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

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Running title: Functional properties of hydroSOStainable table olives

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Abbreviations

AA: antioxidant activity

AW: applied water

dw: dry weight

ETc: crop evapotranspiration

FAMEs: fatty acids methyl esters

fw: fresh weight

GAE: gallic acid equivalents

H-AA: hidrofilic antioxidant activity

L-AA: lipophilic antioxidant activity

MUFA: monounsaturated fatty acids

PUFA: polyunsaturated fatty acids

RDI: regulated deficit irrigation

RID: refractive index detector

TGR: trunk growth rate

TPC: total polyphenols content

ABSTRACT

The influence of 3 irrigation treatments (T0, no stress; T1, soft stress; and, T2, moderate stress) on the key functional properties [fatty acids, sugars, organic acids, minerals, total polyphenols content (TPC), and antioxidant activity (AA)], sensory quality, and consumers' acceptance of table olives, cv. "Manzanilla", was evaluated. 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 color, the content of linoleic acid, but decreased the content of phytic acid and some minerals. The final conclusion is that soft RDI conditions are a good option for the cultivation of olive trees because they are environmental-friendly and simultaneously maintains or even improves the functionality, sensory quality, and consumer acceptance of table olives.

Keywords: consumers; functional; hydrosostainable; Olea europaea L.; water stress; "Manzanilla".

63 # 1. INTRODUCTION

64

65 Olive (Olea europaea L.) is the most extensive tree crop of the Mediterranean
66 basin and has been traditionally cultivated in marginal areas with low density under
67 rainfall conditions (Iniesta, Testi, Orgaz, & Villalobos, 2009). The aridity of the
68 climate and the persistent shortage of water resources in the Mediterranean
69 agrosystems are aggravated by strong competition for the available water with
70 other nonagricultural users, for example intense use in touristic areas during
71 summer time (Collado-González et al., 2015a). These problems have led to
72 development of new water saving techniques, such as regulated deficit irrigation
73 (RDI). This technique in olive trees (drought tolerant plant) is mainly based on
74 scheduling a water deficit period during pit hardening; it has been proved that this
75 stage is a non-critical phenological period (Goldhamer, 1999). In this way, it is
76 possible to save water in the irrigation of olive trees but with a minimum impact on
77 yield and fruit quality (Janick & Naor, 2006).

78 Table olives are prepared from the fruit of the olive tree because fresh olives
79 are not edible (Boskou, Camposeo, & Clodoveo, 2015). Table olives are probably
80 the most important fermented food in the Mediterranean countries and are very
81 valuable because of their highly appreciated taste and rich nutritional composition
82 leading to interesting health benefits (Aktas, Ozen, Tokatli, & Sen, 2014).
83 Therefore, the daily consumption of table olives will contribute in an important way
84 to the intake of healthy substances, such as phenolic compounds, which are highly
85 recommended because their antioxidant properties (Fabiani et al., 2011).

86 Although irrigation normally has positive impact on olive production, it is also
87 known that different water regimes can affect its nutritional, antioxidant and quality
88 components (Servili et al., 2007; Gómez-Rico et al., 2007). Cano-Lamadrid, Girón,
89 Pleite, Burló, Corell, Moriana & Carbonell-Barrachina (2015) concluded that RDI can
90 affect the quality of Manzanilla table olives, including fruit size, color, texture,
91 volatile and fatty acids profiles, and even consumer satisfaction. Simultaneously,

92 Collado-González et al. (2015b) using raw olives, from the same RDI treatments,
93 showed the effects of RDI on some functional components, phytoprostanes. In
94 these two studies, the RDI was applied during the pit hardening period. However,
95 there are no studies about the effects of RDI on antioxidant activity, mineral
96 composition, or sugars and organic acids profiles.

97 Table olives cultivated under RDI conditions are considered as
98 "hydroSOStainable" products, and have a solid identity (higher contents of essential
99 components, higher intensity of key sensory attributes, etc.); besides, they can be
100 a good alternative for this type of crop and reduce the economic and environmental
101 costs linked to irrigation, optimizing the use of a very valuable resource in the
102 word, water (Cano-Lamadrid, et al., 2015).

