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Influence of sustained deficit irrigation and foliar kaolin application on almond kernel composition

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ABSTRACT

Deficit irrigation and kaolin foliar application are agronomic practices that have been demonstrated to improve productivity and physiological response in almond trees but there is no consistent information on the effects on the kernel composition. The objective of this study was to evaluate the effect of different Sustainable Deficit Irrigation (SDI) strategies and kaolin application on some physicochemical composition of the cv Ferragnès almond (*Prunus dulcis* (Mill.) D.A. Webb). A randomized block design with five treatments was developed: non-irrigation (NI), non-irrigation with foliar kaolin application (NI+K), full irrigation (FI), and two levels of SDI receiving 70% and 35% of evapotranspiration (SDI75 and SDI35). Pomological parameters, volatile profile and oil composition were analyzed. SDI effect on kernel weight was reduced against full irrigation (FI) but substantially improved in comparison with the rainfed treatments (NI and NI+K). Irrigation treatments showed a lower volatile compounds concentration in comparison with rainfed treatment. The fatty acid composition of the oil was significantly affected, with all the irrigated treatments having higher oleic acid contents than the rainfed treatments, and therefore an expected increased shelf life. Kaolin application had no positive effects on the non-irrigated trees, other than an apparent increase in the total volatiles, a tendency also observed in the NI. Moderate water stress levels (SDI35) improved the synthesis of tocopherols compared to NI, FI and SDI70, which could be related to the adaptation of the almond tree to drought. SDI35 is therefore regarded as a very interesting approach, with significant improvements in comparison with non-irrigated trees, and a clear saving on water against full irrigation without significant pomological and chemical alterations.

1. Introduction

Almond tree cultivation has increased worldwide in the latter years (International Nut and Dried Fruit Council, 2021), particularly in the countries of the Mediterranean basin, where it is expected to have economic relevance in rural areas in the years to come. Portugal is no exception, increasing its cultivation area by 26% between 2010 and 2020 (FAOSTAST, 2020), with this crop representing an important economic resource in some of the most disadvantaged rural areas, producing a multitude of direct and indirect jobs.

Despite having a significant tolerance to periods of water stress, it is generally consensual that irrigation is essential to improve almond tree crop performance (Egea et al., 2013; García Tejero et al., 2018a) and that among the agricultural practices, irrigation seems to be the most critical factor affecting almond yield and quality (Lipan et al., 2018). For this reason, different strategies to manage irrigation water consumption and crop yields have been implemented worldwide: deficit irrigation, orchard groundcover management, mulches, foliar application of reflective or antitranspirant film protection, etc. (Girona et al., 2005; Rosati et al., 2006; Galindo et al., 2020; García-Tejero et al., 2018a; Lipan et al., 2018; Gutiérrez-Gordillo et al., 2019; Lepsch et al., 2019). Thus, it is already known that the almond tree has an optimal response in terms of productivity under non-limiting water conditions (García-Tejero et al., 2018a) and a strong tolerance to drought (Goldhamer et al.,

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2006). Between these two extreme behaviors, the concept of Deficit Irrigation (DI) was born both for the almond tree and for other crops such as vineyards and olive tree.

DI consists of a controlled application of irrigation water, always below the optimum water needs of the crop (Fereres and Soriano, 2007). With DI is possible to improve the water status of a dryland crop, increasing its productivity, while reducing the consumption of irrigation water that would be used in a crop with full irrigation. There are many DI strategies, depending on the pattern of water stress to which the crop is subjected, whether it is temporal, spatial or a combination of both (Egea et al., 2011; García-Tejero et al., 2018a). One of the most used DI in almonds is Sustained Deficit Irrigation (SDI), where the water is applied below the crop evapotranspiration evenly throughout the irrigation season, maintaining the plant with a level of water stress (Nanos et al., 2002; Girona et al., 2005). Another type of DI is Regulated deficit irrigation (RDI), that consists of the imposition of water deficits, or even completely stop irrigation, during specific phenological stages, which are less sensitive to water stress and supplying full irrigation in critical phenological periods (Ruiz-Sánchez et al., 2010). Some works have reported the effect of DI techniques on almond tree productivity, growth and physiological response (Girona et al., 2005; Goldhamer et al., 2006; Egea et al., 2010; Egea et al., 2013; García-Tejero et al., 2018b; Gutiérrez-Gordillo et al., 2019). Nanos et al. (2002) evaluated some physicochemical parameters of almonds of the Ferragnès cultivar, including kernel weight, moisture, sugars and fatty acids contents in an experiment with comparing one irrigation treatment and one non-irrigation treatment. They concluded that the irrigation effect on kernel fresh weight was reduced but it modified the sugar profile, with higher amounts of sucrose and raffinose and lower of inositol and other sugars than almonds from dry plots. Regarding almond oil, irrigation improved its quality based on the composition of fatty acids. However, Zhu et al. (2015) also compared different RDI and SDI treatments on the Nonpareil cultivar and concluded that SDI did not impair its quality while. Lipan et al. (2019a,b) compared the volatile profile of almonds from Vairo cultivar grown under different RDI and SDI treatments and highlighted that moderate DI led to almonds with a higher total content of volatile compounds (potentially linked with almond odor, aroma, and flavor). Gutierrez-Gordillo et al. (2020) also analyzed the composition of the almond from three cultivars (Marta, Guara and Lauranne) submitted to different SDI treatments and a control treatment with full irrigation, concluding that all cultivars improved the quality of the fruit under SDI irrigation. Based on the literature search, we have not found evidence of any investigation comparing in the same experiment the effects of SDI, full irrigation and rainfed treatments on almond composition.

Another strategy to mitigate adverse effects caused by elevated temperature and high irradiance in almond and other Mediterranean crops (walnuts, hazelnut, vineyard, etc.) is the application of foliar reflective films (e.g., kaolin and pinolene) (Brillante et al., 2016; Brito et al., 2019; Gharaghani et al., 2018; Dinis et al., 2018). Some works evaluated the effect of kaolin on productivity and its interactions with the physiological activity of different crops (walnut and vineyard) (Rosati et al., 2006; Gharaghani et al., 2018; Dinis et al., 2018; Mahmoudian et al., 2021), but as far as we know, no study on almonds has focused on the effect on almonds physicochemical composition. Based on the positive effect on olive oil quality achieved by Khaleghi et al. (2015), with the application of kaolin particle film (0%, 3% and 6%; w/v) to mature cv 'Zard' olive trees, with an increased oleic acid content, and the improvement of the quality of walnuts sprayed with kaolin by Gharaghani et al. (2018) this strategy is worth to be explored.

Therefore, based on the reduced studies comparing the effect of deficit irrigation in the same field experiment with full irrigation and rainfed treatments and the little knowledge of the effects of kaolin foliar application in almonds composition, this work aims to study the impact of different SDI techniques and the effect of kaolin application on some physicochemical parameters of almond kernels under Mediterranean semi-arid conditions. The physicochemical parameters included pomological and compositional ones (volatile compounds, lipid composition in terms of fatty acids, tocopherols and tocotrienols). The Ferragnès cultivar was chosen, due to its relevance throughout all the producing countries of Europe, such as Greece, Italy, France and Spain, due to its late flowering, high production and good seed quality (Muncharaz, 2017).

2. Materials and methods

2.1. Experimental site and orchard management

The trial was conducted in a commercial almond orchard (*Prunus dulcis* (Mill.) D.A. Webb cv. Ferragnès), located in Alfândega da Fé, NE Portugal (latitude 41° 21′ N; longitude 6° 56′W; 555 m a. s. l.). Trees were grafted onto GF-677 rootstock and planted in 2002, spaced by 6 × 4 m and drip irrigated using one pipeline with an emitter of 3.6 L h^{-1}, spaced at 1 m intervals. Trees were trained in the open vase and pruned to preserve form and balance on the canopy tree. The soil is a loamy textured dystric Regosol (WRB, 2015). It had a pH_{H2O} of 5.2 and the organic matter content was 1.5%. All trees received the same annual amount of fertilizers: 66 kg N, 45 kg P_2O_5 and 45 kg K_2O per hectare. Weed growth in the orchard was controlled by mechanical clearing between the lines spacing and with herbicide on the line.

