Research Article

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Antioxidant capacity, fatty acids profile, and descriptive sensory analysis of table olives as affected by deficit irrigation

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Abstract

BACKGROUND: The influence of three irrigation treatments (T0, no stress; T1, soft stress; and, T2, moderate stress) on the key functional properties [fatty acids, sugar alcohols, organic acids, minerals, total polyphenols content (TPC), and antioxidant activity (AA)], sensory quality, and consumers' acceptance of table olives, cv. 'Manzanilla', was evaluated.

RESULTS: A soft water stress, T1, led to table olives with the highest oil and dry matter contents, with the highest intensities of key sensory attributes and slightly, although not significant, higher values of consumer satisfaction degree. Besides, RDI in general (T1 and T2) slightly increased green colour, the content of linoleic acid, but decreased the content of phytic acid and some minerals.

CONCLUSION: The soft RDI conditions are a good option for the cultivation of olive trees because they are environmentally friendly and simultaneously maintain or even improve the functionality, sensory quality, and consumer acceptance of table olives.

Keywords: consumers; functional foods; hydrosustainable products; *Olea europaea* L; water stress; 'Manzanilla'

INTRODUCTION

Olive (*Olea europaea* L.) is the most extensive tree crop of the Mediterranean basin and has been traditionally cultivated in marginal areas with low density under rainfall conditions.[1] The aridity of the climate and the persistent shortage of water resources in the Mediterranean agrosystems are aggravated by strong competition for the available water with other non-agricultural users, for example intense use in tourist areas during summer time.[2] These problems have led to the development of new water saving techniques, such as regulated deficit irrigation (RDI). This technique in olive trees (a drought tolerant plant) is mainly based on scheduling a water deficit period during pit hardening; it has been proved that this stage is a non-critical phenological period.[3] In this way, it is possible to save water in the irrigation of olive trees but with a minimum impact on yield and fruit quality.[4]

Table olives are prepared from the fruit of the olive tree because fresh olives are not edible.[5] Table olives are probably the most important fermented food in the Mediterranean countries and are very valuable because of their highly appreciated taste and rich nutritional composition leading to interesting health benefits.[6] Therefore, the daily consumption of table olives will contribute in an important way to the intake of healthy substances, such as phenolic compounds, which are highly recommended because of their antioxidant properties.[7]

Although irrigation normally has a positive impact on olive production, it is also known that different water regimes can affect its nutritional, antioxidant and quality components.[8,9] Cano-Lamadrid *et al.*[10] concluded that RDI can affect the quality of 'Manzanilla' table olives, including fruit size, colour, texture, volatile and fatty acids profiles, and even consumer satisfaction. Simultaneously, Collado-González *et al.*[11] using raw olives, from the same RDI treatments, showed the effects of RDI on some functional components, phytoprostanes. In these two studies, the RDI was applied during the pit hardening period. However, there are no studies about the effects of RDI on antioxidant activity, total

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phenolic compounds, mineral composition, or sugar alcohols and organic acids profiles.

Table olives cultivated under RDI conditions are considered as 'hydrosustainable' products, and have a solid identity (higher contents of essential components, higher intensity of key sensory attributes, etc.); besides, they can be a good alternative for this type of crop and reduce the economic and environmental costs linked to irrigation, optimising the use of a very valuable resource in the world, water.[10]

Considering all the above, the main aim of this work was to evaluate the effects of RDI conditions on key functional properties of 'Manzanilla' table olives. The functionality of table olives was studied by evaluating their (1) nutritional composition: fatty acids, sugar alcohols, organic acids, and minerals profiles, and (2) antioxidant properties: DPPH$^{\bullet}$, FRAP, ABTS^{+}, and total polyphenols content. These analyses were completed by evaluating the effects of RDI on (1) morphology: yield per tree, weight, and size, and CIE *L*a*b** colour, and (2) sensory quality: descriptive profile using a trained panel, and consumer acceptance using an affective panel.

EXPERIMENTAL

Plant material, growing conditions and experimental design

Fresh green olives were produced at the experimental farm 'The Hampa' located in Coria del Río (Seville, Spain); this farm is the property of the Spanish Higher Council for Scientific Research (CSIC). The plot has an area of 0.5 ha and consists of olive trees (44 years of age) of the variety 'Manzanilla de Sevilla'.

Depending on the phenological stages of the trees and the water stress established in each of these stages, two types of RDI were applied together with a control treatment. The water stress levels in the RDI treatments were controlled using indicators of trunk diameter fluctuations.[12] The specific indicator selected in this work was the trunk growth rate (TGR, difference between two consecutive maximum values in the cycles of shrinkage and swelling of the trunks). This indicator was considered as the most accurate one in olive trees,[13] and was selected to characterise the water status of this field experiment.