103 Considering all the above, the main aim of this work was to evaluate the
104 effects of RDI conditions on key functional properties of Manzanilla table olives. The
105 functionality of table olives was studied by evaluating their (i) nutritional
106 composition: fatty acids, sugars, organic acids, and minerals profiles, and (ii)
107 antioxidant properties: DPPH, FRAP, ABTS, and total polyphenols content. These
108 analyses were completed by evaluating the effects of RDI on (i) morphology: yield
109 per tree, weight, and size, and CIEL*a*b* color, and (ii) sensory quality:
110 descriptive profile using a trained panel, and consumer acceptance using an
111 affective panel.

2. MATERIALS AND METHODS

2.1. 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 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 (Moriana, Corell, Girón, Conejero, Morales, Torrecillas, & Moreno, 2013). 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 (Moriana, & Fereres, 2002), and was selected to characterize the water status of this field experiment.

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

- Control (T0): Irrigation was applied to supply the estimated crop evapotranspiration (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): (i) 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 $\mu m\ day^{-1}$ (this is half of the value found in trees under fully irrigated conditions) (ii) same conditions as in stage I;

and, (iii) finally at the stage III, enough water was applied to reach a water status similar to that of T0 trees.

- RDI-2 (T2, moderate stress): (i) during the stage I, olive trees were under low water deficit conditions; trees were only irrigated when the TGR was lower than 10 mm day^{-1}; (ii) trees were not irrigated during stage II; and, (iii) finally at the stage III, enough water was applied to reach a water status similar to that of T0 trees.

A randomized complete-block design was used with 3 blocks per treatment and 2 trees per block. Irrigation scheduling was controlled with the measurements of 6 trees per treatment (2 per block) along the growing season.

2.2. 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; the details of this methods can be found in Cano-Lamadrid, et al., (2015).

2.3. 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).

2.3.1. 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 (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).

169 2.3.2. Instrumental color

170 Instrumental color measurements were made using a Minolta Colorimeter CR-
171 300 (Osaka, Japan), at 25 ± 2 °C. This spectrophotometer uses an illuminant D_{65}
172 and a 10° observer as references. Color data are provided as CIEL*a*b*
173 coordinates, which define the color in a three-dimensional space. Color analyses
174 were run in 3 batches of 25 fruits, making a total of 75 fruits per treatment.

175 2.3.3. Oil content and fatty acids

176 Oil was extracted by sonication using a 1 L ultrasonic Selecta bath model
177 3000512 JP (Barcelona, Spain). A 2 g of ground olive flesh was mixed with 3 mL of
178 cyclohexane and the mixture was sonicated at room temperature for 3 h. Then, the
179 mixture was centrifuged, and the oil was recovered after the evaporation of the
180 cyclohexane in a nitrogen stream.

181 The fatty acids methyl esters (FAMEs) were prepared, identified, and
182 quantified using the method recently described by Cano-Lamadrid, et al., (2015).

183 2.3.4. Mineral analysis

184 Approximately 1 g of milled table olive were digested, for 3 h a temperature
185 below 130ºC, in a multi-place digestion block, Selecta Block Digest 20 (Barcelona,
186 Spain) after the addition 5 mL of concentrated, 65% (w/v), HNO_3 (Carbonell-
187 Barrachina, García, Sánchez-Soriano, Aracil, & Burló, 2002). Samples were left to
188 cool down to room temperature, transferred to volumetric flask and dilutions 1:10
189 and 1:50 were prepared using ultrapure deionized water, 18 MΩ (Milli-Q® system,
190 Millipore Corporation, Madrid, Spain).

191 Determination of macro-nutrients (Ca, Mg, and K) and micro-nutrients (Cu,
192 Fe, Mn, and Zn) in previously mineralized samples was performed using a Unicam
193 Solaar 969 atomic absorption-emission spectrometer (Unicam Ltd., Cambridge,
194 U.K.). All minerals were analyzed using atomic absorption except K that was
195 measured using atomic emission.