The climate in the study area is classified as Csb according to the Köppen climate classification (Peel et al., 2007). Summers are hot and dry, and winters have moderate temperatures and changeable rainy weather. Meteorological data were collected from an automatic weather station (CR800, Campbell Scientific, Logan, Utah) located in the experimental trial. The climatic conditions have been measured during the two years of the experiment and are reported in Fig. 1. Significant variability in terms of accumulated precipitation between the years 2016 (820.6 mm) and 2017 (326.4 mm) can be highlighted. Regarding temperatures, no notable events were observed between the two years.

2.2. Irrigation treatments and experimental design

SDI and kaolin application was first applied to the almond orchard in 2015 and it continued during 2016 and 2017. Many agronomic factors, such as irrigation and fertilization, greatly influence the proportion of fully differentiated dormant flower buds on a tree and, therefore, the productivity of the following year (Lamp et al., 2001; Girona et al., 2005). For these reasons, to consider the residual effect of irrigation on the sequent years in the trees, the almonds for the analysis have been collected only in 2017.

Four different irrigation treatments were implemented based on crop evapotranspiration (ETc): Full Irrigation (FI), with 100 % of crop evapotranspiration (ETc); two Sustainable Deficit Irrigation treatments: SDI70 and SDI35, with 70 and 35%, of ETc, respectively, and non-irrigated regime (NI). In addition, a foliar application of kaolin was tested on a second group of non-irrigated trees (NI+K) to test its potential to protect trees and crops from severe drought stress. A dose rate of 2 L/tree of aqueous kaolin suspension (4%) (BAS 24000 F, SURROUND® – 95%) was applied, at the beginning of the summer, on 16th June in 2015; 7th of June in 2016 and on the 22nd June in 2017. Table 1 shows the start and end dates of irrigation, as well as the amount of water applied for each treatment and year. The experimental design was a randomized block with 12 trees for each block and two blocks for each treatment with which we had 24 monitored trees for each treatment. The trees were selected according to the homogeneity criteria concerning their size and represented the entire plot. All trees were in perfect health conditions maintained during the growing season. However, the lower temperatures and a late frost event on March 15th, when the almond trees were in the phenological stage - 6.5 Full flowering: 50% of flowers open, first petals falling- (BBCH scale), affected pollination and fruit set. These extreme weather conditions equally affected the almond trees monitored in all plots. The same irrigation treatment

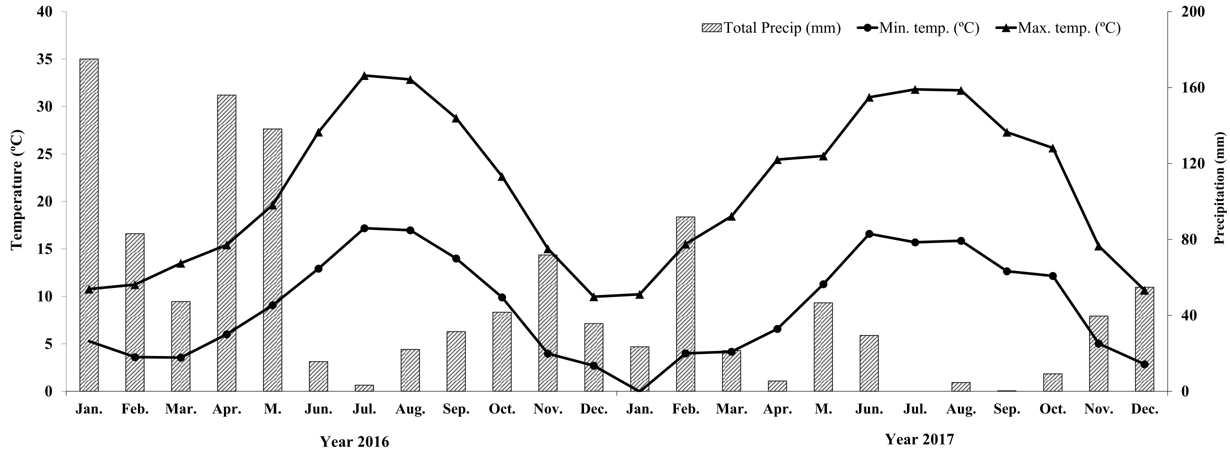

Fig. 1. Average monthly minimum and maximum temperatures and accumulated precipitation recorded during the experimental period (2016 and 2017) from an automatic weather station located in the field trial.

Table 1
Dates of the first and last irrigation and total water applied in the three treatments on the three years of the assay.

Growing season	Annual Rainfall (mm)	Irrigation Dates		Water applied (mm)				
		First irrigation	Last irrigation	FI	SDI70	SDI35	NI	NI+K
2015	643.2	9 Jun	8 Sep	325.9	217.3	108.6	-	-
2016	820.6	24 Jun	5 Sep	294.3	196.2	98.1	-	-
2017	326.4	6 Jun	4 Sep	352.7	235.1	117.6	-	-

FI: Full Irrigation, SDI70: Sustainable Deficit Irrigation at 70%, SDI35: Sustainable Deficit Irrigation at 35%, NI: Non-Irrigation and NI+K: Non-Irrigation with foliar application of Kaolin.

was applied to a plot for each successive growing season.

The weekly volume of irrigation water to be applied was calculated each week according to the previous week's total ETc and effective precipitation using the following equation:

$$SDI = (K \times ETc - Pe)/Er$$

where Pe is the effective precipitation; Er is the irrigation efficiency of the irrigation system (0.95), and K value represents the fraction of the ETc for the different irrigation regimes (1.0 for FI; 0.7 for SDI70, and 0.35 for SDI35). ETc was estimated using the FAO Penman-Monteith equation for reference evapotranspiration (ETo) and a crop coefficient (Kc) of 0.9 (for the mid-season stage) (Allen et al., 1998). The meteorological data were obtained from an automatic weather station placed near the almond orchard.

The midday stem water potential was measured with a pressure chamber (Model 1000, PMS Instrument Company, Albany, USA). Measurements were made by taking single leaves near the trunk covered with a small bag of black polyethylene covered by silver foil for at least one hours before measurements. The measurements were made on six leaves per treatment along two different periods on growing season.

Nuts were harvested at commercial maturity when the fruit mesocarp was thoroughly dried and split along the fruit suture. Each of the 24 trees monitored per treatment was collected separately. The harvest of all monitored trees for each treatment was carried out on 11th September 2017. The phenological state was 8.9 - "Detachment of exocarp and mesocarp, fruit ripe for harvest", according to the BBCH scale. Collected almond were processed manually to remove the hulls and leaves. After that, cleaned almonds were left to air-dry and weighed once a humidity content of about 5-6% was reached. Thus, the yield and all yield compounds were calculated with this moisture percentage. From each of the twenty-four trees analyzed per treatment, 250 g of almonds were collected, mixed and taken to the laboratory.. Subsequently, they were stored under controlled conditions (4°C) until the analysis was carried out.

2.3. Pomological measurements

The weight of the nut and kernel and the shelling percentage are considered essential traits by producers. pomological parameters of the nut (with shell) and dehulled (kernels) were determined (weight, length, width, thickness). A caliper (Powerfix® Electronic Digital Caliper, Model: Z22855, Paget Trading Ltd, London, UK) and precision balance (RADWAG, AS 220.R2, Poland) with a reading accuracy of 0.0001 was used. The kernel percentage was calculated using the equation: kernel percentage (%) = kernel mass (g)/nut mass (g) x 100.

