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Effects of Summer Water Deficit Stress on Olive Fruits and Oil Quality

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Abstract: The Mediterranean basin is the leading worldwide region for olive production. Extreme weather is increasingly frequent in this region, and how these climate extremes will affect olive products and quality remains unknown. We aim to study the effects of the water deficit stress, which occurred in the summer of 2017, on olive fruit and oil quality from a 30-year-old orchard. Fruits from olive trees standing on (i) one hydrated and (ii) one dry area of an orchard at the north of Portugal were harvested. Fruits' water content, oil yield and quality, fruit carbohydrates, and fruit and oil phenolic metabolite profiles were analyzed. Fruits from the dry area presented low water availability and increased carbohydrates, oleuropein, oleoside, and elenolic acid glucoside abundance. Oil yield was lower in the dry area, but the abundance of oleacein increased, together with traits of some sensory sensations. Climate stress events can reduce oil yield but stimulate the accumulation of bioactive compounds that improve oil quality and nutritional value.

Keywords: climate change; flavonoids; secoiridoids; *Olea europaea*; drought; sensorial quality; olive metabolome

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1. Introduction

Olea europaea L. is a pivotal crop in the Mediterranean basin, the leading worldwide region in olive production. Since 2001, the area occupied by olive orchards in the Mediterranean increased ≈25%, reaching in 2019 around 10.5 Mha [1], which shows high consumer demand for olives and olive oil. However, the Mediterranean basin and its agriculture are threatened by climatic extremes [2]. For instance, in the summer of 2017, the Mediterranean region suffered prolonged drought and heatwaves [3,4]. These extreme weather events are expected to be more frequent and intense in the next decade, representing losses in crop productivity [2,5].

Olives and olive oil are rich in fatty acids, phenolic compounds, carbohydrates, sterols, terpenes, proteins, minerals, and vitamins [6–9]. The levels of these compounds have a significant influence on the organoleptic properties of olives and olive oil. The monounsaturated fatty acid (MUFA) oleic acid and the polyunsaturated fatty acid (PUFA) linoleic acid are present in high amounts in both fruit and oil [9]. Other fatty acids, such as the saturated fatty acids (SFAs) palmitic and stearic acids, are also abundant in these olive products [9]. Additionally, olive fruits and oil have several secoiridoids and flavonoids known to be

beneficial for human health, like preventing cancer and cardiovascular diseases [10]. Within the secoiridoids, oleuropein, which can scavenge reactive oxygen species, is one of the most abundant phenolic compounds and occurs in all olive plant organs [11]. Regarding flavonoids, luteolin has an essential role, as this compound has a catechol group on its B-ring, which can detoxify H_2O_2 and H_2O_2-generated hydroxyl radicals [12]. Olive fruits also contain sugars and polyols that provide energy for metabolic adjustments and are precursors for the biosynthesis of olive oil [13]. Several factors might influence the phenolic compounds present in olive oil, for instance, the cultivar, fruit maturation, environmental factors, altitude, and cultivation practices [14]. Additionally, the crushing of fruits and the malaxation process for oil extraction can impact the quality of oils due to the phenolic compounds released and the volatile compounds formed [15]. During these processes, several reactions involving enzymes like β-glucosidase and lipoxygenase act on the phenolic compounds and fatty acids, influencing the oil flavor and aroma [15].

Abiotic stresses like drought and heat affect the metabolite composition of olive fruits and oils, changing their quality [10]. For instance, drought conditions seem to increase oleuropein and tyrosol levels in olive fruits but reduce the accumulation of amino acids, terpenes, fatty acids, and sugar levels [10,11]. Heat can induce fatty acid desaturases, which might change the profile of olives' fatty acids [16]. The combination of drought and heat is described as increasing the pool of sugars, oleic acid, and hydroxytyrosol-glucoside and decreasing hydroxytyrosol, apigenin-7-*O*-glucoside, and luteolin-7-*O*-glucoside in olive fruits [10]. Concerning olive oil quality, drought does not seem to affect oil-free acidity, peroxide value, and UV absorbances (232 and 270 nm), but increases the levels of 3,4-DHPEA-EDA and 3,4-DHPEA-EA [17]. High temperature decreases the content of oleic acid and volatile compounds (e.g., *E*-2-hexenal, hexan-1-ol, and *Z*-3-hexen-1-ol) in olive oils [17]. The reaction cascades that abiotic stresses induce in olive fruit and oil quality are still unknown, and studies are needed to help predict the future of oliviculture in future climate change scenarios.