196 In each analytical batch, at least one reagent blank and one spike were
197 included to assess precision and accuracy for chemical analysis. Calibration curves
198 were used for the quantification of minerals and showed good linearity ($R^2 \geq 0.999$).
199 Analyses were run in triplicate.
200 2.3.5. Sugars and organic acids
201 Organic acids and sugars were quantified according to Sánchez, Calín-
202 Sánchez, Carbonell-Barrachina, Melgarejo, Hernández, & Martínez (2014). Briefly,
203 for each sample, 2 g of table olives were homogenized in 5 mL of 50 mM phosphate
204 buffer pH= 7.8. The mixture was centrifuged at 10000 g for 20 min at 4ºC (Sigma
205 3–18K, Osterode and Harz, Germany). Then, 1 mL of supernatant was filtered
206 through a 0.45 µm filter and injected into a Hewlett-Packard HPLC series 1100
207 (Wilmington Del., U.S.A.). The elution buffer consisted of 0.1% phosphoric acid
208 with a flow rate of 0.5 mL min^{-1}. Organic acids were isolated using a Supelco
209 column (SupelcogelTM C-610H column 30 cm × 7.8 mm) and Supelguard (5 cm x
210 4.6 mm, Supelco, Inc., Bellefonte, PA) and absorbance was measured at 210 nm
211 using a diode-array detector (DAD). These same HPLC conditions were used for the
212 analysis of sugars; however, the detection was conducted using a refractive index
213 detector (RID). Standards of organic acids (phytic, ascorbic, citric, malic, tartaric,
214 quinic, shikimic, lactic, and oxalic acids) and sugars (glucose, fructose, sucrose,
215 sorbitol, maltitol, and glycerol) were obtained from Sigma (Poole, Dorset, UK).
216 Calibration curves, obtained by triplicate injection of standard solutions, were
217 conducted and showed good linearity ($R^2 > 0.999$). Results were expressed in g kg^{-1}
218 fw (fresh weight) of table olives.
219 2.3.6. Antioxidant activity (ABTS, DPPH and FRAP methods) and total
220 polyphenols
221 For the antioxidant activity determination, a methanol extract was prepared
222 for each sample to be analyzed. Approximately 0.5 g of freeze-dried table olives
223 were mixed with 10 mL of MeOH/water (80:20, v/v) + 1 % HCl, and the mixture
224 was sonicated at 20 ºC for 15 min and left for 24 h at 4 ºC. 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 $DPPH^{\bullet}$ radical (2,2-diphenyl-1-picrylhydrazyl) method, as described by Brand-Williams, Cuvelier, & Berset (1995) 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 $DPPH^{\bullet}$ 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, Mercers Row, 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, Pellegrini, Proteggente, Pannala, Yang, & Rice-Evans (1999) and Benzie & Strain (1996), 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, Mercers Row, Cambridge, UK). 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 ($R^2 \geq 0.998$). Results were expressed in mmol Trolox kg^{-1} 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, Cano, & Acosta (2001). 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 Folin–Ciocalteu colorimetric method described previously by Gao, Ohlander, Jeppsson, Björk, & Trajkovski (2000). 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

254 temperature. The absorbance of the resulting blue color solution was measured at
255 765 nm using an UV-Visible spectrophotometer (Helios Gamma model, UVG 1002E,
256 Mercers Row, Cambridge, UK). Quantification was done with respect to the
257 standard curve of gallic acid. The results were expressed as gallic acid equivalents
258 (GAE), g kg^{-1} fw (fresh weight).

259 2.4. Sensory Analyses

260 2.4.1. Sensory evaluation with trained panel

261 Eight trained panelists (aged 30 to 55 years; 4 female and 4 male) from the
262 department of Agro-Food Technology (UMH) participated in this study. Samples
263 were served into odor-free, disposable 90 mL covered plastic cups, at room
264 temperature and were coded using 3 digit numbers. Unsalted crackers and
265 distillated water were provided to panelists to clean their palates between samples.

