723a | 3.067a | 2.999a |
| Hydroxytyrosol acetate | NS | 0.000 | NS | 13.067a | 14.418a | 5.435c | 20.695a | 22.072a | 7.084c | 13.428b | 13.444a | 14.041a |
| o-Coumaric acid | NS | NS | 0.010 | 1.262a | 1.556a | 1.786 | 1.211 | 1.208 | 1.412 | 1.428 | 1.630a | 1.188b |
| Oleuropein | NS | 0.000 | 0.000 | 3.288a | 3.366a | 6.891a | 2.999b | 1.847c | 3.375b | 1.522c | 3.832a | 2.821b |
| Tyrosol acetate | NS | 0.000 | 0.000 | 3.545a | 3.581a | 5.847a | 2.453c | 2.850c | 2.646c | 4.018b | 2.556b | 4.569a |
| Cinnamic acid | NS | 0.000 | 0.001 | 1.473a | 1.329a | 1.633b | 1.316bc | 2.768a | 0.832cd | 0.455d | 1.087b | 1.715a |
| Luteolin | NS | 0.000 | NS | 16.551a | 16.763a | 31.426a | 20.825b | 7.050d | 7.432d | 16.552c | 17.150a | 16.164a |
| Apigenin | NS | 0.000 | NS | 5.108a | 5.113a | 9.903a | 6.323b | 1.793e | 2.888d | 4.643c | 4.853a | 5.367a |
| ΣOrtho-diphenols | NS | 0.000 | 0.009 | 27.12a | 27.91a | 14.76c | 27.39b | 28.58b | 14.02c | 52.80a | 30.27a | 24.75b |
| ΣSecoiridoids | NS | 0.000 | 0.000 | 234.3a | 246.9a | 425.2a | 309.6b | 150.0d | 131.7d | 186.6c | 223.5b | 257.7a |
| Total phenolic content | NS | 0.000 | 0.019 | 307.5a | 299.8a | 496.8a | 377.2b | 202.1d | 168.6e | 273.6c | 291.6b | 315.7a |

Irrig.: irrigation; Loc.: location; and CS: crop season. NS = not significant at $p < 0.05$. Different letters mean significant differences between levels of the treatment according to the LSD test $p < 0.05$. Number of replicates = 3.

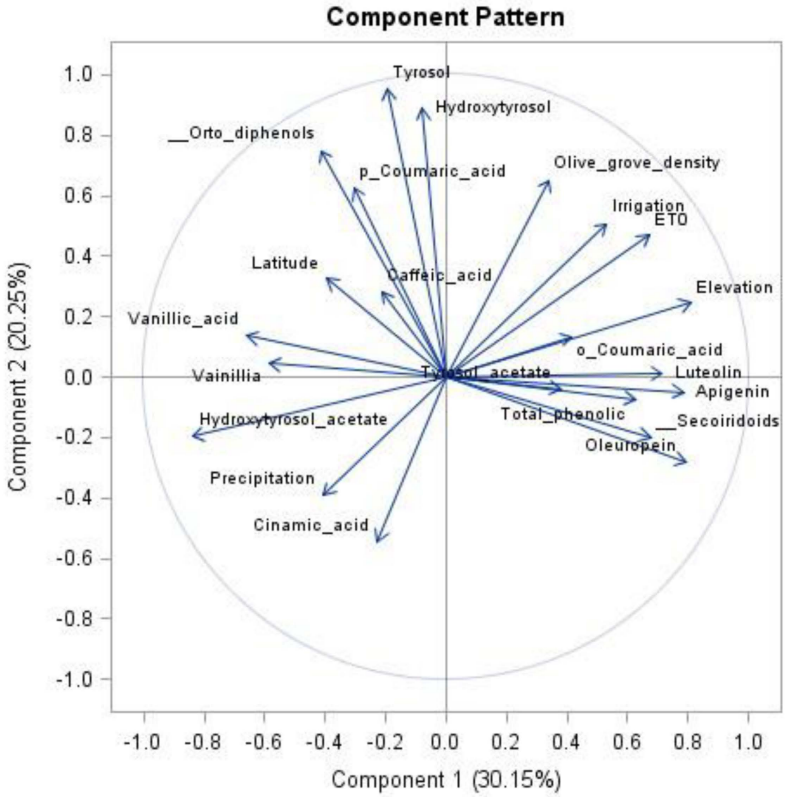



**Figure 4.** Principal component analysis (PCA) biplot (50.40% of explained variance) after Varimax rotation including main meteorological and orchard characteristics and the phenolic compounds.