To our knowledge, this is the first study in a real scenario that evaluates the effects of water deficit in olive fruits and oil quality in adult trees. We hypothesize that climate conditions, particularly water deficit, induce an adjustment of important compounds that alter olives and olive oil nutritional profiles and increase oil quality. We reported previously [18] that the climatic conditions of the summer of 2017 induced stress in olive trees from a dry area of an orchard, leading to metabolic and physiological adjustments. Here, we aim to focus on the effects of this stress period on the olive fruit and oil quality.

2. Materials and Methods

2.1. Study Site and Plant Material Conditions of Harvest

The selected olive orchard for this study consists of olive trees about 30 years old from the cultivar Cobrançosa, a highly representative cultivar in Portugal. The grove is in the north of Portugal, in Suções-Mirandela (41°30′39.5″ N 7°15′28.1″ W), and is managed as dryland farming, which is the regional standard of olive crop management. The climate is characterized as having dry and hot summers and rainy winters [19]. In 2017, the Mediterranean area was affected by extreme heatwaves, and Portugal was under severe/extreme drought between April and December [20].

A meteorological station monitored temperature and precipitation in the orchard. From June to the end of September, the monthly average precipitation reached values below 20 mm (typical of a dry summer according to the Köppen climate classification [19]) and the monthly air temperature reached values above 30 °C (Figure 1). Two adjacent distinct areas were selected: one area without a water supply that showed olive trees with visible dehydration symptoms in leaves and fruits (dehydrated trees, dry area), and another area where olive trees showed leaves and fruits without signs of dehydration due to their location near a groundwater supply (hydrated area) and based on tree water status determination [18]. Five blocks of ten plants were selected in two plots, hydrated and dry. On the second day of October 2017, between 14:00 and 16:00, olive fruits (with

perfect sanitary conditions) at a state of maturation that varied between M2.5 and M3 (according to the guidelines defined by the International Olive Oil Council, available at https://www.internationaloliveoil.org/wp-content/uploads/2019/11/COI-OH-Doc.-1-2011-Eng.pdf, accessed on 7 April 2021) were harvested for oil processing. Olive fruit water content was determined, and the fruits were used for metabolite analysis (lipophilic and phenolic profiles) and to extract oil that was then analyzed in terms of quality, oxidative status, and metabolites (lipophilic and phenolic profiles).

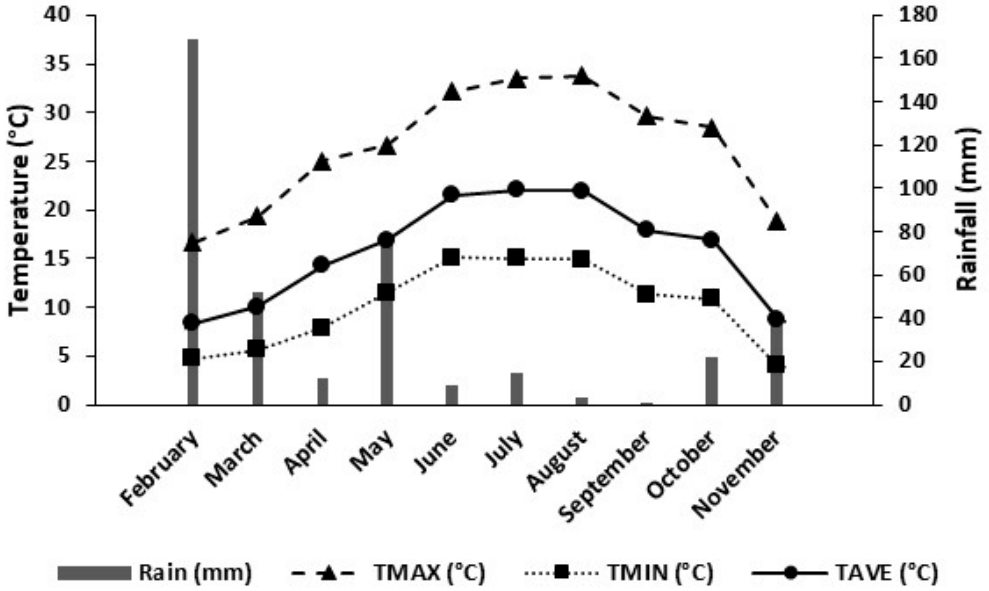

Figure 1. Monthly average precipitation (rainfall) and temperature (minimum—TMIN, average—TAVE, and maximum—TMAX) from January to December 2017 in the orchard.

