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Influence of irrigation regime and seasonal temperatures on nut quality and the oil fatty acid profile of walnuts (*Juglans regia* L.)

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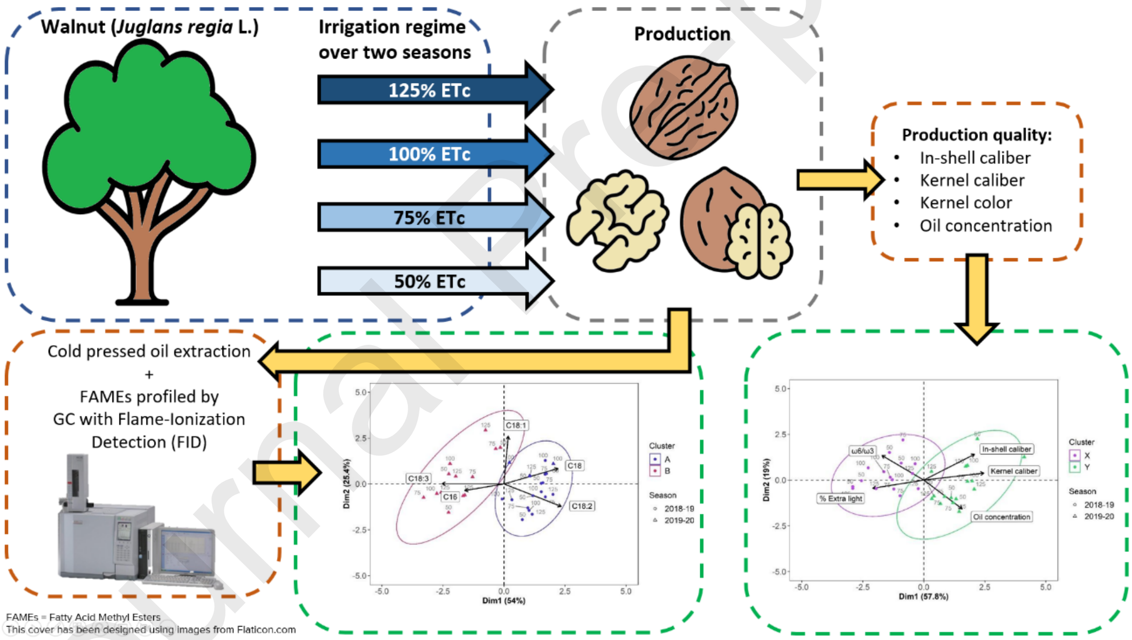

Influence of irrigation regime and seasonal temperatures on nut quality and the oil fatty acid profile of walnuts (*Juglans regia* L.)

Abstract

Walnut production is expanding worldwide due to the high demand for natural products with proven nutraceutical properties. This expansion includes new growing areas, such as central–western Argentina, where crop water requirements have yet to be determined and little is known about the response in terms of production quality to the water regime. The aim of this study was to assess the effects of four irrigation regimes (at 50, 75, 100, and 125 % of crop evapotranspiration) and the weather conditions over two consecutive seasons in a young Chandler walnut orchard in terms of in–shell and kernel caliber, kernel color, oil concentration, and fatty acid profile. Quality production characteristics were not significantly affected by irrigation regimes within each season. In contrast, the quality parameters achieved were significantly different between seasons. The first season (S1) was wetter (462mm) and cooler (17.05 °C mean air temperature in the oil accumulation period) and produced more extra–light kernels (92%). The second season (S2) was drier (326 mm) and warmer (19.02 °C) and produced a lower proportion of extra–light kernels (82.2%). In addition, during the second season, walnuts produced larger kernels with a higher oil concentration (62.7%; +3.5% of S1) and a better omega–6/omega–3 ratio (3.25 in S2 vs. 3.70 in S1). These results suggest that kernel quality was more sensitive to the seasonal temperature than the irrigation studied regimes. The nutritional quality of walnuts may increase significantly in warmer seasons/environments despite the deterioration of kernel color.

Keywords

Argentina; Chandler; water deficit; kernel color; omega–6/omega–3 ratio; linolenic acid

Abbreviations: ETc, crop evapotranspiration; ET0, reference evapotranspiration; ω6 = omega–6; ω3 = omega–3; masl, meter above sea level; PCA, principal component analysis; HCPC, hierarchical clustering on principal components; C16, Palmitic acid; C18, Stearic acid; C18:1, Oleic acid; C18:2, Linoleic acid; C18:3, Linolenic acid.