2.4. Volatile characterization

The characterization of volatiles from almonds was performed by headspace solid-phase microextraction (HS-SPME) followed by gas-chromatography-mass spectrometry (GC/MS).

2.4.1. Extraction of volatile compounds by HS-SPME

After the end of the drying period, approximately fifteen days after harvesting, a sample of ten almonds for each treatment was selected at random. These fruits were shelled and immediately crushed in a porcelain mortar with a pestle to obtain a homogenous flour. In 50 mL vials, 3 g of almond flour plus 6 mL of distilled water were spiked and 5μL the internal standard (2-methyl-4-pentanol; Sigma Aldrich, Germany) (10 ppm in methanol) and volatiles were extracted with an SPME fiber coated with divinylbenzene/carbonex/polydimethylsiloxane (DVB/CAR/PDMS 50/30 μm) (Supelco, Bellefonte, USA). The sampling conditions were determined based on a conjunction of the methodologies presented by Malheiro et al. (2018) and Oliveira et al. (2019b).

Before analysis, the fiber was conditioned in the injector port of the chromatography system at 270°C for one hour, as recommended by the supplier. The vials with the respective samples were placed for 5 min at 40 °C for volatiles release and equilibrium. After this period, the SPME fiber was exposed for 30 min for the adsorption of the compound in the headspace. The fiber was then inserted into the injection port of the chromatography system. For each irrigation and kaolin foliar treatment the HS-SPME analysis was performed in fivefold.

2.4.2. Gas-chromatography mass-spectrometry analysis (GC–MS)

The retained compounds were desorbed from the fiber in the injection port for 1 min at 220°C, using manual injection in splitless mode, maintained for further 10 minutes for cleaning and conditioning. The gas chromatographer used was a Shimadzu GC-2010 Plus equipped with a mass spectrometer Shimadzu GC/MS-QP2010 SE detector. A TRB-5MS (30 m × 0.25 mm × 0.25 μm) column (Teknokroma, Spain) was used, with helium (Praxair, Portugal) at a linear velocity of 30 cm/s and a total flow of 24.4 mL/min. The oven temperatures were: 40°C (1 min); 2°C/min until 220°C (30 min). The ionization source was maintained at 250°C with ionization energy of 70 eV and with an ionization current of 0.1 kV. All mass spectra were acquired by electron ionization, turned off during the first 2 min. The MS spectra fragments were compared with those obtained from a database (NIST 11), and with those of pure compounds. All mass spectra were acquired by electron ionization, being the spectra fragments identified by comparison with the database of the NIST 11 Library (National Institute of Standards and Technology, Gaithersburg, MD, USA) and with the spectra of commercial standards. The areas of the chromatographic peaks were determined by integrating the re-constructed chromatogram from the full scan chromatogram using the ion base (m/z intensity 100%). In this study, the volatile compounds were identified based on the linear retention index (LRI) and the structural information. For each compound the Kovats retention index was calculated using a commercial standard mixture of n-alkanes (C6- C20, Sigma Aldrich, Germany), and the identification was confirmed by comparing the LRI obtained and those reported in the literature (Adams, 2007). For semi-quantification purposes, the amounts of the identified volatiles were calculated considering the ratio of each base ion peak area to the area of the internal standard base ion peak area, assuming a response factor equal to one. The amounts were converted to mass equivalents based on the IS mass used following the methodology proposed by Malheiro et al. (2018).

2.5. Almond oil characterization

2.5.1. Extraction

The lipid extraction conditions applied were those reported by Fernandes et al. (2015). For each treatment, 5 g of almonds were crushed in a mortar with a pestle. Anhydrous sodium sulfate was added to remove moisture remains. The lipid fraction was obtained by Soxhlet extraction with petroleum ether with 0.01% BHT (2,6-di-tert-butyl-4-methylphenol, Sigma) to prevent oxidation for a 24 h period. The solvent was removed with a rotary evaporator RE300DB (Stuart, Stone, United Kingdom) and the samples were stored at -20°C until analysis. Each treatment was extracted in triplicate.

2.5.2. Fatty acid composition

A 50 mg portion of the oil was used, converting the glycerides to fatty acid methyl esters (FAMEs) by alkaline transesterification with a 2 M methanolic potassium hydroxide solution, following ISO 12966-2:2017. The fatty acid profile was obtained using a Select FAME column (50 m x 0,25μm, Agilent, USA), with an optimizes temperature gradient between 120 and 220 °C, on a Chrompack CP 9001 chromatograph (Chrompack, Middelburg, Netherlands) equipped with a flame ionization detector (FID). The results are expressed in relative percentage of chromatographed fatty acids areas, after calibration of the detector response with a certified reference material (TraceCERT, Supelco CRM47885, Merck Life Science, Portugal).

2.5.3. Vitamin E profile

Tocopherols and tocotrienol measurements were based on the ISO 9936:2016 standard, with the addition of tocol as an internal standard. An accurate sample amount was weighted, followed by the internal standard addition and dissolution in hexane. Separation was achieved on a Luna Silica column (3 μm, 100 × 3.0 mm, Phenomenex, USA), operating at constant room temperature (23°C) with a mixture of n-hexane and 1,4-dioxane (97:3) (v/v) at a flow rate of 0.7 mL/min as eluent, in a Jasco system equipped with a fluorescence detector (Jasco FP-2020 Plus) with excitation wavelength at 290 nm and emission wavelength at 330 nm. The concentrations were expressed as mg/kg of oil using calibration curves of tocopherol and tocotrienol standards (Sigma-Aldrich, USA).

2.6. Statistical analysis

The statistical analysis was carried out using Statgraphics Centurión XVI software. The normality and homogeneity of variance were always checked by the Shapiro-Wilk and Levene Tests, respectively, when analyzing the volatile composition. When both conditions failed, the nonparametric Kruskal-Wallis test was applied, followed by multiple comparisons of order means. On the contrary, when normality and homogeneity of variances were observed, an ANOVA followed by Tukey post-hoc test was used. All statistical tests were performed at a 5% significance level.

Principal component analysis (PCA) was applied to reduce the number of variables in the five treatments to a smaller number of newly derived variables (principal component or factors) that adequately summarize the original information, i.e., the effect of sustainable deficit irrigation and kaolin application on the main fatty acids, tocopherols and tocotrienols of kernels and their relation to stem water potential. Variables corresponding to 13 components, of the most abundant individual fatty acids (palmitic, stearic, oleic and linoleic), total polyunsaturated, monounsaturated and saturated fatty acids and tocopherol and tocotrienol compounds. These were combined with all treatments using the SPSS software, version 21.0 (IBM Corporation, New York, U.S.A.).

3. Results and discussion

3.1. Tree water status

The leaf water potential results obtained at different periods of the almond growth cycle during 2017 are detailed in Fig. 2. In the first measurement, on the 6th of June, all the trees presented the same level of water stress, around -1.5 MPa. In most of the Mediterranean regions, rainfall is scarce at the end of spring and the beginning of summer and the soil water reserves available for plants is drastically reduced, causing water stress in almond trees (García-Tejero et al., 2018b). Irrigation began on 6th of June, after leaf water potential measurements, calculating the amounts of water provided for each treatment as referred to in the Materials and Methods section. Subsequently, three measurements were made throughout the summer, obtaining significant differences in all of them. Thus, in the first measurement of water potential was made six weeks after starting irrigation, on July 18th, significant differences between treatments were already perceived. Water stress increased as the amount of water supplied by irrigation decreased, with the two treatments without irrigation, NI and NI + K, presenting the most negative values (between -2.5 and -3 MPa). This trend was maintained through measurements of water potential throughout the summer. These results agree with other studies that applied DI and monitored the level of water stress in almond trees (Egea et al., 2010; Gutiérrez-Gordillo et al., 2019). The kaolin treatment did not influence the level of water stress, as in other works (Rosati et al., 2006).

