Physico-chemical, sensorial and nutritional quality during the harvest season of 'Tango' mandarins grafted onto Carrizo Citrange and Forner-Alcaide no. 5.

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Abstract

'Tango' mandarin is becoming one of the most demanded varieties in the Mediterranean Region. However, no information on the quality of 'Tango' fruit in this citrus area has been reported. In this study, the physico-chemical, nutritional and sensorial quality of 'Tango' mandarins grafted onto Carrizo Citrange and FA5 rootstocks from two locations (Sevilla and Huelva) was evaluated by harvest season. The fruit from Sevilla exhibited lower levels of acids and sugars than those from Huelva, which was associated with a higher sandy soil percentage in the Huelva orchard. In both orchards, the FA5-produced fruit had higher sugars and acids. Flavonoids were affected mainly by location, and the Huelva fruit exhibited the highest levels. The highest vitamin C was for the FA5 fruit. The decreased antioxidant capacity observed throughout the harvest season was related to reduced vitamin C. The sensorial evaluation corroborated changes in the quality parameters.

Keywords: Biocomponents, harvest, sugars, acidity, physico-chemical quality, sensorial quality.

1. Introduction

'Tango' mandarin is a recent cultivar that was developed at the University of California, Riverside from an irradiated bud of the diploid mandarin cultivar 'W. Murcott' (USPP17863; Roose & Williams, 2007). It is characterised by its mid-late harvest period and having high-quality seedless fruits. Currently this cultivar is becoming one of the most demanded mandarin varieties in the world's main citrus production areas.

In Spain, the 'Tango' cultivar is being introduced as an interesting alternative to be commercialised when the Clementine season ends. Nowadays, most 'Tango' plots are located in Andalucia (S. Spain), although there is interest in extending its production throughout the Mediterranean Region. The harvest period in this area roughly starts at the end of December and continues to the end of February. Presently, there is no information on the changes that 'Tango' fruit undergoes during this period, or if an optimal harvest time exists when this fruit exhibits optimal quality.

It has been reported that fruit quality can be influenced by biotic and abiotic factors (Iglesias et al., 2007). In citrus, one important aspect to consider is the rootstock onto which a specific cultivar is grafted because it may influence several tree growth and development aspects, including yield, fruit quality and tolerance to stress caused by biotic and abiotic factors (Filho, Espinoza-Núñez, Stuchi & Ortega, 2007)

Likewise, the nutritional composition of citrus fruit can vastly vary depending on the growth conditions of groves, including rootstock, soil composition and elevation (Zhang, Breksa III, Mishchuk & Slupsky, 2011). Indeed, the nutritional quality of citrus fruit juice has been reported to be directly linked with rootstock effects on plant-water relations regulating sucrose transport (Barry, Castle & Davies, 2004).

The most widely used rootstock in Spain is Carrizo Citrange (CC) [*Citrus sinensis* (L.) Osb. x *Poncirus trifoliata* (L.) Raf.]. This rootstock is tolerant to CTV (citrus tristeza virus), and induces good productivity and fruit quality, but frequently presents iron chlorosis and salinity problems (Forner, Forner-Giner & Alcaide, 2003). The search for new better performing citrus rootstocks than those normally used is one of the citrus industry's main challenges in many countries. In this context, an ambitious programme for breeding citrus rootstocks is being undertaken at the Institute Valenciano de Investigaciones Agrarias (IVIA) (Valencia, Spain). As a result of this programme, different rootstocks have been selected and released. One of the most interesting obtained rootstocks has been Forner-Alcaide no. 5 [*Citrus reshni* Hort. Ex Tan. x *Poncirus trifoliata* (L.) Raf.], (FA5). FA5 is a hybrid of the Cleopatra mandarin [*Citrus reshni* Hort. ex Tan) x *Poncirus trifoliata* (L.) Raf.] obtained by traditional hybridisation. This rootstock offers high productivity and good fruit quality of the scion cultivar. It is tolerant to CTV, salinity and lime-induced chlorosis, and is resistant to *Phytophthora* sp. and citrus nematode *Tylenchulus semipenetrans* Cobb (Forner et al., 2003; Forner-Giner, Primo-Millo & Forner, 2009). For all these reasons, it would be very interesting to know the behaviour of the 'Tango' cultivar grafted onto FA5. In this context, it is important to know the influence of this rootstock on physico-chemical and nutritional fruit quality as it has been reported that rootstock can also affect internal quality, organoleptic and nutritional quality, and the concentration of some bioactive compounds (Legua, Forner, Hernández & Forner-Giner, 2014).

The aim of the present work was to evaluate the changes in the physico-chemical, sensorial and nutritional quality during harvest of 'Tango' mandarins from trees grafted onto Carrizo Citrange or Forner-Alcaide no. 5 under Mediterranean conditions.

2. Material and Methods

2.1. Plant material

Experiments were conducted with the 'Tango' mandarins taken from the trees grafted onto Carrizo Citrange (CC) [*Citrus sinensis* (L.) Osb. x *Poncirus trifoliata* (L.) Raf.], and also grafted onto Forner-Alcaide no. 5 (FA5) [*Citrus reshni* Hort. Ex Tan. x *Poncirus trifoliata* (L.) Raf.] in two commercial orchards located in different growing areas of Andalucia. One of the orchards is located inland in Sevilla and has loamy soil (45% sand; 36% lime; 19% clay). This orchard had EC1-5 <70 µS cm-1 (20ºC) and a pH of 6.19. The other orchard is found in Huelva on the Mediterranean coast with sandy loam soil (80% sand, 10% lime; 10% clay). This orchard had EC1-5 <106 µS cm-1 (20ºC) and a pH of 6.75.

