Quality Attributes of Table Olives as Affected by Regulated Deficit Irrigation

Cano-Lamadrid M.[a], Girón I. F.[b, c], Pleite R.[d], Burló F.[a], Corell M.[c, e], Moriana A.[c,e], Carbonell-Barrachina A.A.[a,*]

[a] Universidad Miguel Hernández de Elche (UMH). Department of Agro-Food Technology. Ctra. Beniel, km 3.2. 03312-Orihuela, Alicante, Spain

[b] Instituto de Recursos Naturales y Agrobiología (CSIC), P.O. Box 1052, E-41080 Sevilla, Spain

[c] Unidad Asociada al CSIC de Uso sostenible del suelo y el agua en la agricultura (US-IRNAS). Carretera de Utrera, km 1, 41013-Sevilla, Spain.

[d] Global Olive Consulting. 7 Contador Street. 41530-Morón de la Frontera, Sevilla, Spain.

[e] Departamento de Ciencias Agroforestales, ETSIA, Universidad de Sevilla, Carretera de Utrera, km 1. 41013-Sevilla, Spain.

Running title: Quality of hydroSOStainable table olives

*Corresponding author: Tel.: +34 966749754; fax: +34 966749677 E-mail address: angel.carbonell@umh.es

ABSTRACT

Regulated deficit irrigation (RDI) allows us to decrease the amount of water to apply without significantly affecting yield and fruit quality. The influence of 3 irrigation treatments [T0: control (no stress); T1: moderate stress during pit hardening; and, T2: low stress at the end of flowering stage and moderate during pit hardening) on the quality of table olives, cv. "*Manzanilla"*, was evaluated. The parameters evaluated in table olives (after processing) were: weight, size, texture, color, fatty acids, volatile compounds and sensory quality. T1 olives had the highest weight and size, and were rounded. Color coordinates *L** and *b** had the highest values in T2 olives. Aldehydes and monounsaturated fatty acids predominated in T0 olive fruits, while terpenes and polyunsaturated fatty acids predominated in T1 fruits, and finally saturated fatty acids were abundant in T2 olives. Finally, the results of sensory studies indicated that global acceptance was higher for T1 olive, obtaining better satisfaction degrees for fresh olive flavor, crunchiness, and global satisfaction. Deficit irrigation is effective and can be a good alternative for this type of crop, "*Manzanilla"* table olives.

Keywords: consumers; sensory quality; *Olea europaea* L.; water stress; "*Manzanilla"*.

INTRODUCTION

The olive is the fruit of the olive tree (*Olea europea L.*) belonging to the family of Oleaceae. According to FAO, 10,000,000 ha worldwide are olives orchards, with Spain having the highest surface with 2,500,000 ha; this surface is mainly located in Andalusia and Extremadura (FAOSTAT, 2013). There is a big difference between surface dedicated to table olives and oil olive in Spain; the average in the last 6 seasons is 165,762 and 2,461,700 ha, respectively (MAGRAMA, 2014a). Irrigated olive farming experienced a big increase at the beginning of the 1990 decade; for instance, 40% of the land dedicated to table olives was irrigated in 2010. Now olive

55 tree is the most important crop grown under irrigated conditions in Spain
56 (MAGRAMA, 2014b). Among the total production of olives (391,350 t), the variety
57 used in this study, *"Manzanilla"*, represents about 33% of the total production
58 (129,810 t) (MAGRAMA, 2014c).

59 The olive tree is drought tolerant because of its specific morphological
60 mechanisms (extensive root system, stomata located on the undersides of the
61 leaves, etc.) (Orgaz, & Fereres, 1997). Despite being one of the most resistant
62 species, olive tree physiology is also affected by lack of soil water. The effects of
63 Regulated deficit irrigation (RDI) depend on the phenological stage of the plant and
64 modify fruit size and oil content (Orgaz, & Fereres, 1997; Moriana, Orgaz, Pastor, &
65 Fereres, 2003). The olive development consists of three periods: (i) *stage I*: it
66 starts at the beginning of the fruit growth ending at the beginning of massive pit
67 hardening; (ii) *stage II*: period in which pit hardens; and finally, (iii) *stage III*:
68 period of oil accumulation and maturation. However, this last stage was very short
69 (2-3 weeks) because fruits were harvested early because they have been used for
70 green table olives manufacturing. Under the conditions, this period is mainly used
71 for trees rehydration (Goldhamer, 1999).

72 RDI is an irrigation scheduling that was developed in the early 80s in peaches
73 (Chalmers, Mitchell, & Jerie, 1985), and is a system of managing water supply by
74 imposing some water deficits in specific phenological stages, which have been
75 found to be less sensitive, with no or low reduction in economic benefits
76 (Behboudian, & Mills, 1997). Goldhamer (1999) was the first researcher describing
77 the use of RDI in olive orchards. Later, many studies have evaluated the
78 physiological responses and performance of olive trees grown under different water
79 regimes (D'Andria, Lavini, Morelli, Sebastiani, & Tognetti, 2009), and also the
80 overall development and composition of the fruits (Chaves et al., 2010). All these
81 changes, however, lead only to minor changes in the flavor of the resulting oil
82 (Lavee, 2011); but perhaps the changes in the flavor of the table olives could be
83 more pronounced. Cultivation of olive trees under water stress conditions are linked

to: increased contents of total phenolic compounds (Marsilio et al., 2006), changes in the phenolic composition (D'Andria et al., 2009), and high proportion of unsaturated fatty acids (Gómez-Rico, Salvador, & Fregapane, 2009).

