Antioxidants (carotenoids and phenolics) profile of cherry tomatoes as influenced by deficit irrigation, ripening and cluster

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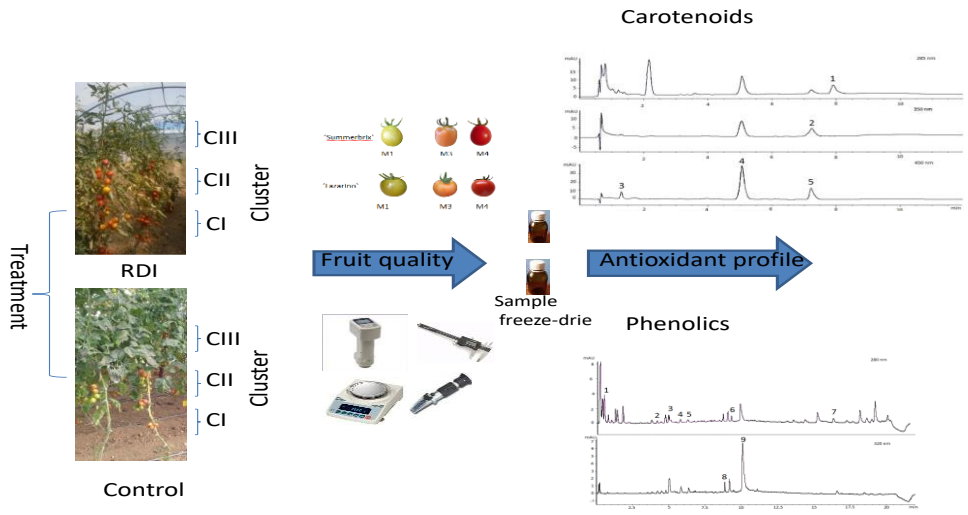
Abstract

The purpose of this study was to assess the relationship between the effect of regulated deficit irrigation, cluster, developmental stages and two seasons (autumn 2015 and spring 2016) on the commercial and functional quality (carotenoids and plenolics levels) in 'Lazarino' and 'Summerbrix' tomatoes. Autumn had a positive effect on the commercial quality, with larger fruits (22% in 'Summerbrix'; 26% in 'Lazarino') and higher soluble solids (16% in 'Summerbrix'; 12% in 'Lazarino'). Total carotenoids did not change significantly with irrigation and variety while total phenolics did with the cluster and season. In most cases, the main amounts of carotenoids and phenolic were found were found in the higher cluster and carotenoids in ripe fruit. Thus, irrigation of such varieties could be reduced drastically (ca. 80%) without affecting considerably the overall quality of their fruits (changes not greater than 30%).

Graphical abstract



***Key words**:* functional foods, antioxidant compounds, lycopene, phytoene, chlorogenic acid, cherry tomatoes, water potential

Highlights

- Deficit irrigation can be used to save water for the cultivation of cherry tomatoes.
- In some cases the treatment affected the quality parameters studied.
- The parameters were affected frequently with plant height, ripening and seasons.
- 80% water can be saved without causing marked changes in the parameters studied.

1. INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is an important vegetable crop in much of the world. Cherry tomatoes are characterized by their small size and are being increasingly demanded. Despite their smaller size compared to other tomato genotypes, their nutritional value can be higher (Figás, et al., 2015). The tomato fruit contains a complex mixture of nutrients and other compounds of nutritional interest including carotenoids, flavonoids and other phenolic compounds, vitamins and minerals (Kimura & Rodriguez-Amaya, 2002; Meléndez-Martínez, Fraser, & Bramley, 2010). Tomato quality is the sum of quality attributes of different nature. Thus, it does not only includes weight, shape, colour, soluble solids, sugar and organic acid (parameters much related to the commercial quality), but also other compounds of nutritional interest and storage characteristics (Wang, Kang, Du, Li, & Qiu, 2011), among others.

The main carotenoids in tomato are lycopene, phytoene, phytofluene, β-carotene and lutein, the fruits also containing diverse phenolics as gallic acid, p-hydroxybenzoic acid, chlorogenic acid, caffeic acid, p-coumaric acid and quercetin (Stinco, et al., 2013; Meléndez-Martínez, Fraser, & Bramley, 2010). Indeed tomatoes are one of the best known dietary sources of the colourless carotenoids phytoene and phytofluene, which have not been extensively studied and are attracting much attention recently (Meléndez-Martínez, Mapelli, Benítez, & Stinco, 2015; Meléndez-Martínez, Stinco, Liu, & Wang, 2013). Both carotenoids and phenolics attract much attention as they may have health-promoting properties (Wang X.-D. , 2012; Shadini & Ambigaipalan, 2015; Meléndez-Martínez, et al., 2013). The biosynthesis of these compounds is dependent on many factors, like the genotype, growth conditions, developmental stage, environmental conditions and abiotic

and biotic stress (Wang, Kang, Du, Li, & Qiu, 2011; Meléndez-Martínez, Fraser, & Bramley, 2010; Liu, Shao, Zhang, & Wang, 2015).

From an agricultural point of view, the lack of water is an important factor to address as it represents a severe environmental problem in dry regions worldwide that is aggravated with non-agricultural users, for instance in tourist areas in summertime (Cano-Lamadrid, Girón, Pleite, Burló, Corell, & Moriano, 2015). Furthermore, reduced irrigation can have an impact in the overall fruit quality, as tomato has a high requirement of water (Cantore, et al., 2016). Currently, the efficient uses of water include regulated deficit irrigation as a strategy of water-saving. Water deficit usually leads to decreased photosynthesis, plant growth and crop productivity and beneficial effects on some fruit quality parameters, like for instance increased antioxidant compound levels and higher sugar accumulation (Ripoll, Urban, Brunel, & Bertin, 2016). It is thought that water deficit increases the temperature in the plant and that carotenoids can help dissipate excess heat in chloroplasts while phenolic compounds can be important in plant stress as signaling molecules and antioxidants (Atkinson, Dew, Orfila, & Urwin, 2011). On the other hand, there are also reports indicating that water stress may reduce the acid, sugar, carotenoid and phenolic content and increase fruit quality (Ripoll, Urban, Brunel, & Bertin, 2016).

