Combined effects of deficit irrigation and fresh-cut processing on quality and bioactive compounds of nectarines

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Abstract

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Water scarcity makes necessary the use of tools for increasing water productivity such as regulated deficit irrigation strategies (RDI). The effect of RDI on fresh-cut white flesh extra-early nectarine was studied. Initially, the soluble solids content of nectarines from RDI was higher than in control and over-irrigated fruits $(9.72 \pm 0.20 \text{ vs. } 8.57 \pm 0.05^{\circ}\text{Brix}$, respectively) but after shelf-life storage no differences were found. During storage, RDI fruits had a more stable antioxidant capacity and soluble phenolic content. This treatment showed 10% more vitamin C than the other irrigation treatments, due to an increase in dehydroascorbic acid content. No pathogenic bacteria growth was found and all microbial counts were below the European legal limits. For all the irrigation treatments, the final sensorial scores were above the limit of marketability. Overall, RDI enabled savings of about 1,260 m³ of water/ha and year, without negatively affecting the quality of the fruit, allowing for their use as a fresh-cut product with a shelf-life of 8 days at 5°C.

Keywords: Prunus persica; minimally processed; microbial and sensory quality

Presently, an increasing commitment for optimizing water productivity is being developed, especially in dry areas (PAÇO et al. 2013). For example, in the Mediterranean basin, agriculture is limited by water scarcity and often by the poor quality of this resource. Besides a structural water deficit, competition from other uses (tourist and residential development) adds to the problem (ABRISQUETA et al. 2010). In addition, irrigation management is set to shift from emphasizing production per unit

area towards maximizing the production per unit of water consumed, namely, water productivity (Fereres, Soriano 2007). Within stone fruit cultivars, peaches and nectarines (*Prunus persica* (L.) Batsch) are the fourth more important fruit crop in the world and the second one in Europe, with Spain being the fourth largest world producing country (FAOSTAT 2015). Growing stone fruit requires large amount of water, which is not always available in these areas, as they sometimes suffer emergency

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drought situations (VERA et al. 2012). Therefore, it is necessary to search for new strategies for a more efficient management of irrigation water to obtain max. water productivity and min. environmental impact (Fereres, Soriano 2007). One of these strategies is the application of water below the total crop evapotranspiration requirements (ETc) during certain phenological periods, known as regulated deficit irrigation (RDI) (CHALMERS et al. 1981). Previous studies have shown that in whole stone fruits, such as peach, nectarine or apricot, quality attributes are maintained using RDI strategies (Gelly et al. 2004; Pérez-Pastor et al. 2007). It has also been reported that water deficit promotes and allows for the concentration of bioactive compounds in whole (BARBAGALLO et al. 2013; Laribi et al. 2013; Navarro et al. 2015) and cut products (Peña et al. 2013). Bioactive compounds, such as ascorbic acid (AsA), phenols, anthocyanins, flavonoids and carotenoids are part of the plant's defence system, and many of them have human health-promoting effects that are linked to the prevention of cancer and cardiovascular diseases (Boeing et al. 2012; Haminiuk et al. 2012; BARBAGALLO et al. 2013). Moreover, RDI is more interesting in extra-early varieties, as their maximum irrigation requirements occur in periods of relatively low evaporative demand. In recent years there has been also an increasing demand for freshcut (FC) fruits and vegetables all over the world due to the changes in lifestyle as well as the health benefits associated with their consumption, which is attributed to their content in phytochemical compounds, which scavenge free radicals (RICO et al. 2007). In fact, the fresh-cut industry has become a multi-billion dollar international industry (Santos et al. 2014). For all these reasons, the main aim of

the present work was to study how RDI affects the overall quality and content of bioactive compounds of FC extra-early nectarines which were stored for 8 days at 5°C.