266 After careful study of the lexicon developed by the International Olive Oil
267 Council, IOOC (2011), the panel evaluated only the following attributes: (flavor)
268 green-olive flavor, sourness, bitterness, saltiness, sweetness, and aftertaste; and
269 (texture) hardness, crunchiness, fibrousness, and pit removal. The panel used a
270 numerical scale for quantifying the intensity of the olives attributes where 0
271 represents none and 10 extremely strong with 0.5 increments. This scale is the
272 most logical and easy-to-use by Spanish panelists, as previously stated by Galindo
273 et al. (2015).

274 2.4.2. Sensory evaluation with consumer panel

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

281

282 2.5. Statistical Analyses

283 Results are provided as the mean ± standard error. First, data was subjected
284 to one-way (factor= RDI treatment) analysis of variance (ANOVA) and later data
285 was also subjected to Tukey's multiple-range test to compare the means.
286 Differences were considered statistically significant at $p<0.05$. All statistical
287 analyses were performed using StatGraphics Plus 5.0 software (Manugistics, Inc.,
288 Rockville, MD).

289 # 3. RESULTS AND DISCUSSION

290

291 ## 3.1. Field experiment and tree parameters

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

299 The irrigation of control trees (T0) was around the ETc needs, except during
300 stage I in which the rainfall was considered. In this treatment (T0), TGR presented
301 the maximum values during stage I (15.1 µm day^{-1}), which corresponded with the
302 period of vegetative growth. During stage II, pit hardening, vegetative growth is
303 stopped, even in full irrigated conditions, and TGR was around 0 (1.6 µm day^{-1})
304 until the end of the season (3.8 µm day^{-1} at stage 3). T1 (RDI-1) was scheduled
305 only with TGR data, and this irrigation scheduling led to a water saving of around
306 44% in comparison to control. TGR values of T1 indicated that trees water status
307 were also under full irrigated conditions, with values being equivalent to those of
308 the T0 trees, 19.0, 4.7, and 7.4 µm day^{-1} at stages I, II, and III, respectively.
309 Finally, T2 (RDI-2) presented the greatest reduction in irrigation (71% as compared
310 to control), and this water saving was produced along the season. However, such
311 irrigation reduction affected tree water status leading to lower TGR values during
312 stages I and II. The greater values of TGR in this treatment than control and T1
313 during stage III are related with the need to recover the water plant status.

314 The effect of RDI in the olive tree yield in an isolated season is not always
315 clear, especially because of the biennial cycles of olive trees. In this particular
316 season, although no statistically significant differences were found, there was a

317 clear trend of yield reduction in T2 (6.7 t ha^{-1}) in comparison to T1 (8.2 t ha^{-1}) and
318 T0 (9.0 t ha^{-1}).

319 3.2. Morphological and physico-chemical analysis

320 3.2.1. Weight and size

321 Table 2 shows the results of the weight and size (longitudinal and equatorial
322 diameters) of "Manzanilla" tables olives as affected by regulated deficit irrigation
323 (RDI) treatments. It can be observed that T2 olives had highest weight ($p<0.001$)
324 of all treatments, 4.35 g, although their weight was statistically equivalent to that
325 of T0 fruits. It is generally admitted that the weight of "Manzanilla" table olives
326 must be in the range from 2.1 to 4.9 g to have an appropriate or good size (IOOC,
327 2014); the experimental values found in this study were at the upper part of this
328 range, specifically between 4.0 and 4.4 g. The working hypothesis of all RDI studies
329 is that the treatments will slightly decrease the yield but will improve the quality of
330 the fruits (Cano-Lamadrid, et al., 2015; Carbonell-Barrachina et al., 2015). This is
331 exactly the case observed in the table olives weight; a slight reduction on the yield
332 makes that the fruits of the treated trees have more nutrients available for them
333 and will grow bigger, and have a higher weight, as observed. However, the size of
334 the table olives as described by the longitudinal (d_l, length) and equatorial (d_e,
335 thickness) diameters was not significantly affected by the RDI treatments;
336 however, a trend can be found in which T2 fruits had the highest values of both
337 diameters, although all values were statistically equivalent. The ratio d_l/d_e took
338 values of 1.22, 1.14, and 1.16, respectively, meaning that T1 and T2 fruits were
339 more rounded than those of T0.