1. Introduction

The global walnut production has increased significantly since the 2000s, from 1.2 million t to 3.3 million t in 2020 (FAOSTAT, 2023). This increase in production can be attributed to the demand of natural foods with proven nutraceutical properties (Chatrabnous et al., 2018; Ros et al., 2021). To meet the high demand for walnuts the crop is actively expanding in new areas, such as Argentina's semi–arid central–west. In Argentina, the area cultivated with walnuts has increased by 400% in the last two decades, reaching 18,235 ha with a production of 21,500 t in 2021 (FAOSTAT, 2023). In central–western Argentina, the mean annual rainfall is limited, ranging from 200 to 600 mm year^{-1} (Rubí–Bianchi and Cravero, 2010), below the walnut water demand close to 1,000 mm year^{-1} (Goldhamer, 1998). Consequently, walnut production in this region is possible only under irrigation, using water from underground aquifers. Rivera et al. (2021) studied the water balance of the region and identified, in the period 2010–2020, a significant reduction in snow reserves in the Andes and in the main basin flows so that the recharge of underground aquifers is also limited and could lead to overexploitation by agriculture. The application of irrigation doses lower than maximum crop requirements has been widely studied in other dry fruit crops such as almond (García–Tejero et al., 2020) and pistachio (Carbonell–Bachina et al., 2015). It is also applied to fruit trees because it provides increases in crop water productivity, with the additional advantage of improving fruit quality (Yang et al., 2022). In walnuts, studies on the irrigation strategies are limited and mainly focused on improving irrigation scheduling, but their effects on productivity and quality of walnuts and oil must be evaluated.

The commercial quality of walnuts is determined by in–shell caliber and kernel color. As walnuts have a larger equatorial diameter and a lighter color in their kernel pellicle, they will be better appreciated. Apart from the importance of walnut production per area unit, the kernel/in–shell weight ratio (ranging between 50 and 60%) and kernel oil concentration (ranging between 50 and 70%) are key to defining productivity (Martínez et al., 2010). The in-shell walnut caliber is determined between female flowering and endocarp hardening (Ramos et al., 1978), while active oil accumulation reaches the maximum rate around with the endocarp hardening phase and ends with the physiological maturity of the kernel (Jin et al., 2023). Some studies suggest that an early–season water deficit can significantly affect the nut's final size (Ramos et al., 1978; Cohen et al., 1997; Fields et al., 2020). On the other hand, there is evidence that a water deficit towards maturity can affect kernel color due to the increase in temperatures caused by the lack of hydraulic conductivity at maturity and the consequent chemical and enzymatic oxidations of the phenols present in the kernel pellicle (Pakrah et al., 2021).

Walnut kernel oil is rich in essential fatty acids, proteins, and minerals (Pakrah et al., 2022; Özcan and Lemiasheuski, 2020). The proportions of linoleic (ω6 = C18:2) and linolenic (ω3 = C18:3) acid reported from walnut orchards growing in Argentina were 57.9 ± 2.8% and 13.9 ± 2.1% (Martínez et al., 2010), meanwhile 55.3 ± 1.1 and 8.7 ± 0.7 were reported in walnut orchards growing in Turkey (Özcan, 2009), respectively, according to the cultivar. It is well documented that an unbalance of consumption in the ω6/ω3 ratio, which should ideally be in the range of 1:1 to 4:1 but in western diets reaches values around 15:1, is related to cardiovascular, autoimmune, rheumatic diseases, diabetes, cancer, obesity, asthma, and depression (Simopoulos, 2016). In this sense, walnut oil has one of the best ω6/ω3 ratios, around 4:1 (Zec and Glibetic, 2018; Özcan et al., 2010). An increase in walnut consumption has been linked to the prevention and treatment of obesity and metabolic syndrome and to the modulation of coronary heart disease risk (Zec et al., 2020; Zibaeenezhad et al., 2017). Regarding the influence of the water regime on oil composition, some studies suggest that a water deficit in sunflower (Akbari et al., 2020), soybean (Carrera and Dardanelli, 2017), and olive (Dag et al., 2015) results in an increase in the concentration of polyunsaturated fatty acids. In this context, under deficit irrigation conditions, the crop is also subject to weather conditions (mainly temperature) to achieve commercially appreciated yields, so the selection of growing areas with adequate water supply and temperatures throughout the season becomes relevant.

This study aims to evaluate the effects of seasonal–long application of four water regimes at 50, 75, 100 and 125 % of ETc and the weather conditions on in–shell and kernel caliber, kernel color, oil concentration and fatty acid profile of a young walnut orchard in central western Argentina.

2. Material and Methods

2.1. Experimental design

The experiment was carried out on a 6–year–old commercial walnut cv. Chandler orchard in Guanchín (29° 10′ S; 67° 40′ W; 1750 masl), La Rioja province, Argentina, over two seasons: 2018–2019 (from October 1, 2018, to March 31, 2019) and 2019–2020 (from October 1, 2019, to March 31, 2020). The trees were spaced at 7 m x 5 m and irrigated with one microjet per tree. The mean annual temperature and rainfall in the 2010–2020 decade were 13.55 °C (mean minimum air temperature = 7.45 °C; mean max air temperature = 20.20 °C) and 534 mm (in the range from 265 mm to 763 mm), respectively. The rainfall was mainly concentrated in the summer months (December to March in the Southern Hemisphere). Daily meteorological data, including air temperature, relative humidity, solar radiation, wind speed, rainfall, and reference evapotranspiration (ET0) were collected at a weather station near the experimental site. Sixteen experimental plots were selected, which consisted of 12 trees (3 rows x 4 trees per row), in which the two central trees (similar in height, canopy volume, and trunk cross sectional area) were kept unaltered until harvest. The irrigation treatments consisted of replenishing the equivalent of 50 % (T50), 75 % (T75), 100 % (T100) and 125 % (T125) of crop evapotranspiration (ETc) throughout both seasons. The irrigation system, irrigation frequency and orchard management are fully described in Calvo et al. (2022).