MATERIAL AND METHODS

The study was conducted in the 2010-2011 growing season in a commercial orchard located in Murcia (38°8'N, 1°13'W). The experimental plot had an area of 2 ha and consisted of 11-year-old extra-early nectarine trees cv. VioWhite 5 grafted onto plum cv. Puebla de Soto 101 rootstock at a spacing of 6×3.5 m. This cultivar was chosen because of its high commercial value due to its early picking date, which commands a high market price, and its suitable size for European sales. The electrical conductivity of the irrigation water varied between 1.5 and 2.5 dS/m, depending on the water source used (irrigation canal, well or a mix of both). The drip irrigation system had two lines per tree row and nine pressure-compensated emitters (1.6 l/h) per tree placed every 75 cm. Irrigation was scheduled weekly and applied daily at night throughout the study period. During the experiment, three different irrigation treatments were applied: (i) Control (CTL), irrigated at 110% of ETc during the whole season in order to avoid limiting soil water conditions, determined from the crop reference evapotranspiration (ET₀ Penman-Monteith method, Allen et al. 1998); (ii) regulated deficit irrigation (RDI), irrigated at 110% ETc during the critical periods (second rapid fruit growth period and two months after harvest) and at 80 and 60% CTL during the second fruit growth stage in March and late postharvest (from July until the end of the previous growing season), respective-

Table 1. Precipitation and irrigation applied to control (CTL) regulated deficit irrigation (RDI) and over-irrigation (OI) treatments in each phenological period

Phenological period	Period (days)	Precipitation (m ³ /ha)	Irrigation applied (m ³ /ha)		
			CTL	RDI	OI
Early postharvest	134–182 (2010)	440	1,320	1,320	1,210
Late postharvest	183-304 (2010)	720	2,940	1,720	2,760
Winter dormancy	305 (2010)-31 (2011)	810	350	390	440
Bloom and fruit set	32-59 (2011)	125	180	180	240
Fruit growth I and II	60-94 (2011)	422	480	390	550
Fruit growth III	95–126 (2011)	95	750	740	850
Season	134 (2010)-126 (2011)	2,620	6,010	4,750	6,050

ly, and (iii) over-irrigated treatment (OI), irrigated according to the criteria followed by the farmer, at 135% CTL and 115% CTL during the first and the last two fruit growth stages, respectively. In total, the amount of water applied in the CTL treatment during the season was 6,010 m³/ha and year, 4,750 m³/ha and year for RDI and 6,050 m³/ha and year in OI treatment. The water saving in RDI was 1,260 m³/ha and year. Precipitation and irrigation for each treatment and phenological period are shown in Table 1. The experimental design consisted of three replicates per treatment, following a randomized block design distributed within the orchard. Each replicate had three adjacent tree rows and contained 15 trees per row. Measurements were taken in trees from the central row, the other trees serving as borders. Nectarines (mean fruit calibre – 62 mm, mean fruit mass - 92.7 g) were picked at the beginning of ripening (21st April) taking into account their maturity index averaged to 6.11. The fruit was immediately transported to the Pilot Plant in the Technical University of Cartagena in a ventilated vehicle. Nectarines were refrigerated in a cold room at 0-1°C and 90-95% of the relative humidity (RH). Individual pieces of fruit were selected by eliminating those with defects or bruises. Sound fruit was cut vertically into 6 to 8 wedges with a sharp stainless steel knife. Then, the nectarine wedges were dipped into a water solution at 5°C with 150 ppm NaClO (pH 6.0) for 2 min and rinsed out for 1 min in tap water at 5°C. The pieces (250 g) were then gently dried with filter paper and packaged in 2-l rigid polypropylene (PP) trays. All trays were heat-sealed (Barket, Befor Model, Chassieu, France) on top with a 35 μm-thick oriented PP film (Plásticos del Segura S.L., Murcia, Spain). Each 250 g tray was considered a replicate and all data are means of three replicates. To maintain near-ambient oxygen concentration in the trays, a 0.45-mm hole was punched through the film of each tray. All the trays were stored in a cold room at 5°C for 8 days to simulate shelf-life. The quality parameters of the FC nectarines were monitored on days 0, 5 and 8. For the analysis of bioactive compounds, nectarines were cut into small cubes, frozen in N₂, ground to a fine powder with a mincer (IKA, A 11 basic, Berlin, Germany) and kept at -80°C until analysed.