It is important to describe the different stages of the development of the olive fruit: (1) *stage I*: which starts at the beginning of the fruit growth and ends at the beginning of the massive pit hardening; (2) *stage II*: the period in which the pit hardens; and finally, (3) *stage III*: the period of oil accumulation and maturation. The irrigation treatments under study were:

- *Control* (*T0*): Irrigation was applied to supply the estimated crop evapo-transpiration (ETc); this means that a full replenishing of all the extracted soil water was conducted by addition of irrigation water.
- *RDI-1* (*T1, soft stress*): (1) Olive trees were under low water deficit conditions; in this way, trees were only irrigated when the TGR (trunk growth rate) was lower than 10 µm day^{-1} (this is half the value found in trees under fully irrigated conditions); (2) the same conditions as in stage I; and (3) finally at stage III, enough water was applied to reach a water status similar to that of T0 trees.
- *RDI-2* (*T2, moderate stress*): (1) During stage I, olive trees were under low water deficit conditions; trees were only irrigated when the TGR was lower than 10 µm day^{-1}; (2) trees were not irrigated during stage II; and (3) finally at stage III, enough water was applied to reach a water status similar to that of T0 trees.

A randomised complete-block design was used with three blocks per treatment and two trees per block. Irrigation scheduling was controlled with the measurements of six trees per treatment (two per block) along the growing season.

Sample processing

'Manzanilla' olives from the three RDI treatments were hand-harvested in mid-September at their optimal mature-green stage. All fruits from all the trees of each RDI treatment were systematically mixed and a sample of approximately 50 kg per treatment was used to prepare table olives. Fruits were transported the day after their picking at the farm to the Cooperativa Nuestra Señora de las Virtudes (La Puebla de Cazalla, Seville, Spain) to be processed as table olives according to the Spanish style method. Initially, raw olives were treated with NaOH (0.6 mol L^{-1}), later olives were washed with abundant water, and finally fermented using different concentrations of NaCl (from 0.17 to 0.09 mol L^{-1}). Further details of this method can be found in Cano-Lamadrid *et al.*[10]

Morphological and physico-chemical analysis

All physico-chemical analyses were only conducted on fermented table olives. Approximately 5 kg of table olives per treatment were used; this means that about 1000–1250 fruits per treatment were evaluated (4.0–4.5 g per fruit).

Weight and size

One hundred table olives from each treatment were randomly selected and the weight of the whole fruit was measured using a scale Mettler Toledo model AG204 (Mettler Toledo, Barcelona, Spain). Later, the two dimensions (equatorial and longitudinal diameters) of the olives were measured using a digital caliper Mitutoyo 500-197-20 (Mitutoyo, Kawasaki, Japan).

Instrumental colour

Instrumental colour measurements were made using a Minolta Colorimeter CR-300 (Minolta, Osaka, Japan), at 25 ± 2 °C. This spectrophotometer uses an illuminant D_{65} and a 10° observer as references. Colour data are provided as CIE *L*a*b** coordinates, which define the colour in a three-dimensional space. Colour analyses were run in three batches of 25 fruits, making a total of 75 fruits per treatment.

Oil content and fatty acids

Oil was extracted by sonication using a 1 L ultrasonic Selecta bath model 3000512 JP (Selecta, Barcelona, Spain). A 2 g sample of ground olive flesh was mixed with 3 mL of cyclohexane and the mixture was sonicated at room temperature for 3 h. Then, the mixture was centrifuged, and the oil was recovered after the evaporation of the cyclohexane in a nitrogen stream.

The fatty acids methyl esters (FAMEs) were prepared, identified, and quantified using the method recently described by Cano-Lamadrid *et al.*,[10] but using a SupraWax-280 column, 100% polyethylene glycol (Teknokroma S. Co. Ltd., Barcelona, Spain; 30 m × 0.25 mm × 0.25 µm film thickness). Analyses were carried out using helium as carrier gas at a flow rate of 1.1 mL min^{-1} in a split ratio of 1:10 and a program: (1) initial temperature 80 °C, hold for 2 min, (2) rate of 8.0 °C min^{-1} to 160 °C; (3) rate of 4 °C min^{-1} from 160 to 220 °C and hold for 13 min, and (4) rate of 10 °C min^{-1} from 220 to 260 °C and hold for 6 min. Injector and detector temperatures were held at 230 and 260 °C, respectively; 0.5 µL of the extract was injected.

Mineral analysis

Approximately 1 g of milled table olive were digested, for 3 h a temperature below 130 °C, in a multi-place digestion block, Selecta Block Digest 20 (Selecta, Barcelona, Spain) after the addition 5 mL of concentrated, 65% (w/v), HNO_3.[14] Samples were left to cool down to room temperature, transferred to volumetric flask and dilutions 1:10 and 1:50 were prepared using ultrapure deionised water, 18 MΩ (Milli-Q® system; Millipore Corporation, Madrid, Spain).