3.2. Pomological characteristics and yield

Table 2 shows the results obtained for the studied pomological properties and kernel and nut yield. The pomological characteristics recorded (weight, length, width, thickness,) were comparable to those

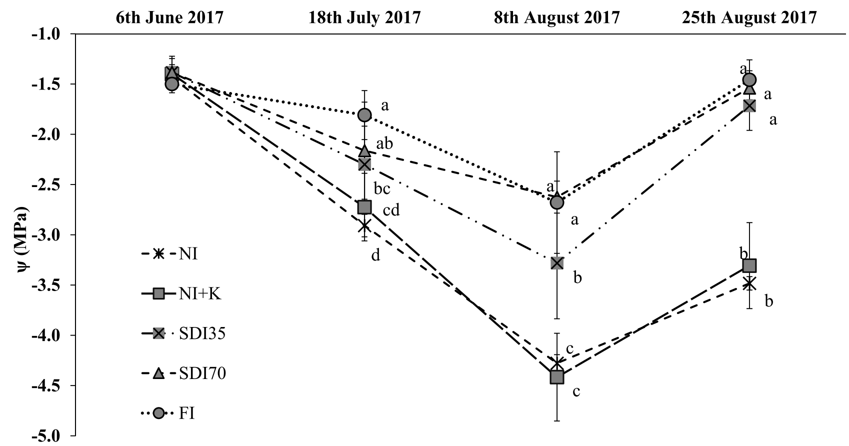

Fig. 2. Seasonal evolution of midday stem water potential (ψ) in shaded leaves during the experimental period of 2017. FI, SDI70, SDI35, NI, NI+K: treatments are those explained in the Material and Methods section. For the same data, different letters differ significantly ($p < 0.05$).

Table 2
Average values of pomological characteristics of almond nuts and kernels and kernel and nut yield grown under different irrigation and foliar kaolin application treatments (n = 100; mean ± standard deviation).

Pomological parameters		Treatments NI	NI+K	SDI 35	SDI 70	FI	*P*-value*
Nut	**Weight (g)**	2.26 ± 0.63 a	2.26 ± 0.50 a	3.16 ± 0.52 b	3.18 ± 0.60 b	3.27 ± 0.61 b	< 0.0001
	Length (mm)	31.65 ± 2.20 a	31.57 ± 1.95 a	32.72 ± 1.96 b	32.49 ± 2.34 b	33.09 ± 1.90 b	< 0.0001
	Width (mm)	19.23 ± 1.41 a	19.40 ± 1.80 a	20.08 ± 1.38 ab	20.11 ± 1.67 ab	20.81 ± 4.23 b	< 0.0001
	Thickness (mm)	13.23 ± 1.06 a	13.40 ± 0.98 ab	13.40 ± 1.03 ab	13.85 ± 1.11 bc	14.07 ± 2.00 c	< 0.0001
Kernel	**Weight (g)**	0.72 ± 0.22 b	0.60 ± 0.28 a	0.92 ± 0.23 c	0.95 ± 0.20 c	0.96 ± 0.23 c	< 0.0001
	Length (mm)	22.58 ± 2.00 b	21.13 ± 3.12 a	23.96 ± 1.87 c	24.21 ± 2.00 c	24.22 ± 1.85 c	< 0.0001
	Width (mm)	11.11 ± 1.04 a	10.87 ± 1.51 a	12.17 ± 0.94 b	11.99 ± 0.79 b	12.19 ± 0.82 b	< 0.0001
	Thickness (mm)	5.95 ± 0.93 b	5.11 ± 1.50 a	6.77 ± 0.85 c	7.12 ± 0.89 c	6.93 ± 1.02 c	< 0.0001
Kernel percentage (%)		32.43 ± 7.76 c	25.80 ± 10.32 a	28.94 ± 5.06 b	29.88 ± 3.13 bc	29.52 ± 5.43 b	< 0.0001
Kernel Yield (kg/ha)		327.8 ± 64.8 a	279.4 ± 53.5 a	462.0 ± 80.1 ab	494.1 ± 92.4 b	563.9 ± 96.1 b	< 0.0001

FI, SDI70, SDI35, NI, NI+K: treatments are those explained in the Material and Methods section.
In each row, different letters mean significant differences between samples, at a 5% significance level (P-value < 0.05), where "a" and "d" correspond to the lowest and highest values, respectively, according to multiple comparison Tukey's HSD test.
[*] P-value < 0.05 means that the mean value of the evaluated quality parameter of at least one treatment differs from the others, according to the one-way ANOVA results (in this case, multiple-comparison tests were performed).

reported by other researchers working with the same cultivar, Ferragnès. In this study, the nut weight varied between 2.26 to 3.27g, similar to Oliveira et al. (2018a) (mean nut weight, 3.68 g) and Melhaoui et al. (2019) (nut weight, between 2.65 g to 3.68 g).

A proportional trend was observed in nut and kernel weights and dimensions, with the irrigation treatments presenting the highest measures, and the non-irrigation treatments having the lowest ones. In particular, the highest mean value of nut mass was observed in the treatment with FI (3.27 g), while the lowest one was recorded in the NI treatments, both with 2.26 g. No significant differences were detected between all the irrigation treatments (FI, SD70 or SD35) but this group was significantly higher ($p<0.05$) than the NI group. In terms of nut length, it varied from 31.57 to 33.09 mm, and the width ranged between 19.23 and 20.81 mm, both measures with the same statistical trend as observed with nut mass.

There are several quality standards worldwide for grades of nuts almonds, both in the shell and shelled, as those from the Agricultural Marketing Service of the U.S. Dept. of Agriculture (USDA) or, in Europe, the Shell almond reception standards (2019) prepared by Spanish industries. One of these parameters is the kernel weight, which must be near or above 1.0 g. In the present study, the irrigated treatments presented values near the recommended ones (FI - 0.96 g, SDI 70 - 0.95 g and SDI 35 - 0.92 g), without significant differences between them, followed by the NI (0.72 g) and NI + K (0.60 g) treatments, statistically different between them and clearly below the quality standard. Goldhamer et al. (2006) and Egea et al. (2010) reported similar tendency to ours for other almond cultivars.

Regarding sustainable irrigation treatments, SDI35 and SDI70 did not present significant differences from the FI regarding nut weight, kernel weight, and kernel percentage. In this sense, SDI treatments allow substantial water saving without loss of quality of almond pomological parameters, which are important for consumer acceptability. This finding agrees with other studies that apply deficit irrigation in almond trees, such as Lipan et al. (2019a). They also concluded that water used for irrigation could be reduced without significantly affecting these properties. Saving water and preserving quality characteristics can be a factor in favour of implementing these irrigation techniques.

Regarding the results obtained for kernel yield, the non-irrigated treatments presented the lowest values, with 327.8 kg/ha for the NI treatment and 279.4 kg/ha for the NI+K treatment. The foliar application of kaolin did not show significant differences in kernel yield. The effect of irrigation treatments on kernel yield was statistically significant. There was a 12.4% and 18.1% reduction in kernel yield for SDI70 and SDI35 treatments, respectively, with respect to FI. Similar results were obtained by Girona et al. (2005) and Egea et al. (2010) on almond trees in the same Mediterranean climate conditions. The reduction in kernel yield of non-irrigated almond trees compared to FI was 42% which is a similar reduction to that observed by other authors (Moldero et al., 2022; Mañas et al., 2014).

3.3. Volatile compounds

The volatile composition of the almond of different treatments with irrigation, non-irrigation, and kaolin application is described in Table 5. In total, 18 volatile compounds were identified and distributed by seven chemical classes, namely, alcohols (7); aldehydes (6); alkanes (1); esters (1); ketones (1); terpenes (2). All volatile compounds were detected in all treatments in greater or lesser amounts, and correspond to compounds already identified in previous scientific works, as in Xiao et al. (2014) for the American cultivars Butte and Padre, Kwak et al. (2015) with the cultivar Nonpareil, Lipan et al., (2019b) for the Spanish cultivar Vairo, and both Oliveira et al. (2019b) and Karaat (2019) for the same cultivar as in our research, Ferragnès.