2.2. Fruit Water Content

Olive fruit's fresh weight was determined, and then fruits were dried at 40 °C until a constant weight was achieved. The fruit water content was calculated and expressed as a percentage (%).

2.3. Olive Oil

2.3.1. Olive Fruit Processing for Oil Extraction

Olive fruits were processed in the first 24 h after handpicking in a pilot extraction plant with an Abencor system (Comercial Abengoa S.A., Sevilla, Spain). This system consists of three units: a mill, a thermobeater, and a pulp centrifuge. Olives milled from each area (5 samples per area) were weighted to about 1.2 kg, and 40 g of talc were added. After weighing, the milled olives were put in the thermobeater for 30 min at 25 °C, where malaxation took place, and after this, the pulp was centrifuged. The olive oil was separated by decantation, and the amount of oil was evaluated. The oil was dehydrated with anhydrous sodium sulphate, filtered through Whatman no. 4 paper, and stored in 100 mL dark bottles.

2.3.2. Quality Parameter Determination

Free acidity (FA, in % of oleic acid), peroxide value (PV, in mequiv O_2 kg^{-1}), coefficients of extinction at 232 nm (K_{232}) and 270 nm (K_{270}), and the respective ΔK values were analyzed following the European Union standard methods [Commission Regulation (EEC) No 2568/91 of 11 July 1991 Annexes II and IX]. Six months after oil extraction, olive oil samples were subjected to organoleptic analysis following the methods and standards adopted by the International Olive Council (IOC) for sensory analysis of olive oils, namely, COI/T.20/Doc. no. 15/Rev. 6 and COI/T.30/Doc. no. 17. Each sample (5 samples per area)

was subjected to the judgment of a trained panel, who classified the olfactory sensations, gustatory-retronasal sensations, and final olfactory-gustatory sensations using a modified test sheet following the recommendations of the IOC with a scale from 0 (no perceived sensation) to 10 (highest perceived sensation). For olfactory sensations, the following attributes were evaluated: olive fruitiness, apple, banana, tomato, dry fruits, fresh grass, cabbage, tomato leaves, and harmony. Concerning gustatory-retronasal sensations, the olive fruitiness, sweetness, bitterness, pungency, apple, banana, tomato, dry fruits, fresh grass, cabbage, tomato leaves, and harmony were evaluated. A final olfactory-gustatory sensation for each sample was also considered using the same scale for complexity and persistency. Complexity increases with the number and intensity of the perceived sensations. Persistency is related to the sensation's persistence in the mouth over time.

2.3.3. Oxidative Stability

Oxidative stability (OS) was measured [21] using the 743 Rancimat (Metrohm AG, Herisau, Switzerland). Olive oil (3 g) was heated at 120 ± 1.6 °C, filtered, and cleaned, and dried air was incorporated at a rate of 20 L h^{-1}. The resulting volatiles were collected in water, and the consequent increase in water conductivity was continuously measured. The required time to reach the conductivity inflection was recorded and was assumed as the OS value.

2.3.4. Radical Scavenging Activity—DPPH (2,2-Diphenyl-1-picrylhydrazyl) Assay

The antioxidant activity of olive oil was analyzed by measuring its capacity of scavenging DPPH$^{\bullet}$, according to Rodrigues et al. [21]. Briefly, 1 mL of olive oil in ethyl acetate (10%, *W*/*V*) was mixed with 4 mL of 0.1 mM DPPH$^{\bullet}$ (prepared in ethyl acetate). After being vigorously shaken for 10 s, the mixture was kept in the dark for 30 min, and the absorbance was read at 515 nm (Thermo Fisher Scientific, GENESYS™ 10 UV-Vis, Waltham, MA, USA) against a blank solution.

2.3.5. Total Phenol Content

The total phenol content of olive oil was extracted according to Rodrigues et al. [21]. Olive oil (2.5 g) was diluted with 2.5 mL of *n*-hexane (1:1; *v*/*v*). To the mixture, 2.5 mL of methanol/water (80:20; *v*/*v*) were added, and the mixture was centrifuged for 5 min at 5000 rpm. Two phases formed, and the methanolic phase was extracted. The same procedure was repeated three times. Folin-Ciocalteau reagent (1 mL), 7.5% Na_2CO_3, and ultrapure water (7 mL) were added to the methanolic extract (1 mL). The homogenized mixture was stored overnight in the dark. The absorbance was read at 765 nm, and the concentration of phenols was calculated using a calibration curve with caffeic acid in methanol.