Considering the high water demand of tomato and that there are very few studies addressing how cluster affects tomato fruit quality the main purpose of this study was to determine the effect of regulated deficit irrigation and cluster (CI: first cluster; CIII: third cluster, CV: fifth cluster) on quality parameters (weight, soluble solids, colour, carotenoids and plenolic compounds) of the fruits. To have a wider picture, other factors like the season (autumn 2015 and spring 2016) and the developmental stage (M1: 25% of fruit red; M3: 75% of fruit red; M4: 100% of fruit red) were also considered. For this purpose two

cherry varieties (Summerbrix and Lazarino) were studied, because ‘Lazarino’ was more susceptible to regulated deficit irrigation, while ‘Summerbrix’ more resistant. This was observed in our preliminary studies during the spring in 2015.

2. MATERIALS AND METHODS

2.1 Reagents and standards

Chemical compounds studied in this article: Methanol (PunChem CID: 887), trichloromethane (PumChem CID: 6212) and hydrochloric acid (PumChemCID: 313) were of analytical grade and purchased from Labscan (Dublin, Ireland). HPLC-grade methanol, HPLC-grade acetonitrile (PumChemCID: 6342), HPLC-grade ethyl acetate (PumChemCID: 8857), formic acid (PumChemCID: 284) (Barcelona, Spain). Water was purified in a NANOpureDlamond^{TM} system (Barnsted Inc., Dubuque, IO). β-Carotene (PumChem CID: 5280489) was purchased from Sigma-Aldrich (Taufkirchen, Germany) and lutein and lycopene were obtained from appropriate sources as described elsewhere (Meléndez-Martínez, Vicario, & Heredia, 2007; Meléndez-Martínez, Stinco, Liu, & Wang, 2013). Quercetin (PumChem CID: 5280804), p-coumaric acid (PumChem CID: 637542), gallic acid (PumChem CID: 370) and chlorogenic acid (PumChem CID: 1794427) were purchased from Sigma-Aldrich (Madrid, Spain).

2.2 Plant materials

Two red tomatoes *(Solanum Lycopersicum* L.*)* cherry type varieties (‘Lazarino’ and ‘Summerbrix’) with indeterminate growth were studied. The seeds were provided by Fitó from Spain. ‘Summerbrix’ was a pear small variety and ‘Lazarino’ a round variety. These varieties were grown for 30 days in a nursery seedling and these were transplanted into soil when the seedlings had developed three or four true leaves. They were tested in a greenhouse production at Escuela Técnica Superior de Ingeniería Agronómica (E.T.S.I.A.)

at the Universidad de Sevilla (Seville, South Spain, 37º21'09.71" Lat. N, 5º56'19.13" Long. W, 33 m a.s.l.) during autumn of 2015 (23rd September to 15th December) and spring 2016 (23rd February to 15th June). The transplants of cherry tomatoes were realized on September 23rd 2015 and February 3rd 2016. The plants were set at a distance of 50 cm between plants and 100 cm between rows Flowers were biologically pollinated with bumblebees (BioSur, Spain). Plants were trained and pruned, especially secondary stems and leafs, with the usual practices in tomato crop in greenhouse. A randomized complete-block design was used with 3 blocks per treatment and 21 plants per block. The tomato plants were grown on a soil having the following characteristics: average depth 30 cm; pH 8.11; organic matter oxidizable 2.50%; electric conductivity 1050.00 μS/cm; total nitrogen 0.25%; phosphorus 126.01 mg/Kg; calcium 0.73%; magnesium 0.25%; sodium 0.04% and potassium 0.13%.

The irrigation of the plants was done by dripping, with two daily cycles of irrigation that depended to crop evapotranspiration (ETc) of the plant. The regulated deficit irrigation was applied two weeks after transplantation. Treatments irrigation were: regulated deficit irrigation (RDI), with a threshold of -1 MPa of leaf water potential (82.7 mm of applied water in autumn and 84 mm in spring), and a control treatment with irrigation requirements determined according to daily crop evapotranspiration (ETc) calculated with the FAO Penman-Monteith method (Allen, Pereira, Raes, & Smith, 2006) (398.7 mm of applied water in autumn and 458,7mm in spring). The measurements performed on the growth were plant height, number of leaves, inflorescences and fruits, amount of water supplied, leaf water potential with pressure chamber (PMS Instrument Company, USA).

Harvesting of the tomatoes was made between January 8th to February 26th on 2015 and May 20th to June 9th on 2016. Fruits with different developmental (visual assessment on fruits colour) were harvested for the analysis (Figure 1).

Figure 1. Photographs of tomato at different developmental stages

147 Samples included fruits representative of seven plants, of three different experimental
148 blocks collected at three clusters (first, third and fifth cluster) and at three different
149 developmental stages. The developmental stages corresponded to fruits with 25% red
150 (M1), 75% red (M3) and 100% red (M4). Samples included a mix of Sixty-three tomato
151 fruits of each cluster and developmental stage, previously characterized. This mix was
152 divided into two samples for the quantification of functional quality. The seeds and inside
153 locular tissues were removed, cut and quickly frozen at -80 °C, before being freeze-dried
154 with a Cryodos system (Telstar, Japan). The dried samples were ground in a basic IKA A
155 11 mill, then stored in a dark glass bottle and hermetically sealed under nitrogen
156 atmosphere. The samples were stored in a freezer at -21 °C until their analysis.