Soluble solids content (SSC) measurements. Five of the nectarine wedges from each tray were homogenized in a commercial blender (Moulinex, Barcelona, Spain), with the juice used to measure the SSC, as described in FALAGÁN et al. (2014).

Microbial analysis. To determine microbial growth (psychrophilic bacteria, mold and yeast), 10 g of FC nectarine tissue were collected from trays and homogenized for 1 min in 90 ml of sterile peptone-buffered water (Scharlau, Barcelona, Spain) in a sterile stomacher bag with a Colorworth Stomacher 400 (Steward Laboratory, London, UK). Serial dilutions were prepared in the same peptone solution. The presence of pathogenic bacteria (Escherichia coli, Listeria monocytogenes and Salmonella spp.) was also determined and evaluated according to the European legislation for FC plant commodities (EC Regulation 1441:2007 on microbiological criteria for food stuffs). The agar medium and incubation conditions were as described in Tomás-Callejas et al. (2011). All samples were carried out in triplicate. Microbial counts were reported as \log_{10} colony forming units per g of sample (log CFU/g).

Sensory evaluation. A trained seven-person panel (aged 24–67) carried out the sensory evaluation in a room at 20°C. Before the evaluation session, the panelists agreed on those attributes that better described sensory changes. Dehydration, off-flavours and browning were scored on a nine-point scale of damage incidence and severity (1 – extreme, 3 – severe, 5 – moderate, limit of marketability, 7 – slight, 9 – none). Flavour, texture and overall quality were evaluated by using a nine-point scale (1 – extremely poor, 5 – fair, limit of usability, and 9 – excellent).

Analysis of bioactive compounds. Materials, extractions and quantification for soluble phenolic content (SPC), antioxidant capacity (AC) using the Ferric Reducing Ability of Plasma reagent, and vitamin C were as described by Falagán et al. (2014). SPC was expressed as mg gallic acid equivalents (GAE)/100 g fresh weight (f.w.). AC results were expressed as mg ascorbic acid equivalent (AsAE)/100 g f.w. Total vitamin C was expressed as the sum of both mg of ascorbic acid (AsA) and mg of dehydroascorbic acid (DHA) in 100 g f.w. All quality measurements were evaluated using three trays per treatment.

Statistical analysis. A randomized design with three replicates per treatment was used where each tray constituted a replicate. To determine the effect of RDI and storage time on each dependent variable, a two-way analysis of variance (ANOVA, P < 0.05) was carried out (Statgraphic Plus, version 5.1, Manugistic Inc, Rockville, USA). Mean values were compared by Fisher's LSD test to identify significant differences and interactions among treatments and factors.

RESULTS AND DISCUSSION

SSC measurements

Simple factors such as irrigation treatment and time of storage and their interaction affected the SSC (data not shown). On initial evaluation, samples under RDI had a higher SSC (9.8 ± 0.05°Brix) than CTL (8.6 \pm 0.24°Brix) and OI samples (8.2 \pm 0.20°Brix). At the end of storage period, all treatments showed similar SSC values (8.2 to 8.4°Brix). Previous researchers have reported that the reduction of water supply leads to a higher initial SSC in whole apricot (Pérez-Pastor et al. 2007) or pomegranate (LARIBI et al. 2013). The changes observed during storage were due to the consumption of sugars and organic acids as respiratory substrates in the fruit pieces. This is generally observed during climacteric fruit ripening and senescence (CRISOS-TO, KADER 2000), such as nectarines and peaches.

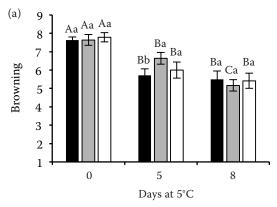
Microbial analysis

The presence of pathogenic bacteria was not detected in any irrigation treatment or evaluation day. Throughout the storage period, mold and yeast counts were lower than 2 log CFU/g. Similar counts were obtained for psychrophilic bacteria at days 0 and 5. However, at the end of the shelf-life period, the psychrophilic bacterial counts were 2.61 \pm 0.14 log CFU/g for CTL pieces, 2.36 \pm 0.17 log CFU/g for OI treatment and < 2 log CFU/g in RDI wedges. The counts obtained were under the Euro-

pean Union legal limits (EC Regulation 1441:2007) in all cases. These low counts and little growing of the colonies could be due to hygienic management during the minimal processing stage, and the proper storage temperature. Moreover, cv. VioWhite 5 is an acidic variety (pH 3.3) which complicates bacterial growth (Devlieghere et al. 2004).