The fruit from six trees grafted onto each rootstock in both orchards were used for the physico-chemical evaluations. On seven harvest dates, 20 fruit were picked in both orchards from two trees grafted onto each rootstock to perform three replicates: 5 January (H1); 9 January (H2); 17 January (H3); 24 January (H4); 30 January (H5); 6 February (H6) and 13 February (H7). After each harvest, fruit were transported to the IVIA where the physico-chemical, nutritional and sensorial analyses were carried out.

2.2. Determination of the physico-chemical parameters

The external peel colour of fruit was measured by a Minolta colorimeter (model CR-300, Minolta Co. Ltd, Osaka, Japan) over 15 fruit per lot by taking two measurements from the opposite equatorial sides of each fruit. The mean values for the 'L', 'a' and 'b' Hunter parameters were calculated with each fruit and expressed as the Citrus Colour Index (CCI) (CCI=1000a/Lb) (Sdiri, Navarro, Monterde, Benabda & Salvador, 2012a).

Firmness measurements were taken by a Universal Testing Machine (model 3343, Instron Limited, Buckinghamshire, England) using 15 fruit per lot. The results were expressed as the percentage of millimetres of fruit deformation that resulted from a 10 N force on the longitudinal axis at constant speed (Sdiri et al., 2012a).

In each lot of fruit, three samples of five fruit each were squeezed by an electric juice extractor with a rotating head (Lomi®, Model 4, Lorenzo Miguel, S.L., Madrid, Spain). Juice yield was measured and expressed as a percentage, calculated by dividing the volume of juice by the total fruit weight. Titratable acidity (TA) was determined by titration with 0.1 N NaOH solution using phenolphthalein as the indicator, expressed as g citric acid/100 mL of juice. The total soluble solids content (TSS) in juice was measured by a digital refractometer (Atago PR-1, Atago Co., Ltd, Tokyo, Japan) and data were expressed as ºBrix. The maturity index (MI) was calculated as the TSS/TA ratio.

The ethanol content from each juice was quantified by a headspace analysis in a gas chromatograph (model 1020, Perkin Elmer Corp., Norwalk, CT, USA) following the method described by Sdiri et al. (2012a), expressed as mg ethanol/100mL juice.

2.3. Biocomponents analysis

2.3.1. Extraction and analysis of sugars

The extraction method was the same as the procedure by Bermejo, Pardo, Morales and Cano (2016). Carbohydrates were analysed by HPLC according to the method described by Bermejo, Pardo and Cano (2011). The results were expressed as g/L.

2.3.2. Extraction and analysis of organic acids

Organic acids extraction was carried out according to the method described by Bermejo, et al. (2016), and compounds were analysed by HPLC-DAD and HPLC-MS under electrospray ion negative conditions. An ICSep ICE-COREGEL 87H3 column (Transgenomic) was used with an isocratic mobile phase of 0.1% H_2SO_4 solution at a flow rate of 0.6 mL/min. The injection volume was 5 µL. The HPLC-MS analysis was carried out according to Bermejo et al. (2016). The standard compounds came from Sigma (Sigma Co., Barcelona, Spain). The results were expressed as g/L.

2.3.3. Extraction and analysis of flavonoids

The main flavonoids were extracted following the procedure described by Bermejo, Pardo and Cano (2012b), with some modifications to adapt the method to a microlitre format (Bermejo et al., 2016). Narirutin, hesperidin and didymin were analysed by HPLC-DAD and HPLC-MS under electrospray ion positive conditions according to the method by Bermejo et al. (2011). The standards came from Extrasynthesis (Genay, France), Sigma (Sigma Co., Barcelona, Spain) and ChromaDex (Irvine, CA, USA). The results were expressed as mg/100 mL.

2.3.4. Extraction and analysis of vitamin C

Total vitamin C was extracted according to the method reported by Sdiri, Bermejo, Aleza, Navarro and Salvador (2012b). *DL*-dithiothreitol (DTT) was used as the reducing reagent of dehydroascorbic acid to ascorbic acid. Ascorbic acid quantification was performed by HPLC-DAD as described by Bermejo et al. (2012b). L-ascorbic acid was obtained from Sigma (Sigma Co., Barcelona, Spain) and DTT from Fluka (Sigma Co., Barcelona, Spain). The results were expressed as mg/100 mL.

2.3.5. Instruments for the HPLC analysis

An HPLC analysis were performed in an Alliance liquid chromatographic system (Waters, Barcelona, Spain), equipped with a 2695 separation module, a 2996 photodiode array detector and a ZQ2000 mass detector. Samples were detected at 5ºC, and the column temperature was set at 25ºC or 35ºC. The sugars analyses were carried out with a Waters 515 HPLC pump, a 2414 refractive index detector and a 20 µL loop Rheodyne injector.

Data were acquired and processed by the Empower 2 software (Waters, Spain).

Grade solvents and Milli-Q water were used in all the biocompound HPLC analyses.

2.3.6. Analysis of total antioxidant activity (AA)

The antioxidant activity (AA) of citrus juice was evaluated by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical-scavenging method and the ferric reducing antioxidant power (FRAP) assay. The DPPH free radical-scavenging activity measurement was assessed according to the procedure described by Sdiri et al. (2012b). Briefly in glass 96-well reaction plates, 30 µL of the diluted samples in 100% methanol were combined with 270 µL of methanolic DPPH (0.025 g/L in 100% methanol). Following incubation at 20 ºC for 50 min, absorbance at 515 nm was read by a Multiscan Spectrum microplate reader (Thermo Electron Corporation, SA, Finland). The ability of scavenging the DPPH radical was calculated by the following equation:

$$\% \text{ Inhibition} = [(A_0 - A_x)/ A_0] \times 100$$

where A_0 is the absorbance of a DPPH blank and A_x is the absorbance of the juice solution.