157 **2.3 Physico-chemical analyses**

158 The measurements performed were equatorial and longitudinal diameter (cm), fresh weight
159 (W, expressed in grams), soluble solids (SS, expressed as °Brix), firmness and colour
160 parameters (L*, C_{ab}^*, h_{ab}). The soluble solids were measured using a Hand-refractometer
161 RHC-200ATC (Huake, China). The fruit firmness was analyzed using a PCE-PTR 200
162 Forge Gauce penetrometer (PCE-Inst., Spain) and the fruit colour was analyzed using a
163 CM-700d colorimeter (Minolta, Japan). For this purpose the whole visible spectrum (380 –
164 770 nm) was recorded with a bandwidth of 1 nm. The colour parameters corresponding to
165 the uniform colour space CIELAB were obtained directly from the apparatus. Illuminant
166 D_{65} and 10° observer were considered as references. The humidity was determined using
167 Dry Big oven (Selecta, Barcelona) with air circulation at 110ºC.

168 **2.4 Carotenoid analysis**

169 **Sample preparation**

170 Individual carotenoids were determined as described elsewhere (Borghesi, et al., 2011)
171 with slight modifications. Approximately 20 mg of homogenized freeze-dried powder were

used for the extractions. The powder was mixed with 250 µL of methanol, 500 µL of trichloromethane and 250 µL of MiliQ-water and then vortexed, sonicated for 2 min and centrifuged at 14 000x g for 3 min to remove the aqueous phase. After recovering the colored fraction, 500 µL of trichloromethane were added, and the mixture was vortexed, sonicated and centrifuged again. These operations were repeated until colour exhaustion. The organic coloured fractions were evaporated to dryness at a temperature below 30 °C in a vacuum concentrator and stored under N_2 at -20 °C until analysis.

Rapid-resolution liquid chromatography (RRLC)

The dry residue was re-dissolved in 40 µL of ethyl acetate prior to their injection in the RRLC system. The RRLC analysis was carried out using the method reported by Stinco et al. (2014) (Stinco, Benítez, Hernanz, Vicario, & Meléndez-Martínez, 2014) on an Agilent 1260 system equipped with a diode-array detector, C_{18} Poroshell 120 column (2.7 µm, 5 cm x 4.6 mm) (Agilent, Palo Alto, CA). The injection volume was 1 µL and the flow rate 1 mL/min at 30 ºC. The mobile phase consisted of acetonitrile (solvent A), methanol (solvent B) and ethyl acetate (solvent C) with the following linear gradient elution: 85% A +15% B, 0 min; 60%A +20%B, + 20%C, 5 min; 60%A+20%B+20%C, 7 min; 85% A+ 15% B , 9 min; 85% A + 15% B, 12 min. The chromatograms were monitored at 285, 350 and 450 nm with the open lab ChemStation software. Quantification was carried out by external calibration. The limits of detection (LOD) and quantification (LOQ) were calculated as three and ten times, respectively, the relative standard deviation of the analytical blank values calculated from the calibration curve, using Microcal Origin ver 3.5 sofware (OriginLab Corporation, Northampton, MA, USA). LOD and LOQ were established on the basis of signal to noise (S/N) ratio of 3 and 10, respectively. LODs ranged from 0.002 µg

195 in phytoene to 0.070 µg in lycopene. LOQs ranged from 0.007 µg to 0.232 µg (for
196 phytoene and lycopene, respectively). The samples were analyzed in duplicate.

197 Total carotenoids were calculated as the sum of individual carotenoids.

198 **2.5 Analysis of phenolic compounds**

199 **Sample preparation**

200 Approximately 0.5 g of freeze-dried material were extracted with 15 mL of acidified
201 methanol 0.1%, the mixture was vortexed and sonificated for 15 min, and centrifuged at
202 4190 g for 7 min at 4 °C; the supernatant was collected and the residue subjected to the
203 same process twice, using only 5 mL of acidified methanol 0.1%. The supernatant were
204 finally pooled. The extract was stored at -20 °C until analysis. The samples were analyzed
205 in duplicate.

206 **Chromatography analysis by UHPLC**

207 The extracts were filtered through Millipore membranes (0.45 µm pore, 15 mm diameter)
208 (Agilent Technologies, Spain). The UHPLC analyses were carried out on an Agilent 1290
209 chromatograph equipped with a diode-array detector (Agilent Technologies, Palo Alto,
210 CA. USA) and an Eclipse Plus C_{18} column (1.8 µm, 2.1 x 5mm). The injection volume was
211 5 µL, the flow 1 mL/min and the column was kept at 30 °C. The chromatograms were
212 monitored at 220-500 nm. The mobile phase consisted of 0.01% of formic acid in water
213 (solvent A) and acetonitrile (solvent B) with the linear gradient elution: 100% A, 0 min;
214 95%A + 5%B, + 20%C, 5 min; 50%A+50%B, 20 min; washing and re-equilibration of the
215 column, 22 min. The chromatograms were monitored at 280, 320 and 370 nm with the
216 open lab ChemStation software. Phenolic compounds were identified by comparing their
217 retention time and UV-vis spectra with those of standards. Quantification was carried out

by external calibration. LODs ranged from 0.006 µg in chlorogenic acid to 0.012 µg in *p*-hydroxybenzoic acid. LOQs ranged from 0.014 µg to 0.041 µg (for chlorogenic acid and *p*-hydroxybenzoic acid, respectively). LOD and LOQ were established on the basis of signal to noise (S/N) ratio of 3 and 10, respectively. All the extracts were injected twice and the concentration expressed in mg/100 g dry weight (DW).

