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# Analysis of the changes in quality in mandarin fruit, produced by deficit irrigation treatments

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#### 1. Introduction

## Citrus trees are cultivated widely in semi-arid climates, where the scarcity of water resources is the major factor limiting irrigated agriculture. One way to optimise water resources is to employ deficit irrigation (DI) strategies, in which moderate water stress is applied during part of the seasonal cycle of plant development ([Kriedemann & Goodwin, 2003](#page-5-0)).

The fruit quality of citrus is affected by water stress, due to changes in juice properties, such as increases in sugar concentrations and acidity [\(Chartzoulakis, Michelakis, & Stefanoudaki,](#page-5-0) [1999\)](#page-5-0), which reduce, in some cases, the fruit quality [\(Mougheith,](#page-5-0) [El-Ashram, Amerhom, & Madbouly, 1977](#page-5-0)). For example, Clementine citrus trees exhibit decreased fruit quality, depending on the phenological stage at which the DI is applied ([Romero et al.,](#page-5-0) [2006](#page-5-0)). Also, water stress resulting from mechanical injury inflicted on secondary scaffolds could improve the quality of citrus ([Moon,](#page-5-0) [Mizutani, Rutto, & Bhusal, 2001](#page-5-0)).

Fruit quality is also influenced by the rootstock ([Albrigo, 1977;](#page-5-0) [Barry, Castle, & Davies, 2004](#page-5-0)). Inherent rootstock differences affecting plant–water relations are associated with differential sugar accumulation of citrus fruits, which are proposed as a primary

### ABSTRACT

'Clemenules' mandarin citrus trees, grafted on Cleopatra and Carrizo rootstocks, were subjected to three irrigation treatments: the control (100% ETc), and phases II and III treatments (non-irrigation during phases II and III, respectively). The two deficit irrigation (DI) treatments affected in different ways some fruit quality parameters and these effects were also dependent on the rootstock. Although phase II treatment increased total soluble solids (TSS) and titratable acidity (TA), it delayed the maturation process. Phase II-stressed fruits on Carrizo had more fructose and glucose but less sucrose in relation to control fruits, thus increasing the reducing sugars. Phase II-stressed fruits on Cleopatra had more glucose, fructose and sucrose, for osmotic adjustment, also increasing the reducing sugars in these fruits. Phase IIIstressed fruits had greater acidity, TSS, total phenolics, lycopene, glucose and sucrose than control fruits, but the same maturation state. In conclusion, DI in phase III improved fruit quality, by increasing the values of important quality parameters related to taste, flavour and nutritional benefits, but DI in phase II produced a drastic delay in the maturation process that made fruits non-commercial.

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cause of differences in quality among citrus rootstocks [\(Barry](#page-5-0) [et al., 2004](#page-5-0)). The tree foliage supplies carbohydrates to the fruit but the rootstock determines the amount ([Gardner, 1969](#page-5-0)). It is not clear how rootstocks exert their influence on the juice quality of Citrus species, but plant–water relations are important factors in this [\(Castle, 1995](#page-5-0)). 'Carrizo' citrange and 'Cleopatra' mandarin, the most common rootstocks employed in Spain, have differing characteristics, resulting in different responses of quality [\(Pérez-Pérez,](#page-5-0) [Romero, Navarro, & Botía, 2008; Romero et al., 2006\)](#page-5-0).

In order to apply the optimum DI strategy, a good knowledge of the effects of DI in different phenological stages on fruit quality is necessary. To evaluate the sensitivity of different fruit-growth phases to DI in a semi-arid environment, some important fruit quality parameters were evaluated in field-grown 'Clementine' citrus trees grafted on 'Cleopatra' mandarin or 'Carrizo' citrange, two rootstocks with differing sensitivities to drought.

