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Olive Oil Composition of Cv. Cobrançosa Is Affected by Regulated and Sustained Deficit Irrigation †

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Abstract: The aim of this study was to evaluate the effect of different irrigation strategies on cv. Cobrançosa olive oil main components, in a semiarid region in the Northeast of Portugal (Alfândega da Fé, 2019)—regulated (RDI) and sustained deficit (SDI) irrigation against well-irrigated controls (FW). Total polyphenols (Folin) were higher in RDI than SDI and FW treatments. Among the phenolic components, hydroxytyrosol and tyrosol derivatives (HPLC, after acid hydrolysis), were higher in olive oils obtained under SDI, potentially complying with the nutrition allegation allowed in Regulation (EU) No 432/2012, ("polyphenols in olive oil contribute to the protection of blood lipids against undesirable oxidation"), while the amounts in FI_{120} and RDI_{100} olive oils were 10% lower to the threshold. Olive oil vitamin E (mainly α-tocopherol) was also higher in oils obtained from SDI deficit irrigation treatments while oils from RDI had values very close to FI treatments. Olive oil bitterness, evaluated by K_{225}, was highly positively correlated with TP ($r^2 = 0.94$, $p < 0.01$). The fatty acidy profile was not affect by the irrigation regime. Results are preliminary and need to be continued to extract solid conclusions.

Keywords: irrigation; extra-virgin olive oil; biophenols; vitamin E; oil bitterness

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1. Introduction

Olive trees are usually grown in areas with limited available water resources. Although they can grow and produce adequate yields under low annual rainfall, irrigation is crucial to attain high yields and ensure production stability between years [1,2]. However, the increasing water scarcity due to climate changes and increased competition of water for other uses in our society has caused pressure to reduce the irrigation [3]. Therefore, great emphasis is placed on irrigation management in arid regions with the aim of increasing water use efficiency leading to adopt deficit irrigation (DI) approaches to save water. Important savings in the levels of irrigation without an associated penalty in yield have been reported for olive under sustained (SDI) and regulated deficit irrigation (RDI) strategies [2].

In relation to olive oil composition, there is a controversy on the effect of irrigation on the quality of virgin olive oil (VOO), as the effect on the physico-chemical composition is cultivar dependent and its interaction with edaphic and environmental conditions. High irrigation rates are associated with a decrease mainly in minor compounds of virgin olive oil (VOO) as they are total polyphenols (TP), orto-diphenols (OD), tocopherols (TC) volatile compounds (VC) [4,5] that have an important role in nutritional value, biological proprieties, and organoleptic characteristics of VOO. Unlikeness SDI strategies often have better olive oil quality compared to well irrigated [6]. In relation of RDI strategies when applied in summer it was observed a significantly increase in polyphenol concentration and oil oxidative stability [7]. These compounds are of great interest because they influence the quality and the palatability of olive oils and increase their shelf life by slowing the formation of polyunsaturated fatty acid hydroperoxides [8]. Thus, DI strategies would allow improving the overall chemical-sensory quality and ensuring that the legal requirements concerning the health claim are fulfilled. However, there is still uncertainty about which deficit irrigation strategies are better, although a wide array of alternatives exists, particularly regarding whether the application is constant (CDI or sustained SDI) or regulated to specific periods (RDI). On the other hand, no studies have been performed on the effect of DI strategies in the health claim fulfillment. In this context, this study aims to evaluate the effect of different SDI and RDI strategies on olive oil composition and quality of cv. Cobrançosa and to assess the contribution to the fulfilment of the European Food Safety Authority health claim [9].