Total phenolics were calculated as the sum of individual phenolic compounds.

2.6 Statistical analysis

Results are provided as the mean ± standard deviation. In order to study the effect deficit irrigation, developmental stages, clusters and their interactions on the different quality parameters of tomato, statistical differences were determined by analysis of variance (simple and factorial ANOVA). The STATGRAPHICS Centurion XVII software was used for statistical analyses.

3. RESULT AND DISCUSSION

In this study several growth parameters were observed. Thus, maximum values of plant height were 2.5 m, 29 leaves and 11 inflorescences were reported in autumn, while 2.3 m of plant height, 33 leaves and 11 inflorescences in spring. On the other hand, average values of 19 fruit in the CI, CIII and CV cluster in autumn, while 24 in the CI, 42 in the CIII and 47 in the CV cluster was observed.

3.1 Climate trend and irrigation variables

The integral thermal and light data during autumn 2015 and spring 2016 are summarized in Figure 2. The integral light showed decrease in autumn while in spring increased keeps similarity with the studied season. Contrastingly, the values found in the

present study for integral thermal, these keep relationship with those found in other studies (ranging from 3000 to 4400ºCd) for large varieties (Serrano, 2014)while higher values compared with others studied that reported 60 days for ripening time of round cherry varieties grown at 25 ºC in a glasshouse (Atkinson, Dew, Orfila, & Urwin, 2011). On the other hand, it was observed that high temperatures at the beginning of the vegetative development increased the thermal integral on the cultivation of tomato with decreased of days necessary for fruit develop, while low temperatures decreased thermal integral with increased of days required for fruit develop. These dates keep relation with other studies that presented similar conclusion (Klaring, Klopotek, Krumbein, & Schwarz, 2015).

Figure 2 . Integral thermal and light ….

The leaf water potential, in the vegetative development on the plant ranging from -0.6 to -0.2 MPa in autumn, while in spring -0.6 to -1.0 MPa, were observed. At harvest the leaf water potential in autumn was -0.6 MPa in the RDI treatment and -0.3 MPa in the control sample for ‘Summerbrix’, while -0.4 MPa and -0.3 MPa respectively for ‘Lazarino’. Although in spring, ‘Summerbrix’ showed values of leaf water potential of -0.8 MPa in the RDI treatment and -0.5 MPa in the control sample, while -0.7 MPa and -0.5 MPa respectively.

Plant water status during the experiment in ‘Summerbrix’ had 21% reduction of leaf potential water in autumn while in spring reduction of 27%. Although, ‘Lazarino’ in autumn had 14% of reduction to leaf potential water while 23% in spring. These data showed that water consumption depends on the variety, keeps similarity with other studies (Serrano, 2014), which suggest that water volume in crop tomato depend of variety and seasons.

3.2 Physico-chemical analyses

Data on the values of commercial fruit quality parameters (weight, soluble solids and colour) as a function of the factors studied are summarized in Table 1. On the other hand, average values between 29 to 38 mm, 37 to 44 mm, 89 to 94.6 % and 3.7 to 12.7 Kg/cm^2 for equatorial and longitudinal diameter, humidity and firmness respectively were observed in autumn. In addition, in spring average values between 27 to 36 mm, 37 to 46 mm, 92.7 to 96.9 % and 5.0 to 14.2 Kg/cm^2 for equatorial and longitudinal diameter, humidity and firmness respectively were observed. Thus, size, humidity and firmness changed as a function of the treatment, developmental stages, cluster, variety and season, except the humidity in developmental stages.

3.2.1 Weight

Fruit weight values in normal water regime (ranging from 17 to 32 g) were higher than the data reported in other studies for round cherry varieties (ranging from 3 to 8 g) (Figás, et al., 2015). These suggest that the growth conditions in this study were adequate for the growth in two varieties. In most cases, the weight of the control (well-irrigated) samples was higher than that of treated samples in both seasons. These results agree well with the data reported in other studies for small tomatoes in reduced, normal and none water regime (17.4; 16.6 and 12.7 g respectively) (Pernice, et al., 2010) and were expected, as it is accepted that the weight of the fruit in cherry varieties decreases with the water stress **(Ozbahce & Tari, 2010)**.

On the other hand, the cluster in most cases, in the case of ‘Summerbrix’, the highest weight values in the two seasons and the different developmental stages were observed in CIII cluster in both control and RDI samples. In the case of ‘Lazarino’ higher values were observed in the control samples in the two seasons. These data are similar to those reported

287 by other authors that studied weight in cherry tomatoes in function of the clusters height,
288 and did not show specify behavior (Choi, et al., 2016).

289 With regard to the developmental stages, significant effects were observed in
290 'Summerbrix' in the control and RDI samples, in all clusters, and in the two seasons.
291 'Lazarino' did not show statically significant differences in the CI cluster of autumn in the
292 control and RDI samples

293 Overall, the weights changed as a result of the treatment, cluster and developmental
294 stages in both seasons and varieties. The weight showed higher values in the CV cluster.
295 Thus, the treatment showed greater changes in spring with increments between 7 to 20 %.
296 Although, the cluster and developmental stages presented greater changes in autumn with
297 increments from 25 to 32% and 13 to 35 %, respectively was observed. In general highest
298 values of weight were observed in autumn. These data seem to indicate that the effect can
299 be dependent on the integral thermal with reduced cell division and cell expansion rate in
300 low temperatures, as suggested elsewhere (Klopotek & Klaring, 2014). Thus, in autumn
301 the weight is greater with 2900 ºCd and in spring it was lower with 2400 ºCd.