#### 2. Materials and methods

#### 2.1. Plant material

The study was carried out during 2003 in Murcia (southern Spain), on 10-year-old citrus trees (Citrus reticulata Blanco) grafted on two rootstocks, 'Cleopatra' mandarin (Citrus reshni Hort. ex





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Tanaka) and 'Carrizo' citrange (*Citrus sinensis L., Osbeck*  $\times$  *Poncirus* trifoliata L.). Two DI treatments were applied: the phase II treatment consisted of non-irrigation during this period of the fruit growth (mid-June to early October) and the phase III treatment, non-irrigation during this period (early October to the end of November). The control treatment involved irrigation at 100% of crop evapotranspiration (ETc), over the entire crop season. The layout took the form of three completely-randomised, selected plots. Each treatment was applied to nine trees (three trees per treatment in each plot).

To determine the levels of water stress, the midday xylem water potential ( $\Psi_x$ ) was measured, periodically, at noon (12:00–14:00), using a pressure chamber according to the [Schölander, Hammel,](#page-5-0) [Bradstreet, and Hemmingsen \(1965\)](#page-5-0) technique and following the recommendations of [Romero et al. \(2006\).](#page-5-0)

#### 2.2. Samples

When fruits had reached commercial size, 15 fruits per tree were collected randomly from the nine trees per treatment, for analysis of fruit quality. The fruits were squeezed and filtered juice was used for chemical analysis. Combined flavedo samples from the fifteen fruits were used for chlorophyll determination.

#### 2.3. Chemicals

The 2,2'-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS) was obtained from Fluka (Buchs, Switzerland) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) from the Sigma–Aldrich Chemical Co. (St. Louis, MO). Other reagents were of analytical grade.

#### 2.4. Fruit colour

The external fruit colour was measured in fifteen fruits per tree, using a tristimulus colour difference meter (Minolta CR-300 colorimeter), at three locations around the equatorial plane of the fruit. The Hunter parameters a and b were used, and colour was expressed as a/b, since this ratio has a high correlation with the visual appreciation of the fruit colour.

For determination of chlorophylls and carotenoids, fresh samples of flavedo (250 mg) were homogenised using an UltraTurrax homogeniser; then 20 ml of acetone:hexane (4:6) solvent were added and mixed in a test tube. Five millilitres of fruit juice were also mixed with 20 ml of acetone:hexane (4:6). Automatically, two phases separated and an aliquot was taken from the upper solutions for measurement of optical density at 663, 645, 505 and 453 nm in a spectrophotometer. Chlorophyll a, lycopene and b-carotene were calculated according to the [Nagata and Yamashita](#page-5-0) [\(1992\)](#page-5-0) equations.

#### 2.5. Maturity index determination

The content of total soluble solids (TSS) in the juice was measured with a refractometer and titratable acidity (TA) was determined by titration with NaOH and phenolphthalein indicator. The maturity index (MI) was expressed as 10  $\times$  TSS/TA.

#### 2.6. Sugar determination

The concentrations of soluble sugars (glucose, fructose and sucrose) in the fruit juice were determined by HPLC (Merck-Hitachi). The HPLC analysis was performed using a LiCrospher 100 NH<sub>2</sub> 5 lm column coupled with a differential refractometer detector. The mobile phase was acetonitrile:water (85:15), with a flow rate of 1 ml/min.

#### 2.7. Ascorbic acid determination

For ascorbic acid determination, the samples obtained for sugar analysis were used. The ascorbic acid concentration was then determined by HPLC (Merck-Hitachi), using a Chromsil C18 (10  $\mu$ m) 25  $\times$  0.4 cm column, coupled with a UV–Vis wavelength detector set at 245 nm. The mobile phase was di-ammonium hydrogen phosphate (20 g/l), adjusted to pH 2.8 with ortho-phosphoric acid. The flow rate was 0.9 ml/min.

#### 2.8. Total phenolics determination

The total phenolics content was determined in fruit juice samples using the Singleton and Rossi colorimetric procedure, with phosphotungstic–phosphomolybdic acid reagent, and measurement of the optical density of samples at 660 nm ([Singleton & Ros](#page-5-0)[si, 1965\)](#page-5-0). The total phenolics were determined by comparison of the values obtained with a standard curve of p-coumaric acid.

#### 2.9. Antioxidant activity determination

The test used to determine the antioxidative capacity of the fruit juice was the ABTS<sup>+</sup> radical cation assay, using Trolox for standardising the system ([Miller, Rice-Evans, Davies, Gopinathan,](#page-5-0) [& Milner, 1993\)](#page-5-0).