2. Material and Methods

2.1. Study Site Conditions

The study was conducted during the season of 2019, in a 25-years-old commercial olive orchard (*Olea europaea* L. cv "Cobrançosa"), with a tree spacing of 6 m × 6 m, located at Vilariça Valley (41.33° N, 7.04° W; 240 m altitude) a typical olive growing area of Northeast of Portugal, with a Csa climate by Köppen-Geiger classification [10]. The irrigation of the experimental plot of 2.5 ha was scheduled on the basis of daily evapotranspiration of the crop (ET) accumulated during the previous week. ET values were estimated by multiplying reference evapotranspiration (ET_o), calculated with the Penman-Monteith methodology, via a monthly local crop coefficient (K_c), according to [1]. The correction coefficient for ground cover (K_r) was done, according to [11]. Six irrigation treatments were tested: (i) full irrigated (FI), the control treatment, that received an amount of water equivalent to 100% of estimated evapotranspiration (ET); (ii) over full irrigated (FI_{120}) that received 120% of estimated ET; (iii) two sustained deficit irrigation (SDI) with 60% (SDI_{60}) and 30% (SDI_{30}) of FI, and (iv) two regulated deficit irrigation, one irrigated equally to FI (RDI_{100}) except in the pit hardening period in which irrigation was reduced to 10%, and in the other, irrigation was cut off at pit hardening period (end of July to the third week of August), after that was irrigated equally to SDI_{60}. Irrigation started at June 16th and stopped at October 1th, and olive trees were irrigated with a drip line emitters (± 4 L/h). Plant water status was evaluated periodically by shoot water potential and relative water content according to the methodology reported in [12].

2.2. Olive Oil Samples

Harvest was performed at the end of November. Each tree was manually harvested and the yield weighed at the site. The olive ripeness index was determined in a sample of 100 fruits/tree that were randomly collected around the canopy of the tree and three replications per treatment were sampled; the fruits were classified using a 0 to 7 scale according to skin color [13]. Subsamples of around 3 kg of each tree/treatment in seven olive trees were collected and mixed to complete a sample of 30 kg which was used for oil extraction by the system Oliomio (Oliomio 50) hammer mild. The paste underwent malaxation at room temperature for 30 min and the oil extracted with a two-phase decanter. The olive oils were put in 750 mL dark glass and stored in the dark at room temperature. Analysis were carried out after 5 months of extraction. All the assays were carried out in triplicate.

2.3. Evaluation of Quality Parameters

Olive oils were analyzed according to the methodologies described by the European Union standard methods [14]. Free acidity (FA), given as % of oleic acid, peroxide value (PV), expressed as mEq of active oxygen per kg of oil ($mEqO_2/kg$), and the specific coefficients of extinction at 232 nm and 270 nm (K_{232} and K_{270}) were analyzed.

2.4. Index K_{225}

Bitterness index (K_{225}) was determined by the method described by [15] which consists of the extraction of the bitter components from a sample of 1.0 ± 0.01 g of oil dissolved in 4 mL of hexane passed through a C18 column (Cromabond spe, Macherey_Nagel Inc., USA) previously activated with methanol and washed with hexane. After elution, 10 mL of hexane was passed to eliminate the oil residues 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.

2.5. Polyphenol Content

Phenolic compounds were isolated and extracted using the modified method described by [16]. The oil sample (10 g) dissolved in n-hexane (25 mL), was extracted (10 mL) in triplicate with methanol/water (60:40). The aqueous fractions were collected in a volumetric flask (50 mL) and completed with distilled water to obtain the total polyphenol extract. The concentration of total polyphenol was estimated with the Folin-Ciocalteau reagent at 725 nm using a calibration curve ($R^2 = 0.9996$) of gallic acid in methanol (0.78–25 mg/L). Results were expressed as mg of gallic acid per kg of oil.

2.6. Total Content of Hydroxytyrosol and Tyrosol Derivatives: Acid Hydrolysis of Secoiridoids

Phenolic compounds from olive oils were also analytically extracted and analyzed according to the method proposed by [17] with some modifications according to [18]. All samples were analytically extracted and injected in duplicate totalizing 12 chromatographic results (for the 6 irrigation treatment) The total hydroxytyrosol or tyrosol contents after hydrolysis were expressed as the individual sum in mg of hydroxytyrosol or tyrosol equivalents, respectively, per kg of oil. Hydroxytyrosol and tyrosol calibration curves ($R^2 = 0.9992$ and 0.9990, respectively) were prepared in methanol/water (80:20, *v*/*v*) in a concentration range from 0.0005 to 0.02 mg/mL. Because after hydrolysis only the tyrosol and hydroxytyrosol moieties are quantified, loosing information on the molecular weight of the original molecules, the original bound forms were estimated using the correction factors proposed in the literature for hydroxytyrosol (2.2) and tyrosol (2.5) [19,20].