Fruits and vegetables, including olives, cultivated under RDI are called *"hydroSOStainable" products*, and have a solid identity (higher content in bioactive compounds, higher intensity of some sensory attributes, etc.); besides, they are environmentally-friendly because optimize the use of a very valuable resource in the world, water (Carbonell-Barrachina, Memmi, Noguera-Artiaga, Gijón-López, Ciapa, & Pérez-López, 2015).

For all the above reasons, the main aim of this work was to evaluate the effects of RDI conditions on the main quality parameters of table olives. The quality of the samples was studied from different points of view (i) *morphological*: yield per tree, weight, and size, (ii) *physico-chemical*: CIE*L*a*b** color, fatty acids profile, and profile of volatile compounds, and (iii) *sensory*: descriptive profile using a trained panel, and consumer acceptance using an affective panel.

MATERIALS AND METHODS

Plant Material, Growing Conditions and Experimental Design

Olives belong to the experimental farm "The Hampa", from the Higher Council for Scientific Research (CSIC). This farm is located in Coria del Rio (Seville, Spain). The plot has an area of 0.5 ha, and olives come from olive trees, variety "*Manzanilla de Sevilla*", of 43 years of age. Irrigation water is obtained from an existing well on the property.

Two types of RDI were evaluated depending on the stress level and the phenological stage of the trees, together with a control treatment. Water stress levels in RDI treatments were scheduled according to trunk diameter fluctuations indicators (Moriana et al., 2013) in order to obtain the low or moderate levels. Briefly, trunk diameter fluctuations are a daily cycle of shrinkage and swelling which are measured continuously with dendrometer (DF 2.5, Solartorn, UK). The indicator

selected in this work was the trunk growth rate (TGR, difference between two consecutive maximum); TGR is the most accurate indicator in olive trees (Moriana, & Fereres, 2002) and was selected for characterizing the water status of the field experiment. Irrigation treatments were:

- Control (T0): Irrigation to supply the estimated crop evapotranspiration (ETc), i.e., based on fully replenishing all the extracted soil water.
- RDI-1 (T1): (*i*) stage I, trees irrigated under non-limited conditions; *(ii*) stage II, trees under moderate water deficit conditions, they were no irrigated during this period; and, *(iii*) stage III, water applied in order to provide a water status similar to T0 treatment.
- RDI-2 (T2): (*i*) stage I, trees under low water deficit conditions. Trees were irrigated only when TGR was lower than 10 μm day^{-1}; this is half of the TGR in fully irrigated conditions *(ii*) stage II, trees under moderate water deficit conditions, they were no irrigated during this period; and, *(iii*) stage III, water applied in order to provide a water status similar to T0 treatment.

A randomized 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

All "*Manzanilla"* olives from the three RDI treatments were completely hand-harvested at their mature-green stage in mid-September. The fruit of all trees for each of the three RDI treatments were systematically mixed and a sample of around 45 kg per treatment was used in the industrial processing. Fruits were transported next day to *Cooperativa Nuestra Señora de las Virtudes* (La Puebla de Cazalla, Seville, Spain), to be processed as table olives according to the Spanish style method. This delay between harvest and processing (1 day) is common in order to prevent the skin from sloughing or bursting during alkaline treatment (IOOC, 1990).Initially, raw olives were treated with a solution of NaOH (0.6 mol L^{-1})

until the lye penetrates three quarters through the flesh to remove oleuropein and increase the permeability of the fruits. Later, olives were washed with water to remove completely the NaOH residues. Finally, the fermentation was carried out for 4 months using different concentration of brine; it started with 0.17 mol L^{-1} NaCl and ended with 0.09 mol L^{-1}; the pH used was 4.5.

Physico-chemical Analyses

All physico-chemical analyses were conducted in processed table olives. Approximately 2 kg of table olives per treatment were used to evaluate the quality attributes, this means that about 450 fruits per treatment were evaluated.

Weight and size

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

Instrumental color

Color determinations were made, at 25 ± 1 °C, using a Minolta Colorimeter CR-300 (Osaka, Japan). This spectrophotometer uses an illuminant D_{65} and a 10° observer as references. Color data are provided as CIE*L*a*b** coordinates, which define the color in a three-dimensional space. Color analyses were run in 20 replicates.

Puncture and Magness-Taylor tests

The puncture and Magness-Taylor (PT, MTT) tests were conducted using a Texture Analyzer TA-XT2 (Stable Micro Systems, Surrey, UK). Puncture test (force) was measured using a stainless-steel needle probe P/2N (2 mm thickness) which

was applied in the center of the olive fruit. This parameter is related to the peel firmness of olives. This probe moved at a speed of 0.5 mm s^{-1}, and penetrated 7 mm or until the stone was reached. The parameter evaluated was the maximum force of rupture in the registry of curve force versus time (Szychowski, Frutos, Burló, Pérez-López, Carbonell-Barrachina, & Hernández, 2015). Magness-Taylor test is an empirical flesh hardness indicator of the olive fruit; MTT was measured using a stainless-steel cylindrical probe P/MT of 8 mm diameter. Penetration rate was 0.33 mm s^{-1} and the probe penetrated 8 mm or until the stone was reached (Szychowski et al., 2015). The tests was performed in 25 replicates, 1 replicate per fruit, and results were expressed in N.