2.2. Harvest and walnut processing

The darkening of the packing tissue and husk splitting indicated harvest maturity. At this stage, one of the central trees was hand–harvested. The husks were manually removed, and the walnuts were dried in a forced–air oven at 25 °C until the kernel moisture content reached 4 %, monitored every 8 hours with a Precisa XM60 moisture analyzer. Next, the in–shell diameter was measured with a 0.01–mm precision vernier caliper. Afterwards, a sample of 100 walnuts per treatment was taken, manually cracked, and the diameter of the kernels was measured. A USDA standard color chart was used to determine the color proportions of the 100–kernel sample. Finally, until the oil concentration extraction, the kernels were stored in Ziplock bags at −20 °C.

2.3. Oil concentration and extraction

Oil concentration was estimated by the weight difference before and after solvent oil extraction. The solvent extraction was performed with an automated soxhlet extractor (Ankom XT10) according to AOAC 920.39 methodology, which consisted of grinding 30 kernels per treatment while 2 g samples were taken in duplicate (Thiex et al., 2003). The subsamples were dried until constant weight in a forced–air oven at 70 °C. Solvent extraction was carried out with petroleum ether in a cyclic extraction program of 60 minutes. On the other hand, the oil of 30 kernels was extracted by cold pressing with a manual hydraulic press at 40 MPa; then, the oil extracts were centrifuged at 5,000 g for 10 minutes and filtered (14 µm pore size). The oil samples were stored at −20 °C until chromatography.

2.4. Fatty acid profiling by gas chromatography

The fatty acid profile was determined according to the methodology proposed by the International Olive Council (IOC/T.20/Doc.34/Rev.1). Briefly, 0.1 g of oil was weighted, 2 mL of heptane was added, and the mixture was vortexed for 10 seconds. Subsequently, 0.2 mL of a 2 N methanol potassium hydroxide solution was added and vortexed for 10 seconds. Finally, the mixture was centrifuged at 10,000 g for 5 min. The supernatant was transferred to a vial for chromatography. 1µL of the fatty acid methyl esters was injected into a Shimadzu GC2010 Plus gas chromatograph with a flame ionization detector (FID) and a Phenomenex ZB–FAME column (60 m x 0.25 mm x 0.2 µm). H_2 was used as the carrier gas, with a flow rate of 1.2 mL min^{-1}; the detector was set to 260 °C, and the injector was set to 240 °C (split ratio 1:10). The results were expressed as the relative proportion of the total area of fatty acid methyl esters.

2.5. Statistical analysis

Data analysis was performed in the R v.4.1.2 environment for statistical computing, using the nlme and emmeans packages for univariate analysis. A linear mixed model with the treatments as a fixed effect and the blocks as a random effect was assumed, as was another linear mixed model with a season as a fixed effect and treatment as a random effect. Principal component analysis (PCA) and Hierarchical Clustering on Principal Components (HCPC) were performed with the FactoMineR and factoextra packages.

3. Results

3.1. Weather conditions and irrigation water use

The weather conditions during 2018–2019 and 2019–2020 seasons (Fig. 1) were divided into two periods: the first one between bloom–endocarp hardening (B–EH), and a later period between endocarp hardening–harvest maturity (EH–H). The mean temperature and ET0 in B–EH were similar in both seasons: 16.50 °C and 330 mm in 2018–2019 and 16.81 °C and 350 mm in 2019–2020, respectively. The accumulated rainfall in the B–EH period was 157 mm in 2018–2019 and practically half (71 mm) in 2019–2020. In the EH–H period, mean temperatures were 17.05 °C in 2018–2019 and 19.02 °C in 2019–2020. ET0 and accumulated rainfall along the EH–H period reached 305 and 255 mm, respectively for the 2018–2019 season, and 337 and 256 mm, respectively for the 2019–2020 season. When comparing overall seasonal weather conditions, a distinct pattern of rainfall distribution can be observed. In 2018–2019, rainfall was distributed uniformly between December and April, while in the 2019–2020 season it was mainly concentrated between January and February. The total water applied (irrigation + effective rainfall) to irrigation treatments T50, T75, T100, and T125 was 447 mm, 586 mm, 730 mm, and 895 mm in 2018–2019, distributed in 19 irrigation events throughout the season. In the 2019–2020 season, the volumes applied to the same treatments were 443 mm (T50), 563 mm (T75), 716 mm (T100), and 893 mm (T125) in 21 irrigation events. Effective rainfall is the portion of total rainfall that the crop can intercept, and it was the same in all treatments. It was estimated according to the following equation: rainfall event > 12 (mm) → [rainfall (mm) – 12 (mm)] * 0.8. Rainfall less than 12 mm was not considered (Calvo et al., 2022).