2.7. Vitamin E Content

Vitamin E content was obtained by adding the individual tocopherol contents (α-, β- and γ-), which were determined according to the ISO 9936 (2006), with some modifications [21]. Tocopherols standards and the internal standard 2-methyl-2-(4,8,12-trimethyltridecyl) chroman-6-ol (tocol) were respectively from Sigma (Spain) and Matreya Inc. (USA). Fifty mg of filtered olive oil and tocol were dissolved in n-hexane and centrifuged at 13,000 rpm during 5 min being then the supernatant analyzed by HPLC. A liquid chromatograph comprised a data unit (Jasco, Japan), a Pump (PU-1580) and a fluorescence detector ($\lambda exc = 290$ nm; $\lambda em = 330$ nm). The equipment and the analysis conditions are described in detail by [21].

2.8. Fatty Acids Composition

Fatty acids were determined following the European Community Regulation EEC/2568/91 from 11th July. According to Rodrigues et al. (2020) a Chrompack CP 9001 chromatograph with a split-splitless injector, a FID detector and a Chrompack CP-9050 autosampler was used. Fatty acids separation was achieved using a Select FAME fused silica capillary coated column (50m × 0.25mm i.d.) (Agilent). Helium was used as carrier gas (internal pressure equal to 110 kPa). The detector and injector temperatures were equal to 250 °C and 230 °C, respectively. A split ratio of 1:50 was used being injected 1 μL. Fatty acids levels were expressed in relative percentage, determined by internal normalization of the chromatographic peak area eluting between myristic and lignoceric methyl esters. Peaks identification and quantification were carried out using a control (olive oil 47118, from Supelco) and a certified fatty acids methyl esters standard mixture (Sigma, Spain).

3. Results

3.1. Plant Water Status

At the start of the irrigation season (DOY 165) no differences were observed in midday shoot water potential (Ψ_{MD}) and relative water content (RWC) between treatments (Figure 1 and Table 1) and the values are in the range of that reported for well-watered conditions in previous studies in this cv. [12]. During the irrigation season, Ψ_{MD} remains almost constant and higher than −2.5 MPa in FI, FI_{120} treatments, although the values in the later treatment are slight higher. The behavior of RWC was similar to Ψ_{MD} and values are usually higher than 90%. In the RDI_{100} treatment no differences were observed in both plant water status indicators (Ψ_{MD} and RWC) in relation to well-watered treatments until the DOY 211, afterward the values of Ψ_{MD} dropped drastically reaching values of −3.9 MPa and 85.0% for Ψ_{MD} and RWC, respectively, due to the reducing of amount of irrigation applied to 10% of FI during the pit hardening period (from the day of the year 206 until 230). After this phase, irrigation was replaced to values similar to FI and the values either to Ψ_{MD} and RWC were recovered to values in the same range of FI. However, in early autumn, after the end of irrigation the values fallen again reaching −3.5 MPa for Ψ_{MD} and 84.1% for RWC. The RDI_{60} showed a similar trend than RDI_{100} along the irrigation season, although the values of Ψ_{MD} and RWC are lower and statistically different from FI, FI_{120} and RDI_{100}. In the irrigation cut off period it was observed values very low either for Ψ_{MD} and RWC, −6.2 MPa and 70.8 %, respectively. For the SDI_{60} irrigation treatment, it was observed that the values of plant water status indicators (Figure 1 and Table 1) were slight lower than that observed for the control treatment (FI) until the DOY 211, and as drought intensify during the season these values showed a progressive decrease reached minimal values (Ψ_{MD} = −4.1 MPa; RWC= 79.6% in DOY 252) in early Autumn and they were statistically different from the control.