Oil content and fatty acids

A 1 L ultrasonic Selecta bath model 3000512 JP (Barcelona, Spain) was used to extract the oil by sonication. A 2 g 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 by evaporating the cyclohexane using a nitrogen stream.

Fatty acids methyl esters (FAMEs) were prepared according to the method described by Majdi, Barzegar, Jabbari, and AghaAlikhani (2012) with some modifications. Extracted oil (50 mg) was saponified with 100 µL dichloromethane (Cl_2CH_2), and 1 mL methanolic NaOH solution by refluxing for 10 min at 90°C. After addition of 1 mL BF_3-methanolic, the sample was boiled for 10 min. The FAMEs were extracted from a salt saturated mixture by adding 600 µL hexane. The organic layer was separated and used for GC–MS analysis. The GC-MS set up (GC-17A and GCMS-QP5050A), previously described for volatile compounds was used for the identification and quantification of fatty acids methyl esters. Injector and detector were held at 230 and 300ºC, respectively. The GC program was as follows: (i) initial temperature 80ºC for 2 min, (ii) rate of 8ºC min^{-1} from 80 to 160ºC, (iii) rate of 4ºC min^{-1} from 160 to 240ºC and hold for 30 min. Identification of peaks was

made by comparison with FAME standards from Sigma-Aldrich. Analysis of FAMEs was run in triplicate.

Extraction of volatile compounds

Headspace solid phase micro-extraction (HS-SPME) was the method selected to study the volatile composition of the samples under analysis. After several preliminary tests to optimize the extraction system, 5 g of finely chopped olives plus 15 mL of ultrapure water were hermetically placed into 50 mL vials with polypropylene caps and PTFE/silicone septa. A magnetic stirring bar was added, together with NaCl (0.26 mol L^{-1}) and the vial was placed in a water bath with controlled temperature and stirring. Vials were equilibrated during 15 min at 40ºC (to simulate the mouth temperature during the chewing process) and after this equilibration time, a 50/30 µm DVB/CAR/PDMS fiber was exposed to the sample headspace for 50 min at 40ºC. This type of fiber was chosen for its high capacity of trapping fruits volatile compounds (Vázquez-Araújo, Koppel, Chambers, Adhikari, & Carbonell-Barrachina, 2011). After sampling, desorption of the volatile compounds from the fiber coating was carried out in the injection port of the GC-MS during 3 min.

Chromatographic analyses

The identification of the volatile compounds was performed on a gas chromatograph, Shimadzu GC-17A (Shimadzu Corporation, Kyoto, Japan), coupled with a Shimadzu mass spectrometer detector GC-MS QP-5050A. The GC-MS system was equipped with a TRACSIL Meta X5 column, 95% dimethyl-polysiloxane and 5% diphenyl-polysiloxane (Teknokroma S. Co. Ltd., Barcelona, Spain; 30 m × 0.25 mm i.d., 0.25 μm film thickness). Analyses were carried out using helium as carrier gas at a flow rate of 0.6 mL min^{-1} in a split ratio of 1:5 and a program: (a) initial temperature 80ºC, (b) rate of 3.0ºC min^{-1} to 210ºC and hold for 1 min; (b) rate of 25ºC min^{-1} from 210 to 300ºC and hold for 8 min. Injector and detector

229 temperatures were held at 230 and 300ºC, respectively. 1 µL of the extracts was
230 injected.

231 Most of the compounds were simultaneously identified by using 3 analytical
232 methods: 1) retention indices, 2) GC-MS retention times [authentic standards
233 (SAFC, 2011)], and 3) mass spectra (authentic chemicals and NIST05 spectral
234 library collection; NIST 2011). Identification was considered tentative when it was
235 based only on mass spectral data. The volatile composition analysis was run in
236 triplicate and results were expressed as percentage of the total area represented by
237 each one of the volatile compounds.

238

239 **Sensory Analyses**

240 *Sensory evaluation with trained panel*

241 Eight trained panelists (aged 20 to 55 years; 5 female and 3 male) from the
242 research group Food Quality and Safety (UMH) participated in this study. Each of
243 the panelists had more than 600 h of testing experience with a variety of food
244 products; the panel received further orientation on table olives (three sessions of 1
245 h).

246 Samples were served into odor-free, disposable 90 mL covered plastic cups.
247 Half cup filled with olives was served to each panelist. All samples were served at
248 room temperature and were coded using three digit numbers. Unsalted crackers
249 and distillated water were used to clean palates between samples. The testing room
250 was at ~21ºC; the illumination was a combination of natural and non-natural
251 (fluorescent) light.

252 Three 2 h-sessions were held for samples evaluation, all samples were
253 evaluated in each session and thus, each sample was tested in triplicate (3
254 sessions). The panel started to work with the lexicon developed by the International
255 Olive Oil Council, IOOC (2011) but, after the orientation sessions, the panel agreed
256 to evaluate only the following attributes: (*appearance*) color and size; (*flavor*)
257 green-olive flavor, sour, bitter, salt, sweet, and aftertaste; (*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.

Sensory evaluation with consumer panel

Consumer acceptance was studied, on May 2014, at UMH. Sixty consumers (60% female) were recruited via e-mails for a central location test. The consumers had to complete a screener stating their gender, age, and diet restrictions or allergies. The consumers were asked about olives consumption frequency and willingness to taste olives. Consumers, who stated that they were 18-60 years old, ate some kind of olives at least twice per week, had no diet restrictions or allergies, and were willing to taste olives, were recruited for testing.