Regarding the total amount of volatile compounds per treatment, irrigation significantly reduced the amounts of volatiles. Thus, the FI treatment presented the lowest average value (6.8 mg kg^{-1}), followed by SDI35 (7.8 mg kg^{-1}) and SDI70 (7.7 mg kg^{-1}), and the non-irrigated ones presented the highest values: NI with 14.1 mg kg^{-1} and NI+K with 17.7 mg kg^{-1}. We have not found any references on almond volatile compounds that compare deficit irrigation treatments with control on rainfed conditions. Some works compare different deficit irrigation treatments with full irrigation, as Lipan et al. (2019b) and García-Tejero et al. (2020), both observed similar trends with full irrigation treatments presenting lower concentrations of volatile compounds compared to deficit irrigation treatments. However, these same authors found differences between treatments with moderate deficit irrigation (which would be equivalent to our SDI70) and severe deficit irrigation (our SDI35), referring that severe water stress reduced the abundance of transcripts of the enzymes involved in the production of volatile compounds (Deluc et al., 2009). In our case, this does not happen since no significant differences were found between our two SDI treatments and the treatments without irrigation, with higher water stress, were those with the highest concentration of total volatiles. Additionally, it seems that kaolin caused an increase in the amount of volatiles compared to the control treatment (NI).

The most abundant chemical class of compounds were alcohols in all treatments. This is so because they are the main group of volatiles in raw almonds (Franklin and Mitchell, 2019; King et al. 2019), crucial in

Table 3
Volatile compounds and their concentrations (mg kg^{-1}) for each treatment in almonds (n = 5; mean ± standard deviation).

Chemical class	Compound	LRI[1]	LRI lit[2]	QI (m/z)[3]	ID[4]	Treatments (mg kg^{-1} kernel)[5] NI	NI+K	SDI 35	SDI 70	FI	*P*-value*
Alcohols	3-Methyl-1-butanol	740	740	55	MS	2.93 ± 0.41b	4.43 ± 1.09c	1.74 ± 0.34a	1.70 ± 0.34a	1.13 ± 0.12a	<0.0001
	3-Methyl-2-butenol	780	773	71	MS	0.74 ± 0.11ab	0.94 ± 0.19b	0.59 ± 0.10a	0.59 ± 0.12a	0.50 ± 0.10a	0.0003
	2,3-Butanediol	788	789	45	MS	2.18 ± 0.50b	0.66 ± 0.12a	1.81 ± 0.30b	0.95 ± 0.20a	0.80 ± 0.14a	<0.0001
	Hexanol	873	870	56	S/MS	0.58 ± 0.24b	1.38 ± 0.30c	0.22 ± 0.03a	0.27 ± 0.05ab	0.27 ± 0.06ab	<0.0001
	Benzyl alcohol	1032	1031	79	MS	2.87 ± 0.5b	3.10 ± 0.65b	0.44 ± 0.07a	0.27 ± 0.02a	0.54 ± 0.10a	<0.0001
	Octanol	1073	1068	41	S/MS	0.04 ± 0.02ab	0.05 ± 0.01b	0.04 ± 0.01ab	0.03 ± 0.00a	0.03 ± 0.00a	0.0050
	Phenyl ethyl alcohol	1108	1106	91	MS	3.23 ± 1.22ab	3.64 ± 0.67b	2.15 ± 0.60a	2.73 ± 0.64ab	2.32 ± 0.28ab	0.0186
Aldehydes	Hexanal	798	801	44	S/MS	0.53 ± 0.19bc	0.70 ± 0.06c	0.35 ± 0.08ab	0.32 ± 0.04a	0.21 ± 0.01a	<0.0001
	Heptanal	899	902	44	MS	0.05 ± 0.01ab	0.05 ± 0.01ab	0.06 ± 0.01b	0.05 ± 0.01ab	0.03 ± 0.01a	0.0009
	Benzaldehyde	960	960	77	S/MS	0.14 ± 0.04a	2.22 ± 0.27b	0.17 ± 0.04a	0.10 ± 0.02a	0.18 ± 0.04a	<0.0001
	Octanal	1000	998	41	S/MS	0.06 ± 0.0	0.06 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.06 ± 0.00	0.3692
	Nonanal	1101	1100	41	S/MS	0.13 ± 0.03	0.10 ± 0.00	0.12 ± 0.02	0.11 ± 0.03	0.15 ± 0.04	0.0917
	Decanal	1202	1201	41	S/MS	0.02 ± 0.01	0.02 ± 0.00	0.03 ± 0.01	0.02 ± 0.00	0.03 ± 0.01	0.5246
Alkanes	Dodecane	1200	1200	57	MS	0.03 ± 0.0	0.03 ± 0.00	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.8263
Esters	Hexyl acetate	1015	1009	43	S/MS	0.06 ± 0.01	0.06 ± 0.00	0.05 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.2963
Ketones	5 Hepten-2-one, 6-methyl-	986	985	43	S/MS	0.15 ± 0.02bc	0.22 ± 0.03d	0.10 ± 0.01ab	0.09 ± 0.02a	0.16 ± 0.03c	<0.0001
Terpenes	p-Cymene	1024	1024	119	MS	0.23 ± 0.03ab	0.23 ± 0.03ab	0.21 ± 0.03a	0.27 ± 0.03b	0.21 ± 0.04ab	0.0437
	Limonene	1028	1029	68	S/MS	0.09 ± 0.00b	0.08 ± 0.01ab	0.06 ± 0.01a	0.08 ± 0.01ab	0.07 ± 0.01a	0.0014
∑ of Alcohols						12.6 ± 2.0b	14.2 ± 2.1b	6.6 ± 1.1a	6.5 ± 0.7a	5.6 ± 0.4a	<0.0001
∑ of Aldehydes						0.93 ± 0.15a	3.15 ± 0.69b	0.77 ± 0.07a	0.67 ± 0.06a	0.66 ± 0.06a	<0.0001
∑ of Terpenes						0.32 ± 0.03a	0.31 ± 0.03a	0.27 ± 0.03a	0.34 ± 0.03a	0.28 ± 0.05a	0.0297
Total volatiles						14.1 ± 2.1b	17.7 ± 2.4c	7.8 ± 1.1a	7.7 ± 0.7a	6.8 ± 0.4a	<0.0001

[1] LRI—linear retention index obtained
[2] LRI Lit—linear retention index reported in the literature (Adams, 2007)
[3] QI (m/z) - Quantification ions
[4] ID - Identification method (S - identified with standard; MS - identified by comparing mass spectrum with database NIST 11)
[5] Values are from semi-quantification using 4-methyl-2-pentanol as internal standard
FI, SDI70, SDI35, NI, NI+K: treatments are those explained in the Material and Methods section.
[*] In each row different letters mean significant differences between samples, at a 5% significance level (P-value < 0.05), where "a" and "d" correspond to the lowest and highest values, respectively, according to multiple comparison Tukey's HSD.

promoting consumer acceptance, as they are part of raw almonds' natural fruity and sweet aroma. The second class are aldehydes, where benzaldehyde was the main compound, associated with the typical almond "bitter oil" scent and used as an artificial aroma for almond oil. Both 3-methyl-1-butanol, benzyl alcohol and benzaldehyde were higher in the non-irrigated treatments, the main contributors to the increased volatile content. Hexanal, associated with lipid oxidation, presented variation between treatments, but without a clear pattern or statistical significance.