Each sample was analysed in triplicate by measuring absorbance at 734 nm and referenced to a Trolox dose–response curve. The Trolox Equivalent Antioxidant Capacity (TEAC index) was calculated as the concentration of Trolox (mM) in phosphate buffer and shown as the antioxidative potential equivalent to the millilitres of juice used, in the 30th reaction minute.

#### 2.10. Statistical analysis

The data were analysed using analysis of variance (ANOVA) procedures and means were separated by Tukey's multiple range test, using the SPSS software package (SPSS 7.5.1 for Windows, standard version, 1996; SPSS Inc., Chicago, IL).

#### 3. Results and discussion

#### 3.1. Soluble sugars

Plants under moderate water-deficit stress accumulate more carbohydrates than unstressed plants, independent of fruit size and juice content, since passive dehydration of juice sacs and concentration of soluble solids is not the primary cause of differences in sugar accumulation among irrigation treatments ([Barry et al., 2004;](#page-5-0) [Yakushiji, Morinaga, & Nonami, 1998; Yakushiji et al., 1996](#page-5-0)). The two deficit irrigation treatments produced differing increases of sugars in the juice of fruits on the different rootstocks ([Table 1\)](#page-2-0). Phase II-stressed fruits on Cleopatra had significantly more fructose, sucrose and glucose, and consequently more total sugars, than control fruits. However, fruits from phase II-stressed trees on Carrizo had increased fructose and glucose but decreased sucrose, so that they showed no increase of total sugars relative to the control. This differing behaviour could be related to the different plant– water relations of the two rootstocks, which are closely related to citrus juice quality [\(Barry et al., 2004; Romero et al., 2006](#page-5-0)). Under drought stress turgor is maintained in juice sacs by decreased osmotic potential as a result of sucrose hydrolysis, which increases the concentrations of hexose sugars (osmotically-active solutes) ([Yakushiji et al., 1996, 1998\)](#page-5-0). The great drought stress suffered by phase II-stressed trees on Carrizo  $(-2.8 \text{ MPa})$  increased the concentrations of fructose and glucose, and decreased sucrose, suggesting

#### <span id="page-2-0"></span>Table 1

Effect of DI and rootstock on the concentrations of total sugars, fructose, glucose and sucrose (g/l) and on the non-reducing/reducing sugars ratio in the fruit juice of Clemenules mandarin.



 $\ast$ ,  $\ast\ast$  and  $\ast\ast\ast$  indicate significant differences between means at the 5%, 1% and 0.1% levels of probability, respectively, ns = not significant. Values are the means of nine replicates. Values followed by the same letter within a column are not significantly different at the 0.05 level of probability, according to the Tukey test.

that sucrose hydrolysis took place in juice sacs during the process of osmotic adjustment, to maintain the osmotic gradient ([Barry et al.,](#page-5-0) [2004; Hockema & Etxeberria, 2001; Yakushiji et al., 1996\)](#page-5-0). The hydrolysis of sucrose could have been also stimulated by the high acidity of these fruits (150% higher than control fruits, Table 1), since the degree of conversion of sucrose to glucose and fructose depends on the pH, so, higher juice acidity should mean a greater proportion of reducing sugars ([Gónzalez-Sicilia, 1960; Pennisi,](#page-5-0) [1985\)](#page-5-0). The lower stress experienced by phase II-stressed trees on Cleopatra  $(-2.2 \text{ MPa})$  produced an active accumulation of glucose, fructose and also sucrose, the three sugars contributing to the fruit osmotic adjustment, as was observed under mild water-deficit stress by [Yakushiji et al. \(1996, 1998\)](#page-5-0).