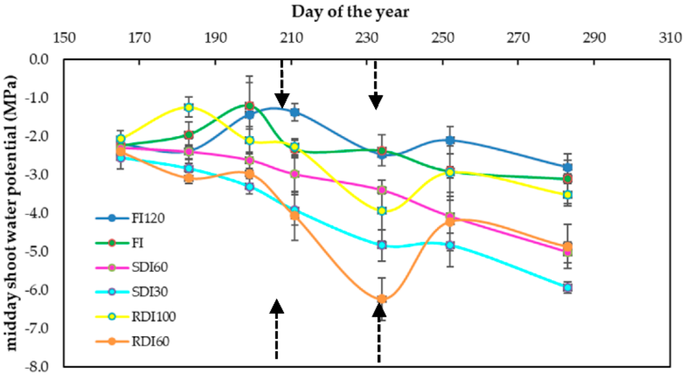

Figure 1. Seasonal time course of midday shoot water potential (Ψ_{MD}) for FI-full irrigation, FI_{120}-over full irrigation, sustained deficit irrigation (SDI_{30} and SDI_{60}) and regulated deficit irrigation

(RDI$_{100}$ and RDI$_{60}$) treatments during the irrigation season of 2019 (mean ± standard deviation, n = 3). The arrows indicated the period of irrigation cut of in RDI$_{60}$ and irrigation reduction in RDI$_{100}$.

Table 1. Mean values of relative water content (RWC, %) for FI-full irrigation, FI$_{120}$-over full irrigation, sustained deficit irrigation (SDI$_{30}$ and SDI$_{60}$) and regulated deficit irrigation (RDI$_{100}$ and RDI$_{60}$) treatments during the irrigation season of 2019 (mean ± standard deviation, n = 5).

Treat.	**Day of the Year (from June 14th to October 25th)**							
	165	**183**	**211**	**234**	**240**	**252**	**283**	**298**
FI$_{120}$	92.9 ± 0.8	89.5 ± 3.2	87.8 ± 1.1	91.5 ± 2.5	91.3 ± 2.5	88.1 ± 3.4	88.5 ± 1.8	95.7 ± 1.0
FI	94.5 ± 1.8	90.0 ± 2.3	89.5 ± 1.5	90.5 ± 1.8	88.9 ± 0.9	90.2 ± 0.7	85.9 ± 3.5	95.2 ± 1.7
SDI$_{60}$	91.4 ± 2.9	86.2 ± 5.0	85.5 ± 4.0	82.7 ± 0.8	89.7 ± 2.1	80.8 ± 1.1	76.4 ± 1.6	96.8 ± 0.6
SDI$_{30}$	90.7 ± 1.6	81.4 ± 3.5	73.5 ± 4.7	71.5 ± 5.2	82.6 ± 5.2	68.7 ± 5.1	61.9 ± 5.1	95.2 ± 1.7
RDI$_{100}$	93.0 ± 1.1	91.4 ± 0.3	86.7 ± 2.1	85.0 ± 2.6	90 ± 1.8	91.4 ± 5.8	84.1 ± 3.1	96.1 ± 2.7
RDI$_{60}$	92.6 ± 1.1	86.8 ± 2.3	79.3± 3.0	70.8 ± 4.4	88.6 ± 2.4	79.6 ± 6.0	72.2 ± 6.7	93.1 ± 1.8

Comparing RDI$_{60}$ with SDI$_{60}$, was observed that plant water status indicators was always lower in RDI$_{60}$ and it was noticeable that olive trees experienced a water deficit more pronounced. In the SDI$_{30}$ treatment, it is possible to observe a gradual decrease during the first 4 weeks after the start of irrigation, with values very close to those observed in SDI$_{60}$ and RDI$_{60}$, but which decrease sharply with the intensification of the water deficit (RWC = 61.9% and Ψ_{MD} −5.9 MPa) being statistically different from the others irrigation treatments.