340 3.2.2. Color

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

344 The color of T2 olives was lighter (L*) and had higher green intensity (a*)
345 than control (T0) and T1 fruits. In a previous study with table olives "Manzanilla de

346 Sevilla" it was concluded that water stressed fruits had higher intensity of yellow
347 color (up to 10 units) than control ones (Cano-Lamadrid, et al., 2015). Besides,
348 Pastor et al. (1999) reported a decrease in the intensity of the yellow color in
349 Arbequina olive oil when olive trees were stressed. In any case, the differences in
350 color among the RDI treatments in this study can be considered of limited real
351 significance because changes of less than 2 units will not cause noticeable visual
352 differences (Navarro et al., 2011; Galindo et al., 2015).

353 3.2.3. Dry matter and oil contents

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

363 As regard to the oil content, the highest value (404 g dw kg^{-1} fw) was found
364 in table olives grown under moderate RDI conditions (T1). Additionally, no
365 statistical significant differences were found between the oil contents of fruits from
366 the other two treatments, T0 and T2. According to Lavee, Hanoch, Wodner, &
367 Abramowitch (2007) a moderate water stress will lead to an increased
368 accumulation of oil in Muhasan olives grown in Israel.

369 The trend shown in oil content completely agreed with the initial hypothesis
370 sustained in our experiments. This is, under soft water stress (T1), the plant or tree
371 metabolism seems to get activated resulting in a highest accumulation of oil and
372 DMC, as previously other authors concluded in table olives Cano-Lamadrid, et al.,
373 2015) or pistachios (Carbonell-Barrachina, et al., 2015). However, under a more
374 severe water stress or a longer period of stress, the plant metabolism is damaged

375 and after an initial increase in the accumulation of oil and DMC, the contents start
376 to be reduced, as seen in T2 olives.

377 3.2.4. Fatty acids

378 The relative abundance of fatty acids observed in table olives followed the
379 order: C18:1 (mean of all treatments 73.1%) >> C16:0 (17.0%) > C18:2 (4.1%)
380 ≈ C18:0 (3.6%) > C16:1 (1.6%) > C20:0 (0.4%) ≈ C20:1 (0.2%) (Table 3).
381 Linoleic (C18:2) and oleic (C18:1) acids were significantly affected by the RDI
382 treatments (Table 3). The most important result is that severe RDI conditions (T2)
383 significantly increased the content of linoleic acid, an ω-6 fatty acid, which must be
384 ingested through food due to the fact that human body is not able of produce it and
385 therefore is called "essential fatty acid" (Lunn & Theobald 2006; FAO 2010). As a
386 result of the changes in linoleic and oleic acid, T2 table olives experienced a
387 significant increase of PUFAs (polyunsaturated fatty acids) and a simultaneous
388 decreased of MUFAs (monounsaturated fatty acids), with this being important
389 because PUFAs are beneficial to human health (FAO, 2010). A similar trend, but
390 only valid for moderate stressed Manzanilla de Sevilla olives was recently reported
391 by Cano-Lamadrid et al. (2015).

392 3.2.5. Minerals content

393 Only the content of the macro-nutrient calcium (Ca) was significantly affected
394 by the RDI treatments; with the highest content being found in fruits from the
395 control trees, T0 (Table 4). The contents of the macro-nutrients followed the
396 order: Ca (mean of all treatments 2.4 g kg^{-1}) > K (1.6 g kg^{-1}) > Mg (0.4 g kg^{-1}).
397 Water stress caused a lower accumulation of Ca in T1 and T2 fruits, this is in water
398 stressed olives; it is important to mention that Ca is taken up by the plant and
399 transported primarily through the xylem, along with water (Giliham, Dayod,
400 Hocking, Xu, Conn, Kaiser, Leigh & Tyerman, 2011). Therefore, the absorption of
401 Ca is directly related to plant transpiration; besides, Ca follows the transpiration
402 stream and consequently for this mineral is difficult to reach plant organs with low
403 transpiration rate, such as fruits (Giliham, et al., 2011). Sodium (Na) was not

404 analyzed because is one of the major ingredients used during the processing of
405 table olives.