3.2. Walnut quality parameters

In–shell walnut and kernel dry yields did not vary between treatments in both seasons (Table 1). The variables in–shell caliber, kernel caliber, percentage of extra–light kernels, and oil concentration (Table 2) did not show significant differences between irrigation regimes within each season but were affected significantly by the season. In–shell caliber was significantly higher in the second season, reaching 32.9 mm, while in the first season it was 32.2 mm. Similarly, the kernel caliber was 1.1 mm higher in 2019–2020 than 2018–2019. Both variables, in–shell and kernel calibers, were linearly related ($y = 0.75x$, $R^2 = 0.66$; Fig. 2). Likewise, oil concentration was 62.7 % higher in the 2019–2020 season. The amount of extra–light kernels was 11.6 % higher in the first season, reaching 93.8 %, whereas in the second season, it reached 82.2 %. The number of extra–light kernels was significantly negatively related to in–shell caliber in 2019–2020 but not in 2018–2019 (Fig. 3).

3.3. Fatty acid profile

The fatty acids found in proportions greater than 0.1 % (Table 3) were: palmitic (C16), stearic (C18), oleic (C18:1), linoleic (C18:2), and linolenic (C18:3) acids. Similar to quality parameters, there were no differences in the proportions of fatty acids between irrigation regimes. Regarding saturated fatty acids, palmitic acid did not show significant differences between seasons. On the contrary, stearic acid presented significant differences, accumulating 0.13 % more in 2018–2019 (1.94 %) than in 2019–2020 (1.81 %, Fig. 3). All the unsaturated fatty acids presented significant differences between seasons. Oleic and linolenic acids reached a higher concentration in the second season (15.73 and 18.01 %, respectively in 2018–2019, and 15.41 and 16.31 %, respectively in 2019–2020). Meanwhile, linoleic acid reached its maximum value in the first season (60.30 % in 2018–2019 and 58.31 % in 2019–2020). The ratio between unsaturated and saturated fatty acids did not show significant differences between treatments or seasons. In contrast, the ratio between monounsaturated and polyunsaturated fatty acids (MUFA/PUFA) was different between seasons, reaching a value of 0.21 in the second season (vs. 0.20 in 2018–2019). Finally, the ω6/ω3 ratio remained less than 4:1 in both seasons, with 3.70:1 in 2018–2019 and 3.32:1 in 2019–2020, respectively. This last quality parameter (ω6/ω3) presented, only in 2019–2020, two significant correlations with other quality parameters: a positive relationship with in–shell caliber and a negative relationship with the percentage of extra–light kernels (Fig. 3; Table 3).

3.4. Principal component analysis

A principal component analysis (PCA) was performed to evaluate the clustering patterns of the fatty acid profiles (Fig. 4). The first two dimensions explained 79.4 % of the total variance, with dimensions 1 and 2 (Dim1 and Dim2) accounting for 54.0 % and 25.4 %, respectively (Fig. 4). Stearic, linoleic, and linolenic acids contributed significantly (greater than 20 %) to Dim1. Oleic acid was the best–represented variable in Dim2. Meanwhile, palmitic acid contributed only 15 % to Dim1. Individuals from 2018–2019 are clearly distributed in quadrants I–IV, whereas those from 2019–2020 are distributed in quadrants II–III of the two–dimensional plane, respectively. Applying the Hierarchical Clustering on Principal Components (HCPC) algorithm, two clusters were formed that respected the seasons. The 2018–2019 season was grouped in Cluster–A, distinguished by a higher concentration of stearic and linoleic acids. The 2019–2020 season repetitions were grouped in Cluster–B, distinguished by a higher concentration of palmitic, oleic, and linolenic acids. No significant variation was observed among the selected parameters between irrigation regimes, leading to no cluster formation.

Fig. 5 shows a PCA performed to evaluate the behavior of variables related to walnut quality. Dim1 and Dim2 accumulated a total of 76.6 % of the total variability. The best–represented variables in Dim1 were kernel caliber and quantity of extra–light kernels, and in Dim2, oil concentration and ω6/ω3 ratio, whereas in–shell caliber contributed more than 20% of the variability in both dimensions. Similarly to Fig. 4, the clusters were separated by season rather than irrigation regime. The first season (2018–2019) was clustered on Cluster–X, with quadrants II and III correlated primarily by a high proportion of ω6/ω3 and extra–light kernels, respectively. Whereas the second season (2019–2020) was clustered on Cluster–Y, with in–shell and kernel caliber (quadrant I) and oil concentration (quadrant IV). The descriptor variables of each cluster were highly correlated and opposite to the descriptor variables of the other group.

4. Discussion

The application of four irrigation regimes at 50, 75, 100, and 125 % of ETc did not affect in-shell size, kernel quality or fatty acid profile. The fact that yield was not affected under the 50 % and 75 % ETc regimes suggests that a water deficit threshold of economic losses was never reached. Neither increases in quality nor yield were found at 125 % of ETc. In this sense, we consider that the Kc proposed by Goldhamer et al. (1998) overestimates the water demands of walnut trees in central–western Argentina, so we suggest making a local estimate to avoid excess irrigation and thus increase water use efficiency. In spite of no differences observed in the different irrigation regimes applied, there is a clear effect of weather seasonal conditions on the quality of production.