Regarding the treatment with kaolin (NI+K), it presented the highest amounts of total volatiles, with the highest amounts of alcohols, aldehydes and ketones, all statistically relevant ($p<0.05$) but the differences with the NI treatments were reduced, imposed by the high variation in the analytical data in the replicates. In this case, we can say that this tendency of the kaolin can result from an accumulation of effects, derived first from the water stress, as observed with the NI against all the irrigated treatments, and probably also from the Kaolin treatment, but we have not found literature that analyzes volatile compounds in almond trees with foliar application of kaolin. Song et al. (2012) studied the volatile composition of Merlot grapes between deficit irrigation treatments and foliar application of kaolin, concluding that kaolin did not show any significant effect.

3.4. Fatty acids

Table 3 presented the results of the main individual fatty acids and their sum as saturated (SFA), monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA). The predominant fatty acid of all treatments was oleic acid (C18:1), followed by linoleic acid (C18:2), palmitic acid (C16:0), and stearic acid (C18:0). Other authors reported the predominance of these fatty acids for the same cultivar Ferragnès, in variety characterization studies in Spain (Kodad et al., 2008) and Portugal (Barreira et al., 2012, Oliveira et al., 2019a).

In detail, oleic acid ranged from 63.4% (NI+K) to 74.3% (FI), slightly lower than the amounts reported by Nanos et al. (2002) (74.7% to 80.8%) and Oliveira et al. (2019a) with 77.9%, but in line with those of Barreira et al. (2012), with an average of 68%, all for the same cultivar. These differences for the same cultivar could be related to environmental or agronomic factors, such as the geographical area, climatic conditions, and the use of a different rootstock or the level of soil fertility (Yada et al., 2011). No significant differences ($p<0.05$) between the total irrigation treatment FI (74.3%) and the deficit irrigation treatments, SDI70 (73.4%) and SDI35 (73.5%) were observed, but the oleic acid proportion was reduced in the non-irrigated treatments, with a proportional increase in linoleic acid and saturated ones (palmitic and stearic). These results follow the trend described by Zhu et al. (2015) in a study carried out with several SDI treatments in which the oleic acid content decreased with the water deficit. This trend was similar to that of Nanos et al. (2002) for the same cultivar, Ferragnès, but these authors only compared an irrigated and a non-irrigated treatment. Another study, conducted by Gutiérrez-Gordillo et al. (2020) on three distinct almond cultivars (Marta, Guara and Lauranne), found a significant increase in oleic acid content in treatments with SDI compared to a control treatment under FI. Lipan et al. (2019a) in an investigation on almond trees of the Vairo cultivar in which they compared four irrigated treatments found no significant differences.

Among the treatments under rainfed conditions, kaolin showed a significant reduction on the oleic acid proportion compared to the treatment without application (from 61.9% to 64.3%, respectively). We cannot suggest that these differences could be related to water stress since there were no differences throughout the growing season between the two treatments (Fig. 2). Khaleghi et al. (2015), for two consecutive seasons, observed that olive trees with foliar application kaolin increased oleic acid content of its olives which is opposite to the preset achievements.

Linoleic acid was the second most abundant fatty acid, with the NI + K treatment presenting the highest content (24.4%), followed by the NI (22.7%), while the lowest values were registered in the irrigated treatments (15.9% to FI; 16.4% to SDI70 and 16.2% to SDI35) (Table 3). These values were similar to those found in other investigations with the same cultivar: Barreira et al. (2012) reported 22% and Oliveira et al. (2019a) 13.6%. There were no significant differences between the FI treatment and the treatments with sustainable deficit irrigation (SDI70 and SDI35). However, a significant increase in the linoleic acid content was observed in the rainfed treatments (NI and NI+K) compared to the irrigated treatments. These results agree with those obtained by Nanos et al. (2002) for the same cultivar, where the rainfed treatment showed a significant increase in linoleic acid content compared to the irrigated treatment. In investigations with different deficit and control irrigation treatments, with maximum irrigation, Lipan et al. (2019a) did not find any significant difference, and Zhu et al. (2015), in the second season of the experiment, showed that linoleic acid increased its concentration with greater water deficit. Thus, we can suggest that the increased water stress in almond trees causes an increase in the activity of the oleyl-CoA-desaturase enzyme that converts oleic acid into linoleic acid (Sadras and Villalobos, 2021). In terms of almond oil quality, an increase in linoleic acid can, if not adequately protected by antioxidants, result in increased rancidity and lower shelf life. The oleic/linoleic ratio is used as an indicator of quality, with all irrigated regimes having more protective scores than the non-irrigated ones (Table 3).

As for the other two major fatty acids, both saturated, palmitic (C16:0) and stearic (C18:0) showed a similar trend to linoleic acid, with a significant increase in its proportion in rainfed treatments, compared to irrigated treatments. The SFA sum showed a significant trend, causing an increase in the SFA content when water stress increased. Thus, the treatment with full irrigation (FI) reported the lowest content (8.0%), followed by the treatments with sustainable deficit irrigation (SDI70 and SDI35, both with 8.4%) and finally, in the rainfed treatments, NI showed a content of 9.5% and the treatment with kaolin (NI+K) the highest content with 10.0%.

Thus, the irrigation resulted in almonds with superior oil quality as the oil had higher MUFA and PUFA content and less SFA content than almonds from non-irrigated trees. These results agree with those obtained in previous works for the same cultivar, Ferragnès, such as in the case of Nanos et al. (2002).

3.5. Tocopherol and tocotrienol

In the present work, six isoforms of tocopherols and tocotrienols were identified and quantified in the almond oils, namely α-, β-, γ-tocopherol, and α-, β-, γ- tocotrienol (Table 4). The analysis of variance showed that the irrigation effect was significant for all the compounds ($p < 0.05$).

The NI treatment presented the lowest value of total tocopherols (275 mg kg^{-1}) compared to all irrigated ones (383 mg kg^{-1}; 379 mg kg^{-1} and 411 mg kg^{-1} respectively to FI, SDI70, and SDI35). These values are in line with reported values for Ferragnès cultivar, as Kodak et al. (2010) with almonds from different varieties and production countries (Spain and Morocco) and mean values from 301 to 462 mg kg^{-1} oil or Yildirim et al., (2016) with of 318 and 542 mg kg^{-1} oil for the Ferragnès cultivar, depending on the year.

As far as we know, there are few works with clear conclusions on the relationships between water stress, deficit irrigation and tocopherol content in almond trees. Zhu et al. (2015) also studied the content of tocopherols in almonds in an experiment with different deficit irrigations during two consecutive years but did not find a clear trend. Ortega-Farias et al. (2020), in an experiment conducted to evaluate the effect of irrigation cut-off on hazelnut, also found no significant differences between the α-tocopherol kernel content among treatments. Nevertheless, in the literature, we found works with other crops, as well, García et al. (2020) observed that olive trees with more water-stressed olive trees produced oils with the highest contents of tocopherol and Steven

Table 4
Fatty acids profile (relative percentage, %) of almond oils extracted from each treatment (n = 6; mean ± standard deviation).