The FRAP method was assessed according to the method described by Sdiri et al. (2012b). Briefly, the FRAP reagent was freshly prepared by mixing 25 mL of 300 mM acetate buffer (pH 3.6), 2.5 mL of 10 mM TPTZ (2,4,6-Tris(2-pyridyl)-s-triazine) solution and 2.5 mL of 20 mM ferric chloride solution. The assay was carried out by placing 30 µL of appropriately diluted samples in a 96-well microplate and then adding 270 µL of FRAP reagent. Absorbance was read at 593 nm After 10 min of incubation at 37 ºC and shaking. The results were compared with a standard curve prepared with different ascorbic acid concentrations and were expressed as an mM ascorbic acid equivalent (AAe).

2.4. Sensory evaluation

Panellists were employees from the Postharvest Technology Centre, who were classified as semi-experts given their ample experience in serving on citrus sensory panels. The sensorial evaluation was made up of between 15-17 panellists. Sensory sessions were carried out in a specifically adapted room, where panellists sat in individual evaluation booths, and samples were presented through a small door in front of the booths. Three segments from three different mandarins were presented per sample to compensate for biological variability. Room temperature distilled water was provided to cleanse palates between samples. Samples were served in random order to panellists in 50-mL stainless steel soufflé cups, identified by a unique three-digit number. Each fruit was tasted by at least three panellists, who gave a hedonic score (liking) for each sample, which ranged from 1 to 9: 1 was “dislike very much” and 9 was “like very much”. Panellists were also asked about their purchase intention on a 5-point scale, which went from 1 “definitely would not buy” to 5 “definitely would buy”.

Sweetness and acidity levels were also evaluated using 5-point scales that went from 0 “very low” to 4 “very high”. Finally, the off-flavour level was evaluated from 0 “absence” to 4 “a very high off-flavour”.

2.5. Statistical analysis

Statistical procedures were performed using a statistical software (Statgraphics plus 5.1. Manugistics, Inc., Rockville, MD, USA). All the data were subjected to an analysis of variance, and means were compared by an LSD test at $P \leq 0.05$.

3. Results and Discussion

3.1. Physico-Chemical quality

‘Tango’ fruit is characterised by having an intense reddish external colour (USPP17863). In the present study, the fruit from the Sevilla orchard were harvested with high values close to 20 at the beginning of January (H1). Slight changes for the following harvests were observed. The fruit from the trees grated onto FA5 showed slightly higher values than those grafted onto CC (Fig. 1). Nevertheless, in the fruit from the Huelva orchard, the differences between the two rootstocks were more marked. While the CCI values of the CC fruit were similar to those observed in the fruit from Sevilla, the fruit from FA5 had lower values during the first harvest (CCI=17.4). Although a gradual increase was observed during the following harvests, the maximum value came close to 19.

The ‘Tango’ mandarins from both orchards had high firmness values, with a percentage of deformation values close to 4.5-5% throughout the harvest period (data not shown). No influence of rootstock was observed on this parameter in any case. The juice yield of the fruit in all cases was higher than 33%, which is the minimum to commercialise hybrid mandarins according to EU Quality Citrus Standards (EU Regulation No. 543/2011). The juice yield content was higher in the fruit from the Huelva orchard (45-50%) than those from Sevilla (43-46%) throughout the study, with no differences among harvests (data not shown). Rootstock did not influence the juice yield content in any case.

Regarding total soluble solids content (TSS), at first harvest, the TSS values of the Sevilla orchard fruit were lower (between 10.3 ºBrix and 11.2 ºBrix) than those for the fruit from Huelva (between 12.3 ºBrix and 13.0 ºBrix; Fig. 2A). In both orchards, the highest TSS values were shown in the fruit from the trees grafted onto FA5. In all cases, TSS remained practically constant until 24 January (H4), after which time a slight gradual increase in later harvests took place. This increase was more marked in the fruit from Sevilla, with values close to 11 ºBrix and 12 ºBrix in the fruit from the trees on CC and FA5, respectively. A rise in total sugars during fruit ripening has been previously described in the pulp of other citrus fruit (Ladaniya, 2008; Emmanouoilidou & Kyriacou, 2017). Our TSS values coincided with the value set out in the patent of the variety (USPP17863), and fell within the range of other mandarin varieties (Goldenberg, Yaniv, Kaplunov, Doron-Faigenboim, Porat & Carmi, 2014)

Similarly to that observed for TSS, at each harvest fruit acidity (TA) was higher in the fruit from Huelva than from Sevilla. Although the fruit from FA5 in both orchards also obtained higher values, the differences between rootstocks were more marked in the Huelva orchard. The fruit from both rootstocks from Sevilla had stable acidity values as no changes were observed throughout the study season, and only a slight decrease was noted for the last harvest. In Huelva, a gradual decrease in fruit acidity took place throughout the harvest with values coming close to 1.1 g citric acid/100 mL juice in the fruit from FA5, and close to 0.9 g citric acid/100 mL juice in CC-fruit at the end of the harvest season. According to the patent text of 'Tango' mandarin (USPP17863), fruit acidity values between 0.54 and 1.19 g citric acid/100 mL juice are reported, which are slightly lower than those obtained for our assayed conditions.

The Maturity Index (MI), calculated as the TSS/TA ratio, is the parameter included in most quality standards for citrus fruit. In our case, the high TA of the fruit values led to lower MI values than we expected when considering the high TSS value. In all cases, a slight increase was observed in the first four harvests, which then became more marked.

In both orchards, the fruit from trees grafted onto CC, with the lowest TSS and TA values, showed the highest MI throughout the harvest period. The fruit from Sevilla, with lower TSS and TA values, obtained higher values than those from Huelva.

It is worth mentioning that in all cases the MI of fruit exceeded the minimum value for mandarins and hybrids (7.5), established by The European Regulation for the commercialisation of citrus fruits (EU Regulation No. 543/2011).