In the period from bloom to endocarp hardening (B–EH), in–shell and kernel calibers are defined simultaneously (Ramos et al., 1978); in this sense, the kernel caliber was 0.75 times the equatorial diameter of the in–shell walnut in both seasons (Fig. 2). Meanwhile, the distribution of precipitation in the B–EH period varied by seasons; in 2018–2019 40 % of rainy days (daily precipitation > 0.1 mm) were accumulated, while only 23 % were accumulated in 2019–2020 (Fig. 1). This rainfall pattern could limit the amount of photosynthetically active radiation (PAR) intercepted by the canopy and decrease source activity. In relation to these findings, Wang et al. (2022) found that reducing source activity (modifying the leaf–to–fruit ratio) reduced the fresh and dry weight of the walnut and its kernels but not kernel oil or protein concentration. The non–relationship between the in–shell or kernel calibers with oil concentration (Figs. 3a and 3b) had been previously reported by Martínez and Maestri (2008). Although there were statistically significant in–shell walnut caliber differences, these were less than 1 mm (Table 2), falling into quality category III from SENASA Res. 453/2013 (32 mm < in–shell caliber ≥ 34 mm) of the Argentine export standard, evidencing no differences at the commercial level.

In the endocarp–hardening to harvest (EH–H) period, high temperatures can affect the kernel pellicle color (Table 2) through an increase in phenolic oxidations and Maillard reactions (Pakrah et al., 2021; Fields et al., 2020). In line with other reports, kernel color was lighter in 2018–2019 (93.8 % of extra–light kernels), a season in which the mean temperature in the EH–H period reached 17.05 °C, which in comparison was 1.97 °C lower than in the 2019–2020 season (19.02 °C), impacting an 11.6 % increase in extra–light kernels. The relationship between in–shell caliber and kernel color was significant only in the second season (2019–2020), with a Pearson correlation of −0.73 (Fig. 3; $p < 0.0001$). Thus, in warmer and water–limited environments, it may be worthwhile to sacrifice in–shell fruit size by using deficit irrigation rates in the B–EH period and then try to take care of kernel color in the EH–H period. Moreover, in the EH–H phase, oil accumulation in 2019–2020 reached a maximum of 62.7 %, which was 3.5 % higher than that of 2018–2019, contrary to what happened with kernel color, which reached the maximum extra–light percentage in the cooler season (2018–2019). García–Inza et al. (2016) observed the opposite behavior in olive seeds, with olive seed oil concentration decreasing 1.2 % for every 1 Celsius degree increase in mean air temperature along the oil accumulation phase.

The fatty acids profiled (Table 3) were as expected for the walnut species and within the ranges reported for cv. Chandler (Cittadini et al., 2020; Pakrah et al., 2021). Larger differences were found in linoleic (higher in 2018–2019) and linolenic acid (higher in 2019–2020) proportions. In olive seeds, pistachios, and annual crops the concentration of linoleic (C18:2) and linolenic (C18:3) acids are negatively related to increases in mean daily temperature along the oil accumulation period (García–Inza et al., 2016; Roozban et al., 2006). Otherwise, Martinez et al. (2006) reported significant differences in oil accumulation in successive years for the walnut Criolla variety (66.88 % in 2004 and 67.61 % in 2005); also in this report, the season with the highest oil accumulation had a higher concentration of linolenic acid (15.61 % in 2004 vs. 11.88 % in 2005). These findings, which agree with ours, point to a possible link between oil concentration and linolenic acid accumulation, which could be related to high–temperature induced overexpression of FAD3 (in charge of C18:2 to C18:3 unsaturation) genes (He and Ding, 2020).

5. Conclusions

On the basis of the results obtained from this work, we can infer that productions coming from i) season 1 (Fig. 4, Cluster–X, cooler and wetter season) would be better appreciated by the market only for kernel color, and ii) season 2 (Fig. 5, Cluster–Y, warmer and dryer season) would be better appreciated from a nutritional point of view since it produced larger walnuts with a higher oil concentration and with a better ω6/ω3 ratio. At this point, more extensive studies on the ecophysiological responses of the crop to different water and weather regimes are needed.

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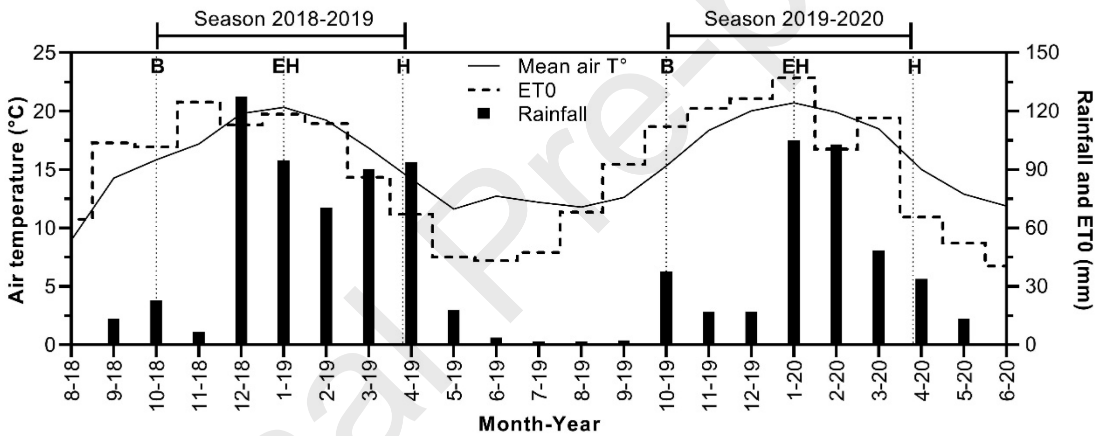

Fig. 1. Weather conditions of 2018-2019 and 2019-2020 seasons in La Rioja province, Argentina. The letters B, EH and H indicate bloom, endocarp hardening and harvest dates, respectively.