Determination of macro-nutrients (Ca, Mg, and K) and micro-nutrients (Cu, Fe, Mn, and Zn) in previously mineralised samples was performed using a Unicam Solaar 969 atomic absorption-emission spectrometer (Unicam Ltd, Cambridge, UK). All minerals were analysed using atomic absorption except K, which was measured using atomic emission.

In each analytical batch, at least one reagent blank and one spike were included to assess precision and accuracy for chemical analysis. Calibration curves were used for the quantification of minerals and showed good linearity ($R^2 \geq 0.999$). Analyses were run in triplicate.

Sugar alcohols and organic acids

Organic acids and sugar alcohols were quantified according to Sánchez *et al.*[15] Briefly, for each sample, 2 g of table olives were homogenised in 5 mL of 50 mmol L^{-1} phosphate buffer pH = 7.8. The mixture was centrifuged at 10 000 × *g* for 20 min at 4 °C (Sigma 3–18 K; Sigma Laborzentrifugen, Osterode and Harz, Germany). Then, 1 mL of supernatant was filtered through a 0.45 µm filter and injected into a Hewlett-Packard HPLC series 1100 (Wilmington, DE, USA). The elution buffer consisted of 0.1% phosphoric acid with a flow rate of 0.5 mL min^{-1}. Organic acids were isolated using a Supelco column (SupelcogelTM C-610H column 30 cm × 7.8 mm) and Supelguard (5 cm × 4.6 mm; Supelco, Bellefonte, PA, USA) and absorbance was measured at 210 nm using a diode-array detector (DAD). These same HPLC conditions were used for the analysis of sugar alcohols; however, the detection was conducted using a refractive index detector (RID). Standards of organic acids (phytic and lactic) and sugar alcohols (mannitol and glycerol) were obtained from Sigma (Poole, UK). Calibration curves, obtained by triplicate injection of standard solutions, were conducted and showed good linearity ($R^2 > 0.999$). Results were expressed in g kg^{-1} fw (fresh weight) of table olives.

Antioxidant activity ($ABTS^+$, $DPPH^•$ and FRAP methods) and total polyphenols

For the antioxidant activity determination, a methanol extract was prepared for each sample to be analysed. Approximately 0.5 g of freeze-dried table olives were mixed with 10 mL of MeOH/water (80:20, v/v) + 1% HCl, and the mixture was sonicated at 20 °C for 15 min and left for 24 h at 4 °C. Then, the extract was again sonicated for 15 min, and centrifuged at 10 000 × *g* for 10 min. The radical scavenging activity was evaluated using the $DPPH^•$ radical (2,2-diphenyl-1-picrylhydrazyl) method, as described by Brand-Williams *et al.*[16] with a modification in the reaction time. Briefly, 10 µL of the supernatant were mixed with 40 µL of MeOH and added to 950 µL of $DPPH^•$ solution. The mixture was shaken and placed under dark conditions for 15 min. The decrease in absorbance was measured at 515 nm using a UV–visible spectrophotometer (Helios Gamma model, UVG 1002E; Helios, Cambridge, UK). Additionally, the $ABTS^+$ [2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] radical cation and ferric reducing antioxidant power (FRAP) methods were also employed, according to Re *et al.*[17] and Benzie and Strain,[18] respectively. Briefly, 10 µL of the supernatant were mixed with 990 µL of $ABTS^+$ or FRAP solutions. After 10 min of reaction, the absorbance was measured at 734 nm for $ABTS^+$ and 593 nm for FRAP. The absorbance was measured using a UV–visible Spectrophotometer (Helios Gamma model, UVG 1002E). Calibration curves, in the range 0.5–5.0 mmol Trolox L^{-1} were used for the quantification of the three methods of antioxidant activity showing good linearity ($R^2 = 0.998$). Results were expressed in mmol Trolox kg^{-1} fresh weight (FW).

Besides, the antioxidant activity (AA) was measured, for the first time, separately in hydrophilic (H-AA) and lipophilic (L-AA) fractions. AA was quantified by spectrophotometry as described by Arnao *et al.*[19] In both cases, AA was determined in each extract using the $ABTS^+$ method. Results (mean ± SE) were expressed as mmol Trolox kg^{-1} FW.

Total polyphenols content (TPC) was quantified using the Folin–Ciocalteu colorimetric method described previously by Gao *et al.*[20] The extracts of freeze-dried table olives (0.1 mL) were mixed with 0.2 mL of Folin–Ciocalteu reagent and 2 mL of H_2O. Then, the mixture was incubated at room temperature for 3 min and 1 mL of 20% sodium carbonate was added to the mixture. The TPC was determined after 1 h of incubation at room temperature. The absorbance of the resulting blue colour solution was measured at 765 nm using an UV–visible spectrophotometer (Helios Gamma model, UVG 1002E). Quantification was done with respect to the standard curve of gallic acid. The results were expressed as gallic acid equivalents (GAE), g kg^{-1} FW.