Once consumers were selected, samples were served under the same preparation conditions described above in the section on Sensory Evaluation with Trained Panel. Consumers had to complete a questionnaire about their global satisfaction degree for the samples under evaluation. Consumers responded using a 9-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 $p<0.05$. Pooled standard variance has been used to estimate the analyses precision. All statistical analyses were performed using StatGraphics Plus 5.0 software (Manugistics, Inc., Rockville, MD).

RESULTS AND DISCUSSION

Irrigation

Treatments produced clear differences in the applied water, AW (**Table 1**); about 3 times more water was applied to T0 than to T1-T2 trees. The water status of control trees (T0) was not affected according to the trunk growth rate (TGR), and presented an almost constant value with time (mean of 2.44 μm day^{-1}), which is common in high fruit load years (Moriana et al., 2013). On the other hand, the restriction of irrigation in the regulated deficit treatments with almost no irrigation during stage II produced water stress conditions in both group of trees (T1 and T2). During stage II, both RDIs treatments presented negative TGR values, which were below the threshold recently suggested for this parameter in deficit irrigation of olive trees, -5 mm day^{-1} (Moriana et al., 2013). Finally, the yield was not significantly affected by RDI treatments (mean of 5.8 t ha^{-1}), although irrigation and water status were affected in some periods of the field experiment. However, an isolated season is not enough for obtaining a conclusion about yield response.

Physicochemical Analyses

Weight and size

The results of the weight and size (longitudinal and equatorial diameters) are shown in **Table 2**. T1 olives had higher weight than control (~4%) and T2. In the T1 treatment, water stress was applied in stage II (pit hardening), the period of recovery (phase III) could have enhanced flesh growth. Lavee, Hanoch, Wodner, & Abramowitch (2007) suggested that a moderate water stress produced a higher oil content, which can be linked with an increase in flesh; however, this statement needs further research. In addition, the mild water stress can cause the plant to react by activating defense mechanisms, improving its metabolism and fruit development, as seems the case of T1 olive trees. However, under a more severe stress or a longer period, the plant cannot react and negative effects are produced, as seen in T2 olives. According to IOOC (2014), "*Manzanilla"* olives must have good

size, ranging from 2.1 to 4.9 g; experimental values were within the upper part of this range, specifically between 4.2 and 4.7 g. The highest longitudinal diameter (d_l, length) was that of T0 fruits, while the highest equatorial diameter (d_e, thickness) was that of T1 fruits. The shape of the olive fruits changed completely from T0 (long but thin fruits) to T2 (thick but short fruits), with T1 fruits being almost rounded (same d_l and d_e). In general, the fact that T1 *"Manzanilla"* olives are rounded is a beneficial effect because this variety of olives is generally used for manufacturing filled table olives and rounded fruits are easy to pit (removal of the pit to fill the hole created with anchovy or pepper paste) (Rejano Navarro, 1999).

Instrumental color

The results of the parameter CIE*L*a*b** coordinates are shown in **Table 2**. The RDI treatments significantly (p<0.001) affected lightness (*L**), and the blue-red coordinate, *b**; however, no significant effects were found in the green-red coordinate, *a**.

The color of T2 olives was lighter and had higher yellow intensity than olives from T0 and T1 trees. *L** and *b** increased as the RDI conditions got more severe; however, no statistical significant differences were found between the first two treatments, T0 and T1.

These results differ from previous studies where olive oils had less intensity of the yellow color when stressed olives were used (Pastor et al., 1999). However, this study was conducted using a different olive variety, *Arbequina,* and a different matrix was studied, exactly oil, while in our study the color of olive fruit skin was evaluated.

Puncture and Magness-Taylor tests

The texture of the olive flesh depends on the fat and fiber contents. According to the IOOC (2014), the flesh of *"Manzanilla"* olives is delicate, flavorful, firm, fleshy, of soft consistency, non-fibrous and the skin is thin. Instrumental texture of both skin (PT) and flesh (MTT) are summarized in **Table 2**.

341 The hardest skin was that of T1 olives, while the hardest flesh was that of
342 control samples (T0). Olives from severely stressed trees (T2) had the softest skin
343 and flesh. The skin of olives present stomata and after fruit set, the skin prevents
344 fruit dehydration (Rapoport, Costagli, & Gucci, 2004). Moderate water stress (T1)
345 could enhance this growth, but more severe conditions (T2) may limit skin
346 development. Regarding flesh hardness (MTT), it seems that it basically depends on
347 the water availability, with flesh being harder when more water is available. MTT is
348 more related to cell turgor than to the number or size of fruit cells, which are better
349 correlated with other attributes, especially fibrousness (Rapoport et al., 2004).
350 *Dry matter and oil contents*
351 There is no doubt that the dry matter content (DMC) of table olives depended
352 on water availability for trees, with control fruits (T0) having the lowest content of
353 DMC [268 g dry weight (dw) kg^{-1} fresh weight (fw)] but the highest content of
354 moisture (**Table 2**). However, mild RDI conditions (T1) significantly activated plant
355 metabolism resulting in the highest oil content (341 g dw kg^{-1} fw) (**Table 3**). If the
356 moisture content is calculated considering the fact that oil+DMC+moisture
357 (%)=100 and transforming to appropriate units, this parameter decreased as the
358 RDI conditions got more severe, taking values of 454, 375, and 358 g H_2O kg^{-1} fw
359 for T0, T1, and T2, respectively.
360 *Fatty acids*
361 As expected the fatty acids profile of table olives was dominated by oleic acid
362 (C18:1), with a mean content of 68.2%, followed by palmitic acid (C16:0), with
363 17.2%, and linoleic acid (C18:2), with 5.9% (**Table 3**). Only 3 out of the 7 fatty
364 acids found in this type of table olives were significantly ($p<0.05$) affected by the
365 RDI treatments, these were palmitoleic (C16:1), linoleic (C18:2) and oleic (C18:1)
366 acids. The most relevant finding is that mild RDI conditions significantly increased
367 the content of linoleic acid from reductions of oleic and palmitoleic acids. This
368 change in T1 fruits resulted also in a significant increase of PUFA and a decreased