The higher TSS and TA levels in the 'Tango' mandarins from Huelva could be attributed to texture soil composition. The Huelva orchard had a higher sandy soil percentage, and sandy soils have been reported to lead to higher radicular development, mainly fibrous roots (Li, Xu & Cohen, 2005). An increase in fibrous roots density improves the plant water status due to more effective water uptake and greater hydraulic conductivity, which induce higher TSS and TA levels (Barry et al., 2004). Rootstock also influences water uptake, nutrients transport and scion budded vigour (Castle, 1995). In the specific case of FA5, the highest TSS and TA values could be explained by their higher photosynthetic rate compared to CC, as reported by González-Mas, Llosa, Quijano and Forner-

Giner (2009). Higher net photosynthetic flux higher photoassimilate compounds are transported from leaves to fruits. Sucrose is the main sugar transported through the phloem in citrus (Jover et al., 2012).

Regarding ethanol content, it is must be noted that it is presented in the aroma of citrus fruits and has been reported to be a natural precursor of aromatic compounds (Sdiri, Rambla, Besada, Granell & Salvador, 2017). In the present study, very low values with no relevant changes throughout the studied harvest period were found in all cases (data not shown). Notwithstanding, the fruit from Huelva exhibited higher values (between 14.3 and 20.14 mg ethanol/100mL juice) than those from Sevilla (between 3.17 and 11.88 mg ethanol/100mL juice).

3.2. Nutritional quality

3.2.1. Sugars and organic acids contents

The main detected sugar was sucrose, followed by fructose and glucose (Table 1). The sucrose-fructose-glucose ratio (2:1:1) was similar for all the 'Tango' mandarins. As previously shown in TSS, the concentration of total sugars in the fruit from Huelva was higher than for the fruit from Sevilla. The values of the three detected sugars agree with those reported in the literature for juices from different mandarins (Bermejo et al., 2016), but are slightly higher than those reported for the parental 'Murcortt' (Sdiri et al., 2012b). No differences in the total sugars concentration were found between H1 and H4. An increment was observed at H7, except in the CC fruit from Sevilla, for which a similar total sugar content was observed in the three harvests. A significant rootstock effect on sugars accumulation was found in the Huelva orchard, where the FA5 fruit obtained higher values for the three individual sugars. In the Sevilla orchard, the rootstock influence was only significant on the sucrose concentration.

The measurements taken of the main organic acids revealed that the major acid was citric acid, followed by malic and succinic acids, as previously reported (Kelebek & Selli, 2011). The citric: malic: succinic ratio (2:1:1) was similar for all the mandarins under study. The citric acid levels found in this study fell within the previously reported range for other mandarins in different ripening stages (Bermejo & Cano, 2012a).

As previously observed for the TA values, a slight decrease in citric acid was shown as season advanced. No effect of harvest was observed on malic acid. A significant decrease in succinic acid was observed only for the FA5 fruit from Huelva throughout the season. The fruit from Huelva always contained higher citric acid contents (8.40-12.10 g/L) than those from Sevilla (7.30-9.38 g/L). Nevertheless, malic and succinic acid levels were higher in the fruit from Sevilla. This fact has been related to the stimulation of converting citrate into malate to maintain the pool of the TCA cycle intermediates constant when respiration increases (Etienne, Génard, Lobit, Mbeguié-A-Mbéguié & Bugaud, 2013). As regards the influence of rootstock on organic acid accumulation, the FA5-fruit had higher citric acid contents than the CC-fruit in both orchards. Conversely, while the malic acid content in the Sevilla orchard was higher in the FA5-fruit, it was higher in the CC-fruit from Huelva. The succinic acid concentration was influenced only by rootstock in the fruit from Huelva, in which the CC-fruit exhibited higher values than the FA5-fruit.

3.2.2. Flavonoids composition

Flavonoids are widely distributed in fruits and each species is characterised by a particular flavanone glycoside pattern. Flavonoids generally contribute to fruit and juice quality in many ways, which influences the appearance, taste and nutritional value of fruits (Bermejo et al., 2016).

Flavanones are the major flavonoids found in mandarin fruits (Bermejo et al., 2016). It was observed that the 'Tango' mandarins under all the study conditions did not differ in terms of individual flavonoid chromatographic profiles. As in other mandarin varieties, the most abundant flavanone glycoside was hesperidin, followed by narirutin and didymin (Sdiri et al., 2012b) (Table 2). The contents of hesperidin (7.67-10.69 mg/100 mL juice), narirutin (3.15-4.85 mg/100 mL juice) and dydimin (1.51-2.15 mg/100 mL juice) were slightly below those reported for other irradiated 'Murcott' mandarin clones, an overall narirutin compound (Bermejo et al., 2012b).

The values of the three identified flavanones were higher in the fruit from Huelva than in those from Sevilla. Harvest time did not affect the content of these compounds, except in fruit from Huelva whose hesperidin content lowered while harvest advanced. Rootstock did not influence the hesperidin content in both orchards. Nevertheless, higher levels of narirutin and didymin were observed in the FA5-fruit, but this rootstock effect was exhibited only on the fruit from Huelva. Although previous studies have observed a rootstock effect on flavonoids composition, this effect can be affected by other preharvest factors (Gil-Izquierdo, Riquelme, Porras, & Ferreres, 2004).

3.2.3. Vitamin C content

Vitamin C is the major antioxidant compound in citrus juice (Gardner, White, McPhail, & Duthie, 2000). Vitamin C content can be influenced by several factors, such as genotypic differences, preharvest factors, climate conditions, cultural practices, environmental stress during fruit development and postharvest treatment (Yang et al., 2011).