Sensory analyses

Sensory evaluation with a trained panel

Eight trained panellists (aged 30 to 55 years; four women and four men) from the department of Agro-Food Technology (UMH) participated in this study. Samples were served into odour-free, disposable 90 mL covered plastic cups, at room temperature and were coded using three-digit numbers. Unsalted crackers and distilled water were provided to panellists to clean their palates between samples.

After careful study of the lexicon developed by the International Olive Oil Council (IOOC),[21] the panel evaluated only the following attributes: (*flavour*) green-olive flavour, sourness, bitterness, saltiness, sweetness, and aftertaste; and (*texture*) hardness, crunchiness, fibrousness, and pit removal. The panel used a numerical scale for quantifying the intensity of the olives attributes where 0 represents none and 10 extremely strong with 0.5 increments. This scale is the most logical and easy-to-use by Spanish panellists, as previously stated by Galindo *et al.*[22]

Sensory evaluation with a consumer panel

One hundred consumers (65% female) were recruited via e-mails for a central location test. Consumers, being 20–60 years old, eating table olives at least twice per week, not having diet restrictions or allergies, were recruited for testing. Samples were served under the same conditions described in the section on Sensory Evaluation with Trained Panel. Consumers responded using a nine-point hedonic scale, where 1 = dislike extremely, and 9 = like extremely.

Statistical analyses

Results are provided as the mean ± standard error. First, data was subjected to one-way (factor = RDI treatment) analysis of

variance (ANOVA) and later data was also subjected to Tukey's multiple-range test to compare the means. Differences were considered statistically significant at $P < 0.05$. All statistical analyses were performed using StatGraphics Plus 5.0 software (Manugistics, Rockville, MD, USA).

RESULTS AND DISCUSSION

Field experiment and tree parameters

In Table 1 several measurements of the field experiment are presented, divided into the considered phenological stages (stage I, stage II, and stage III); for each treatment the applied water [AW (mm)], the yield (t ha^{-1}), and the trunk growth rate [TGR (μm day^{-1})] are presented. Besides, the estimation of the crop evapo-transpiration [ETc (mm)] is also included. The main differences in ETc between phenological stages are related with the duration of each one, stage I (116 days), stage II (57 days) and stage III (30 days).

The irrigation of control trees (T0) was around the ETc needs, except during stage I in which the rainfall was considered. In this treatment (T0), TGR presented the maximum values during stage I (15.1 μm day^{-1}), which corresponded with the period of vegetative growth. During stage II, pit hardening, vegetative growth is stopped, even in full irrigated conditions, and TGR was around 0 (1.6 μm day^{-1}) until the end of the season (3.8 μm day^{-1} at stage 3). T1 (RDI-1) was scheduled only with TGR data, and this irrigation scheduling led to a water saving of around 44% in comparison to control. TGR values of T1 indicated that trees water status were also under full irrigated conditions, with values being equivalent to those of the T0 trees, 19.0, 4.7, and 7.4 μm day^{-1} at stages I, II, and III, respectively. Finally, T2 (RDI-2) presented the greatest reduction in irrigation (71% as compared to control), and this water saving was produced along the season. However, such irrigation reduction affected tree water status leading to lower TGR values during stages I and II. The greater values of TGR in this treatment than control and T1 during stage III are related with the need to recover the water plant status.

The effect of RDI in the olive tree yield in an isolated season is not always clear, especially because of the biennial cycles of olive trees. In this particular season, although no statistically significant differences were found, there was a clear trend of yield reduction in T2 (6.7 t ha^{-1}) in comparison to T1 (8.2 t ha^{-1}) and T0 (9.0 t ha^{-1}).

Table 1. Irrigation and tree parameters [applied water (AW, mm), yield (t ha^{-1}), and trunk growth rate (TGR, μm day^{-1})] of 'Manzanilla' olive trees as affected by regulated deficit irrigation treatment

	Stage		
Irrigation parameter	I	II	III
ETc (mm)	308^{a}	181^{b}	70^{c}
	Irrigation treatment		
Parameter and stage	T0	T1	T2
AW (mm)			
Stage I	108^{a}	72^{b}	62^{b}
Stage II	193^{a}	89^{b}	0^{c}
Stage II	68^{a}	46^{b}	44^{b}
TGR (μm day^{-1})			
Stage I	15.1^{b}	19.0^{a}	6.2^{c}
Stage II	1.6^{b}	4.7^{a}	−5.9^{c}
Stage II	3.8^{c}	7.4^{b}	9.8^{a}
Yield (t ha^{-1})	9.0^{a}	8.2^{a}	6.7^{b}

Values (mean of six replications) followed by the same letter, within the same row, were not significantly different ($P < 0.05$), according to Tukey's least significant difference test.