Sensory evaluation

For all treatments and sampling days, no off-flavours or dehydration were found. With respect to flavour, a decreasing trend with time of storage was registered, with scores falling from 7.6 to 5.8, with no significant differences among treatments (data not shown). A similar trend was found in texture; a loss of firmness in all treatments was found with scores ranging from 7.8 to 6.8 (≈10 to 12 N) although they were still high enough for the consumer's acceptance (Crisosto, Kader 2000). Browning was the most important factor that affected the appearance of the FC fruit (Fig. 1) increasing at day 5 in all treatments and obtaining similar scores among irrigation treatments by the end of the storage period. Enzymatic browning tends to be more noticeable in white flesh nectarines as in this case, and it appears on the cut-edge due to the breakdown of cells and the subsequent interaction of polyphenols and oxygen with polyphenol oxidase enzymes (MISHRA et al. 2012). The overall quality of the nectarine wedges on the initial day showed no significant differences among the different types of irrigation (7.6 to 7.8). At day 8, a decreasing trend in overall quality was



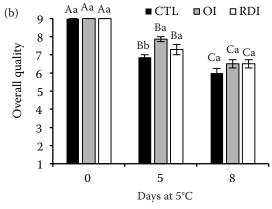


Fig. 1. Sensory scores for (a) browning and (b) overall quality changes of cv. VioWhite 5 nectarines subjected to three irrigation treatments, freshly-cut and stored up to 8 days at 5°C

CTL – control; RDI – regulated deficit irrigation; OI – over-irrigated; bars with the same letters indicate no significant difference at 95% using LSD test; capital letters refer to time of storage, lowercase letters refer to irrigation treatment; data represent means of seven replicates ($n = 7 \pm SE$)

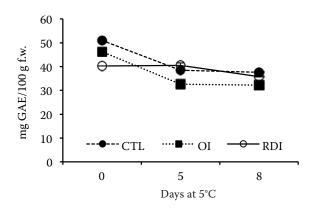


Fig. 2. Changes on soluble phenolic content of cv. VioWhite 5 nectarines subjected to three irrigation treatments freshly cut and stored up to 8 days at 5° C

CTL – control; RDI – regulated deficit irrigation; OI – over-irrigated; vertical bars indicate the standard error of the means ($n = 3 \pm \text{SE}$); LSD (5%) $_{\text{irrigation treatment} \times \text{time}} = 6.70$

found, but was still above the limit of marketability in all irrigation treatments (Fig. 1).

Analysis of bioactive compounds

On harvesting day, nectarine pieces from CTL and OI wedges contained more phenolic compounds than RDI fruits (Fig. 2). At day 5, OI samples had a lower level than CTL and RDI sample pieces. At the end of the storage period, the CTL and RDI treatment pieces reached the highest values vs. the OI treatment. As observed, the results obtained for SPC showed a decreasing trend during shelf-life storage, mainly in CTL and OI fruit pieces, while in wedges from RDI the values registered were much more stable (Fig. 2). According to ROMERO et al. (2002), this fact can be due to the activation of the phenolic biosynthesis pathway. As observed in Tovar et al. (2002), this could have been provoked by the stress suffered under RDI, which leads to an increase in L-phenylalanine ammonia lyase activity, which is the main enzyme in this metabolic pathway.