406 Table olives are a good source of iron (Fe), with the contents of the studied
407 micro-nutrients following the order: Fe (mean of all treatments 11.8 mg kg^{-1}) > Cu
408 (8.0 mg kg^{-1}) > Zn (5.0 mg kg^{-1}) ≈ Mn (4.5 mg kg^{-1}). The irrigation treatments
409 affected the contents of two of these minerals, Zn and Mn; in both cases, the
410 higher the water stress, the lower the minerals contents.

411 3.2.6. Sugars and organic acids

412 Only two sugars (maltitol and glycerol) and two organic acids (phytic and
413 lactic acids) were identified and quantified in "Manzanilla" table olives (Table 5).
414 The only significant effect ($p < 0.05$) of the RDI treatments on the contents of sugars
415 and organic acids, was a reduction of the content of phytic acid [known as inositol
416 hexakisphosphate (IP6)] in T1 and T2 fruits (mean of 6.8 g kg^{-1} fw) as compared to
417 control fruits (14.7 g kg^{-1} fw). Recent investigations have begun to focus on
418 possible beneficial physiological/health effects of food phytates, which until few
419 years were mainly considered as anti-nutrient (Urbano, López-Jurado, Aranda,
420 Vidal-Valverde, Tenorio & Porrs, 2000). The possible beneficial effects of food
421 phytates include lowering of serum cholesterol and triglycerides and protection
422 against certain diseases such as cardiovascular diseases, renal stone formation, and
423 even certain types of cancers (Thompson, 1993; Zhou & Erdman, 1995; Graf,
424 1983). The absence of reducing sugars in table olives was expected because they
425 are major substrates of the lactic fermentation (the only typical spontaneous lactic
426 process followed in Spanish-style green olives).

427 3.2.7. Antioxidant activity and total polyphenols

428 There are different methods for evaluating the antioxidant activity (AA) of
429 foods. This variety of methods is due to the fact that none of them is able to
430 determine exactly the total antioxidant capacity of a product. The measured AA of a
431 sample depends on methodology and on free radical generator or oxidant in the
432 measurement (Cao, Alessio, & Cutler, 1993). Electron-transfer-based assays

433 (ABTS, FRAP, and DPPH) measure the capacity of an antioxidant in the reduction of
434 an oxidant which changes colour when reduced. However, there are differences
435 among them; for instance, ABTS measures both hydrophilic and lipophilic AA, while
436 DPPH only considers lipophilic compounds (Kuskoski, Asuero, Troncoso, Mancini-
437 Filho, & Fett, 2005). For this reason, the antioxidant activity of "Manzanilla" table
438 olives was evaluated using three different analytical methods: ABTS, DPPH, and
439 FRAP (Table 6). The AA and TPC were not significantly affected ($p > 0.05$) by the
440 RDI treatments. The total polyphenols content found in table olives (5.28 g GAE kg^{-1}
441 fw, mean value for all treatments) was higher than that previously reported in the
442 flesh of table olives by Boskou et al. (2006), who reported values ranging from 0.8
443 to 1.7 g caffeic acid kg^{-1}. These authors also identified oleanolic acid, hydroxyl-
444 tyrosol, and tyrosol as the main polyphenols present in Greek table olives. Table
445 olives are highly consumed by the Mediterranean population. The consumption of
446 20 g of table olives (approximately 5 units) provides about 100 mg of polyphenols.
447 Taking into account these results, it can be concluded that Spanish table olives are
448 a very good source of polyphenols and can help in the prevention of many health
449 diseases.

450 3.3. Sensory Analysis

451 The satisfaction degree of 100 Spanish consumers on "Manzanilla" table olives
452 was not affected at all by the RDI treatments (Table 7); neither the global
453 satisfaction degree nor any of the key attributes were affected. T1 olives got the
454 highest values of: (i) typical flavor of green table olives (6.8), and (ii) what it is
455 more important of global satisfaction degree (6.8); however, the differences with
456 the other treatments were not statistically significant. The values of the consumers
457 scores for their satisfaction degree regarding these two parameters (table olive
458 flavor and global) for T0 and T2 fruits had similar values (6.5 and 6.3,
459 respectively). In affective tests consumers normally use only the central part of the
460 scale avoiding the use of extreme values; consequently, the value of 6.8
461 (remember that 7 is "like moderately") obtained by T1 olives for the global

462 satisfaction degree indicate that Spanish consumers really liked T1 “Manzanilla”
463 table olives. Perhaps the number of consumers used, 100, was not high enough to
464 show significant differences among the RDI treatments; this is a topic that will
465 require further research in national and international markets.