Table 2. Morphological parameters and CIE L^* a^* b^* coordinates of 'Manzanilla' table olives as affected by deficit irrigation treatment

Parameter	ANOVA	T0	T1	T2
Fruit weight (g)	***	4.20^{ab}	4.01^{b}	4.35^{a}
Longitudinal diameter (mm)	NS	20.3	19.3	20.3
Equatorial diameter (mm)	NS	16.6	16.9	17.5
L^*	*	50.8^{ab}	50.1^{b}	52.0^{a}
a^*	**	−1.75^{a}	−1.91^{ab}	−2.17^{b}
b^*	NS	26.4	24.9	26.4
DMC (g DW kg^{-1} FW)	***	248^{c}	359^{a}	331^{b}

The number of replications for the analysis of weight, size, instrumental colour, oil content, and dry matter content (DMC) were 100, 100, 75, 3 and 5, respectively.
NS, not significant at $P < 0.05$; *, **, and ***, significant at $P < 0.05$, 0.01, and 0.001, respectively.
Values followed by the same letter, within the same row, were not significantly different ($P < 0.05$), according to Tukey's least significant difference test.

Morphological and physico-chemical analysis

Weight and size

Table 2 shows the results of the weight and size (longitudinal and equatorial diameters) of 'Manzanilla' table olives as affected by regulated deficit irrigation (RDI) treatments. It can be observed that T2 olives had highest weight ($P < 0.001$) of all treatments, 4.35 g, although their weight was statistically equivalent to that of T0 fruits. It is generally admitted that the weight of 'Manzanilla' table olives must be in the range from 2.1 to 4.9 g to have an appropriate or good size;[23] the experimental values found in this study were at the upper part of this range, specifically between 4.0 and 4.4 g. The working hypothesis of all RDI studies is that the treatments will slightly decrease the yield but will improve the quality of the fruits.[10,24] This is exactly the case observed in the table olives weight; a slight reduction on the yield makes that the fruits of the treated trees have more nutrients available for them and will grow bigger, and have a higher weight, as observed. However, the size of the table olives as described by the longitudinal (d_l, length) and equatorial (d_e, thickness) diameters was not significantly affected by the RDI treatments; however, a trend can be found in which T2 fruits had the highest values of both diameters, although all values were statistically equivalent. The ratio d_l/d_e took values of 1.22, 1.14, and 1.16, respectively, meaning that T1 and T2 fruits were more rounded than those of T0.

Colour

Table 2 also shows the results of the parameter CIE $L^*a^*b^*$ coordinates. The RDI treatments significantly affected lightness (L^*), and the green–red coordinate, a^*; however, no significant effects were found in the blue–yellow coordinate, b^*.

The colour of T2 olives was lighter (L^*) and had higher green intensity (a^*) than control (T0) and T1 fruits. In a previous study with table olives 'Manzanilla de Sevilla' it was concluded that water stressed fruits had higher intensity of yellow colour (up to 10 units) than control ones.[10] Besides, Pastor *et al.*[25] reported a decrease in

Table 3. Oil content (g kg^{-1} DW) and fatty acids (% of total area) of 'Manzanilla' table olives as affected by deficit irrigation treatment

Parameter	ANOVA	T0	T1	T2
Oil content (g kg^{-1} DW)	***	261^{b}	404^{a}	278^{b}
C16:0 (%)	NS	17.1	16.4	16.7
C16:1 (%)	NS	1.54	1.69	1.53
C18:0 (%)	NS	3.66	3.65	4.27
C18:1 (%)	*	69.1^{b}	68.6^{b}	70.0^{a}
C18:2 (%)	**	6.10^{b}	7.31^{a}	4.96^{c}
C18:3 (%)	NS	1.25	1.18	1.27
C20:0 (%)	NS	0.81	0.71	0.86
C20:1 (%)	NS	0.53	0.46	0.46
SFA (%)	NS	21.5	20.8	21.8
MUFA (%)	*	71.1^{b}	70.7^{b}	72.0^{a}
PUFA (%)	**	7.34^{b}	8.49^{a}	6.23^{c}
(MUFA + PUFA)/SFA	NS	3.64	3.81	3.59

NS, not significant at $P < 0.05$; *, **, and ***, significant at $P < 0.05$, 0.01, and 0.001, respectively.
Values (mean of three replications) followed by the same letter, within the same row, were not significantly different ($P < 0.05$), according to Tukey's least significant difference test.
SFA, saturated fatty acids (C16:0, C18:0, and C20:0); MUFA, monounsaturated fatty acids (C16:1, C18:1, and C20:1); PUFA, polyunsaturated fatty acids (C18:2).

the intensity of the yellow colour in Arbequina olive oil when olive trees were stressed. In any case, the differences in colour among the RDI treatments in this study can be considered of limited real significance because changes of less than 2 units will not cause noticeable visual differences.[22,26]