3.2. Quality Parameters and Bitterness Index

The results for quality parameters and bitterness index (K$_{225}$) are presented in Table 2. Free acidity (FA) ranged between 0.18% to 0.32%, respectively to SDI$_{30}$ and FI. The values didn't show a consistently trend with plant water status. According to the results obtained for this parameter, it was verified that there is a low global free acidity, below the limit of 0.8% established by Commission Delegated Regulation (EU) 2015/1830 of 8th July for the EVOO category. For the peroxide value (PVs), indicative of oxidation, the lower value was also observed in more stressed treatments, 5.3 and 5.4 mEqO$_2$/kg olive oil (SDI$_{30}$ and SRDI60) and the higher in well-watered treatments with 9.0 mEqO$_2$/kg olive oil in the control treatment (FI). Once again all PVs were below the 20 mEqO$_2$/kg maximum limit established by Commission Delegated Regulation (EU) 2015/1830 of 8th July for the classification of olive oil as EVOO. For the extinction coefficients, namely for K$_{232}$, the values are lower than 1.50, with the lowest observed in RDI$_{60}$ (1.17 ± 0.44) as apposed in FI that presented the highest (1.50 ± 0.27). In relation of K$_{270}$ values are quite similiar in all treatments and less than 0.08. All extinction coefficient values were within the legal limits established by the European Community Regulation EEC/2568/91 from 11th July for EVOO.

Olive oil bitterness measured by the instrumental K$_{225}$ parameter (Table 2), called bitterness index, ranged from 0.12 to 0.45, with higher values in deficit irrigation treatment (RDI$_{60}$, SDI$_{60}$ and RDI$_{100}$), and the lowest was observed in the treatment that received the higher amount of applied water (FI$_{120}$). The values of the control (FI) were 2.6 higher than those of the over full-irrigated (FI$_{120}$) and in deficit irrigation treatment (SDI$_{60}$, RDI$_{60}$) treatments they were around 1.5 higher than that of the control.

Table 2. Free acidity (% oleic acid), peroxide value (mEq O$_2$/kg), specific extinction coefficients (K$_{232}$ and K$_{270}$) and bitterness index (K$_{225}$) of olive oils obtained from olives produced from different irrigation strategies during the year 2019 (mean ± standard deviation).

Treatment	Free Acidity	Peroxide value	K$_{232}$	K$_{270}$	K$_{225}$
FI	0.32 ± 0.01	9.0 ± 0.2	1.50 ± 0.27	0.07 ± 0.02	0.31 ± 0.01
FI$_{120}$	0.31 ± 0.01	7.4 ± 0.2	1.40 ± 0.11	0.08 ± 0.00	0.12 ± 0.01
SDI$_{60}$	0.27 ± 0.01	5.6 ± 0.2	1.25 ± 0.43	0.08 ± 0.02	0.43 ± 0.03
SDI$_{30}$	0.18 ± 0.01	5.4 ± 0.2	1.26 ± 0.33	0.08 ± 0.01	0.21 ± 0.01
RDI$_{100}$	0.23 ± 0.01	7.0 ± 0.3	1.25 ± 0.06	0.08 ± 0.01	0.40 ± 0.00
RDI$_{60}$	0.25 ± 0.01	5.3 ± 0.2	1.17 ± 0.44	0.06 ± 0.02	0.45 ± 0.00

[1] FI-full irrigation, FI$_{120}$-over full irrigation; sustained deficit irrigations (SDI$_{30}$ and SDI$_{60}$) and regulated deficit irrigations (RDI$_{100}$ and RDI$_{60}$).

3.3. Effect of Irrigation Regime in Polyphenols and in the Total Content of Hydroxytyrosol and Tyrosol Derivatives and Health Cliam Evaluation

The obtained results for total phenols (TP) are presented in Table 3. TP varied from 462.6 to 762.1 mg/kg with the lowest value obtained for FI$_{120}$ as the opposite observed in RDI$_{60}$. In general, a tendency of diminution of TP was observed with the increase of water applied.