of MUFA. It is important to highlight that PUFA are beneficial to human health because our body is not able to synthesize these essential compounds (FAO, 2010).

Similar results were obtained by Caruso, Rapoport, and Gucci (2014); these authors reported an increase in the content of PUFA in olives irrigated following moderate RDI conditions during pit hardening. However, other author found no effects of RDI on the content and composition of fatty acids in *Arbequina* olive oil (Morábito, Pérez-Peña, Puertas, & Trentacoste, 2008).

Volatile compounds

A total of 43 compounds were identified in the volatile profile of "*Manzanilla"* table olives (**Table 4**); RDI conditions significantly affected the contents of 30 of these compounds. The five most abundant volatile compounds were: acetic acid (mean value of the three treatments 12.4%), tetrahydrogeraniol (9.6%), 2-decenal (9.3%), 1,4-dimethoxy-benzene (7.4%), and 4,8-dimethyl-1,3,7-nonatriene (mean of 5.4%).

The 43 compounds have been classified into 11 chemical families (**Figure 1**). The volatile profile of the control samples (T0) was predominated by aldehydes (20.4%) and phenolic compounds (19.9%). Farming under RDI conditions led to increases of acids, lineal hydrocarbons, and sulfur compounds, but simultaneous decreases of aldehydes and phenolic compounds. In this way, the volatile profiles of T2 table olives (severe RDI conditions) was predominated by organic acids (17.8%), linear hydrocarbons (15.8%), alcohols (14.2%), and terpenes (12.5%); while the most abundant families in the profiles of T1 table olives (mild RDI conditions) were terpenes (19.3%), aldehydes (16.3%), linear hydrocarbons (13.4%), organic acids (13.3%), and phenolic compounds (12.7%).

In general, alcohols (high in T0 and T2) are associated with fruity and candy flavor notes, aldehydes (highest in T0) with green, vegetable and herbaceous notes, terpenes (highest in T1) with citrus and pine notes, organic acids (highest in T2) with herbaceous and vinegar notes, and phenolic compounds with green, woody, and cheesy notes (SAFC, 2011). It is possible that the synergistic effects

among the simultaneously high contents of aldehydes (16.3%), organic acids (13.3%), phenolic compounds (12.7%) and terpenes (19.3%) found in T1 table olives are responsible for the high intensity of the descriptor "green-olive" that will be reported later in this manuscript.

Sensory Analysis

Table 5 shows that RDI treatments affected most of the table olive sensory parameters, with the exception of sourness (mean of 2.2), crunchiness (mean of 6.0), and fibrousness (mean of 0.1); the fact that the intensity of this last attribute was so low indicated that no elongated particles were perceived by the panel. Control fruits had pits which were easy to remove from the edible portion of the olives (7.9); however, the intensities of all other parameters under study were higher in RDI olives. T1 fruits (mild RDI conditions) were characterized by high intensities of saltiness, bitterness, green-olive flavor, long aftertaste and had higher value of hardness. Finally, T2 had the highest intensity of sweetness.

There was a positive correlation between sensory hardness and the values of the puncture test; however, no such relationship was found between sensory data and values of MTT. Therefore, the hardness of the skin seemed more related to the sensory hardness than that of the flesh in table olives.

The results from the affective study using 60 Spanish consumers proved that T1 table olives were those with higher degree of satisfaction for three of the parameters under study (**Table 6**). T1 fruits got the highest values for typical flavor of green table olives (6.9) and crunchiness (6.9), and what it is more important of global satisfaction degree (6.9). It is important to remember that 6 and 7 mean that consumers like table olives slightly or moderately; besides, in affective tests consumers are well known to use only the central part of the scale avoiding the use of extreme values. Consequently, the value of 6.9 obtained by T1 olives for the global satisfaction degree indicated that Spanish consumers really liked T1 *"Manzanilla"* table olives.

CONCLUSIONS

Moderate regulated deficit irrigation (RDI-T1; these fruits only suffered water stress during pit hardening) had positive effects on the quality and consumer's satisfaction degree of table olives. Table olives from T1 had the highest weight, size, skin hardness, and linoleic acid content; besides, they also had the highest intensities of saltiness, bitterness, green olive note, aftertaste and hardness and finally, obtained the highest values of satisfaction degree for typical flavor of fresh table olives, crunchiness, and global acceptance. It is therefore possible to save water using RDI strategies without jeopardizing the quality of the fruits, in this particular case, of table olives, cv. *"Manzanilla"*.

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552

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 treatments.