2.4. Metabolite Extract and Analysis: Lipophilic and Phenolic Metabolite Profiles

2.4.1. Extract Preparation for Metabolite Analysis

Olive fruits were destoned and milled. Approximately 50 g (fresh weight) of each sample were mixed with 500 mL of *n*-hexane with stirring for 72 h at room temperature. The *n*-hexane was renewed, and a similar extraction was repeated. The solvent was evaporated to dryness using a rotary vacuum evaporator, and the hexane extracts were used for the acidic composition by gas Chromatography-Mass spectrometry (GC-MS) analysis. To extract phenolic compounds, 500 mL of methanol were added to the olives' dry pellets resulting from the hexane extraction. Two cycles of 72 h, each at room temperature, were carried out. Methanol was evaporated to dryness using a rotary vacuum evaporator. The methanol extracts were used for ultra-high-performance liquid Chromatography-Mass spectrometry (UHPLC-MS) analysis.

For the acidic composition by GC-MS analysis, 20 mg of the obtained olive fat were homogenized with 200 µL of dichloromethane. For the phenolic analysis by UHPLC-MS, 5 g of olive oil were mixed with 12 mL of *n*-hexane and then 5 mL of methanol/water

(80:20; *v*/*v*), after which the mixture was shaken for 10 min and centrifuged at 5000 rpm for 20 min at 4 °C to separate the two phases. After this, the methanolic phase was recovered, and the procedure was repeated three times. The solvent was evaporated to dryness using a rotary vacuum evaporator, and the extract was used for UHPLC-MS analysis [22].

2.4.2. Gas Chromatography-Mass Spectrometry and Ultra-High-Performance Liquid Chromatography-Mass Spectrometry Analysis of Fruits and Oils

For GC-MS analysis, 200 µL of the extracts (fruits 40 mg mL^{-1}; oil 20 mg mL^{-1}), 200 µL of internal standard (tetracosane, 0.46 mg mL^{-1}), 50 µL of dichloromethane, 250 µL of pyridine, 250 µL of *N*,*O*-bis(trimethylsilyl)trifluoroacetamide, and 50 µL of trimethylsilyl chloride were mixed in a glass tube and incubated at 70 °C for 30 min. The silylated extracts were then injected (1 µL) into a QP2010 Ultra Shimadzu device (Shimadzu Corporation, Tokyo, Japan) with a FactorFour Capillary Column (VF-5 ms 30 m × 0.25 mm ID DF = 0.25) and the chromatography conditions were performed as described by Araújo et al. [18]. The profile of α-D-mannopyranose, D-mannitol, and D-glucose in the fruits was determined.

For the UHPLC-MS analysis, extracts from fruits (40 mg mL^{-1}) and oil (30 mg mL^{-1}) were resuspended in pure methanol, filtered through a 0.2 µm membrane (Whatman, Medstone, UK), and injected into a Thermo Scientific Ultimate 3000RSLC (Dionex, Sunnyvale, CA, USA) equipped with a Dionex UltiMate 3000 RS diode array detector coupled to a mass spectrometer. The chromatography conditions were performed as described by Araújo et al. [18]. The chromatograms obtained were analyzed at 280, 240, and 230 nm. The phenolic compounds of the extracts were identified based on UV-Vis spectra and MS^n spectra data with those of the closest available reference standards and data reported in the literature. The phenolic profile (desoxyelenolic acid, 1-β-D-glucopyranosyl acyclodihydroelenolic acid, oleoside, elenolic acid glucoside, elenolic acid, oleuropein, tyrosol, hydroxytyrosol, hydroxylated product of the decarboxymethyl elenolic acid, 10-hydroxy-decarboxymethyl oleuropein aglycon, oleacein, hydroxy-oleuropein aglycon, and oleuropein aglycon) of fruits and oils was determined.

The semi-quantification of the individual compounds in the fruit and oil extracts was performed by peak integration at the appropriate wavelength. For each chromatogram, the area of each peak and the total peak area were calculated, and the compound' relative abundance (%) was determined [relative peak area average (%) = (compound peak area/total peak area average) × 100]. For the calculation of the GC-MS compound relative abundance, the area of the internal standard was considered (compound peak area/internal standard).

2.5. Statistical Analysis

The Student's *t*-test was used to analyze the statistical significance between groups (hydrated and dry) at a significance level of 0.05. When the variance failed, a non-parameter test (Mann-Whitney) was performed. Pearson's correlations for fruit parameters and oil parameters were analyzed. All statistical analyses were performed using the SigmaPlot for Windows version 3.1 (Systat Software, San Jose, CA, USA).