The results of vitamin E content (Table 3), mainly α-tocopherol, varied from 266.3 ± 3.0 mg/kg (RDI$_{100}$) to 314.8 ± 0.6 mg /kg (SDI$_{30}$) and didn't showed a pattern with plant water status.

The concentrations of hydroxytyrosol and tyrosol quantified by HPLC-DAD after the acid hydrolysis of the polar fraction and the assessment the oils' health claim fulfillment (European Commission Regulation EU No 432/2012,2012) are presented in Table 3. The concentration of hydroxytyrosol ranged from 51.4 mg /kg (RDI$_{60}$) to 178.6 ± 4.6 mg /kg (SDI$_{30}$). Comparing the more stressed treatments the values observed in SDI$_{30}$ are 347.5% higher than that of RDI$_{60}$. For the SDI$_{60}$ and RDI$_{100}$, that received a seasonal equivalent amount of water but with different distribution along the season, results demonstrated that the values in the former treatment was 130% than RDI$_{100}$. Only SDI treatments presented values higher than the well-watered (FI and FI$_{120}$). The results for tyrosol showed a similar pattern of that reported for hydroxytyrosol except for RDI$_{100}$ that had higher values than well-watered treatments. The concentration of tyrosol ranged from 46.3 mg /kg (RDI$_{60}$) to 155.4 mg /kg (SDI$_{30}$).

The total amounts of hydroxytyrosol and tyrosol derivatives the higher values were obtained in olives oils from SDI treatments, with 5.7 ± 0.1 mg/20g in SDI$_{60}$ and 6.7 ± 0.2 mg/20g in SDI$_{30}$, followed by the control tretament (4.9 ± 0.0 mg/20g) and the lowest value was observed in RDI$_{60}$ (2.0 ±0.0 mg/20g) while RDI$_{100}$ and FI$_{120}$ are values very close (4.6 ± 0.0–4.7 ± 0.1 mg/20g).

Table 3. Total phenols (mg of gallic acid equivalent/kg of olive oil), vitamin E (mg/kg of olive oil), concentrations of hydroxytyrosol (mg of hydroxytyrosol equivalent/kg of olive oil) and tyrosol (mg of tyrosol equivalent/kg of olive oil), and amounts of the sum of both compounds (mg/20 g of oil) after the secoiridoids' acid hydrolysis and determined by HPLC-DAD (280 nm) of olive oils obtained from olives produced from different irrigation strategies during the year 2019 (mean ± standard deviation).

Treatment	Total Polyphenolics	Vitamin E	Hydroxytyrosol	Tyrosol	Hydroxytyrosol + Tyrosol
FI	611.9 ± 12.6	269.2 ± 1.7	128.0 ± 4.6	118.6 ± 0.9	4.7 ± 0.1
FI$_{120}$	462.6 ± 21.3	296.0 ± 8.4	122.9 ± 1.4	113.2 ± 1.5	4.9 ± 0.0
SDI$_{60}$	677.4 ± 17.7	309.2 ± 5.0	139.0 ± 3.0	143.7 ± 0.5	5.7 ± 0.1
SDI$_{30}$	548.6 ± 19.3	314.8 ± 0.6	178.6 ± 4.6	155.4 ± 3.3	6.7 ± 0.2
RDI$_{100}$	735.7 ± 22.9	266.3 ± 3.0	106.8 ± 0.9	123.2 ± 1.0	4.6 ± 0.0

RDI$_{60}$	762.1 ± 20.5	280.3 ± 2.0	51.4 ± 1.0	46.3 ± 0.1	2.0 ±0.0

FI-full irrigation, FI$_{120}$-over full irrigation; sustained deficit irrigations (SDI$_{30}$ and SDI$_{60}$) and regulated deficit irrigations (RDI$_{100}$ and RDI$_{60}$).