Fatty acids composition (relative %)	Treatments NI	NI+K	SDI 35	SDI 70	FI	*P*-value*
SFA						
C14:0 (Myristic)	0.02 ± 0.01 a	0.05 ± 0.03 b	0.02 ± 0.01 a	0.03 ± 0.01 ab	0.02 ± 0.01 a	0.0073
C15:0 (Pentadecylic)	0.01 ± 0.01 a	0.02 ± 0.01 b	0.01 ± 0.01 a	0.01 ± 0.01 a	0.01 ± 0.01 a	0.0681
C16:0 (Palmitic)	7.13 ± 0.20 b	7.71 ± 0.14 c	6.37 ± 0.06 a	6.33 ± 0.23 a	6.08 ± 0.17 a	< 0.0001
C17:0 (Margaric)	0.08 ± 0.00 c	0.08 ± 0.00 c	0.07 ± 0.00 b	0.07 ± 0.00 b	0.06 ± 0.00 a	< 0.0001
C18:0 (Stearic)	2.13 ± 0.05 c	2.02 ± 0.09 bc	1.80 ± 0.14 a	1.89 ± 0.10 ab	1.74 ± 0.12 a	< 0.0001
C20:0 (Arachidic)	0.08 ± 0.00 b	0.08 ± 0.00 b	0.08 ± 0.00 b	0.08 ± 0.00 b	0.07 ± 0.00 a	< 0.0001
C22:0 (Behenic)	0.02 ± 0.00 a	0.02 ± 0.00 a	0.02 ± 0.00 a	0.02 ± 0.00 a	0.02 ± 0.00 a	0.8261
MUFA						
C16:1 (Palmitoleic)	0.70 ± 0.03 c	0.74 ± 0.02 c	0.57 ± 0.03 b	0.51 ± 0.03 a	0.55 ± 0.01 ab	< 0.0001
C17:1 (Heptadecenoic)	0.11 ± 0.00 c	0.10 ± 0.00 bc	0.10 ± 0.00 bc	0.09 ± 0.00 a	0.09 ± 0.01 a	0.0004
C18:1 (Oleic)	65.8 ± 0.85 b	63.4 ± 0.37 a	73.5 ± 0.12 c	73.5 ± 1.15 c	74.3 ± 1.15 c	< 0.0001
C20:1 (Eicosenoic)	0.07 ± 0.01 b	0.06 ± 0.01 a	0.07 ± 0.01 b	0.07 ± 0.01 b	0.07 ± 0.01 b	< 0.0001
PUFA						
C18:2 (Linoleic)	22.67 ± 0.63 b	24.39 ± 0.27 c	16.22 ± 0.23 a	16.36 ± 0.90 a	15.94 ± 1.15 a	< 0.0001
C18:3 (α-Linolenic)	0.06 ± 0.01 b	0.08 ± 0.01 c	0.04 ± 0.01 a	0.04 ± 0.01 a	0.03 ± 0.01 a	< 0.0001
Total SFA	9.5 ± 0.2 c	10 ± 0.2 d	8.4 ± 0.2 b	8.4 ± 0.3 b	8 ± 0.3 a	< 0.0001
Total MUFA	66.7 ± 0.8 b	64.3 ± 0.4 a	74.3 ± 0.2 c	74.1 ± 1.1 c	75.1 ± 1.2 c	< 0.0001
Total PUFA	22.7 ± 0.6 b	24.5 ± 0.3 c	16.3 ± 0.2 a	16.4 ± 0.9 a	16 ± 1.2 a	< 0.0001
Oleic/linoleic	2.9 ± 0.1 a	2.6 ± 0.1 a	4.5 ± 0.1 b	4.5 ± 0.3 b	4.7 ± 0.4 b	< 0.0001

nd – not detected. SFA – saturated fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids. NI, NI+K, SDI35, SDI70, FI treatments are explained in the Material and Methods section.
In each row, different letters mean significant differences between samples, at a 5% significance level (P-value < 0.05), where "a" and "d" correspond to the lowest and highest values, respectively, according to multiple comparison Tukey's HSD test.
* P-value < 0.05 means that the mean value of the evaluated quality parameter of at least one treatment differs from the others, according to the one-way ANOVA results (in this case, multiple-comparison tests were performed).

Table 5
Tocopherol and tocotrienol composition (mg kg^{-1} almond oil) of almond oils extracted from different treatments (n = 6; mean ± standard deviation).

Compounds	Treatments NI	NI+K	SDI 35	SDI 70	FI	P-value*
α-tocopherol	255.16 ± 2.20 a	366.40 ± 9.58 bc	375.76 ± 19.75 c	349.99 ± 8.30 b	355.53 ± 0.58 b	< 0.0001
β-tocopherol	8.55 ± 0.40 a	11.02 ± 0.56 b	10.59 ± 0.95 b	10.36 ± 0.25 b	10.49 ± 0.63 b	< 0.0001
γ-tocopherol	10.90 ± 0.66 b	9.73 ± 0.92 a	24.27 ± 0.21 d	18.62 ± 1.06 c	16.64 ± 0.44 b	< 0.0001
∑-tocopherol	274.6 ± 1.16 a	387.2 ± 9.41 b	410.6 ± 20.26 c	379.0 ± 7.9 b	382.7 ± 0.8 b	< 0.0001
α-tocotrienol	16.00 ± 0.95 a	18.20 ± 0.30 b	16.14 ± 0.18 a	16.86 ± 0.88 a	15.84 ± 0.66 a	< 0.0001
β-tocotrienol	5.51 ± 0.26 b	7.38 ± 0.32 c	4.72 ± 0.50 a	5.23 ± 0.39 ab	4.65 ± 0.13 a	< 0.0001
γ-tocotrienol	5.83 ± 0.10 a	6.85 ± 0.04 c	6.27 ± 0.42 abc	6.70 ± 0.55 bc	6.08 ± 0.32 ab	0.0004
∑-tocotrienol	27.4 ± 1.1 ab	32.4 ± 0.6 c	27.1 ± 0.6 ab	28.8 ± 1.8 b	26.6 ± 1.1 a	< 0.0001
Total	265 ± 2 a	378 ± 10 bc	388 ± 20c	361 ± 8 b	366 ± 1b	< 0.0001

NI, NI+K, SDI35, SDI70, FI treatments are explained in the Material and Methods section.
In each row, different letters mean significant differences between samples, at a 5% significance level (P-value < 0.05), where "a" and "d" correspond to the lowest and highest values, respectively, according to multiple comparison Tukey's HSD test.
* P-value < 0.05 means that the mean value of the evaluated quality parameter of at least one treatment differs from the others, according to the one-way ANOVA results (in this case, multiple-comparison tests were performed).

and Diane (2002) in soybean, where drought stress caused a two to three-fold increase in tocopherols content. This seems to be in contradiction with the results obtained by us.

If we analyze the values for all irrigated treatments, the treatment with the least irrigation (SDI35) presented the highest concentration of total tocopherols. This suggests that treatments with high amounts of irrigation (FI and SDI70) and not very low levels of water stress (between -1 MPa and -2 MPa) impair the synthesis of tocopherols. This follows the trend found by García et al. (2020) in olive trees. When the amount of irrigation water decreases, as occurs in the SDI35 treatment, and the almond trees present more negative values of water potential (between -2 MPa and -3 MPa), the accumulation of tocopherols is more significant. At the other extreme, with treatments without irrigation (NI) and very negative levels of water potential (between -3.5 MPa and -5 MPa), we have lower concentrations of tocopherols in almonds. This could explain the plant's adaptation to water stress and the importance of tocopherols in its metabolic activity. Tocopherols have numerous functions within plants, although one of the main ones is their activity as antioxidants, maintaining the integrity of membranes under environmental stress conditions (Munné-Bosch, 2007; Azzi, 2019). Tocopherol biosynthesis at the cellular level occurs in plasts, such as the chloroplasts of photosynthetic tissues or the chromoplasts of fruits (Munné-Bosch, 2007). In this process, condensation, methylation, and cyclization reactions occur in which different enzymes and hormones play an important role (Cela et al., 2009). The different types of stress that can appear in a plant produce changes in gene expression while participating in the generation of hormones such as Abscisic Acid (ABA), Salicylic Acid (SA) and others (Mahajan and Tujeta, 2005). Under water stress conditions, ABA interferes with stomatal closure to reduce water loss through transpiration, reducing CO_2 availability. This leads to an accumulation of NADPH and ATP because the metabolic cycles are incapable of degrading them, which translates into a saturation of photosynthetic electron transport and the formation of Reactive Oxygen Species (ROS). ROS are a set of free radicals that can produce oxidative damage to cells (Hasanuzzaman et al., 2017). To control and reduce the negative effects of ROS, chloroplasts have developed a set of antioxidants, one of the most important being α-tocopherol (Apel and Hirt, 2004). When a plant is in a water stress situation, α-tocopherol has different mechanisms of action. It can donate phenolic hydrogen to lipid radicals to chemically deactivate them and thus reduce the detrimental effects of lipid peroxidation in membranes (Liebler, 1993) and can also chemically and physically deactivate singlet oxygen ($^1O_2$) (Fahrenholtz et al., 1974). All these mechanisms of action of α-tocopherol involve its degradation in other molecules. Thus, according to some authors (Cela, 2012), ABA seems to have an important role in regulating tocopherol biosynthesis. If we take this to our data, we could suggest that since the almond trees of the FI and SDI70 treatments did not have a low level of water stress, the synthesis of ABA is lower than in the SDI35 treatment with more negative water potential levels and consequently the tocopherol levels. On the other hand, the NI treatment presented a much lower concentration of total tocopherols than the rest of the treatments due to its high degradation throughout the summer to withstand the negative effects of the high level of water stress that it endured.