302 **3.2.2 Soluble solids**

303 In this study, cherry varieties in normal water regime showed similar values (4.7
304 and 8.6 ºBrix respectively) compared with other studies, which reported values ranging
305 from (7.6 to 7.7 ºBrix) of local cherry varieties from the Mediterranean region (Figás, et
306 al., 2015). In most case, in autumn, the soluble solids (SS) did not exhibit differences as a
307 result of the treatment, while in spring changed significantly. Thus, the SS values were
308 higher with the treatment. This showed greater changes in spring with increments from 3 to
309 17%. These observations agreed well with values reported elsewhere, showing values

ranging from 5.5 to 9.1 of SS in normal and reduced water regime respectively in small tomatoes (Pernice, et al., 2010). In this sense, there are previous studies reporting that soluble solids in tomato can increase with the treatment and that the effect depends on the variety (Beckles, 2012).

The SS increased with cluster in autumn showed higher values in the CV cluster, while in spring ‘Summerbrix’ showed some higher values in the CV cluster and ‘Lazarino’ in the CI and CIII clusters. These data are in good agreement with those reported by other authors, who described increases of the SS with the clusters of cherry tomato in spring in some cases (Choi, et al., 2016).

As expected, ‘Summerbrix’ had significant differences as a function of the developmental stages. The same was observed for ‘Lazarino’ in most cases. This showed greater changes in spring with increments from 8 to 30%. As it is common for tomatoes and many other fruits, the SS increased with the developmental stages. The data of the present study agree well with the findings of other authors, who reported increases of SS with the developmental stages (6.2 ºBrix in breaker tomatoes, 7.4 ºBrix in pink tomatoes and 8.5 ºBrix in red tomatoes) (Verheul, Slimestad, & Holta, 2015).

The SS changed as a result of the treatment, cluster and developmental stages in autumn and spring in two varieties. With regard to the season, higher values were observed in autumn with increments between 15 to 23% as a result of the cluster, these data contrasting with those of other authors, who found for pear cherry varieties that the SS in winter were lower than in spring (Wang, Kang, Du, Li, & Qiu, 2011) and others that reported higher concentrations at low temperatures (Klaring, Klopotek, Krumbein, & Schwarz, 2015).

3.2.3 Colour

The colour parameter values reported in the present study (ranging from 33.5 to 39.5 in the case of L*, 11.3 to 27.9 in the case of C^*_{ab} and 41.9 to 83.1 in the case of h_{ab}) were similar to those observed elsewhere in small tomatoes (ranging from 28.0 to 44.6 in the case of L*, 22.0 to 42.8 in the case of C^*_{ab} and 40.7 to 60.1 in the case of h_{ab}) (Gómez, Costa, Amo, Alvarruiz, & Pardo, 2001; Zhang, Liu, Zhang, Zhang, & Wang, 2014; Vinha, Alves, Barreira, Castro, & Costa, 2014). In general, L* and C^*_{ab} increased with the treatment, indicating that the deficit irrigation led to brighter and more vivid colours. On the other hand, h_{ab} decreased in 'Summerbrix' and in some cases in 'Lazarino', indicating a shift towards less reddish hues. These data are in good agreement with those reported in other studies indicating that appropriate deficit irrigation leads to lower values of hue angle (Wang, Kang, Du, Li, & Qiu, 2011). This allows improving the visual quality of the market.

In the present study, L* values were higher in spring in the CV cluster in both varieties with decrements between 1 to 9% as a function of the cluster; C^*_{ab} in spring for 'Summerbrix' and in autumn for 'Lazarino' presented higher values in the CV clusters with increments between 23 to 30%. Concerning h_{ab}, 'Summerbrix' showed higher values in the CV cluster in autumn and lower in the CI cluster in spring.

Concerning the developmental stages and the seasons, the colour parameters, in most cases showed significant differences both in autumn and spring in the control and RDI samples Thus, L* and h_{ab} values decreased as developmental progressed, but the contrary was observed in C^*_{ab}, which agrees well with the observations reported in other studies (Zhang, Liu, Zhang, Zhang, & Wang, 2014).

To sum up, C^*_{ab} changed as a result of the treatment, cluster and developmental stages in autumn and spring in two varieties while L* and h_{ab} only in autumn. On the other hand, did

not change as a result of the treatment in L* for 'Summerbrix' and h_{ab} for 'Lazarino'. Taken together, the data indicated that, from a commercial point of view, the values of weight and soluble solids were better in autumn, whereas the colour values were better for the market in spring. In addition, correlations between qualities parameters were observed. Inverse correlation between weight-C^*_{ab}, SS-L*, SS-h_{ab} and L*-h_{ab} with correlation coefficients values ranging from -0.3 to -0.6.

3.3 Individual carotenoids

Quantitative data pertaining to carotenoids are summarized in Table 2.

3.3.1 Phytoene

The levels of phytoene in normal water regime samples, ranged from 1.7 to 43.11 mg/100 g DW. Phytoene levels ranging from 0.6 to 0.7 mg/100 g FW have been reported for cherry tomatoes by other authors (Pernice, et al., 2010). In 'Summerbrix' the amount of this carotenoid increased between 36 to 47% in the CI cluster and decreased between 6 to 71% in the CV cluster with the treatment in autumn. In the case of 'Lazarino', the levels decreased between 1 to 63% in most cases. In spring, the varieties studied did not show a defined behavior. This is in agreement with other studies about water deficit, which did not show a particular pattern depending on the variety (Pernice, et al., 2010).

The amount of phytoene as a function of the cluster showed low values in the CI cluster in 'Summerbrix' in spring. 'Lazarino' showed high values in the CIII cluster in autumn and in the CV cluster in spring.