3.4. Fatty Acid Composition

The fatty acid composition is presented in Table 4. Oleic acid (C$_{18:1}$) values are < 73% in all treatments and without a consistent behavior with irrigation treatments, that showed a slighted difference between them, hence the values ranged from 71.3% to 72.7%. Similarly, linoleic acid (C$_{18:2}$) showed an inconsistent trend with irrigation treatment and values are < 9% in all treatments.

Table 4. Concentration (%) of the main fatty acid, palmitic acid (C$_{16:0}$); oleic acid (C$_{18:1}$), linoleic acid (C$_{18:2}$), ratio of the total unsaturated to saturated fatty acid (UFA/SAT) and ratio of monounsaturated to polyunsaturated fatty acid of olive oils obtained from olives produced from different irrigation strategies during the year 2019 (mean ± standard deviation).

Treatment	C$_{16:0}$	C$_{18:1}$	C$_{18:2}$	UFA/SAT	MUFA/PUFA
FI	12.7 ± 0.1	71.3 ± 0.1	8.7 ± 0.0	4.7 ± 0.0	7.6 ± 0.0
F$_{120}$	12.6 ± 0.1	72.4 ± 0.1	8.0 ± 0.0	4.8 ± 0.0	8.3 ± 0.0
SDI$_{60}$	11.3 ± 0.0	72.1 ± 0.1	8.4 ± 0.0	4.8 ± 0.0	7.9 ± 0.0
SDI$_{30}$	11.0 ± 0.0	72.1 ± 0.1	8.9 ± 0.1	5.0 ± 0.0	7.5 ± 0.1
RDI$_{100}$	11.7 ± 0.4	71.8 ± 0.3	8.8 ± 0.1	4.9 ± 0.1	7.6 ± 0.0
RDI$_{60}$	11.1 ± 0.0	72.7 ± 0.1	8.3 ± 0.1	4.9 ± 0.0	8.1 ± 0.1

FI-full irrigation, FI$_{120}$-over full irrigation; sustained deficit irrigations (SDI$_{30}$ and SDI$_{60}$) and regulated deficit irrigations (RDI$_{100}$ and RDI$_{60}$).

For palmitic acid (C$_{16:0}$) the higher values was observed in well-watered treatments while the lower in the more stressed treatments (SDI$_{30}$ and RDI$_{60}$), and the diferences between irrigation treatments ranged between 0.1% to 1.7%. The relation between unsaturated (mainly due to C$_{16:0}$) and saturated fatty acids (mainly due to C$_{18:1}$) showed that values of SDI$_{30}$ > RDI$_{100}$- RDI$_{60}$ > FI$_{120}$- SDI$_{60}$ > FI, although difference between treatments are very small (0.3). For the relation of monounsaturated (MUFA) and polyinsaturated (PUFA) fatty acid, mainly due to the ratio C$_{18:1}$/C$_{18:2,}$ it followed the order FI$_{120}$ > RDI$_{60}$ > SDI$_{60}$ > FI-RDI$_{100}$ > SDI$_{30}$ with the highest differences between FI$_{120}$ and SDI$_{30}$.

4. Discussion

The low values for RWC and Ψ_{MD} specifically during summer indicates that olives trees from SDI$_{30}$ experienced a moderate to severe water stress (Fernandes-Silva et al., 2010; 2016). The drought imposed in RDI$_{100}$ treatment, by the reducing of water applied in the pit hardening period, led to a moderate water stress in the olive trees, similar to that of SDI$_{60}$, but after irrigation reestablishment, a fast recovery of plant water status of observed. In the same period, irrigation cut off in RDI$_{60}$ treatment led to the lowest values of all water status indicators recorded in this treatment, that were similar to those reported in rainfed conditions in this cv. in previous works [12]. After the reestablishment of irrigation, plant water status recovered to values similar to those of SDI$_{60}$.