3. Results

3.1. Olive Fruit

3.1.1. Water Content

The fruits from the dry area had significantly less water content than those from the hydrated area (fruit water content from hydrated area 48.7 ± 3.8% and dry area 34.5 ± 0.5%).

3.1.2. GC-MS Profile: Carbohydrate Profile

Fruits from Cobrançosa trees presented α-D-mannopyranose, D-mannitol, and D-glucose (Figure 2). The abundance of these carbohydrates was higher in fruits of the dry area (Figure 2).

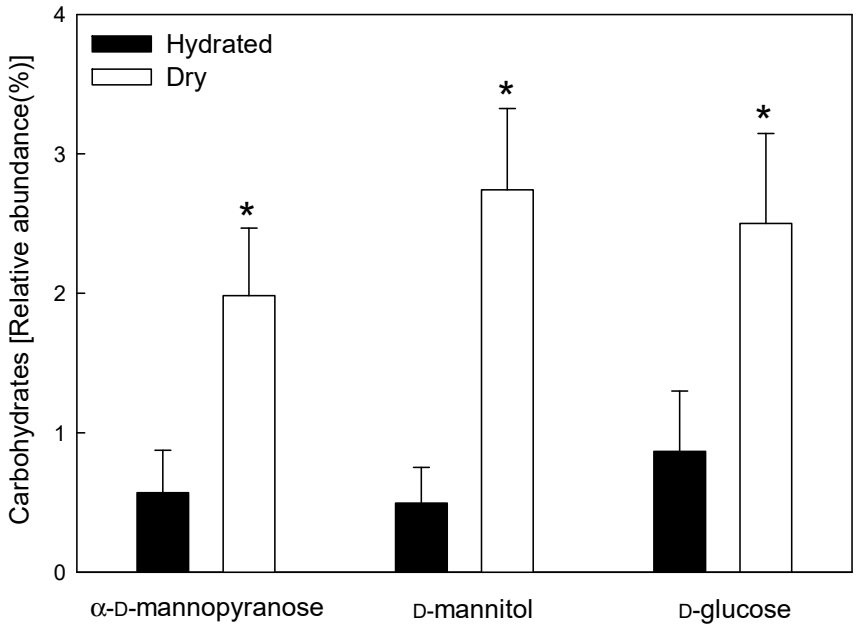

Figure 2. Carbohydrate profile [relative abundance (%)] of olive fruits from Cobrançosa trees from the hydrated and dry areas of the orchard. Values are mean ± standard deviation (n = 4). Asterisk (*) indicates significant differences ($p \leq 0.05$) between areas.

3.1.3. UHPLC-MS Profile

Secoiridoids in the fruits were represented by desoxyelenolic acid, 1-β-D-glucopyranosyl acyclodihydroelenolic acid (GPADHEA), oleoside, elenolic acid glucoside, elenolic acid (EA), and oleuropein (Table 1). GPADHEA and EA reduced their abundance in fruits from the dry area, while oleoside, EA glucoside, and oleuropein increased their abundance in fruits from the same area.

Table 1. Olive fruit phenolic profile [relative abundance (%)] of Cobrançosa trees from the hydrated and dry areas of the orchard. Values are mean ± standard deviation (n = 3). Asterisk (*) indicates significant differences ($p \leq 0.05$) between areas. m/z is presented in negative mode $[M\text{-}H]^-$. R_t—retention time.

RT (min)	Compound	*m/z*	MS^2	Hydrated Area	Dry Area
1.80	Desoxyelenolic acid	225	179	11.7 ± 3.79	19.3 ± 10.7
5.80	1-β-D-glucopyranosyl acyclodihydroelenolic acid	407	389/375/357/313	60.7 ± 2.72	30.4 ± 4.98 *
8.64	Oleoside	389	345/209/121	1.38 ± 0.12	17.7 ± 2.93 *
9.55	Elenolic acid glucoside	403	371/223/179	0.96 ± 0.11	20.2 ± 1.97 *
13.48	Elenolic acid	241	139/127/95/209/101/165/223	23.4 ± 2.78	3.29 ± 2.19 *
14.49	Oleuropein	539	377/307/275/509/507	1.77 ± 0.32	8.48 ± 2.83 *

3.2. Olive Oil

3.2.1. Yield

The olive oil yield extracted from olive plants in the dry area was significantly lower (42 ± 32 mL kg^{-1} of fruits) than from those in the hydrated area (190 ± 13 mL kg^{-1} of fruits).