Regarding the treatment without irrigation and with foliar application of kaolin (NI + K), the values were much higher than the other non-irrigated treatment (NI), 387 mg kg^{-1} and 275 mg kg^{-1}, respectively, despite the application of kaolin did not improve the levels of water stress compared to the rainfed treatment (NI) (Fig. 2). There is little research on the effect of foliar applications of kaolin on tocopherols and tocotrienols in crops. Perri et al. (2006) conducted a study to evaluate whether the foliar application of kaolin could reduce the incidence of the pest *Bactrocera oleae* (Gmelin) in olive trees. At the same time, they analyzed some parameters of the oil composition, concluding that kaolin did not present any effect on tocopherols.

Regarding tocotrienols, the three isoforms, namely α, β and γ-tocotrienol, were identified and quantified. α-Tocotrienol was the major compound, with values ranging from 15.8 mg kg-1 oil to 18.2 mg kg-1 oil. The β-tocotrienol showed contents from 4.6 mg kg-1 oil to 7.4 mg kg-1 oil and γ-tocotrienol 5.8 mg kg-1 oil to 6.8 mg kg-1 oil for the different treatments. Barreira et al. (2012) analyzed the chemical composition of almonds of the Ferragnès cultivar and identified two tocotrienols (α and γ) with concentrations similar to ours. All the tocotrienols showed significant differences between treatments ($p < 0.05$), being the NI+K treatment the one that presented the highest contents for all of them, in line with the highest amounts of linoleic acid. Regarding the total amount of tocotrienols, the content varied from 27.1 mg kg-1 oil for the NI+K treatment to 32.4 mg kg-1 oil for the SDI35 treatment (Table 4). There were no significant differences between the rainfed treatment (NI) and the irrigated treatment or between the deficit irrigation treatments (SDI) and the fully irrigated treatment (FI). With this, we can only suggest that the treatment with kaolin (NI + K) managed to increase the concentration of all the isoforms of tocotrienols, but without a clear explanation, since the levels of water potential of this treatment were similar to the rainfed (NI) treatment. Few data in the literature explain the cause of this trend.

3.6. Effect of water availability on physicochemical composition

Principal components analysis (PCA) was performed on some fatty acids, tocopherols, and tocotrienols for the different treatments (Fig. 3). According to the obtained results, the two main components (PC1 and PC2) exhibited 62.2 and 22.3% of the total variance, respectively (Fig. 3). As can be seen, the samples from the different treatments were separated into three different regions. The samples of NI+K treatment appear in the positive region for the two principal components (PC1 and PC2). The content of tocotrienols and the fatty acids and SFA

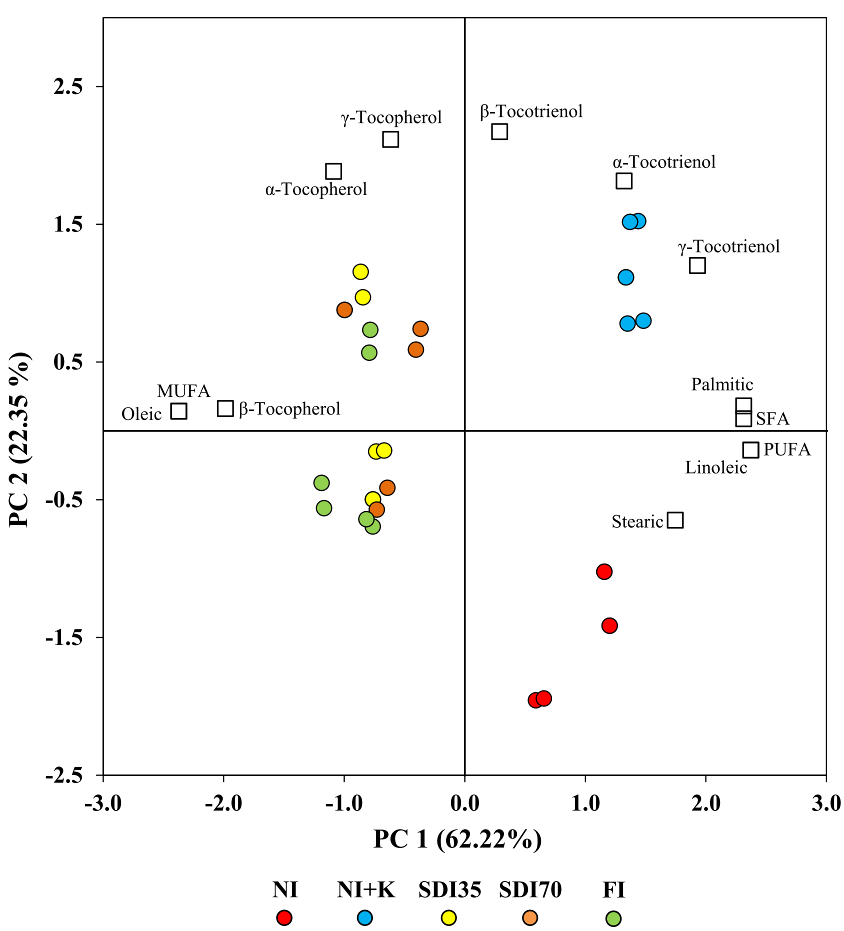

Fig. 3. Principal component analysis obtained from main fatty acids, tocopherols and tocotrienols for the different treatments. The principal components explain 84.57% of the total variance.

concentration mainly describes these samples. In the positive part of PC1 and negative of PC2, we found all the samples from the rainfed control treatment (NI). These samples are linked to a higher amount of stearic and linoleic fatty acids as well as PUFA. Finally, we can highlight a group of samples that includes all irrigation treatments, both FI and SDI, which appear in the positive and negative parts of PC2 and in the negative part of PC1. These samples are better identified with tocopherols, MUFA, and oleic acid concentration. This trend shows a significant separation between irrigated and non-irrigated treatments, given as identified in fig. 3. This suggests that irrigation treatments show few differences between them for the variables studied in PCA and considerable with the control treatment (NI) and the treatment with kaolin application (NI + K).

4. Conclusion

When compared with non-irrigated treatments, sustainable deficit irrigation improved important pomological parameters related to almonds quality, such as the kernel's weight. The oleic acid and tocopherols did not present without significant differences with full irrigation. It was shown that almond trees can grow under deficit irrigation conditions and water stress, achieving savings for water provided by irrigation without reducing some of its major quality indicators in comparison with full irrigation, but also with higher amounts of polyunsaturated fatty acids, indicators of lower oxidation stability, without a clear compensation in terms of vitamin E related compounds production. No positive effects were observed with the application of kaolin in non-irrigated trees.

CRediT authorship contribution statement

David Barreales: Investigation, Data curation, Formal analysis, Writing – original draft. **José Alberto Pereira:** Methodology, Conceptualization, Validation, Writing – review & editing, Funding acquisition. **Susana Casal:** Methodology, Conceptualization, Validation, Writing – review & editing, Funding acquisition. **António Castro Ribeiro:** Conceptualization, Validation, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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