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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 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%).

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 et al., 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, 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 (Liu, Shao, Zhang, & Wang, 2015; Meléndez-Martínez et al., 2010; Wang et al., 2011).

Abbreviations: E.T.S.I.A., Escuela Técnica Superior de Ingeniería Agronómica; a.s.l., above sea level; RDI, regulated deficit irrigation; ETc, crop evapotranspiration; FAO, Food and Agriculture Organization of the United Nations; CI, first cluster; CIII, third cluster; CV, fifth cluster; M0, M1, M2, M3, ripening stages; CIELAB, the Commission International of IEclairage (CIE), defined colour spaces that includes CIE $L^*a^*b^*$; UV–vis, ultravioletvisible; RRLC, rapid resolution liquid chromatography; UHPLC, ultra performance liquid chromatography; Sum, 'Summerbrix'; Laz, 'Lazarino'; W, weight; SS, soluble solids; TC, total carotenoids; TPC, total phenolic content; FW, fresh weight; DW, dry weight; A_T, significance of differences between the RDI and control samples; AC_C, significance of 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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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 et al., 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 et al., 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™ 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, et al., 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, 37°21′09.71″ Lat. N, 5°56′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 458,7 mm 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 (Fig. 1).

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

2.3. Physico-chemical analyses

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

2.4. Carotenoid analysis

2.4.1. Sample preparation

Individual carotenoids were determined as described elsewhere (Borghesi et al., 2011) 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