3.2.2. Quality Parameters

Free acidity, peroxide value, K_{232}, total phenols, and oxidative stability showed no significant differences ($p > 0.05$) between oils from trees in hydrated and dry areas (Table 2). The coefficient K_{270} and ΔK increased in olive oil from the dry area (Table 2). Also, in the olive oil from this area, the radical scavenging activity was decreased (Table 2). The

trained panel perceived 9 olfactory sensations and 12 gustatory-retronasal sensations (Figure 3). Olive oils from the dry area showed higher olfactory sensation scores of "apple", "banana", and "dry fruits" in relation to oils from the hydrated area. Other olfactory sensations like "fruitiness", "tomato", "tomato leaves", and "green" were less perceived in the dry-area oils. The olfactory sensation of "cabbage" was not found in oils from the dry area plants. Harmony was similar ($p > 0.05$) in oils from both areas. As for olfactory sensations, attributes like "apple", "banana", and "dry fruits", as well as "sweet" in gustatory-retronasal sensations, showed higher values in olive oils extracted from the dry-area trees. These olive oils also showed lower gustatory-retronasal sensations of fruitiness, bitterness, pungency, green tomato, and tomato leaves compared to olive oils from the hydrated-area plants. Also, oils from the dry area showed no gustatory-retronasal sensation of cabbage. Harmony was similar ($p > 0.05$) in oils from both areas. Concerning the olfactory-gustatory sensations, oils showed significant differences in complexity and persistence. The hydrated-area oils were evaluated as 7.46 ± 0.35, while dry-area oils were 6.56 ± 0.44 in complexity. Regarding persistency, hydrated-area oils were evaluated as 8.28 ± 0.42, while those from the dry area were 7.84 ± 0.37.

Table 2. Quality parameters, antioxidant activity, total phenols, and oxidative stability of Cobrançosa olive oils extracted from trees in the hydrated and dry areas of the orchard. Values are mean ± standard deviation ($n = 5$). Asterisk (*) indicates significant differences ($p \leq 0.05$) between areas.

Parameter	Hydrated Area	Dry Area
Free acidity (%)	0.29 ± 0.02	0.27 ± 0.02
Peroxide value (mequiv O_2 kg^{-1})	6.81 ± 0.69	6.65 ± 0.83
K_{232}	1.85 ± 0.18	1.68 ± 0.16
K_{270}	0.17 ± 0.03	0.21 ± 0.01 *
ΔK	-0.005 ± 0.00	-0.016 ± 0.00 *
DPPH• (%)	83.0 ± 0.70	79.79 ± 0.78 *
Total phenols (mg CAE kg^{-1})	320.8 ± 34.4	352.7 ± 45.5
Oxidative stability (h)	17.93 ± 3.82	14.4 ± 1.98

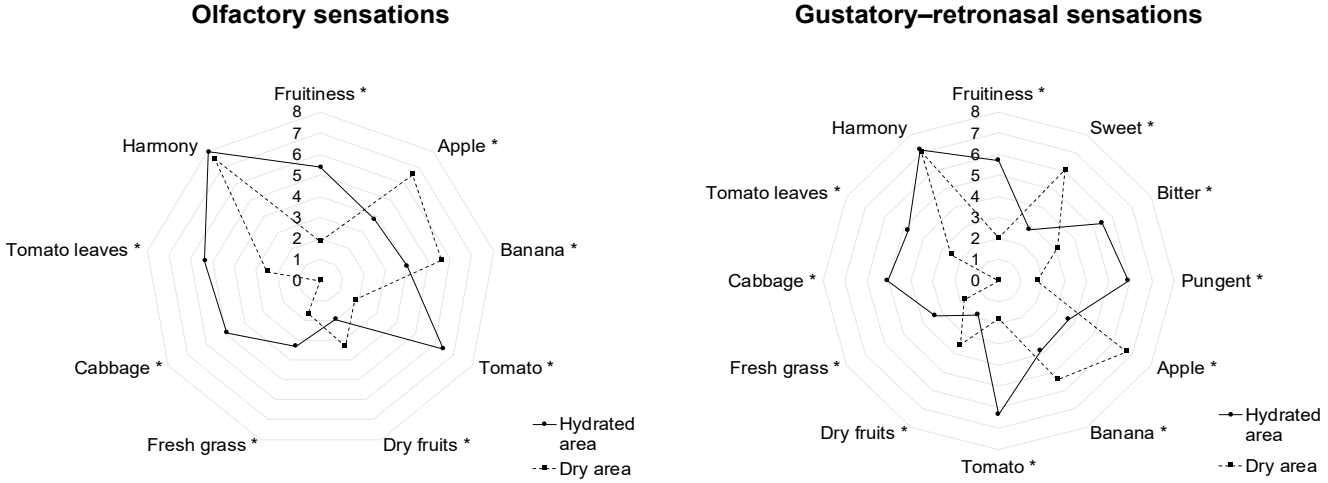

Figure 3. Sensory analysis of Cobrançosa olive oils extracted from trees from hydrated and dry areas of the orchard. Values are mean ± standard deviation. Asterisk (*) indicates significant differences ($p \leq 0.05$) between areas.