### *3.5. Sterol and Triterpene Dialcohol Composition*

Phytosterol level is related to olive oil quality and purity. These compounds are mainly affected by the cultivar [41], the maturity index [42], and the environmental and agronomic

conditions [43,44]. The percentage of campesterol, apparent β-sitosterol, and the triterpene dialcohols were affected by the irrigation treatment, the location, and the crop season (Table 4). Different groups [45,46] found a reduction in total sterols due to differences in irrigation in other cultivars, while no differences were stated in Arbequina [9,47–49] with deficit irrigation regarding FI treatments.

**Table 4.** Three-way ANOVA (prob > F) and least square means of sterols and triterpenes dialcohols compositions for the different factors: Irrig: irrigation (full irrigation: FI; regulated deficit irrigation: RDI); Loc: locality (Mad: Madrid; Lle: Lleida; Tar: Tarragona; Ali: Alicante; and Nav: Navarra); and CS: crop season (2015 and 2016).

| Sterols and Triterpene Dialcohol (%, Except Total Sterols in mg·kg$^{-1}$) | Factor (*p*-Value) | | | Factors | | | | | | | | | Standard of Sterol Composition in EVOOs |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Irrig. | Loc. | CS | Irrig. | | Loc. | | | | | CS | | |
| | | | | FI | RDI | Mad | Lle | Tar | Ali | Nav | 2015 | 2016 | |
| Cholesterol | NS | 0.015 | NS | 0.180 | 0.192 | 0.258a | 0.183b | 0.179b | 0.167b | 0.142b | 0.200 | 0.172 | ≤0.5 |
| Brasicasterol | NS | NS | NS | 0.100 | 0.100 | 0.100 | 0.100 | 0.100 | 0.100 | 0.100 | 0.100 | 0.100 | ≤0.1 |
| 24-Methylene cholesterol | NS | NS | NS | 0.103 | 0.107 | 0.100 | 0.108 | 0.100 | 0.108 | 0.108 | 0.100 | 0.110 | |
| Campesterol | 0.008 | 0.000 | 0.000 | 3.417b | 3.488a | 3.658a | 3.392c | 3.554b | 3.625ab | 3.033d | 3.360b | 3.545a | ≤4.0 |
| Campestanol | NS | NS | NS | 0.105 | 0.100 | 0.100 | 0.112 | 0.100 | 0.100 | 0.100 | 0.105 | 0.100 | |
| Stigmasterol | NS | 0.000 | 0.000 | 0.850 | 0.857 | 0.842b | 0.708c | 0.892ab | 0.892ab | 0.933a | 0.773b | 0.933a | < Campeste. |
| Δ7-Campesterol | NS | NS | NS | 0.103 | 0.107 | 0.117 | 0.100 | 0.100 | 0.108 | 0.100 | 0.100 | 0.110 | |
| Apparent β-Sitosterol | 0.009 | 0.000 | 0.000 | 94.680a | 94.548b | 94.058c | 94.875a | 94.638b | 94.500b | 95.000a | 94.793a | 94.435b | ≥93.0 |
| Δ7-Stigmastenol | NS | 0.000 | 0.000 | 0.182 | 0.203 | 0.300a | 0.179bc | 0.125c | 0.167bc | 0.192b | 0.242a | 0.143b | ≤0.5 |
| Δ7-Avenasterol | NS | 0.000 | 0.001 | 0.450 | 0.480 | 0.650a | 0.433b | 0.425b | 0.375c | 0.442b | 0.437b | 0.493a | |
| Erythrodiol+Uvaol | 0.039 | 0.000 | 0.005 | 1.920b | 2.227a | 2.642b | 1.825c | 1.017d | 1.467cd | 3.417a | 1.857b | 2.290a | ≤4.5 |
| Total sterols | NS | 0.000 | NS | 1319 | 1235 | 967c | 1327b | 1507a | 1547a | 1036c | 1326 | 1228 | ≥1000 |

NS = not significant at $p < 0.05$. Different letters mean significant differences between levels of the treatment according to the LSD test $p < 0.05$. Number of replicates = 3.