Dry matter and oil contents

Table olives have three main components: (1) moisture, (2) oil, and (3) dry matter content (DMC). The water availability for trees (RDI treatments) clearly influenced the contents of these three components of table olives (Table 2 and Table 3). The logical situation would be that control fruits, which have been irrigation with no water restriction, will have the highest content of moisture, but the lowest content of DMC and perhaps of oil; in fact, this theoretical hypothesis was clearly confirmed by the experimental results. The lowest content of DMC [248 g dry weight (DW) kg^{-1} fresh weight (FW) was found in control fruits (T0), followed by T2 and T1 fruits, with contents of 331 and 359 g DW kg^{-1} FW, respectively (Table 2).

As regard to the oil content, the highest value (404 g DW kg^{-1} FW) was found in table olives grown under moderate RDI conditions (T1). Additionally, no statistical significant differences were found between the oil contents of fruits from the other two treatments, T0 and T2. According to Lavee *et al.*[27] a moderate water stress will lead to an increased accumulation of oil in Muhasan olives grown in Israel.

The trend shown in oil content completely agreed with the initial hypothesis sustained in our experiments. This is, under soft water stress (T1), the plant or tree metabolism seems to get activated resulting in a highest accumulation of oil and DMC, as previously other authors concluded in table olives or pistachios.[10,24] However, under a more severe water stress or a longer period of stress, the plant metabolism is damaged and after an initial increase in the accumulation of oil and DMC, the contents start to be reduced, as seen in T2 olives.

Table 4. Mineral content of 'Manzanilla' table olives as affected by deficit irrigation treatment

Parameter	ANOVA	T0	T1	T2
Macro-elements (g kg^{-1} DW)				
Calcium (Ca)	***	2.4^{a}	1.7^{c}	1.9^{b}
Magnesium (Mg)	NS	0.5	0.4	0.4
Potassium (K)	NS	1.7	1.4	1.7
Micro-elements (mg kg^{-1} DW)				
Iron (Fe)	NS	12.1	12.1	11.2
Zinc (Zn)	**	6.0^{a}	5.0^{ab}	4.1^{b}
Copper (Cu)	NS	8.5	7.5	8.1
Manganese (Mn)	**	4.9^{a}	4.4^{ab}	4.1^{b}

NS, not significant at $P < 0.05$; ** and ***, significant at $P < 0.01$ and 0.001, respectively.
Values (mean of three replications) followed by the same letter, within the same row, were not significantly different ($P < 0.05$), according to Tukey's least significant difference test.

Fatty acids

The relative abundance of fatty acids observed in table olives followed the order: C18:1 (mean of all treatments 69.2%) > > C16:0 (16.7%) > C18:2 (6.1%) ≈ C18:0 (3.9%) > C16:1 (1.6%) ≥ C18:3 (1.2%) ≥ C20:0 (0.8%) ≥ C20:1 (0.5%) (Table 3). Linoleic (C18:2) and oleic (C18:1) acids were significantly affected by the RDI treatments (Table 3). The most important result is that soft RDI conditions (T1) significantly increased the content of linoleic acid, ω-6 fatty acid, which must be ingested through food due to the fact that human body is not able of produce it and therefore is called 'essential fatty acid'.[28,29] As a result of the changes mainly in linoleic acid, T1 table olives experienced a significant increase of polyunsaturated fatty acids (PUFAs) and a simultaneous decreased of monounsaturated fatty acids (MUFAs), with this being important because PUFAs are beneficial to human health.[29] A similar trend, but only valid for 'moderate' stressed 'Manzanilla de Sevilla' olives was recently reported by Cano-Lamadrid *et al.*[10]

Mineral content

Only the content of the macro-nutrient calcium (Ca) was significantly affected by the RDI treatments; with the highest content being found in fruits from the control trees, T0 (Table 4). The contents of the macro-nutrients followed the order: Ca (mean of all treatments 2.4 g kg^{-1}) > K (1.6 g kg^{-1}) > Mg (0.4 g kg^{-1}). Water stress caused a lower accumulation of Ca in T1 and T2 fruits, this is in water stressed olives; it is important to mention that Ca is taken up by the plant and transported primarily through the xylem, along with water.[30] Therefore, the absorption of Ca is directly related to plant transpiration; besides, Ca follows the transpiration stream and consequently for this mineral is difficult to reach plant organs with low transpiration rate, such as fruits.[30] Sodium (Na) was not analysed because it is one of the major ingredients used during the processing of table olives.

Table olives are a good source of iron (Fe), with the contents of the studied micro-nutrients following the order: Fe (mean of all treatments 11.8 mg kg^{-1}) > Cu (8.0 mg kg^{-1}) > Zn (5.0 mg kg^{-1}) ≈ Mn (4.5 mg kg^{-1}). The irrigation treatments affected the contents of two of these minerals, Zn and Mn; in both cases, the higher the water stress, the lower the mineral content.