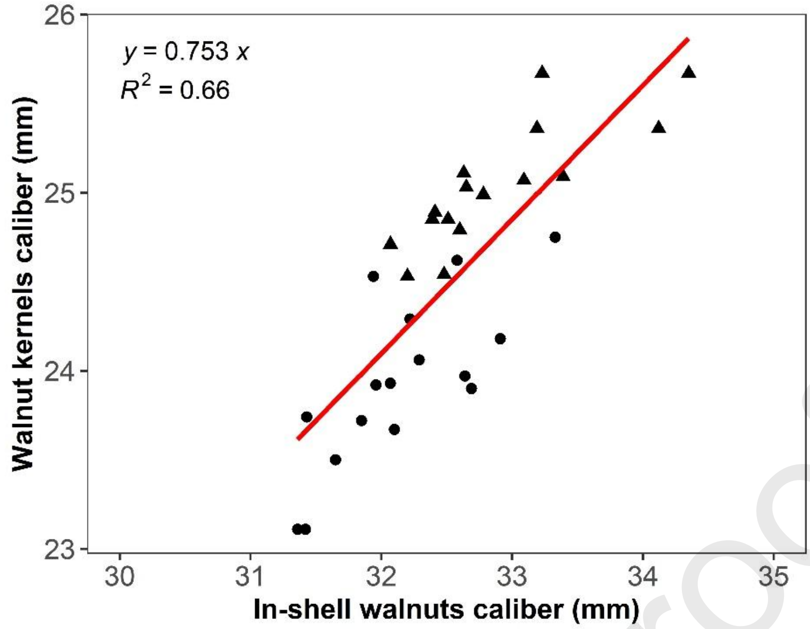

Fig. 2. Relationship between kernel and in-shell calibers of walnut cv. Chandler irrigated with different doses during 2018-2019 (●) and 2019-2020 (▲) seasons.

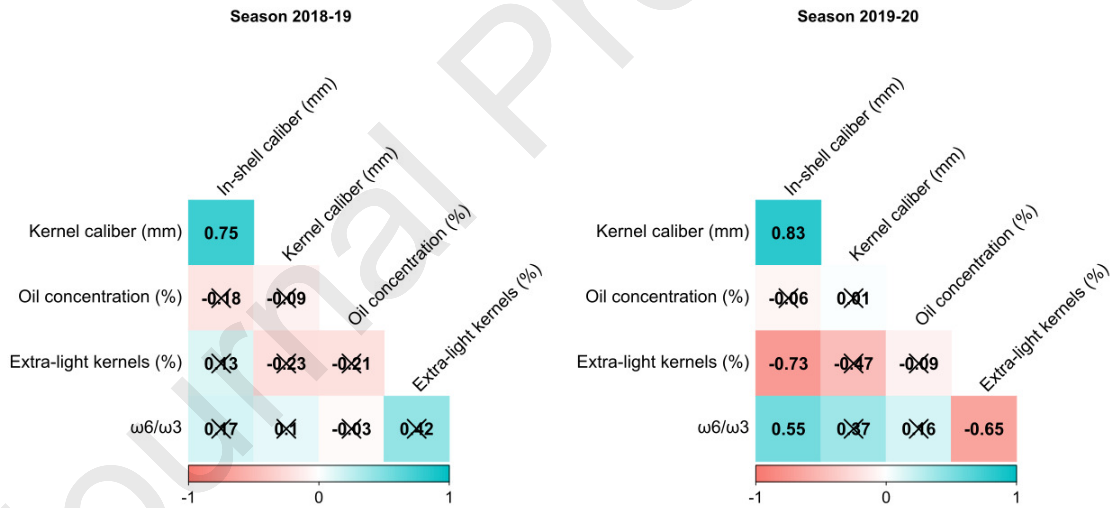

Fig. 3. Pearson correlation matrix of in–shell caliber, kernel caliber, oil concentration, extra–light kernels, quantity and ω6/ω3 of walnuts cv. Chandler in 2018-2019 and 2019-2020 seasons. Non-significant correlations (p>0.05) are marked with X.