Water-deficit stress applied too late in fruit development does not modify sugars concentration but they accumulate when the stress was imposed during the major sugar-accumulation period ([Barry et al., 2004](#page-5-0)). The timing and severity of the water-deficit stress also influenced the fruit juice composition in our experiment. Phase III-stressed fruits had significantly increased glucose and sucrose, and consequently total sugars, independent of the rootstock (Table 1). Unlike phase II-stressed fruits, phase IIIstressed fruits had significantly higher sucrose levels than control fruits. This was probably due to the fact that the stress was lower and was maintained over less time on both rootstocks (two months) than in phase II (more than three months). The increased content of sugars in juice from fruits under mild water-deficit stress occurs in order to achieve osmotic adjustment ([Yakushiji](#page-5-0) [et al., 1996, 1998](#page-5-0)). The increased partitioning of photosynthate to fruit, in spite of a presumed decrease in photosynthesis, is possibly a response to increased sink strength ([Hockema & Etxeberria,](#page-5-0) [2001; Yakushiji et al., 1996, 1998](#page-5-0)).

With maturation, total sugars increase, mainly because nonreducing sugars are at twice the concentration of reducing sugars in mature mandarin fruits ([Gónzalez-Sicilia, 1960\)](#page-5-0). The DI treatments changed this ratio, relative to the control (Table 1). In phase II-stressed fruits on Carrizo, sucrose hydrolysis significantly decreased the concentrations of non-reducing sugars, but they increased in fruits on Cleopatra. Also, fructose and glucose significantly increased, especially in fruits on Cleopatra. As a consequence of these modifications, phase II-stressed fruits on both Carrizo and Cleopatra had similar reductions in the ratio of nonreducing/reducing sugars. In phase III-stressed fruits, this ratio was increased significantly with respect to control fruits, since non-reducing sugars increased more than reducing sugars.

#### 3.2. Ascorbic acid

It has long been recognised that ascorbic acid has a unique and vital beneficial role in the human diet and the juice of citrus fruits provides an important source of ascorbic acid for human nutrition. In the present work, the ascorbic acid content of the fruit juice was dependent on the DI treatment ([Table 2\)](#page-3-0). When DI was applied during phase II, the ascorbic acid content was significantly higher (15%), with respect to the control juice. This effect could be due to both new synthesis of ascorbic acid, in response to water stress suffered by the tree, and to the lower fruit water content, owing to the low plant water potential  $(-3.5 \text{ MPa}$  for the phase II treatment and  $-1.2 \text{ MPa}$  for the control). During phase II of their growth, fruits have higher relative growth and the highest increase in juice content. Drought stress during this phase significantly decreased the juice percentage, by 27%, with respect to control fruits [\(Table 3](#page-3-0)), so this lower juice content could be partially responsible for the increase in ascorbic acid. The ascorbic acid concentration usually decreases in the juice of citrus fruits as they mature [\(Harding &](#page-5-0) [Fisher, 1945](#page-5-0)). So, the higher ascorbic acid content in phase IIstressed fruits confirms that these fruits were in an earlier maturation state than fruits from the control and phase III treatments. In other studies of deficit irrigation on fruit quality, the content of vitamin C in the flesh of peaches was not influenced by deficit irrigation [\(Buendía, Allende, Nicolás, Alarcón, &](#page-5-0) [Gil, 2008](#page-5-0)).

#### 3.3. Total phenolics and antioxidant activity

The antioxidant activity of fruits and vegetables is important for assessing their nutritional value and its measurement removes the need for the analysis of each antioxidant compound. A significant interaction between rootstock and DI treatment was found for the antioxidant activity of the fruit juice since phase II treatment only decreased antioxidant activity on fruits from Cleopatra [\(Table](#page-3-0) [2](#page-3-0)). It is well-recognised that the role of antioxidant molecules is critical in the detoxification of free radicals, so these results indicate that phase II-stress could reduce the beneficial nutritional properties of fruits on Cleopatra, but not those of fruits on Carrizo.

Plants contain phytochemicals that contribute to antioxidant activity, such as phenolic acids, important components that may reduce the risk of human degenerative diseases ([Hasler, 1998](#page-5-0)). Increased total phenolic contents bring increased antioxidant ability.

#### <span id="page-3-0"></span>Table 2

Effect of DI and rootstock on ascorbic acid (mg/l), total phenolics (mg/l), antioxidant activity (TEAC index), lycopene (mg/l) and  $\beta$ -carotene (mg/l) in the fruit juice of Clemenules mandarin.