Nectarines are nutritionally important because of their high acceptance by consumers (Cantín et al. 2009), although they usually have lower antioxidant content than other fruits such as strawberry, apple or orange (Rupasinghe, Clegg 2007). In this work, the initial AC levels ranged from 32 to 34 mg AsAE/100 g f.w. for all treatments. This range was stable during the shelf-life period, with no significant differences as regards to storage time

or type of irrigation (Fig. 3). These levels are in agreement with those reported on different white flesh nectarine cultivars (14 to 104 mg AsAE/100 g f.w.) when evaluated with the FRAP method (GIL et al. 2002). The AC was well maintained in all treatments without decreasing. This was probably due to membrane damage (cuts), which leads to an increase in the biosynthesis of antioxidant metabolites as cell try to compensate for the effects of such damage (VINÃ, CHAVES 2006). The AC highly correlated with SPC ($R^2 = 0.87$, data not shown).

Vitamin C was reported as the combination of mg AsA and mg of DHA, its oxidized form. It is important to measure both molecules because DHA shows the equivalent biological activity to AsA (Spínola et al. 2014). On the initial day and after 5 days of storage, CTL had a higher AsA content than the other irrigation treatments (Fig. 4a). In spite of this, at the end of storage, a difference among irrigation treatments was not found. DHA content had an opposite behavior as compared to AsA content. CTL nectarine wedges had lower levels than OI and RDI samples during the experiment (Fig. 4b). At the end of the storage period, the wedges from the nectarines under RDI showed the highest DHA content than the other irrigation treatments. The results show that water stress affected the oxidation of AsA into DHA, by activating the ascorbate peroxidase enzyme (FALAGÁN et al. 2015). The trend of total vitamin C followed the behaviour observed for DHA, being higher for nectarine wedges under RDI treatment during the entire period of storage (Fig. 4c).

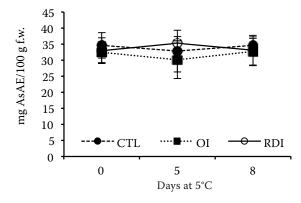
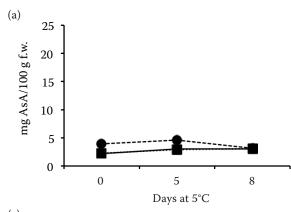
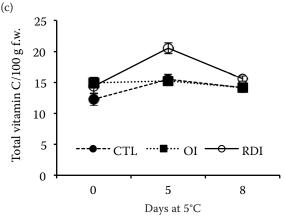


Fig. 3. Total antioxidant capacity changes of cv. VioWhite 5 nectarines subjected to three irrigation treatments, then freshly cut and stored up to 8 days at 5°C

CTL – control; RDI – regulated deficit irrigation; OI – over-irrigated; vertical bars indicate the standard error of the means ($n = 3 \pm SE$); changes were not statistically significant between storage time and type of irrigation at 95%





CONCLUSION

Fresh cut VioWhite 5 nectarines from RDI had the initial SSC higher than CTL samples. Furthermore, during storage, RDI fruits had a more stable antioxidant capacity and soluble phenolic content than those from CTL and OI treatments. This treatment had 10% more vitamin C than the other irrigation treatments, due to an increased dehydroascorbic acid content. The use of RDI achieved an important saving of 1,260 m³/ha and year of water. The results lead us to conclude that RDI did not negatively affect the suitability of cv. VioWhite 5 nectarines for FC processing obtaining a healthy and fresh, readyto-eat fruit and produced by a sustainable system.

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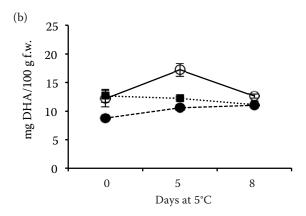


Fig. 4. Vitamin C content (c) as the sum of both mg of ascorbic acid (AsA) (a) and mg of dehydroascorbic acid (DHA) (b) of cv. VioWhite 5 nectarines subjected to three irrigation treatments, freshly cut and stored up to 8 days at 5°C CTL – control; RDI – regulated deficit irrigation; OI – overirrigated; vertical bars indicate standard error of the the means ($n = 3 \pm \text{SE}$); for AsA content changes: LSD (5%) irrigation treatment × time = 0.50; for DHA LSD (5%) irrigation treatment × time = 2.89; for total vitamin C: LSD (5%) irrigation treatment × time = 2.87

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