EVOOs of FI treatment had a slightly higher concentration of apparent β-sitosterol (+0.1%) and lower concentration of campesterol (−0.07%) and erythrodiol+uvaol (−0.3%) than RDI's EVOOs. These parameters are under the European regulation, so the variations due to water stress should be considered. Increased campesterol under RDI treatment was also observed by Berenguer et al. (2006) [9] and Inglese et al. (1996) [50]. Berenguer et al. (2006) [9] also found a decrease in erythrodiol percentage while increasing irrigation in the most extreme treatments.

The EVOO location had a significant effect on the percentage of different sterols and total sterols content. Madrid's EVOOs had a higher percentage of cholesterol, campesterol, Δ7-stigmastenol, and Δ7-avenasterol. They also had a lower content of total sterols (967 mg·kg$^{-1}$); this is below the limit of the European regulation [51] which can cause commercialization issues. The EVOOs with the highest content of total sterols were those from Tarragona and Alicante (1507 and 1541, respectively). These results are opposite to those of Aparicio and García-González (2013) [52] in the Picual cultivar who found a positive effect of altitude and β-sitosterol and negative effect with campesterol, stigmasterol, and total sterols.

A PCA biplot including the main meteorological data (yearly rainfall, yearly ETc, and yearly irrigation) of each crop season, orchard characteristics (latitude, density, and elevation), and the sterols and triterpenes dialcohols compositions is shown in Figure 5. Axis 1 explained 26.08% of the total variance and axis 2 explained 18.59%. The lower the latitude, the higher the campesterol percentage and lower apparent β-sitosterol in EVOOs. A high level in total sterols is related to rainy years and areas, as was previously reported by Arbones et al. [49].

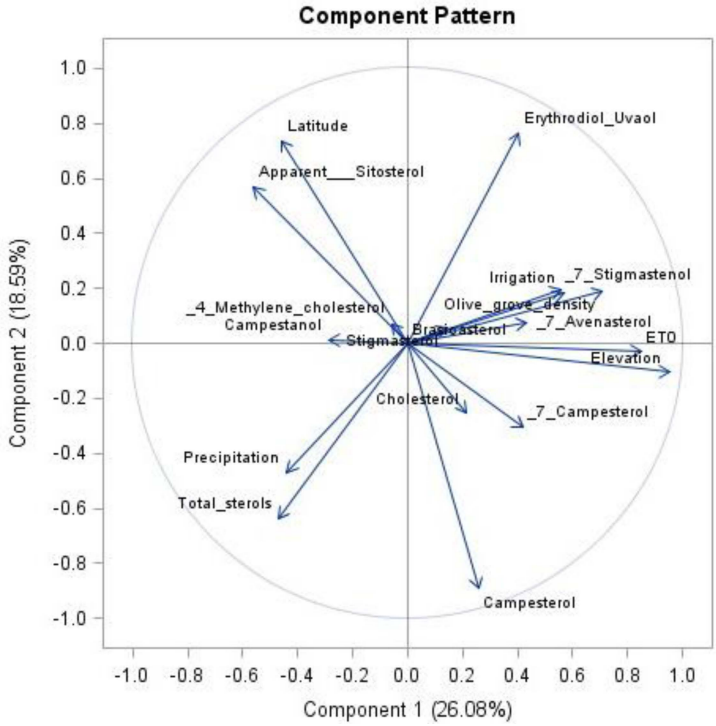



**Figure 5.** Principal component analysis (PCA) biplot (44.67 % of explained variance) after Varimax rotation including main meteorological and orchard characteristics and the sterols and triterpenes dialcohols compounds.

### *3.6. Fatty Acids*

Environmental factors modify fatty acid profile. For instance, high temperatures during fruit development decrease the oleic acid concentration in EVOOs [8] and altitude is related to differences in fatty acid profile [52]. Most of the fatty acids and the oxidative susceptibility were influenced by the irrigation treatment, the location, and the crop season (Table 5). These differences, despite statistically significant, are of small magnitude.