3.2.3. UHPLC-MS Profile

Oils from both areas (hydrated and dry) presented simple phenolics, flavonoids, and secoiridoids (Table 3). Tyrosol and hydroxytyrosol represented the simple phenolics. Hydroxylated products of decarboxymethyl elenolic acid, elenolic acid, 10-hydroxy-decarboxymethyl oleuropein aglycon, oleacein, hydroxy-oleuropein aglycon, and oleu-

ropein aglycon represented the secoiridoids. Luteolin represented the flavonoids. Both oleacein and hydroxylated product of decarboxymethyl elenolic acid increased in oils from the dry area. Luteolin and oleuropein aglycon reduced their abundance in oils from the dry area.

Table 3. Olive oil phenolic profile [relative abundance (%)] from Cobrançosa olive trees in the hydrated and dry areas of the orchard. Values are mean ± standard deviation (n = 4). Asterisk (*) indicates significant differences ($p \leq 0.05$) between areas. m/z is presented in negative mode $[M\text{-}H]^-$. R_t—retention time.

R_t (min)	Compound	m/z	MS^2	Hydrated Area	Dry Area
1.5	Tyrosol	137	137/119	14.6 ± 0.94	17.9 ± 3.57
4.4	Hydroxytyrosol	153	123/153	4.51 ± 1.66	2.65 ± 1.23
6.3	Hydroxylated product of decarboxymethyl elenolic acid	199	181/155/111	0.40 ± 0.13	1.08 ± 0.18 *
8.6	Elenolic acid	241	139/127/95/101/165/209	6.30 ± 1.38	7.75 ± 4.55
14.4	10-Hydroxydecarboxymethyl oleuropein aglycon	335	199/181/155	16.8 ± 1.84	15.0 ± 2.08
15.8	Luteolin	285	285/151	2.79 ± 0.16	1.32 ± 0.46 *
16.2	Oleacein	319	181/199/137/155/111	12.1 ± 2.65	28.0 ± 8.49 *
17.2	Hydroxyoleuropein aglycon	393		12.7 ± 1.63	11.3 ± 2.48
18.8	Oleuropein aglycon	377	307/275	30.5 ± 2.37	15.2 ± 4.75 *

4. Discussion

4.1. Olive Fruit

The Mediterranean Basin is already under the pressure of more intense, more frequent, and longer extreme climatic events [1]. The extreme climatic events verified in Europe in 2017, 2020, and 2021 are examples of climate change already occurring [4]. We reported previously [18] that the environmental conditions of the summer of 2017, particularly the low water availability, induced water deficit stress in olive trees from a dry area of the orchard under study, leading to a reduction in the leaf water availability together with an upregulation of the antioxidant system that was able to control oxidative lipid damage. In the present work, we observed that besides the dehydration of the leaves from these trees of the dry area [18], fruit water content also decreased. A similar response to water deficit was observed in fruits of other cultivars, like Cobrançosa, Frantoio, Corregiola, Leccino, and Koroneiki, growing under stress field conditions [23,24]. Fruit water content is considered an important factor since changes in water availability caused by water deficit can affect fruit and oil quality, as well as the efficiency of extraction during processing [24].

Some sugars and polyols (e.g., mannitol and myo-inositol) are involved in plants' protective mechanisms [18,25], promoting osmoregulation and avoiding tissue dehydration. The accumulation of carbohydrates in olive leaves in response to abiotic stress (e.g., drought and heat) was reported [10,24,26]). Our data are also in line with those findings, as fruits from the dry area showed higher proportions of D-mannitol, α-D-mannopyranose, and D-glucose. Moreover, the strong negative correlation between these carbohydrates and fruit water content (correlation of fruit water content with: α-D-mannopyranose $r = -0.87$ and $p = 0.005$, D-mannitol $r = -0.90$ and $p = 0.002$, and D-glucose $r = -0.84$, $p = 0.009$) supports the accumulation of carbohydrates having been influenced by the reduction in fruit water availability. Despite this response, carbohydrate accumulation seemed not to be used, or was insufficient, to maintain cell turgor since fruit water content decreases. According to Valente et al. [10], carbohydrate accumulation in olives exposed to drought + heat can increase energy availability and even reduce the use of carbohydrates in other metabolic processes, like oil synthesis [13,19].