With respect to the developmental stages, higher values of phytoene in 'Summerbrix' in the developmental stage M4 were observed in all clusters in autumn with increments between 84 to 96%. On the other hand, 'Lazarino' exhibited higher values in the

380 developmental stage M4 in the CIII and CV cluster in autumn. However, no particular
381 behavior was observed in spring.

382 In summary, phytoene levels changed as a result of the treatment, the cluster and
383 developmental stages in autumn and spring in both varieties.

384 **3.3.2 Lutein**

385 The levels of lutein in control samples (ranging from 0.7 to 7.1 mg/100 g DW)
386 were similar compared with those reported in a study where the levels of carotenoids and
387 phenolics were studied in diverse tomatoes and wild relatives along development
388 (Meléndez-Martínez, Fraser, & Bramley, 2010). In most cases, the lutein levels changed
389 significantly with the treatment in all developmental stages. Overall, its levels decreased
390 with the treatment between 1 to 39%.

391 The lutein levels in 'Summerbrix' exhibited lower values in the CV cluster in autumn as a
392 result of the cluster, while 'Lazarino' higher values in spring. On the other hand, lutein in
393 'Summerbrix' decreased in 24% with the cluster in autumn, while in 'Lazarino' the levels
394 increased in 53% in spring. These data keep relationship with the integral light which
395 decreased in autumn and increased in spring (Figure 2).

396 Concerning the developmental stages, statically significantly differences in the amounts of
397 lutein were observed in all cases. These data contrasted with the conclusions of other
398 authors, who showed change with the maturity stages in some varieties (Zhang, Liu,
399 Zhang, Zhang, & Wang, 2014).

400 Altogether, the experiments indicated that the levels of lutein in 'Summerbrix' and
401 'Lazarino' presented change as a result of the cluster and developmental stages in both

seasons. However, ‘Lazarino’ in two seasons and ‘Summerbrix’ in spring did not change with the treatment.

3.3.3 Lycopene

The levels of lycopene in control samples (ranging from 3.1 to 259.5 mg/100 g DW) were higher compared with those reported in other two recent studies, in which they ranged between 100.0 and 370 µg/g DW (Choi, et al., 2014; Verheul, Slimestad, & Holta, 2015). In the case of ‘Summerbrix’ it led to higher values in the two seasons with increments between 16 to 61% with the treatment, while in the case of ‘Lazarino’ higher values were obtained in autumn and lower in spring. This observation is in agreement with the findings of other study in cherry varieties, in which it was concluded that the effect of the treatment was dependent of the variety (Sánchez-Rodríguez, Leyva, Constán-Aguilar, & Ruiz).

With respect the cluster, the levels increased in 54% in ‘Summerbrix’ in spring and in ‘Lazarino’ in 88% in autumn. ‘Summerbrix’ showed lower values of lycopene in the CI cluster in spring and ‘Lazarino’ higher values in the CV cluster in autumn as a function of the cluster. These results contrasted with others reported by other researchers. For instance decreased levels of lycopene with cluster have been described in round cherry varieties (Atkinson, Dew, Orfila, & Urwin, 2011). In the case of ‘Lazarino’ higher values of lycopene in the CV cluster in autumn were observed despite the lower temperatures of that period. In this sense, some authors suggested that low temperatures may be related to decreased lycopene biosynthesis (Dumas, Dadomo, Di-Lucca, & Grolier, 2003), however this variety could be affected by the presence of pests at the end of the crop. It keep similarly with dates of other authors that studied the effect of water stress and root-knot nematode-induce biotic stress on the levels of different parameters and different clusters,

426 who showed that lycopene in the CV cluster decreased in 2% with water stress and
427 increased with nematodes in 22% (Atkinson, Dew, Orfila, & Urwin, 2011; Liu, Shao,
428 Zhang, & Wang, 2015).

429 As expected, statically significant differences with respect to the developmental stages
430 were observed in both varieties. Increased levels of lycopene are one of the features of the
431 developmental of tomatoes as it is well known (Verheul, Slimestad, & Holta, 2015). This
432 changes being more pronounced in spring for 'Lazarino' with 83 % of increments and 98
433 % for 'Summerbrix' in autumn.

434 Overall, the levels of lycopene changed as a result of the cluster and developmental stages
435 in the two seasons. However, 'Summerbrix' in autumn and 'Lazarino' in spring did not
436 change with the treatment. In relation to this, it has been indicated that light and
437 temperature can affect the biosynthesis of lycopene (Jarquín, Mercado, Maldonado, &
438 Lopez, 2013).

439 **3.3.4 β-carotene**

440 The levels of β-carotene subjected to normal water regimen (ranging from 1.8 to
441 37.9 mg/100 g DW) were higher compared to those reported in other recent studies
442 (ranging between 10.0 and 25.1 µg/g DW) (Choi, et al., 2014; Verheul, Slimestad, &
443 Holta, 2015). The treatment in autumn decreased between 2 to 41 % the levels of the
444 compound in most cases, while in spring it led to decreased levels in the developmental
445 stage M1 and increased amounts in the developmental stages M3 and M4 in most cases.

446 With respect to the effect of the cluster, higher values were observed in the CV cluster
447 in autumn and spring. These observations do not contrast with those of others authors,

448 who reported that β-carotene decreased with the cluster from 0.87 to 0.57 mg/100 g FW
449 (Atkinson, Dew, Orfila, & Urwin, 2011).

450 'Summerbrix' exhibited higher values in the developmental stage M3 in autumn and
451 M4 in spring as a function of the developmental stages. Although, 'Lazarino' showed
452 higher values in the developmental stage M4. In the case of 'Lazarino' the amounts of
453 β-carotene increased in 54% with the developmental stages in both seasons in all
454 clusters while in the case of 'Summerbrix' 57 % in the RDI samples in spring.