**Table 5.** Three-way ANOVA (prob > F) and least square means of fatty acids for the different factors: Irrig: irrigation (full irrigation: FI; regulated deficit irrigation: RDI); Loc: locality (Mad: Madrid; Lle: Lleida; Tar: Tarragona; Ali: Alicante; Nav: Navarra); and CS: crop season (2015 and 2016).

| Fatty Acids (%) | Factor (*p*-Value) | | | Factors | | | | | | | | | Standard of Fatty Acids in EVOO |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Irrig. | Loc. | CS | Irrig. | | | Loc. | | | | CS | | |
| | | | | FI | RDI | Mad | Lle | Tar | Ali | Nav | 2015 | 2016 | |
| Myristic acid | 0.014 | 0.000 | 0.000 | 0.011b | 0.014a | 0.010d | 0.012c | 0.013b | 0.017a | 0.010d | 0.014a | 0.011b | ≤0.03 |
| Palmitic acid | 0.039 | 0.000 | 0.009 | 14.095b | 14.329a | 14.273b | 14.396b | 15.080a | 14.553b | 12.757c | 14.087a | 14.336b | 7.50–20.00 |
| Palmitoleic acid | 0.000 | 0.000 | 0.025 | 1.686a | 1.611b | 1.544bc | 1.478c | 2.429a | 1.613b | 1.178d | 1.689a | 1.608b | 0.30–3.50 |
| Margaric acid | 0.003 | 0.003 | 0.003 | 0.099b | 0.110a | 0.104a | 0.109a | 0.097b | 0.109a | 0.104a | 0.098b | 0.111a | |
| Margaroleic acid | 0.000 | 0.000 | NS | 0.220b | 0.233a | 0.217c | 0.221c | 0.235b | 0.247a | 0.213c | 0.226a | 0.227a | |
| Stearic acid | 0.000 | 0.000 | 0.000 | 1.809b | 1.886a | 1.833c | 1.919b | 1.695d | 1.812c | 1.979a | 1.814b | 1.881a | 0.50–5.00 |
| Oleic acid | 0.000 | 0.000 | 0.000 | 72.079a | 71.458b | 73.357b | 71.174c | 69.530d | 68.964e | 75.816a | 72.078a | 71.459b | 55.00–83.00 |
| Linoleic acid | 0.000 | 0.000 | 0.000 | 8.589b | 8.942a | 7.330c | 9.282b | 9.415b | 11.143a | 6.659d | 8.582b | 8.949a | 3.50–21.00 |
| Linolenic acid | NS | 0.000 | NS | 0.501a | 0.506a | 0.451d | 0.495c | 0.559b | 0.595a | 0.417e | 0.499a | 0.508a | |
| Arachidic acid | 0.000 | 0.000 | 0.000 | 0.381b | 0.391a | 0.366c | 0.396a | 0.388b | 0.397a | 0.384b | 0.389a | 0.383b | ≤0.060 |
| Gadoleic acid | NS | 0.000 | NS | 0.307a | 0.296a | 0.289c | 0.301bc | 0.320a | 0.316ab | 0.283c | 0.304a | 0.300a | |
| Behenic acid | NS | NS | NS | 0.136a | 0.138a | 0.136a | 0.130a | 0.149a | 0.143a | 0.127a | 0.137a | 0.137a | ≤0.020 |
| Lignoceric acid | NS | 0.001 | NS | 0.064a | 0.062a | 0.062b | 0.064ab | 0.068a | 0.066ab | 0.055c | 0.064a | 0.062a | ≤1.00 |
| SFA | 0.002 | 0.000 | 0.002 | 16.60b | 16.93a | 16.78b6 | 17.026b | 17.487a | 17.094b | 15.415c | 16.601b | 16.922a | |
| MUFA | 0.000 | 0.000 | 0.000 | 74.299a | 73.603b | 75.406b | 73.184c | 72.516d | 71.153e | 77.498a | 74.297a | 73.606b | |
| PUFA | 0.000 | 0.000 | 0.000 | 9.105b | 9.470a | 7.807c | 9.789b | 10.002b | 11.753a | 7.088d | 9.102b | 9.473a | |
| MUFA/PUFA | 0.000 | 0.000 | 0.042 | 8.651a | 8.225b | 9.7b | 7.8c | 7.3d | 6.4e | 11.0a | 8.521a | 8.354b | |
| OS | 0.001 | 0.000 | 0.000 | 510.9b | 526.6a | 450.3c | 540.3b | 552.1b | 632.1a | 418.9d | 510.4b | 527.1a | |

SFA: Saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, OS: oxidative susceptibility. NS = not significant at $p < 0.05$. Different letters mean significant differences between levels of the treatment according to the LSD test $p < 0.05$. Number of replicates = 3.