In the present study at the beginning of January (H1), 'Tango' mandarins had high ascorbic acid values that range from 25.12 to 27.25 mg/100mL in the fruit from Sevilla, and from 24.74 to 28.11 mg/100 mL in the fruit from Huelva (Table 2). These vitamin concentration values detected in

'Tango' mandarins fell within the range reported for other mandarins and were slightly higher than those found in its parental 'Murcott' (Bermejo et al., 2012b). A reduced vitamin C content in fruit as season advanced was observed. This finding agrees with the results reported in the literature, where less mature fruits have been found to contain the highest vitamin C concentration (Ladaniya, 2008). In this study, rootstock strongly influenced the total amount of vitamin C. Therefore, in both the studied orchards, the fruit grafted onto FA5 had the highest vitamin C content. Different studies have shown that rootstock influences vitamin C levels (Bassal, 2009; Bermejo & Cano, 2012a), and this effect depends on the interaction between each cultivar and each rootstock. This interaction is a thought-provoking approach to adapt citrus cultivars to diverse climatic conditions (Legua et al, 2014).

3.2.4. Antioxidant activity

Antioxidant activity (AA) denotes the ability of a bioactive compound to maintain cell structure and function by effectively clearing free radicals, inhibiting lipid peroxidation reactions, and preventing other oxidative damage (Bravo, 1998). Vitamin C is considered one of the most important nutrients in citrus fruit, and it is an important water-soluble antioxidant that plays a crucial role in suppressing superoxide radicals (Kaur & Kapoor, 2001). Similarly, flavonoids play a direct role in scavenging reactive oxygen species (ROS), which can counteract lipid oxidation *in vitro* and improve the body's antioxidant enzyme activity (Nakao et al., 2011). Rekha et al. (2012) also found that the antioxidant capacity of fruit juices to be directly related to total phenolics and vitamin C content. As a synergistic effect may exist among different antioxidants in juice, the use of more than one assay is highly recommended to evaluate the AA of citrus juice by different assays (Sdiri et al., 2012b). The DPPH radical scavenging test is simple rapid method used to evaluate the AA of a sample by changing the absorbance of the solution after combining antioxidant compositions and DPPH radicals (Alam, Bristi, & Rafiquzzaman, 2013). The FRAP assay measures AA by evaluating the ability of a sample to reduce Fe^{3+} to Fe^{2+} in the medium (Benzie & Strain, 1996). Both methods are based on the electron transfer reaction. However, the DPPH assay is less sensitive than FRAP because the interaction of antioxidant compounds and DPPH radical depends on its structural conformation (Barros, de Castro Ferreira & Genovese, 2012).

In 'Tango' mandarins, AA slightly decreased with harvest date, and changes became more evident when the FRAP method was used (Table 2). AA detriment could be due mainly to reductions in vitamin C as only a few changes in the main flavonoids were observed. It has been reported that ascorbic acid in citrus juice contributes more than 50% total antioxidant capacity (Arena, Fallico, & Maccarone, 2001; Del Caro, Piga, Vacca & Agabbio, 2004; Xu, Liu, Chen, Ye, Ma & Shi, 2007).

Regarding rootstock, the effect on AA, although FA5 gave higher vitamin C values, no rootstock effect was found on antioxidant capacity when evaluated by the DPPH radical scavenging method and the FRAP assay.

3.3. Sensorial Analysis

Fruit taste, one of the most important traits of citrus fruit quality, is principally governed by the levels and ratios of sugars and organic acids in juice sacs (Obenland, Collin, Mackey, Sievert, Fjeld & Arpaia, 2009). In addition to sugars and acids, mandarins possess a typical uniquely rich flavour, attributed to the presence of a mixture of aroma volatiles in pulp (Miyazaki, Plotto, Baldwin, Reyes-De-Corcuera & Gmitter Jr, 2012).

The sensory evaluation revealed that panellists detected higher acidity levels in the fruit from Huelva than in those from Sevilla (Table 3). In the fruit from the Sevilla orchard, the highest values were reported for FA5-fruit. No rootstock effect was found in the fruit from Huelva. In all cases, the fruit acidity scores slightly dropped for the first four harvests, although a more marked acidity loss took place after H4. The acidity scores given by panellists well relate to the above-mentioned TA values.

The sweetness scores were always between 2.3 and 3, which came close to optimal values. Panellists established that the fruit from Huelva were slightly sweeter than those from Sevilla. No effect of rootstock or harvest was perceived by panellists for sweetness.

The liking scores were high in all cases, over 6, with slight changes noted throughout the harvest period. Although these changes are not significant, it is worth noting that while the scores increased as season advanced for the fruit from both rootstocks in Huelva and in the FA5-fruit from Seville, the CC-fruit scores lowered in the Seville orchard. This fact could be related to the changes in the acidity level rather than to sweetness. Thus upon the first harvest in Sevilla, the highest liking scores were obtained for the CC-fruit at H1 (7.13), along with the lowest acidity scores, which were close to 3. At the seventh harvest (H7), this fruit had the lowest liking values when acidity scores fell to 2.4. Conversely, all the other fruit obtained the lowest liking scores at the first harvest, with acidity levels coming close to 4. In all cases, the highest liking scores were obtained when acidity dropped to values close to 3, which happened after the fourth harvest. Purchase intention followed the same pattern as panellists' likings, with maximum scores close to 4 obtained when liking values exceeded 7.

4. Conclusion

In this study, the physico-chemical, nutritional and sensorial quality of 'Tango' mandarins grafted onto two rootstocks during harvest seasons was evaluated in two different Spanish orchards.

The results herein obtained revealed that 'Tango' mandarin displays an attractive external colour and commercial sugars and acidity levels from early January to mid-February for all the studied conditions. Nevertheless, we must take into account that orchard location influenced the main quality parameters as the fruit from Sevilla orchard had lower levels of acids and sugars than those from Huelva. This location influence could be associated with the higher sandy soil percentage in the Huelva orchard. In all cases, the fruit from the trees grafted onto FA5 displayed higher contents of sugars and acids, which is related to the good photosynthetic capacity reported for this rootstock.