455 In summary, β-carotene changed as function of the cluster and developmental stages in two
456 varieties and both seasons. However, 'Summerbrix' in autumn did not change with the
457 treatment.

458 **3.3.5. Total carotenoids**

459 The total carotenoids (TC) as a function of the treatment were found with
460 increments from 20%. In most cases, the treatment led to higher values of TCs in in both
461 seasons in 'Summerbrix' samples. Overall a similar effect was observed for 'Lazarino'
462 samples in autumn. Overall, in most case, the cluster height showed between 50 to 77%
463 increased of TCs. With respect to the developmental stages, as expected, the studied
464 varieties exhibited higher values in the developmental stage M4 in all cases with
465 increments between 68 to 94%. In general, lower levels of individual and total carotenoids
466 were observed in autumn. In relation to this, it is thought that low temperatures and short
467 photoperiod can decrease photosynthesis (Klopotek & Klaring, 2014; Gerszberg,
468 Hnatuszko-Konka, Kowalczyk, & Kononowicz, 2015).

469 Interestingly, direct correlation between quality parameters and individual carotenoids
470 were observed. Thus, weight with lutein and phytoene with lutein and lycopene. This
471 showed coefficient correlation from 0.3 to 0.8 was observed.

472 **3.4 Phenolics**

473 The major phenolic compounds studied were p-hydroxybenzoic acid (p-Hyd),
474 chlorogenic acid (Chlor), gallic acid (Galli), and quercetin (Quer). Quantitative data are
475 summarized in Table 3.

476 **3.4.1 p-Hydroxybenzoic acid**

477 p-Hydroybenzoic acid is a hydroxybenzoic acid derivative. No significant changes
478 were observed in the CI and CV cluster in autumn for 'Summerbrix' and spring for
479 'Lazarino' as a result of the treatment. In spring, important significant increases from 42%
480 were observed in the M1 and M4 stages of the CIII cluster in 'Lazarino'.

481 The cluster led the highest values in the CV cluster in autumn.. In spring, 'Summerbrix'
482 showed lower values in the CI cluster and 'Lazarino' higher values in the CV cluster in the
483 control samples. In most cases, p-Hyd in spring increased with the cluster between 24 to
484 65%.

485 In general, in most case, significant changes in the p-Hyd levels were observed for both
486 varieties as a function of the developmental stage. In summary, p-hydroxybenzoic changed
487 as a function of the cluster and developmental stages in both seasons in two varieties.
488 Although, 'Summerbrix' in both seasons did not show changed with the treatment.

489 **3.4.2 Chlorogenic acid**

Clorogenic acid in control samples (ranging from 13 to 99 mg/100 g DW) keeps similar to those reported by other authors which reported ranging from 23 to 25 mg/100 g FW for cherry varieties (Sánchez-Rodríguez, Ruiz, Ferreres, & Moreno, 2012; Verheul, Slimestad, & Holta, 2015). In general the treatment led to significant changes in the levels of chlorogenic acid without showing defined patterns. These date keep relationship with other studied that suggested which the stress of plant increase chlorogenic acid and immediately decrease phenolic compounds as response to change (Lule & Xia, 2005).

Regarding the cluster the highest values in ‘Summerbrix’ were observed in the CV cluster. In the case of ‘Lazarino’ the highest levels were detected in the CIII (autumn) and CV cluster (spring). This relates well with the integral light in both cases. The data observed for ‘Summerbrix’ and ‘Lazarino’ agree well with those of other authors, which reported that, the chlorogenic acid concentration increased with the cluster, maybe because phenolic levels are influenced by light (Minutolo, Amalfitano, Evidente, Frusciante, & Errico, 2013; Atkinson, Dew, Orfila, & Urwin, 2011).

The developmental stages had a significant impact on the levels of Chlor in most cases. It was frequent that the highest levels of this compound were found in M1 samples. This agreed well with the data reported by other authors, indicating decreases of chlorogenic acid with the developmental stages (Meléndez-Martínez, Fraser, & Bramley, 2010; Verheul, Slimestad, & Holta, 2015).

In summary, it was concluded that, in general the levels of chlorogenic acid changed significantly as a function of the cluster and developmental stages in both autumn and spring in both varieties. Although, ‘Lazarino’ in autumn, did not show changed with the

513 treatment. Increases in the levels of this compound from the CI to the CV cluster between
514 23 to 73% and decreases between 16 to 71% from M1 to M3 were typically observed.

515 **3.4.3 Gallic acid**

516 The concentration of gallic acid did not vary significantly with treatment in many
517 cases, while changed with the cluster was observed. The highest levels of Galli were
518 detected in 'Summerbrix' in the CV cluster in autumn as a function of the cluster. The
519 developmental stages in the Galli level did not led a definite pattern.

520 In summary, in general, gallic acid levels varied significantly in the two seasons with
521 cluster and developmental stages and did not change with the treatment.

522 **3.4.4 Quercetin**

523 Noticeably, the quercetin values observed in normal water regime (ranging from
524 33.6 to 159.2 mg/100 g DW) keep relationship with those reported in other studies for
525 cherry varieties (ranging from 1.07 and 1.71 mg/100 FW) (Pernice, et al., 2010). In this
526 variety, the treatment usually led to decreases between 6 to 30% in the levels of the
527 compound.

528 The cluster led to higher values in the CV and CI cluster in 'Summerbrix' in autumn and
529 spring respectively, although lower values in the control samples in the CI and CV cluster
530 in autumn and spring respectively were observed in 'Lazarino'.

531 In most cases, statically significant differences in the levels of quercetin with the
532 developmental stages were observed in 'Summerbrix', except in the CV cluster in autumn.