466 Table 8 shows that RDI significantly affected several of the key sensory
467 attributes used to describe the quality of “Manzanilla” table olives; however, several
468 attributes were not affected and presented the following mean intensity values:
469 bitterness (5.7), sourness (2.4), sweetness (1.4), crunchiness (7.4), and
470 fibrousness (2.0). One thing that was highlighted by the trained panel while
471 evaluating table olives was that control fruits (T0) had pits which were easier to
472 remove from the edible portion (8.0) than other fruits (T2 = 6.8, and T1 = 7.7). It
473 is possible that the higher water content of control olives helped panelists in
474 removing the stone of these fruits. The most important finding was that T1 fruits
475 had the highest intensities of saltiness (5.8), green-olive flavor (7.9), aftertaste
476 (6.4), and hardness (7.9). It is possible that these higher intensities of T1 olives
477 were due, at least in part, to the production of a thick skin due to the limited water
478 availability (Patumi, d`Andria, Marisilio, Fontanazza, Morelli & Lanza 2002). On the
479 other hand, T2 olives had the lowest intensities of the previous attributes (saltiness,
480 green-olive flavor, aftertaste, and hardness). Finally, the trend shown in descriptive
481 sensory of “Manzanilla” table olives agreed with the initial hypothesis of our study
482 (under soft water stress, T1, the plant metabolism will be activated while under
483 more severe conditions, T2, the metabolism will be damaged.

4. 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 color (a*), and presented significantly lower contents of phytic acid and calcium as compared to control olives. In general, T1 table olives were characterized 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 color, 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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626 Table 1

627 Irrigation and tree parameters [applied water (AW, mm), yield (t ha^{-1}), and trunk
628 growth rate (TGR, µm day^{-1})] of "Manzanilla" olive trees as affected by regulated
629 deficit irrigation treatment.

630

Irrigation Parameter	Stage		
	I	II	III
ETc (mm)	308 a[a]	181 b	70 c
	Irrigation Treatment		
Parameter/ 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

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

634 Table 2

635 Morphological parameters and CIEL* a* b* coordinates of “Manzanilla” table olives

636 as affected by deficit irrigation treatment.

637

Parameter[a]	ANOVA[b]	T0	T1	T2
Fruit weight (g)	***	4.20 ab[c]	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

638 [a] The number of replications for the analysis of weight, size, instrumental color, oil

639 content, and dry matter content (DMC), were 100, 100, 75, 3 and 5 respectively.

640 [b] NS = not significant at $p< 0.05$; *, **, and ***, significant at $p< 0.05$, 0.01, and

641 0.001, respectively.

642 [c] Values followed by the same letter, within the same row, were not significantly

643 different ($p< 0.05$), according to Tukey’s least significant difference test.

644 Table 3

645 Oil content (g kg $^{-1}$ dw) and fatty acids (% of total area) of “Manzanilla” table olives
646 as affected by deficit irrigation treatment.

647

Parameter	ANOVA[a]	T0	T1	T2
Oil content (g kg $^{-1}$ dw)	***	261 b[b]	404 a	278 b
C16:1 (%)	NS	1.69	1.52	1.57
C16:0 (%)	NS	16.9	16.9	17.2
C18:2 (%)	**	2.61 c	3.89 b	5.82 a
C18:1 (%)	*	74.4 a	73.6 a	71.4 b
C18:0 (%)	NS	3.71	3.47	3.54
C20:1 (%)	NS	0.25	0.17	0.12
C20:0 (%)	NS	0.49	0.43	0.37
SFA[c] (%)	NS	21.1	20.8	21.1
MUFA[c] (%)	NS	76.3	75.3	73.1
PUFA[c] (%)	**	2.61 c	3.89 b	5.82 a
(MUFA+ PUFA)/SFA[c]	NS	3.74	3.81	3.74

648 [a] NS = not significant at p< 0.05; *, **, and ***, significant at p< 0.05, 0.01, and
649 0.001, respectively.