The sensorial analysis results were clearly related to the physico-chemical parameters and allowed an optimum harvest time to be established for the different studied conditions. Indeed, the fruit from CC in the Sevilla orchard exhibited the best quality at the first harvest (H1, early January), while the fruit from FA5 did so at the end of January (H5). In the Huelva orchard, the fruit from both rootstocks achieved maximum organoleptic quality in the second half of February.

Regarding the main flavonoids contents, the Huelva fruit had higher levels of hesperidin, narirutin and didymin. Although the rootstocks in both orchards not affect hesperidin content, the FA5 fruit from Huelva had higher narirutin and didymin than the CC-fruit. The vitamin C content in both evaluated orchards was higher in the fruit from the trees grafted onto FA5. The slightly lowering antioxidant capacity values, as reflected mainly by the FRAP method, were related to the drop in the vitamin C values.

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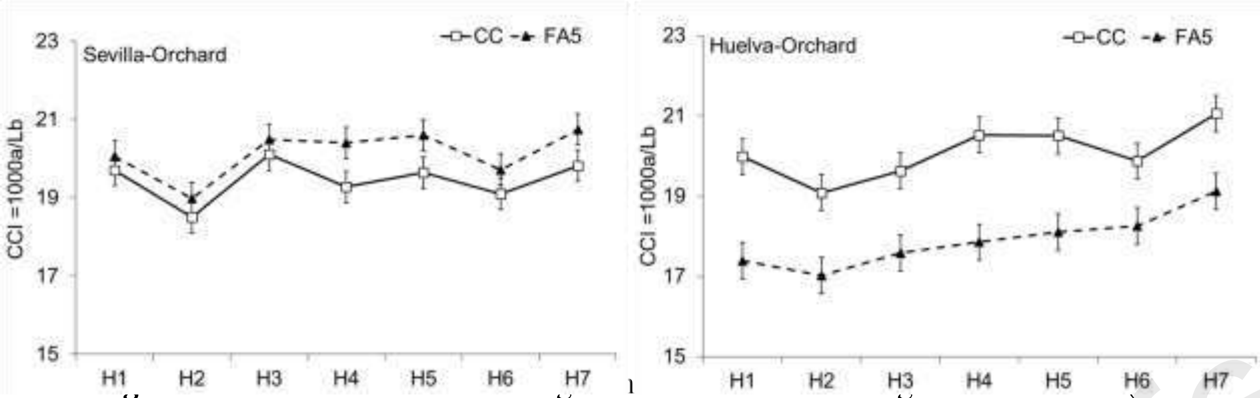
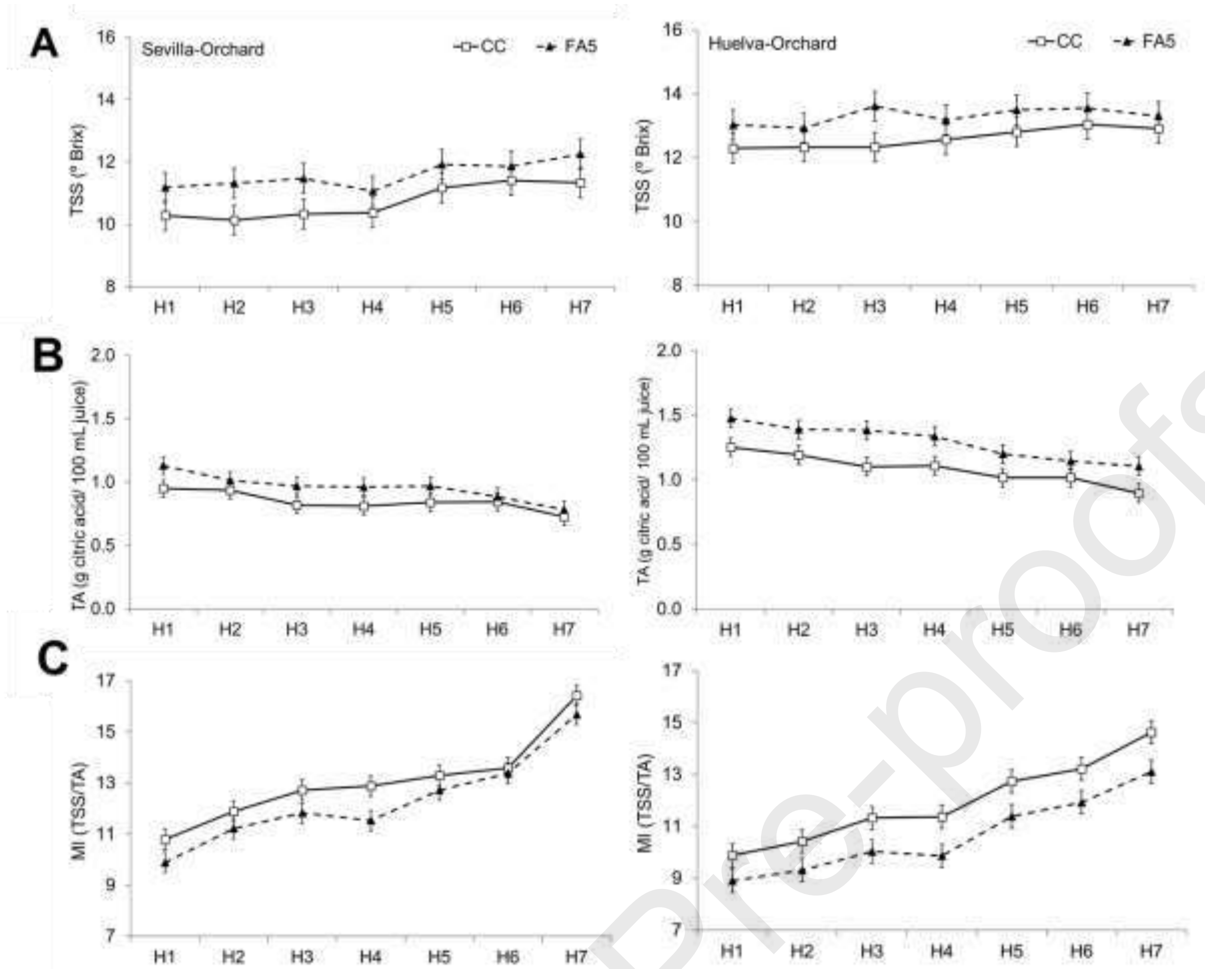


rchard or Huelva-Orchard) grafted onto two different rootstocks (CC and FA5) during harvest seasons from early January to mid-February. The vertical bars represent the interaction rootstock-harvest in each location (least significant difference (LSD) intervals ($P \leq 0.05$)).