Irrigation	Stage			Total	Pooled Std.
Parameter	I	II	III		Variance
ETc (mm)	248 a[†]	186 b	92 c	526	47
Irrigation Treatment					
Parameter/Stage	**T0**	**T1**	**T2**	**Pooled Std. Variance**	
AW (mm)					
Stage 1	229 a	128 b	111 b	17	
Stage 2	214 a	6 b	5 b	15	
Stage 3	97 a	37 b	45 b	21	
TGR (μm day^{-1})					
Stage 1	-2.10 a	-2.60 a	-6.30 a	4.4	
Stage 2	3.34 a	-14.80 b	-20.70 b	6.1	
Stage 3	6.07 b	31.52 a	28.21 a	14.8	
Yield (t ha^{-1})	6.6 a	5.0 a	5.9 a	2.4	

[†]*Values (mean of 6 replicates) followed by the same letter, within the same row, were not significantly different ($p<0.05$), according to Tukey's least significant difference test.*

561 **Table 2**

562 Morphological parameters, CIE*L*a*b** coordinates, and texture parameters of

563 "*Manzanilla"* table olives as affected by regulated deficit irrigation treatments.

Parameter[†]	ANOVA[‡]	T0	T1	T2	Pooled Std. Variance
Fruit weight (g)	***	4.43 b[¶]	4.60 a	4.30 b	0.13
Longitudinal diameter (mm)	***	2.3 a	2.1 b	2.0 b	0.1
Equatorial diameter (mm)	***	1.9 b	2.1 a	1.7 c	0.1
*L**	***	51.51 b	54.62 ab	56.14 a	1.93
*a**	NS	-1.94	-1.82	-1.87	0.57
*b**	***	28.61 b	31.87 b	38.39 a	3.16
Dry matter content (g dw kg^{-1} fw)	***	268 c	284 b	369 a	17
Puncture test, PT (N)	***	0.506 b	0.651 a	0.473 b	0.078
Magness-Taylor test, MTT (N)	**	6.533 a	5.401 b	5.135 b	0.871

564 [†]*The number of replications for the analysis of weight, size, instrumental color, dry*

565 *matter content (DMC), puncture test (PT), and Magness-Taylor test (MTT) were 20,*

566 *20, 20, 5, 25 and 25, respectively*; [‡]*NS = not significant at p< 0.05; *, **, and*

567 ****, significant at p< 0.05, 0.01, and 0.001, respectively.* [¶]*Values followed by the*

568 *same letter, within the same row, were not significantly different (p<0.05),*

569 *according to Tukey's least significant difference test.*

570 **Table 3**

571 Oil content (g kg^{-1} dry weight, dw) and fatty acids profiles (% of total area) of
572 "*Manzanilla"* table olives as affected by regulated deficit irrigation treatments.

Parameter	ANOVA[†]	T0	T1	T2	Pooled Std. Variance
Oil content (g kg^{-1} dw)	**	278 b[¶]	341 a	273 b	51
C16:1	*	2.7 a	1.9 b	2.3 a	0.3
C16:0	NS	16.3	17.8	17.5	2.0
C18:2	***	4.9 b	7.4 a	5.4 b	1.6
C18:1	*	69.3 a	67.1 b	68.1 ab	1.4
C18:0	NS	5.2	4.9	5.2	0.5
C20:1	NS	0.6	0.3	0.5	0.4
C20:0	NS	1.0	0.6	1.0	0.4
SFA[‡]	NS	22.6	23.3	23.6	1.1
MUFA[‡]	*	72.6 a	69.3 b	70.9 ab	2.1
PUFA[‡]	**	4.9 b	7.4 a	5.4 ab	1.6
(MUFA+PUFA)/SFA[‡]	*	3.43 a	3.30 ab	3.23 b	0.13

573 [†]*NS = not significant at p< 0.05; *, **, and ***, significant at p< 0.05, 0.01, and*
574 *0.001, respectively.* [‡]*SFA: Saturated fatty acids (C16:0, C18:0, and C20:0)*; MUFA:
575 *Monounsaturated fatty acids (C16:1, C18:1, and C20:1)*; PUFA: *Polyunsaturated*
576 *fatty acids (C18:2)*. [¶]*Values (mean of 3 replicates) followed by the same letter,*
577 *within the same row, were not significantly different (p<0.05), according to Tukey's*
578 *least significant difference test.*

Table 4

Volatile compounds (% of total area of identified compounds) and descriptors (SAFC, 2011) of "*Manzanilla"* table olives as affected by regulated deficit irrigation treatments.