Table 5. Sugar alcohols and organic acid profiles of 'Manzanilla' olives as affected by deficit irrigation treatment

Parameter	ANOVA	T0	T1	T2
Sugar alcohols (g kg^{-1} FW)				
Mannitol	NS	2.89	2.96	3.07
Glycerol	NS	0.10	0.06	0.07
Organic acids (g kg^{-1} FW)				
Phytic acid	*	14.73[a]	6.09[b]	7.46[b]
Lactic acid	NS	1.62	1.63	1.63

NS, not significant at $P < 0.05$; *significant at $P < 0.05$.
Values (mean of three replications) followed by the same letter, within the same row, were not significantly different ($P < 0.05$), according to Tukey's least significant difference test.

Table 6. Antioxidant activity (mmol Trolox kg^{-1} FW) and total polyphenols content (mg GAE kg^{-1} DW) of 'Manzanilla' table olives as affected by deficit irrigation treatment

Parameter	ANOVA	T0	T1	T2
ABTS^{+} (mmol Trolox kg^{-1} FW)	NS	13.4	13.2	13.4
DPPH$^{\bullet}$ (mmol Trolox kg^{-1} FW)	NS	13.6	13.1	13.2
FRAP (mmol Trolox kg^{-1} FW)	NS	29.1	22.1	28.6
H-AA (mmol Trolox kg^{-1} FW)	NS	10.2	8.61	9.14
L-AA (mmol Trolox kg^{-1} FW)	NS	2.61	2.57	2.56
TPC (g GAE kg^{-1} FW)	NS	5.29	5.28	5.27

NS, not significant at $P < 0.05$.
Values are the mean of three replications.

Table 7. Affective sensory analysis of 'Manzanilla' table olives as affected by deficit irrigation treatment

Parameter	ANOVA	T0	T1	T2
Fresh table olive flavour	NS	6.5	6.8	6.4
Bitterness	NS	6.3	6.4	6.1
Saltiness	NS	6.0	6.4	6.2
Hardness	NS	7.4	7.3	6.9
Crunchiness	NS	7.5	7.3	6.9
After-taste	NS	6.4	6.4	6.2
GLOBAL	NS	6.5	6.8	6.3

Consumers used a 9-point hedonic scale, where 1 = dislike extremely, 5 = neither like nor dislike, 9 = like extremely.
NS, not significant at $P < 0.05$.
Values are the mean of 100 consumers.

Sugar alcohols and organic acids

Only two sugar alcohols (mannitol and glycerol) and two organic acids (phytic and lactic acids) were identified and quantified in 'Manzanilla' table olives (Table 5). The only significant effect ($P < 0.05$) of the RDI treatments on the contents of sugar alcohols and organic acids, was a reduction of the content of phytic acid [known as inositol hexakisphosphate (IP6)] in T1 and T2 fruits (mean of 6.8 g kg^{-1} FW) as compared to control fruits (14.7 g kg^{-1} FW). Recent investigations have begun to focus on possible beneficial physiological/health effects of food phytates, which until a few years ago were mainly considered as anti-nutrient.[31] The possible beneficial effects of food phytates include lowering of serum cholesterol and triglycerides and protection against certain diseases such as cardiovascular diseases, renal stone formation, and even certain types of cancers.[32–34] The absence of reducing sugars in table olives was expected because they are major substrates of the lactic fermentation (the only typical spontaneous lactic process followed in Spanish-style green olives).

Antioxidant activity and total polyphenols

There are different methods for evaluating the antioxidant activity (AA) of foods. This variety of methods is due to the fact that none of them is able to determine exactly the total antioxidant capacity of a product. The measured AA of a sample depends on methodology and on free radical generator or oxidant in the measurement.[35] Electron-transfer-based assays (ABTS^{+}, FRAP and DPPH$^{\bullet}$) measure the capacity of an antioxidant in the reduction of an oxidant which changes colour when reduced. However, there are differences among them; for instance, ABTS^{+} measures both hydrophilic and lipophilic AA, while DPPH$^{\bullet}$ only considers lipophilic compounds.[36] For this reason, the antioxidant activity of 'Manzanilla' table olives was evaluated using three different analytical methods: ABTS^{+}, DPPH$^{\bullet}$, and FRAP (Table 6). The AA and TPC were not significantly affected ($p > 0.05$) by the RDI treatments. The total polyphenols content found in table olives (5.28 g GAE kg^{-1} FW, mean value for all treatments) was higher than that previously reported in the flesh of table olives by Boskou *et al.*,[37] who reported values ranging from 0.8 to 1.7 g caffeic acid kg^{-1}. These authors also identified oleanolic acid, hydroxyl-tyrosol, and tyrosol as the main polyphenols present in Greek table olives. Table olives are widely consumed by the Mediterranean population. The consumption of 20 g of table olives (approximately 5 units) provides about 100 mg of polyphenols. Taking into account these results, it can be concluded that Spanish table olives are a very good source of polyphenols and can help in the prevention of many health diseases.