Many phenolic compounds are found in olive fruits from the Cobrançosa cultivar, with GPADHEA and EA being the most representative compounds. However, the stress condition in the dry area induced a decrease in these compounds (correlations between fruit water content vs. GPADHEA $r = 0.949$ and $p = 0.003$, and fruit water content vs. EA $r = 0.882$ and $p = 0.020$). GPADHEA compounds likely originate from reduction reactions

of EA [27], but their function is still unknown. EA is a product of the hydrolyses of oleuropein by β-glucosidase into oleuropein aglycone, and of this compound into EA and hydroxytyrosol [27]. Interestingly, other phenolic compounds, like oleoside, EA glucoside, and oleuropein, respond differently to the stress, increasing their pools. Moreover, oleuropein, the compound responsible for the fruit bitterness, is a strong antioxidant (potent free radical scavenger), and the stress of the dry area increases olive fruits' oleuropein abundance. This response was also described in other studies using Cobrançosa and Picual cultivars [24,28]. However, it seems that abiotic stresses, like high temperature coupled with water deficit, can have an opposite effect, reducing the levels of oleuropein in fruits from young Cobrançosa plants growing in pots under controlled conditions, as demonstrated by Valente et al. [10].

4.2. Olive Oil

Trees from the dry area produced less olive oil than those in the hydrated area, demonstrating that water availability is important to oil production. According to the European Union on characteristics of olive oil (EU regulation number 2568/91), oils from both areas can be classified as extra-virgin olive oils according to their characteristics of acidity, peroxide value, and UV absorbances. However, oils from the dry area showed higher oxidation (K_{270}), probably due to the higher amount of secondary oxidation products that absorb at this wavelength (270 nm) [29]. This may have influenced the lower antioxidant capacity in oils from the dry area.

Interestingly, although the physicochemical analysis of the oils from the hydrated and dry areas was very similar, the olfactory sensations were quite different between the two areas tested. For instance, oils from the dry area showed higher sensations of apple, banana, and dry fruits. These sensations (apple and dry fruits) were previously attributed to the "*terroir*" of this region [21]. The oils from the hydrated area predominated with sensation of tomato leaves, cabbage, fresh grass, tomato, and fruitiness. Regarding gustatory-retronasal sensations, the same pattern as olfactory sensations was found. In oils from the dry area, sensations of sweet, apple, banana, and dry fruits prevailed, while in the ones from the hydrated area, the sensations of fruitiness, bitterness, pungency, tomato, fresh grass, cabbage, and tomato leaves predominated. The significant gustatory sensations of bitterness and pungency in oils of the hydrated area may be attributed to the high levels of secoiridoids such as oleuropein and other phenolics [30] not detected in this oil of Cobrançosa. The sweet sensation present in the oils of the dry area might be attributable to the breakage of the glycosidic bond in the oleuropein compound that originates oleuropein aglycon and glucose [9].

An important antioxidant compound that can be found in the Cobrançosa oils is luteolin, but the stress imposed in the dry area reduced its production, supporting the data for leaves on the same trees [18]. It is noteworthy that the stress condition augmented oleacein, a known antioxidant and anti-inflammatory phenolic compound. Oleacein is formed when β-glucosidase catalyzes the hydrolysis of oleuropein (which is reduced in response to stress) and ligstroside to form the aglycone forms. These reactions contribute to the formation of oleacein and oleocanthal [31]. These reactions may have contributed to the production of the hydroxylated product of the decarboxymethyl elenolic acid, which also increased as a consequence of water deficit stress.

5. Conclusions

The extreme weather events of the 2017 summer, particularly the water deficit stress, induced fruit dehydration but promoted sugar and polyol accumulation. These stress conditions increased the levels of beneficial compounds in olive fruits, as was the case of oleuropein, oleoside, and eleonic acid glucoside. Regarding olive oil, the stress conditions in the dry area reduced oil yield but improved the levels of the secoiridoid oleacein. Also, the stress conditions promoted some olfactory sensations (like apple, banana, and dry nuts) and gustatory-retronasal sweetness in this oil. This study demonstrates that stress conditions

due to extreme climate events possibly reduce oil yield but stimulate the accumulation of some important bioactive compounds that improve their nutritional and quality value.

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