In terms of olive oil quality parameters, free acidity didn't show a consistently trend with plant water status: the lower values were observed in the more stressed treatments, thought the differences between treatments were very small (≤0.07%). Previous studies in this cv. showed that FA was more influenced by years' conditions than irrigation treatments [6]. In cv. Frantoio [22] reported that FA was unaffected by irrigation, although slightly lower values were measured in oils obtained from severely stressed trees. Several studies showed that SDI irrigation strategies [23,24] and RDI strategies in olive trees do not affect FA [25]. PVs values showed a tendency to decrease with water deficit, as observed in several studies [6,22]. By contrast, many studies have reported no relationship between irrigation and PVs values [22,26]. The presence of conjugated diene content, as indicated by K_{232} showed a tendency of decrease with water deficit values, a similar trend to PVs, which is consistent of others studies [22] while K_{270} was almost constant between treatments. However, the effect of irrigation on these parameters are sometimes contradictory. For example, several studies [25,27] reported that theses indices increased with water deficit and others reported no effect [28].

Olive oil bitterness, measured by the instrumental K_{225} parameter called bitterness index, showed a general decrease with water applied which is in agreement with the results found by Gómez-Rico [27] and was highly positively correlated with TP ($r^2 = 0.94$, $p < 0.01$) as phenolic compounds are responsible for the bitterness of olive oil. It seems that this index was independently of the period of water stress as values obtained in SDI and RDI deficit irrigation are quite similar.

TP increased with water deficit as they are secondary metabolites that are synthetized by plants to protection of free radical scavenging in response of environmental stress. Previous studies on this cv. [29] showed that the polyphenols in fruits decreased with the amount of water applied and that this decrease was highly correlated with the reducing activity of L-phenylalanine ammonia-lyase (PAL). Oils obtained for both regulated deficit irrigation treatments (RDI_{100} and RDI_{60}) showed a higher content than those obtained in sustained deficit irrigation conditions (SDI_{60} and SDI_{30}). Several studies reported a significant linear relationship between total polyphenols and water stress integral [4,6,25]. These results are very important because total polyphenols are also closely related to the oxidative stability of olive oil [6]. Therefore, oil with a high polyphenol content will have a longer shelf life. For vitamin E content, mainly α-tocopherol, results didn't show a pattern with plant water status which is consistent with others studies [6,27] and contradictory to the findings of [30].

The obtained results of hydroxytyrosol and tyrosol derivatives are in the range of values observed to this cv. in different environmental conditions [18,31] and it seems that they were influenced with occurrence of the drought during oil biosynthesis as RDI_{60} had the lowest values while SDI strategies had the highest. Only EVOO from SDI treatments fulfil the health claim [32], while EVOO from FI are very close to health claim.

The fatty acid composition didn't show a consistent behavior with irrigation treatments. Some studies reported that fatty acid composition was unaffected by water stress [7,25], while in others was observed a trend of reduced oleic acid (C18:1) and increased linoleic acid (C18:2) content with increasing water stress. This contrasting behavior may be attributed to the higher influence of varietal factors and climatic conditions on the fatty acid composition than by water status. Among the environmental factors, temperature can play an essential role in fatty acid composition [33].

5. Conclusions

Our preliminary results indicated that the evaluated irrigation cut-off strategies (RDI) and sustained deficit irrigation (SDI) affected the total polyphenols, being the EVOO from RDI richer than SDI. Nevertheless, these differences weren't sufficient enough to express effects on olive oil bitterness (expressed by the index K_{225}) in EVOO from SDI_{60}, that had higher values like RDI treatments; while in EVOO from SDI_{30} they caused a reduction in a half in K_{225}. An opposite pattern was observed in individual phenols, hydroxytyrosol and tyrosol derivatives that are higher in EVOO from both SDI and met the health claim ensuring that their daily intake would contribute to the protection of blood lipids from oxidative stress, while EVOO from FI and RDI_{100} were very close. These preliminary results are interesting and should be investigated in order to extract solid conclusions that help in choosing the irrigation strategy with the best compromise between water use efficiency, oil yield and quality.

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