x (C) of the 'Tango' mandarins from both growth locations (Sevilla-Orchard or Huelva-Orchard) grafted onto two different rootstocks (CC and FA5) during harvest seasons from early January to mid-February. The vertical bars represent the interaction rootstock-harvest in each location (least significant difference (LSD) intervals ($P \leq 0.05$)).

Table 1. Content of individual sugars (sucrose, glucose, fructose and total sugars) and organic acids (citric acid, malic acid, succinic acid and total organic acids) of the ‘Tango’ mandarins from both growth locations (Sevilla-Orchard or Huelva-Orchard) grafted onto two different rootstocks (CC or FA5) at three harvest times (H1, H4 and H7) from early January to mid-February. The letters per each orchard represent the least significant differences (LSD) intervals (P≤0.05).

		Sevilla-Orchard				Huelva-Orchard			
		CC		FA5		CC		FA5	
	Harvest								
Sucrose (g/L)	H1	38.16	a	40.83	ab	46.40	a	49.20	ab
	H4	37.76	a	40.44	ab	47.96	a	50.86	b
	H7	41.82	ab	47.81	b	53.08	b	52.33	b
Glucose (g/L)	H1	13.68	b	13.47	b	13.95	a	16.23	bc
	H4	13.67	b	12.67	a	15.33	ab	15.83	b
	H7	13.90	b	16.58	c	19.06	c	18.27	c
Fructose (g/L)	H1	15.58	ab	15.32	ab	16.47	a	17.75	ab
	H4	15.47	ab	14.23	a	16.90	a	17.51	ab
	H7	16.29	ab	17.97	b	19.52	b	18.52	b
Total Sugars (g/L)	H1	67.42	a	69.62	a	76.83	a	83.18	b
	H4	66.90	a	67.33	a	80.20	ab	84.19	b
	H7	72.01	ab	82.36	b	91.66	c	89.12	c
Citric Acid (g/L)	H1	9.17	b	9.38	b	10.87	ab	12.10	c
	H4	7.14	a	9.38	b	9.22	a	11.46	b
	H7	7.30	a	8.11	ab	8.40	a	10.48	ab
Malic Acid (g/L)	H1	2.10	ab	1.97	a	1.93	b	1.69	a
	H4	1.93	a	2.25	b	1.84	b	1.67	a
	H7	1.92	a	2.17	b	1.70	ab	1.73	ab
Succinic Acid (g/L)	H1	1.97	a	1.76	a	1.92	c	1.64	ab
	H4	1.82	a	1.90	a	1.70	b	1.46	a
	H7	1.82	a	1.79	a	1.55	ab	1.49	a
Total Organic Acids (g/L)	H1	13.24	b	13.10	b	14.72	ab	15.43	b
	H4	10.89	a	13.52	b	12.76	ab	14.60	ab
	H7	11.04	a	12.07	ab	11.65	a	13.69	ab

P-Value	Sucrose	Glucose	Fructose	Total Sugars	Citric	Malic	Succinic	Total Acids
Sevilla-Orchard								
A: Rootstock	0.033 *	0.439	0.667	0.239	0.025 *	0.014 *	0.823	0.087
B: Harvest	0.045 *	0.010 *	0.061	0.089	0.026 *	0.648	0.547	0.264
AB	0.702	0.010 *	0.265	0.231	0.188	0.006	0.174	0.135
Huelva-Orchard								
A: Rootstock	0.017 *	0.019 *	0,040 *	0,001 *	0.025 *	0.012 *	0.006 *	0.063
B: Harvest	0.005 *	0.000 *	0,016*	0,000 *	0.109	0.188	0.008 *	0.073
AB	0.035 *	0.001 *	0,031 *	0,002 *	0.829	0.072	0.321	0.754

Table 2. Content of the main flavonoids (herperidin, narirutin and dydimin), vitamin C (ascorbic acid) and antioxidant capacity (by the DPPH and FRAP methods) of the 'Tango' mandarins from both growth locations (Sevilla-Orchard or Huelva-Orchard) grafted onto two different rootstocks (CC or FA5) at three harvest times (H1, H4 and H7) from early January to mid-February. The letters per each orchard represent the least significant differences (LSD) intervals (P≤0.05).