Compounds	Chemical Family	Retention Indexes		Descriptors	ANOVA[†]	Content (%)			Pooled Std. Variance
		Exp.	Lit.			T0	T1	T2	
Ethanol	Alcohol	496	489		**	4.04 b[¶]	3.70 b	7.14 a	1.72
Dimethylsulfide	Sulfur compound	532	517		***	3.50 c	7.35 b	9.17 a	1.35
Acetic acid	Acid	658	658	Vinegar	***	9.6 b	11.7 ab	15.9 a	3.15
Heptane	Lin. hydrocarbon	693	700		*	4.30 b	7.63 a	5.06 b	1.75
Propionic acid	Acid	716	715		*	0.28 b	0.46 ab	0.60 a	0.26
Ethyl propanoate	Ester	734	725		NS	0.11	0.19	0.17	0.08
Propyl acetate	Ester	737	728	Celery	**	0.09 b	0.34 a	0.14 b	0.07
Octane	Lin. hydrocarbon	799	800		**	3.25 a	4.60 ab	5.73 b	1.43
2-Methylbutanoic acid	Acid	823	831		NS	0.32	0.43	0.40	0.09
Furfural	Furan	842	848	Almond, woody	**	0.85 b	0.70 a	0.15 c	0.12
cis-3-Hexenol	Alcohol	889	882		***	5.99 a	2.33 b	4.76 a	1.48
1-Hexanol	Alcohol	904	888	Green, woody	NS	0.82	0.83	0.52	0.29
cis-2-Heptenal	Aldehyde	935	951		NS	0.24	0.13	0.25	0.11
Hexanoic acid	Acid	960	959	Sour, fatty	NS	0.95	0.68	0.91	0.27
Benzaldehyde	Aldehyde	977	960	Almond, cherry	***	7.71 a	0.57 b	0.48 b	1.68
6-Methyl-5-hepten-2-one	Ketone	985	980	Herbaceous, oily	**	0.18 b	0.29 ab	0.41 a	0.12
β-Pinene	Terpene	990	981	Woody	*	0.10 b	0.13 b	0.25 a	0.09
Octanal	Aldehyde	1006	1006	Fatty, fruity	NS	0.43	0.39	0.31	0.12
Hexyl acetate	Ester	1010	1010	Pear, woody	NS	0.27	0.23	0.33	0.09
p-Cymene	Terpene	1032	1029	Citrus	NS	0.14	0.19	0.10	0.07
Limonene	Terpene	1037	1030	Lemon, orange	**	3.94 a	2.45 b	3.50 a	0.49
trans-β-Ocimene	Terpene	1046	1046		***	0.28 a	0.05 b	0.09 b	0.06
Phenylacetaldehyde	Phenolic compound	1055	1053	Vegetable, green	**	0.30 b	0.46 a	0.36 b	0.08
1-Octanol	Alcohol	1074	1072	Fatty, citrus, waxy	***	2.64 a	0.67 c	1.73 b	0.81

Table 4. Continuation.

Compounds	Chemical Family	Retention Indexes		Descriptors	ANOVA[†]	Content (%)			Pooled Std. Variance
		Exp.	Lit.			T0	T1	T2	
γ-Terpinene	Terpene	1074	1069	Herbaceous, citrus	**	0.46 b	1.86 a	0.34 b	0.26
Guaiacol	Phenolic comp.	1096	1090	Woody, smoky	***	2.53 a	1.71 b	0.47 c	0.63
Undecane	Lin. hydrocarbon	1100	1100		**	0.62 b	1.05 a	0.06 c	0.34
Linalool	Terpene	1104	1101	Lemon, floral, citrus	**	0.23 b	0.19 b	0.50 a	0.05
Nonanal	Aldehyde	1108	1103	Fruity, citrus, nutty	NS	1.62	1.77	1.71	0.28
4,8-Dimethyl-1,3,7-nonatriene[‡]	Other hydrocarbon	1115	1115		*	3.97 c	6.35 a	5.97 b	0.27
Benzeneethanol	Phenolic compound	1160	1159		**	1.75 b	0.82 c	2.33 a	0.43
4-Ethylphenol	Phenolic compound	1170	1171	Alcohol, medicinal	**	1.09 a	0.63 b	0.28 c	0.16
Ethyl octanoate	Ester	1196	1193	Apricot, banana	NS	0.66	1.17	0.72	0.32
1,4-Dimethoxy-benzene[‡]	Phenolic compound	1199	1205		NS	7.97	6.25	8.07	1.92
Tetrahydrogeraniol[‡]	Terpene	1205	1196		**	8.58 b	13.7 a	6.61 c	1.07
α-Citronellol[‡]	Terpene	1210	1212		NS	0.82	0.51	0.57	0.26
Bornyl acetate[‡]	Terpene	1242	1268		NS	0.41	0.20	0.55	0.31
2-Decenal	Aldehyde	1264	1264		***	9.97 b	11.8 a	6.20 c	1.65
5-Hydroxymethylfurfural	Furan	1267	1261		**	0.72 b	0.99 ab	1.13 a	0.18
2-Decenal	Aldehyde	1279	1278		**	0.54 b	1.58 a	0.48 b	0.11
Tridecane	Lin. hydrocarbon	1300	1300		**	1.49 b	0.12 c	4.91 a	0.87
Anethole	Phenolic compound	1300	1285	Anise, spicy	***	6.25 a	2.83 b	0.69 c	2.02

[†]*NS = not significant at p< 0.05; *, **, and ***, significant at p< 0.05, 0.01, and 0.001, respectively.* [‡]*Tentatively identified (only identified by retention indexes and NIST spectral database, 2000).* [¶]*Values (mean of 3 replicates) 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 5

Descriptive sensory analysis of "*Manzanilla"* table olives as affected by regulated deficit irrigation treatments. The scale used ranged from 0 = no intensity to 10 = extremely strong intensity.