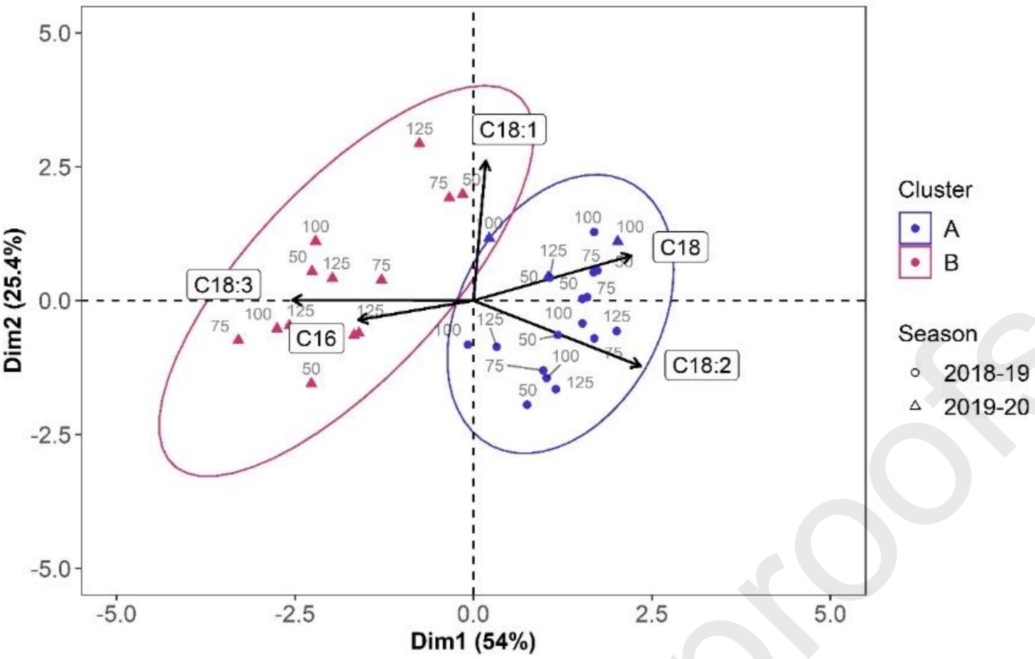

Fig. 4. Principal component analysis-biplot and hierarchical clustering of walnut cv. Chandler fatty acid profile of 2018-2019 (n=16) and 2019-2020 (n=16) seasons. The fatty acids profiled were: palmitic (C16), stearic (C18), oleic (C18:1), linoleic (C18:2), and linolenic (C18:3).

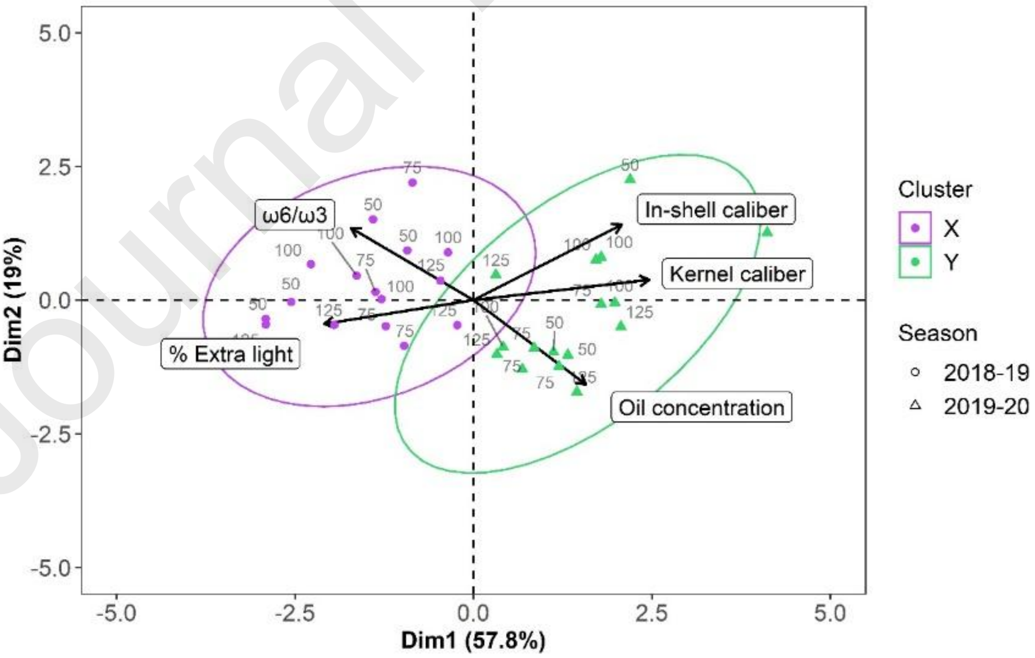

Fig. 5. Principal component analysis-biplot and hierarchical clustering (ellipses) of walnut cv. Chandler quality characteristics of 2018-2019 (n=16) and 2019-2020 (n=16) seasons.

Table 1

Walnut cv. Chandler yield and yield components (mean ± standard deviation, n = 4) under different irrigation regimes in the 2018-2019 and 2019-2020 seasons in La Rioja province, Argentina. Adapted from Calvo et al. (2022)

Season	Irrigation regime (% of ETc[1])	in–shell yield (t ha^{-1})	Kernel yield (t ha^{-1})	Nuts per tree (#)
2018-2019	50	2.50±0.98	1.05±0.45	805±320
	75	2.97±0.65	1.24±0.30	956±213
	100	3.14±1.05	1.39±0.50	1002±321
	125	3.28±0.89	1.43±0.42	1088±330
	p-value	0.5751	0.6522	0.5312
2019-2020	50	3.13±0.90	1.35±0.90	1048±335
	75	3.50±0.60	1.51±0.23	1173±217
	100	3.75±1.16	1.65±0.56	1208±406
	125	3.83±1.35	1.67±0.67	1270±417
	p-value	0.5919	0.7891	0.6678

[1] ETc: Crop evapotranspiration

P-values > 0.05 are not significantly different between means according to the LSD test. Different letters indicate statistical differences between means.