650 [b] Values (mean of 3 replications) followed by the same letter, within the same row,
651 were not significantly different (p< 0.05), according to Tukey’s least significant
652 difference test.

653 [c] SFA: Saturated fatty acids (C16:0, C18:0, and C20:0); MUFA: Monounsaturated
654 fatty acids (C16:1, C18:1, and C20:1); PUFA: Polyunsaturated fatty acids (C18:2).

655 Table 4

656 Minerals content of “Manzanilla” table olives as affected by deficit irrigation
657 treatment.

658

Parameter	ANOVA[a]	T0	T1	T2
Macro-elements (g kg^{-1} dw)				
Calcium (Ca)	***	2.4 a[b]	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

659 [a] NS = not significant at $p< 0.05$; *, **, and ***, significant at $p< 0.05$, 0.01, and
660 0.001, respectively.

661 [b] Values (mean of 3 replications) followed by the same letter, within the same row,
662 were not significantly different ($p<0.05$), according to Tukey’s least significant
663 difference test.

664 Table 5

665 Sugars and organic acid profiles of Manzanilla olives as affected by deficit irrigation
666 treatment.

667

Parameter	ANOVA[a]	T0	T1	T2
Sugars (g kg^{-1} fw)				
Maltitol	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[b]	6.09 b	7.46 b
Lactic acid	NS	1.62	1.63	1.63

668 [a] NS = not significant at $p< 0.05$; *, **, and ***, significant at $p< 0.05$, 0.01, and
669 0.001, respectively.

670 [b] Values (mean of 3 replications) followed by the same letter, within the same row,
671 were not significantly different ($p<0.05$), according to Tukey's least significant
672 difference test.

673 Table 6

674 Antioxidant activity (mmol Trolox kg^{-1} fw) and total polyphenols content (mg GAE

675 kg^{-1} dw) of "Manzanilla" table olives as affected by deficit irrigation treatment.

676

Parameter	ANOVA[a]	T0	T1	T2
ABTS (mmol Trolox kg^{-1} fw)	NS	13.4	13.2	13.4
DPPH (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

677 [a] NS = not significant at $p< 0.05$; *, **, and ***, significant at $p< 0.05$, 0.01, and

678 0.001, respectively.

679 [b] Values are the mean of 3 replications.

680 Table 7

681 Affective sensory analysis of "Manzanilla" table olives as affected by deficit
682 irrigation treatment. Consumers used a 9-point hedonic scale, where 1 = dislike
683 extremely, 5 = neither like nor dislike, 9 = like extremely.

684

Parameter	ANOVA[a]	T0	T1	T2
Fresh table olive flavor	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
Aftertaste	NS	6.4	6.4	6.2
GLOBAL	NS	6.5	6.8	6.3

685 [a] NS = not significant at $p < 0.05$; *, **, and ***, significant at $p < 0.05$, 0.01, and
686 0.001, respectively.

687 [b] Values are the mean of 100 consumers.

688 Table 8

689 Descriptive sensory analysis of "Manzanilla" table olives as affected by regulated
690 deficit irrigation treatment. Trained panelists used a scale from 0 = no intensity to
691 10 = extremely strong intensity.

692

Parameter[a]	ANOVA[b]	T0	T1	T2
FLAVOR				
Saltiness	**	4.8 b[c]	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 flavor	*	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

693 [a] Attributes included in this profile are based on IOOC (2011);

694 [b] NS = not significant at $p < 0.05$; *, **, and ***, significant at $p < 0.05$, 0.01, and
695 0.001, respectively.

696 [c] Values (mean of 10 trained panelists) followed by the same letter, within the same
697 row, were not significantly different ($p < 0.05$), according to Tukey's least significant
698 difference test.