 $\ast$ ,  $\ast\ast$  and  $\ast\ast\ast$  indicate significant differences between means at the 5%, 1% and 0.1% levels of probability, respectively, ns = not significant. Values are the means of nine replicates. Values followed by the same letter within a column are not significantly different at the 0.05 level of probability, according to the Tukey test.

Table 3 Total soluble solids (TSS), titratable acidity (TA), maturity index (MI) and juice content (%) in juice of Clemenules mandarin fruit, in relation to the rootstock (Cleopatra or Carrizo) and the DI treatment.

Main effects		<b>TSS</b>	TA	MI	Juice $(\%)$	
Rootstock	Carrizo	13.8	13.3	11.9	39.6	
	Cleopatra	13.6	13.1	12.0	39.8	
Treatment	Control	12.4c	8.6c	14.5a	42.3 <sub>b</sub>	
	Phase II	14.7a	21.2a	7.0 <sub>b</sub>	30.9 $c$	
	Phase III	14.0 <sub>b</sub>	9.9 <sub>b</sub>	14.3a	45.8a	
Rootstock $\times$ treatment						
Carrizo	Control	12.6	8.7	14.5	42.3	
	Phase II	14.5	21.0	7.0	30.1	
	Phase III	14.4	10.2	14.2	46.3	
Cleopatra	Control	12.3	8.4	14.6	42.3	
	Phase II	14.8	21.4	7.0	31.8	
	Phase III	13.7	9.5	14.4	45.3	
Analysis of variance						
Rootstock		ns	ns	ns	ns	
Treatment		***	***	***	***	
Rootstock $\times$ treatment		ns	ns	ns	ns	

<sup>\*\*\*</sup> indicates significant differences between means at the 0.1% levels of probability, respectively. ns = not significant. Values are the means of nine replicates. Values followed by the same letter within a column are not significantly different at the 0.05 level of probability, according to the Tukey test.

However, our results do not show a correlation of antioxidant activity in fruit juice and total phenolic content. The juice of Clemenules mandarin had a significantly increased level of total phenolics when DI treatments were applied (Table 2). Phase II-stress gave fruit juice with more total phenolics (111% more, relative to the control) than when stress was imposed during phase III (17% more). As for the ascorbic acid concentration, the increase of total phenolics with the phase II treatment could be due both to the lower juice content and to new synthesis. However, for the phase III treatment, juice percentage increased with respect to the control, so fruit accumulation of total phenolics was due to new synthesis. The water stress implies an activation of the biosynthesis of phenolic compounds in fruit suffering deficit irrigation ([Roby,](#page-5-0) [Harbertson, Adams, Matthews, & Matthews, 2004\)](#page-5-0). The accumulation of anthocyanins and other phenolic compounds has been associated in olive with an increase in the activity of L-phenylalanine ammonia lyase as a response to water stress [\(Tovar, Romero, Giro](#page-5-0)[na, & Motilva, 2002](#page-5-0)).

#### 3.4. Lycopene and  $\beta$ -carotene

Carotenoids not only add visual appeal but also nutritional benefits. Lycopene, the main dietary precursor of vitamin A, plays a role in the prevention of cancer and chronic disease as a strong dietary antioxidant, and exhibits significant tumour-suppression activity, which has attracted interest in its pharmaceutical potential ([Edge, McGarvey, & Truscott, 1997\)](#page-5-0). Citrus fruits are complex sources of carotenoids, with the largest number of carotenoids found in any fruit [\(Gross, 1987\)](#page-5-0). Deficit irrigation treatments increased the lycopene and  $\beta$ -carotene found in the fruit juice (Table 2). The juice percentage of phase II-stressed fruits was decreased significantly relative to the control (Table 3); so, the increases of lycopene and  $\beta$ -carotene found in the juice of these fruits (Table 2) could have been due to both the water content reduction and new synthesis. However, phase III-stress induced de novo synthesis of lycopene in the fruit, since its concentration increased in spite of the juice increase. Lycopene is accumulated when the synthesis of lutein is blocked ([Ronen, Cohen, Zamir, & Hirschberg, 1999](#page-5-0)). Since lutein is intimately linked with photosynthesis, as part of the lightharvesting system ([Hornero-Méndez, Gómez-Ladrón, & Mínguez-](#page-5-0)[Mosquera, 2000\)](#page-5-0), and drought stress inhibits photosynthetic processes in citrus ([Romero et al., 2006](#page-5-0)), the stress treatments could have decreased the photosynthetic activity and lutein levels, producing an increase in the lycopene concentration.