Table 2

Walnut cv. Chandler characteristics (mean ± standard deviation, n = 4) under different irrigation regimes in the 2018-2019 and 2019-2020 seasons in La Rioja province, Argentina

Season	Irrigation regime (% of ETc[1])	in–shell caliber (mm)	Kernel caliber (mm)	Extra–light kernels (%)	Oil concentration (%)
2018-2019	50	32.1±0.8	23.7±0.5	95.8±5.3	56.9±0.9
	75	32.3±0.8	24.2±0.4	93.8±9.5	59.9±3.9
	100	32.2±0.3	24.1±0.6	93.2±5.7	59.2±0.8
	125	32.0±0.5	23.8±0.6	92.2±8.7	61.0±2.5
	p-value	0.9114	0.4869	0.8251	0.1549
2019-2020	50	33.4±1.0	25.2±0.4	76.5±13.8	62.3±2.2
	75	32.5±0.5	24.9±0.4	85.2±0.5	63.3±2.2
	100	33.0±0.4	25.2±0.4	82.2±11.2	62.4±3.0
	125	32.6±0.2	24.9±0.3	84.7±9.8	62.8±4.8
	p-value	0.1857	0.3157	0.6163	0.9633
2018-2019	-	32.2±0.6 **b**	23.9±0.5 **b**	93.8±6.8 **a**	59.2±2.6 **b**
2019-2020		32.9±0.6 **a**	25.0±0.3 **a**	82.2±9.8 **b**	62.7±2.9 **a**
	p-value	**0.0019**	**<0.0001**	**0.0012**	**0.0030**

P-values > 0.05 are not significantly different between means according to the LSD test. Different letters indicate statistical differences between means.

[1] ETc: Crop evapotranspiration

Table 3

Fatty acids (mean ± standard deviation, n = 4) of walnut cv. Chandler oil under different irrigation regimes in the 2018-2019 and 2019-2020 seasons in La Rioja province, Argentina

Season	Irrigation regime (%ETc[1])	Palmitic acid (C:16)	Stearic acid (C:18)	Oleic acid (C18:1)	Linoleic acid (C18:2)	Linolenic acid (C18:3)	MUFA/PUFA [2]	UFA/SFA [3]	ω6/ω3 [4]
2018-2019	50	6.06±0.18	2.02±0.07	15.28±0.48	60.16±0.31	16.49±0.32	0.20±0.01	11.39±0.26	3.65±0.07
	75	5.96±0.14	1.97±0.10	15.42±0.37	60.31±0.20	16.36±0.39	0.20±0.01	11.63±0.33	3.69±0.07
	100	6.19±0.16	1.92±0.17	15.57±0.53	60.33±0.49	15.99±0.81	0.20±0.01	11.34±0.49	3.78±0.19
	125	5.96±0.15	1.87±0.07	15.37±0.35	60.40±0.56	16.41±0.30	0.20±0.01	11.77±0.22	3.68±0.10
	p-value	0.1793	0.1924	0.7982	0.8618	0.4880	0.7865	0.2591	0.4576
2019-2020	50	6.07±0.17	1.86±0.08	15.60±0.77	58.40±0.89	18.07±1.16	0.20±0.01	11.61±0.14	3.25±0.25
	75	6.13±0.09	1.77±0.17	15.61±0.50	58.15±0.55	18.36±0.84	0.20±0.01	11.67±0.19	3.17±0.16

	100	6.13± 0.23	1.85± 0.19	15.89 ±0.40	58.60 ±0.95	17.53 ±1.08	0.21± 0.01	11.53 ±0.08	3.36± 0.26
	125	6.22± 0.15	1.77± 0.09	15.83 ±0.74	58.08 ±0.44	18.10 ±0.41	0.21± 0.01	11.51 ±0.14	3.21± 0.07
	p-value	0.6516	0.5488	0.8689	0.4990	0.5195	0.8515	0.1960	0.4418
2018-2019	-	6.04± 0.17	1.94± 0.11 **a**	15.41 ±0.41 **b**	60.30 ±0.38 **a**	16.31 ±0.49 **b**	0.20± 0.01 **b**	11.53 ±0.35	3.70± 0.12 **a**
2019-2020		6.14± 0.17	1.81± 0.11 **b**	15.73 ±0.42 **a**	58.31 ±0.40 **b**	18.01 ±0.48 **a**	0.21± 0.01 **a**	11.58 ±0.14	3.25± 0.19 **b**
	p-value	0.1257	**<0.0001**	**0.0243**	**<0.0001**	**<0.0001**	**0.0282**	0.6142	**<0.0001**

P-values > 0.05 are not significantly different between means by LSD test. Different letters indicate statistical differences between means.

[1] ETc: Crop evapotranspiration

[2] MUFA/PUFA: monounsaturated fatty acids/polyunsaturated fatty acids

[3] UFA/SFA: unsaturated fatty acids/saturated fatty acids

[4] ω6/ω3: linoleic acid/linolenic acid ratio