		Sevilla-Orchard				Huelva-Orchard			
		CC		FA5		CC		FA5	
	Harvest								
Hesperidin (mg/100 mL Juice)	H1	7.77	a	7.81	a	10.33	b	10.69	b
	H4	7.95	a	7.19	a	9.70	ab	9.04	a
	H7	7.67	a	7.89	a	9.02	a	9.36	a
Narirutin (mg/100 mL Juice)	H1	3.15	a	3.31	a	3.95	ab	4.63	c
	H4	3.36	a	3.33	a	4.04	b	4.73	c
	H7	3.41	a	3.35	a	3.45	a	4.85	c
Didymin (mg/100 mL Juice)	H1	1.51	a	1.67	a	1.81	ab	2.13	b
	H4	1.62	a	1.62	a	1.91	ab	2.15	b
	H7	1.58	a	1.69	a	1.60	a	2.08	b
Ascorbic Acid (mg/100mL Juice)	H1	25.12	b	27.25	c	24.74	bc	28.11	d
	H4	25.06	b	25.58	b	23.38	b	25.12	c
	H7	22.50	a	24.71	b	20.93	a	22.53	b
Inhibition DPPH %	H1	24.39	a	23.40	a	23.14	b	23.38	b
	H4	24.23	a	22.57	a	21.45	ab	22.66	ab
	H7	22.97	a	22.42	a	20.75	a	22.81	ab
FRAP mM AAe	H1	5.87	b	5.93	b	5.87	b	5.73	b
	H4	5.43	b	5.33	b	5.03	b	5.03	b
	H7	4.33	a	4.13	a	4.07	a	4.13	a

P-Value	Hesperidin	Narirutin	Didymin	Ascorbic Acid	Inh. DPPH	FRAP
Sevilla-Orchard						
A: Rootstock	0.983	0.461	0.239	0.004 *	0.768	0.663
B: Harvest	0.782	0.795	0.899	0.001 *	0.279	0.000 *
AB	0.247	0.771	0.660	0.284	0.816	0.822
Huelva-Orchard						
A: Rootstock	0.971	0.000 *	0.001 *	0.000 *	0.309	0.921
B: Harvest	0.023 *	0.365	0.199	0.000 *	0.004 *	0.000 *
AB	0.444	0.102	0.488	0.062	0.704	0.931

Table 3. Sensorial evaluation (acidity, sweetness, likely and purchase intention) of the 'Tango' mandarins from both growth locations (Sevilla-Orchard or Huelva-Orchard) grafted onto two different rootstocks (CC or FA5) at three harvest times (H1, H4 and H7) from early January to mid-February. The letters per each orchard represent the least significant differences (LSD) intervals (P≤0.05).

		Sevilla-Orchard				Huelva-Orchard			
		CC		FA5		CC		FA5	
	Harvest								
Acidity (1-5)	H1	3.38	b	4.13	c	4.00	b	4.25	b
	H2	2.75	ab	3.63	c	3.38	ab	4.25	b
	H3	2.73	ab	3.70	bc	3.50	ab	4.11	b
	H4	2.90	ab	3.40	bc	3.50	ab	3.80	b
	H5	2.88	ab	3.00	ab	3.36	ab	3.25	ab
	H6	2.40	a	2.70	ab	3.10	a	3.20	a
	H7	2.44	a	2.63	a	2.78	a	3.20	a
Sweetness (1-5)	H1	2.63	a	2.50	a	2.88	a	2.63	a
	H2	2.63	a	2.50	a	2.75	a	2.63	a
	H3	2.09	a	2.40	a	2.70	a	2.78	a
	H4	2.30	a	2.60	a	2.70	a	2.90	a
	H5	2.25	a	2.88	ab	2.75	a	2.88	a
	H6	2.40	a	2.80	ab	2.90	a	2.80	a
	H7	2.44	a	3.13	b	3.11	a	3.00	a
Likely (1-8)	H1	7.13	a	6.63	a	6.88	ab	6.38	a
	H2	7.00	a	7.00	a	7.50	b	6.25	a
	H3	6.27	a	6.70	a	6.90	ab	6.44	a
	H4	6.80	a	6.70	a	7.20	ab	6.70	ab
	H5	6.25	a	7.13	a	7.50	b	7.38	ab
	H6	6.30	a	7.50	a	7.40	ab	7.40	ab
	H7	6.67	a	7.00	a	7.22	ab	7.60	b
Purchasing (1-5)	H1	4.00	a	3.63	a	3.50	a	3.63	ab
	H2	3.75	a	4.00	a	3.75	ab	3.35	a
	H3	3.55	a	3.60	a	3.80	ab	3.22	a
	H4	4.10	a	3.90	a	3.90	ab	3.80	ab
	H5	3.38	a	4.38	a	4.63	b	4.63	b
	H6	3.60	a	4.20	a	4.40	b	4.30	b
	H7	3.78	a	4.38	a	4.22	b	4.50	b

P-Value	Acidity	Sweetness	Likely	Purchasing
Sevilla-Orchard				
A: Rootstock	0.001 *	0.067	0.506	0.661
B: Harvest	0.000 *	0.255	0.427	0.376
AB	0.162	0.456	0.232	0.227
Huelva-Orchard				
A: Rootstock	0.507	0.977	0.365	0.605
B: Harvest	0.002 *	0.708	0.039 *	0.011 *
AB	0.145	0.465	0.694	0.557

Highlights

Nutritional and physicochemical changes in ‘Tango’ during harvest season is provided
Growth location and rootstock influence the fruit quality of ‘Tango’ mandarins
The trees grafted onto rootstock FA5 displayed higher contents of sugars and acids
Vitamin C content was higher in the fruit from trees grafted onto FA5
On the main flavonoids the effect of location was more marked than rootstock

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Cristina Besada: Methodology sensorial analyses.
Pilar Navarro: Methodology physico-chemical analyses.
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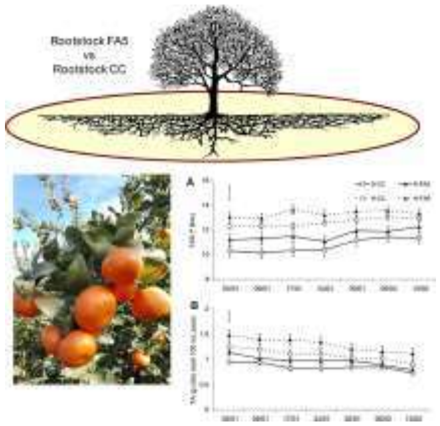
Rootstock FA5
vs
Rootstock CC