Sensory analysis

The satisfaction degree of 100 Spanish consumers on 'Manzanilla' table olives was not affected at all by the RDI treatments (Table 7); neither the global satisfaction degree nor any of the key attributes were affected. T1 olives had the highest values of: (1) typical flavour of green table olives (6.8), and (2) what it is more important of global satisfaction degree (6.8); however, the differences with the other treatments were not statistically significant. The values of the consumers' scores for their satisfaction degree regarding these two parameters (table olive flavour and global) for T0 and T2 fruits had similar values (6.5 and 6.3, respectively). In affective tests consumers normally use only the central part of the scale avoiding the use of extreme values; consequently, the value of 6.8 (remember that 7 is 'like moderately') obtained by T1 olives for the global satisfaction degree indicate that Spanish consumers really liked T1 'Manzanilla' table olives. Perhaps the number of consumers used, 100, was not high enough to show significant differences among the RDI treatments; this is a topic that will require further research in national and international markets.

Table 8 shows that RDI significantly affected several of the key sensory attributes used to describe the quality of 'Manzanilla' table olives; however, several attributes were not affected and presented the following mean intensity values: bitterness (5.7), sourness (2.4), sweetness (1.4), crunchiness (7.4), and fibrousness

Table 8. Descriptive sensory analysis of 'Manzanilla' table olives as affected by regulated deficit irrigation treatment

Parameter	ANOVA	T0	T1	T2
Flavour				
Saltiness	**	4.8^{b}	5.8^{a}	4.9^{b}
Bitterness	NS	5.3	5.8	6.1
Sourness	NS	2.3	2.6	2.2
Sweetness	NS	1.3	1.4	1.4
Green-olive flavour	*	7.0^{ab}	7.9^{a}	6.3^{b}
Aftertaste	*	5.4^{ab}	6.4^{a}	5.2^{b}
Texture				
Hardness	**	7.0^{ab}	7.9^{a}	6.4^{b}
Crunchiness	NS	7.1	7.9	6.9
Fibrousness	NS	2.1	1.8	1.9
Pit removal	*	8.0^{a}	7.7^{ab}	6.8^{b}

Trained panellists used a scale from 0 = no intensity to 10 = extremely strong intensity.
Attributes included in the Parameter profile are based on IOOC information.[21]
NS, not significant at $P < 0.05$; * and **, significant at $P < 0.05$ and 0.01, respectively.
Values (mean of 10 trained panellists) followed by the same letter, within the same row, were not significantly different ($P < 0.05$), according to Tukey's least significant difference test.

(2.0). One thing that was highlighted by the trained panel while evaluating table olives was that control fruits (T0) had pits which were easier to remove from the edible portion (8.0) than other fruits (T2 = 6.8, and T1 = 7.7). It is possible that the higher water content of control olives helped panellists in removing the stone of these fruits. The most important finding was that T1 fruits had the highest intensities of saltiness (5.8), green-olive flavour (7.9), aftertaste (6.4), and hardness (7.9). It is possible that these higher intensities of T1 olives were due, at least in part, to the production of a thick skin due to the limited water availability.[38] On the other hand, T2 olives had the lowest intensities of the previous attributes (saltiness, green-olive flavour, aftertaste, and hardness). Finally, the trend shown in descriptive sensory of 'Manzanilla' table olives agreed with the initial hypothesis of our study (under soft water stress, T1, the plant metabolism will be activated while under more severe conditions, T2, the metabolism will be damaged.

CONCLUSIONS

This is the first study investigating the content of nutrients, antioxidant activity and sensory quality of table olives obtained after regulated deficit irrigation (RDI). Table olives obtained after RDI treatments (T1 and T2) were more rounded than those of the control treatment (T0), had higher intensity of green colour (a^*), and presented significantly lower contents of phytic acid and calcium as compared to control olives. In general, T1 table olives were characterised by the highest dry matter and oil contents, higher intensities of key sensory attributes, and high satisfaction degree among Spanish consumers. In addition, T2 treatment resulted in the highest percentage of polyunsaturated fatty acids (linoleic acid), green colour, and weight. Regarding the antioxidant activity, although no significant effect was observed after the RDI treatments, it can be concluded that Spanish table olives are a very good source of polyphenols and consequently have high antioxidant activity. As the final conclusion, it can be stated that 'soft' RDI is an effective and good alternative for the irrigation of olive trees, 'Manzanilla de Sevilla', because it reduces the economic and environmental costs, and maintains or even increases, in some cases, its functionality and its sensory quality and consumer acceptance.

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