Parameter	ANOVA[†]	T0	T1	T2	Pooled Std. Variance
FLAVOR[‡]					
Saltiness	*	5.8 ab[¶]	6.9 a	5.5 b	1.0
Bitterness	**	4.8 b	6.8 a	4.4 b	1.2
Sourness	NS	1.6	2.3	2.7	1.0
Sweetness	**	1.9 b	1.9 b	2.9 a	0.6
Green-olive	**	6.8 a	7.1 a	5.7 b	0.9
Aftertaste	***	6.5 b	7.9 a	6.1 b	1.1
TEXTURE[‡]					
Hardness	**	6.3 b	7.8 a	6.0 b	1.1
Crunchiness	NS	6.5	6.0	5.4	0.8
Fibrousness	NS	0	0.1	0.1	0.1
Pit removal	*	7.9 a	6.9 b	6.9 b	0.8

[†]*NS = not significant at p< 0.05; *, **, and ***, significant at p< 0.05, 0.01, and 0.001, respectively.* [‡]*Attributes included in this profile are based on IOOC (2011).* [¶]*Values (mean of 8 trained panelists) followed by the same letter, within the same row, were not significantly different (p<0.05), according to Tukey's least significant difference test.*

598 **Table 6**

599 Affective sensory analysis of "*Manzanilla"* table olives as affected by deficit
600 irrigation treatments. Panelist used a 9-point hedonic scale, where 1 = dislike
601 extremely, 5 = neither like nor dislike, 9 = like extremely.

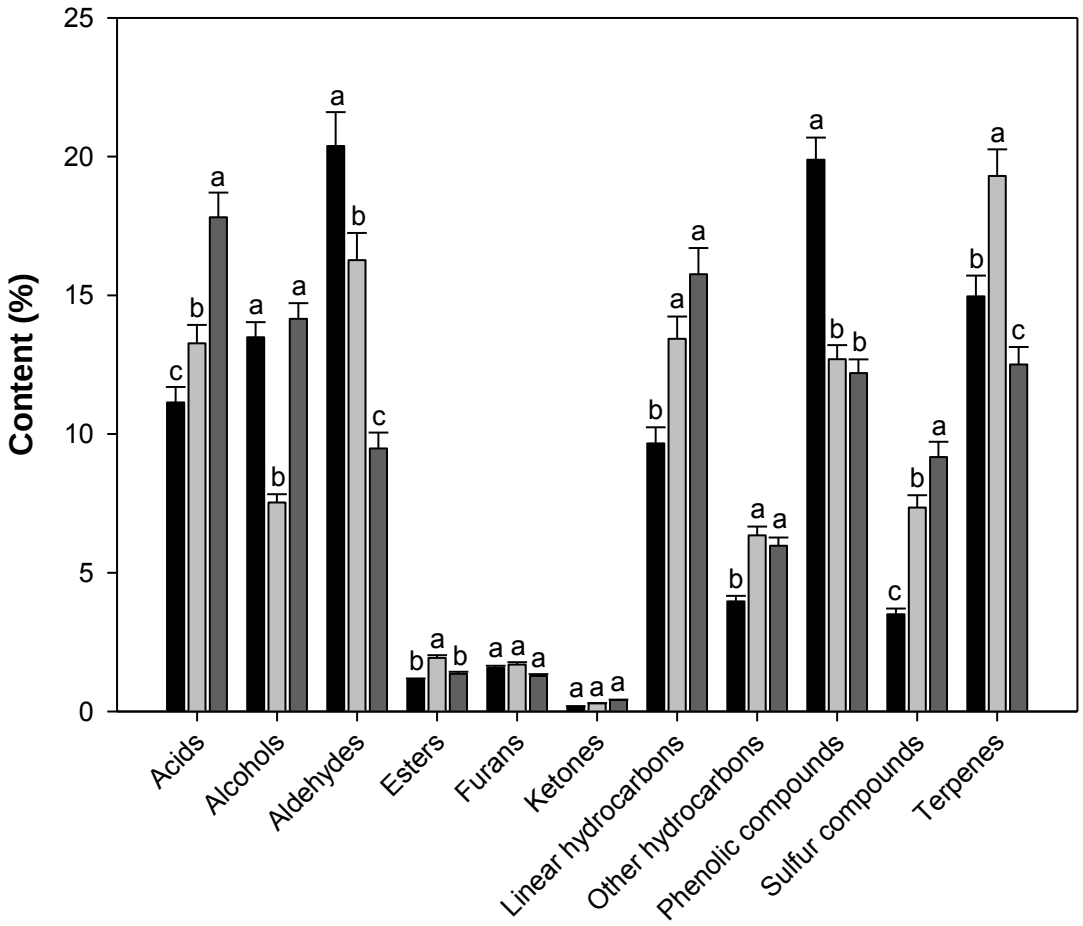
602

Parameter	ANOVA[†]	T0	T1	T2	Pooled Std. Variance
Flavor (table olive)	*	6.3 ab[‡]	6.9 a	5.8 b	0.8
Bitterness	NS	6.1	6.7	5.9	0.7
Saltiness	NS	6.0	6.7	6.1	0.7
Hardness	NS	6.5	6.8	6.3	0.8
Crunchiness	*	6.2 ab	6.9 a	6.0 b	0.7
Aftertaste	NS	6.5	6.3	5.8	0.9
GLOBAL	*	6.5 ab	6.9 a	6.0 b	0.8

603 [†]*NS = not significant at p< 0.05; *, **, and ***, significant at p< 0.05, 0.01, and*
604 *0.001, respectively.* [‡]*Values (mean of 60 consumers) followed by the same letter,*
605 *within the same row, were not significantly different (p<0.05), according to Tukey's*
606 *least significant difference test.*

607

608 **Figure 1.** Chemical families of the volatile compounds (results from **Table 3**) found
609 in *Manzanilla* table olives as affected by deficit irrigation treatments. Black bars =
610 T0; light gray bars = T1; dark gray bars = T2. Bars with the same letter, within the
611 same chemical family, were not significantly different at $p<0.05$.
612



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