#### 3.5. Total soluble solids, total acidity and maturity index

During phase II, fruit growth is due to the higher juice content, so acidity decreases with maturation, due to a dilution effect as juice percentage increases. However, when stress was applied during this phase, trees reached high stress levels  $(-2.6 \text{ MPa})$  compared with the control trees  $(-1.0 \text{ MPa})$ , which was maintained for more than three months, producing a passive dehydration of the juice sacs and giving a juice percentage significantly lower, increasing TSS and TA with respect to the control treatment (TA was 2.5-fold higher; Table 3). It is also possible that de novo synthesis of organic acids occurred, to achieve osmotic adjustment in the fruit. The lower water stress reached in the phase III-stress treatment  $(-1.9 \text{ MPa})$ , maintained only for two months, produced a lower increase of TA than in phase II-stressed fruits. This increase of TA could only have been due to new synthesis, since dehydration seems to have been absent (Table 3). High titratable acid contents of the juice have been found on trees subjected to low-moisture conditions ([Mukai, Takagi, Teshima, & Suzuki, 1996\)](#page-5-0).

The balance between the TSS and the acidity is a useful method for determining the fruit maturation process, and it is also the best criterion in correlating fruit quality with consumer acceptance ([Harding & Fisher, 1945](#page-5-0)). In this experiment, fruits were harvested at the same time, so the maturity index shows the state of the maturation process of the fruit in each treatment. Phase III-stressed fruits had a MI similar to that of control fruits ([Table 3](#page-3-0)), and therefore had the same maturation state. However, phase III-stressed fruits reached higher values of juice TSS and TA than control fruits, and they could have improved the flavour of the juice and therefore the commercial quality and consumer acceptance. However, the MI dropped below 50% of the control value in phase II-stressed fruits, due to their high TA. This value was below the level suitable for early harvesting (8-to-1 ratio), indicating an important delay in the maturation process that could make these fruits of unsuitable commercial quality. This MI value was also very low for acceptance of consumers since a pleasant taste is achieved when it is greater than 10.

In spite of Cleopatra being a less-invigorating rootstock than Carrizo, no differences in TSS or TA were found between fruits from trees on Cleopatra and those on Carrizo ([Table 3](#page-3-0)). Trees on invigorating rootstocks experience less water stress than trees on lessinvigorating rootstocks and may influence the TSS through the degree of dilution. Unlike other studies [\(Wagner, Laborem, Marín,](#page-5-0) [Medina, & Rangel, 2002](#page-5-0)), no differences were found in MI between the two rootstocks [\(Table 3](#page-3-0)).

#### 3.6. External fruit colour

The low a/b ratio of the fruit peel found in phase II-stressed fruits, relative to the control and phase III treatments (Fig. 1), shows a delay in the external maturation process of these fruits. This delay of maturation was greater in fruits from trees on Cleopatra than in those on Carrizo rootstock. During the maturation process (end of phase II and all of phase III), the peel colour changes markedly, since chloroplasts, containing carotenoids and chlorophyll, are transformed into chromoplasts, having only carotenoids. In the last phase of this process, chlorophyll and carotenoid levels are strongly modified, and a massive accumulation of carotenoid takes place [\(Rodrigo, Marcos, & Zacarías, 2004](#page-5-0)). Fruits from the phase II-stress treatment had significantly more chlorophyll  $\alpha$  and less  $\beta$ -carotene than fruits of the phase III treatment (Fig. 1), indicating that they were subsequently in an earlier stage of the maturation process.

#### 4. Conclusions

The phase II treatment increased the TSS, but the maturity index decreased strongly, due to the high TA, indicating an important delay in the maturation process. This delay of maturation was observed on the outside of the fruit also, as the low  $a/b$  ratio and the high chlorophyll a level found in the flavedo. On Carrizo, sucrose was hydrolysed, due to the high acidity, and for osmotic adjustment to combat the great drought stress. Fructose and glucose increased but sucrose decreased, increasing reducing sugars. On Cleopatra, glucose, fructose and sucrose increased, for osmotic adjustment, also increasing reducing sugars. The ascorbic acid, total phenolics, lycopene and  $\beta$ -carotene contents increased but not the antioxidant activity.