533 In summary, significant effects with the treatment, cluster and developmental stage were
534 observed in the quercetin levels in autumn and spring in 'Lazarino'. Although,

535 ‘Summerbrix’ did not change with the treatment in both seasons and developmental stage
536 in autumn, except for the treatment in both seasons and developmental stage in spring for
537 ‘Summerbrix’ . These date keeps relationship with other studies which reported seasonal
538 variation in phenolic compounds, thus in April, August and December the values were 5,
539 11 and 8mg/kg FW, respectively (Slimestad & Verheul, 2009).

540 **Total phenolics**

541 In most cases, ‘Summerbrix’ did not have statistically significant differences in the
542 total phenolics (TP) as a function of the treatment. The treatment led to more significant
543 effects in the case of ‘Lazarino’ especially in autumn. The cluster led to significant
544 changes in all the cases although not a consistent behavior was observed with increments
545 between 12 to 56%. Regarding the developmental stages did not lead a definite pattern.

546 In the case of ‘Lazarino’ higher values of chlorogenic acid, gallic acid, quercetin and total
547 phenolics, were observed, which may be due in part to that high temperatures can increase
548 the photosynthesis, as suggested elsewhere (Klopotek & Klaring, 2014). However, in this
549 study it was also observed that the effect was dependent on the variety as ‘Summerbrix’
550 had different behavior. In general, it can be stated that phenolic compounds levels are
551 related to environmental conditions, water deficit and variety of tomato, which is in
552 agreement with other studies (Minutolo, Amalfitano, Evidente, Frusciante, & Errico,
553 2013).

554 Interestingly, direct correlation between quality parameters, carotenoids and individual
555 phenolics were observed. Thus, weight with p-hydroxybenzoic acid; SS with quercetin;
556 C^*_{ab} with gallic acid and total phenolics; phytoene with p-hydroxybenzoic acid and total
557 phenolics; lutein with p-hydroxybenzoic acid and chlorogenic acid; lycopene with p-

hydroxybenzoic acid, gallic acid and total phenolics; total carotenoids with p-hydroxybenzoic acid, gallic acid and total phenolics; p-hydroxybenzoic acid with gallic acid; chlorogenic acid with quercetin were observed. These showed coefficient correlation from 0.3 to 0.5. These data keeps correspondence with other studies, who suggest that there is a relationship between SS and phenolics in special flavonols (Stakhova, Ladygin, & Stakhov, 2001).

4. CONCLUSION

A comprehensive study on the effect of deficit irrigation, cluster, developmental stage and two seasons on several organoleptic and functional qualities of two cherry tomatoes varieties have been carried out. The study of the effect of cluster is particularly interesting due to the scarcity of studies in this respect. It has been concluded that the commercial quality fruit parameters exhibited changes with the irrigation treatment, cluster, developmental stages, season and variety. The colour parameters showed change as a function of the treatment and season. The levels of carotenoids and phenolics exhibited changes more frequently as a function of the cluster, developmental stages, variety and season, while the irrigation treatment did not affected. Autumn had a positive effect on the commercial fruit quality as in general larger fruits and higher soluble solids contents were observed in the studied varieties. On the other hand, colour parameters more appropriate in commercial terms were observed in spring. However in spring, higher levels of carotenoids were observed in ‘Summerbrix’ and lower levels of phenolics in ‘Lazarino’. Certainly, the effect of the combined conditions in the concentrations of carotenoids and phenolics would have been difficult to predict because antioxidants compound are dependent on factors of different nature as agronomic, developmental, season and genotype. It is therefore

581 concluded that the deficit irrigation affect the commercial quality parameters with changes
582 not greater than 30%.

583 However, the results of this study are interesting beyond an agronomic point of
584 view. Thus, they are important in the context of the provision of reliable health-promoting
585 compositional data, more specifically in the context of functional foods. Although it is well
586 known that the developmental stage and the season have an important impact in the content
587 of plant metabolites, much little is known concerning the effect of deficit irrigation and the
588 location of the fruit in the plant. These are variables that should be taken into account when
589 generating such data. Similarly, it appears reasonable to advice to consider the location of
590 the clusters in the plant when sampling to carry out comparative studies (for example wild
591 type *vs* GMO, conventional *vs* organic, etc.). This is important as if the samples to be
592 compared are taken from clusters located at different heights additional sources of
593 variability are introduced.

594 **ABBREVIATIONS USED**

595 E.T.S.I.A., Escuela Técnica Superior de Ingeniería Agronómica; a.s.l., above sea level;
596 RDI, regulated deficit irrigation; ETc, crop evapotranspiration; FAO, Food and Agriculture
597 Organization of the United Nations; CI, first cluster; CIII, third cluster; CV, fifth cluster;
598 M0, M1, M2, M3, ripening stages; CIELAB, the Commission International of IEclairage
599 (CIE), defined colour spaces that includes CIE L*a*b*; UV-vis, ultraviolet-visible; RRLC,
600 rapid resolution liquid chromatography; UHPLC, ultra performance liquid
601 chromatography; Sum, 'Summerbrix'; Laz, 'Lazarino'; W, weight; SS, soluble solids; TC,
602 total carotenoids; TPC, total phenolic content; FW, fresh weight; DW dry weight; A_T,
603 significance of differences between the RDI and control samples; AC_C significance of
604 difference between clusters in the control samples; $ARDI_C$ significance of difference

between cluster in the RDI samples ;A_M significance of difference between ripening stages; Phy, phytoene; Lut, lutein; Lyc, lycopene; β-car, β-carotene; p-Hyd, p-Hydroxybenzoic; Chlor, chlorogenic acid; Galli, gallic acid; Quer, quercetin.

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Notes

The authors declare no competing financial interest

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