EVOOs from olive groves under RDI had a lower percentage of monounsaturated fatty acids (73.6%) than those under FI (74.3%), and thus the oxidative susceptibility was affected (527 with RDI and 511 with FI). These results are opposite to those described by Hernández et al. (2018) that found a reduction in linoleic acid with an RDI of 30% of ETc [23], but not with an RDI of 60% regarding FI; or the results of Garcia-Garvi et al. (2022) [36] that reported an increase in 3.3% of MUFA under the most restricted irrigation and no differences with the intermediate irrigation treatments. This former group also found important differences between crop seasons in all the fatty acids studied.

Navarra's EVOOs showed the highest oleic acid content, with almost +7% than Tarragona's EVOOs (Table 5). Navarra's EVOOs also had the lower linoleic acid level, while Alicante's EVOOs had the highest. These localities are settled at 283 masl and 87 masl (Table 1), which disagrees with the results reported by Aparicio and García-González (2013) [52] in the Picual cultivar, who found a positive relationship between linoleic acid and altitude. Thus, other factors could affect fatty acid composition on a major scale than altitude.

Figure 6 shows the PCA biplot including the main meteorological data (yearly rainfall, yearly ETc, and yearly irrigation) of each crop season, orchard characteristics (latitude, density, and elevation) in every olive orchard, and the fatty acid composition. Axis 1 explained 30.66 % of the total variance and axis 2 explained 24.13%. The more irrigation is applied, the higher the oleic acid percentage of EVOOs. On the other hand, rain was linked to linoleic, palmitic, and palmitoleic acids. Olive orchards at lower altitudes seem to have higher percentages of myristic, margaroleic, or linolenic acids.

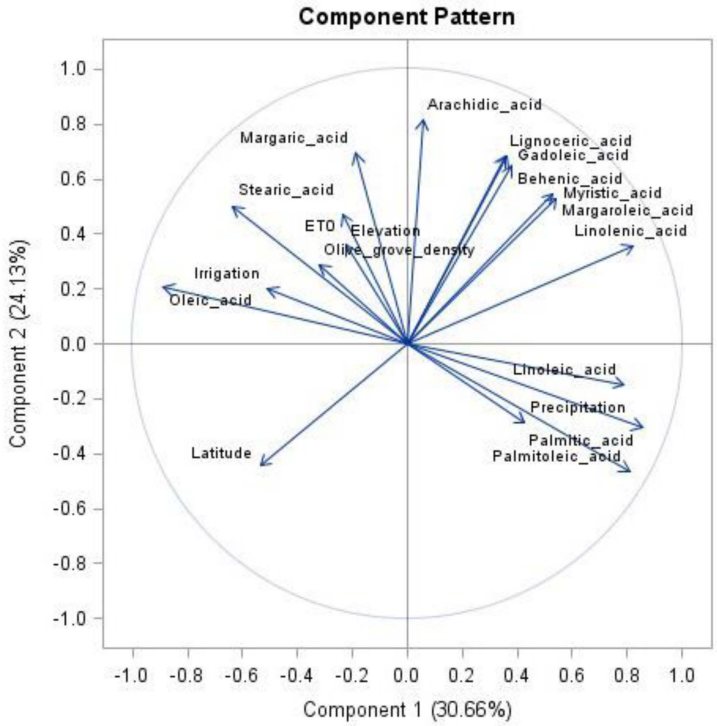



**Figure 6.** Principal component analysis (PCA) biplot (54.79 % of explained variance) after Varimax rotation including main meteorological and orchard characteristics and the fatty acids.

## 4. Conclusions

The edaphoclimatic conditions of the olive orchard had a great influence on different quality parameters of the olive fruits, even more than the irrigation treatment applied in this work. The regulated deficit irrigation (RDI) treatment with the application of 40% of water needs after massive pit hardening during phase II of growing fruit, and 100% during the rest of the year, in 5 different locations of central, eastern, northeast, and northern of Spain is an interesting irrigation management. This strategy contributes to water saving (more

than 20% on average in two crop seasons in the 5 localities), maintaining the crop load and olive oil quality. The most sensitive compounds to this irrigation regime were some sterols (campesterol and apparent β-sitosterol), triterpenic dialcohols, and the majority of the fatty acids. Despite statistically significant differences being detected, these were of small magnitude, so we can conclude that the RDI strategy applied is appropriate to save water and to keep the high standard of extra virgin olive oil.

It is crucial to deepen research among genotype–environment relationships due to the effects on virgin olive oil composition, and to perform long-term trials to thoroughly assess the impact of RDI on olive fruit and virgin olive oil yield.

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