Phase III treatment increased the acidity and TSS (improving the juice flavour and taste), but the maturity index of these fruits was similar to that of the control, exhibiting the same maturation state. These fruits had more glucose and sucrose than control fruits, and



Fig. 1. External fruit colour (Hunter  $a/b$ ) and flavedo content of chlorophyll a and  $\beta$ carotene, in relation to the rootstock (Cleopatra or Carrizo) and DI treatment. Data are means  $(n = 9)$ .

consequently more total sugars and an increased non-reducing/ reducing sugars ratio. In these fruits, total phenolics and lycopene also increased. In general, this DI treatment was beneficial, increasing the fruit quality without delaying maturation.

Phase III-stress improved the fruit quality by increasing the values of some important quality parameters regarding taste, flavour and nutrition, without altering the maturation process, with re<span id="page-5-0"></span>quality parameters but produced a drastic delay in the maturation process that made the fruits non-commercial.

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#### References

- Albrigo, L. G. (1977). Rootstocks affects ''Valencia" orange fruit quality and water balance. Proceedings of the International Society of Citriculture, 1, 62–65.
- Barry, G. H., Castle, W. S., & Davies, F. S. (2004). Rootstocks and plant water relations affect sugar accumulation of citrus fruit via osmotic adjustment. Journal of the American Society for Horticultural Science, 129, 881–889.
- Buendía, A., Allende, A., Nicolás, E., Alarcón, J. J., & Gil, M. I. (2008). Effect of regulated deficit irrigation and crop load on the antioxidant compounds of peaches. Journal of Agricultural and Food Chemistry, 56, 3601–3608.
- Castle, W. S. (1995). Rootstocks as a fruit quality factor in citrus and decidious fruit crops. New Zealand Journal of Crop and Horticultural Science, 23, 383–394.
- Chartzoulakis, K., Michelakis, N., & Stefanoudaki, E. (1999). Water use, growth, yield and fruit quality of 'Bonanza' oranges under different soil water regimes. Advances in Horticultural Science, 13, 6–11.
- Edge, R., McGarvey, D. J., & Truscott, T. G. (1997). The carotenoids as antioxidants a review. Journal of Photochemistry and Photobiology B, 41, 189–200.
- Gardner, F. E. (1969). A study of rootstock influence on citrus fruit quality by fruit grafting. Proceedings of the First International Citrus Symposium, 1, 359–364.
- Gónzalez-Sicilia, E. (1960). El cultivo de los agrios. INIA. Ministerio de Agricultura. Madrid. p. 806.
- Gross, J. (1987). Carotenoids: Pigments in fruits. London: Academic Press.
- Harding, P. L., & Fisher, D. F. (1945). Seasonal changes in Florida grapefruits. United Stated Department of Agriculture, Technical Bulletin, 886.
- Hasler, C. M. (1998). Functional foods: Their role in disease prevention and health. Food Technology, 52, 63–69.
- Hockema, B. R., & Etxeberria, E. (2001). Metabolic contributors to drought-enhanced accumulation of sugars and acids in oranges. Journal of the American Society for Horticultural Science, 126, 599–605.
- Hornero-Méndez, D., Gómez-Ladrón, R., & Mínguez-Mosquera, M. I. (2000). Carotenoid biosynthesis changes in five red pepper (Capsicum annuum L.) cultivars during ripening. Cultivar selection for breeding. Journal of Agricultural and Food Chemistry, 48, 3857–3864.
- Kriedemann, P. E., & Goodwin, I. (2003). Regulated deficit irrigation and partial rootzone drying. Irrigation insights no. 3. In: A. Currey (Ed.), Land and water Australia (p. 102).
- Miller, J., Rice-Evans, C., Davies, M. J., Gopinathan, V., & Milner, A. (1993). A novel method for measuring antioxidant capacity and its application for monitoring the antioxidant status in premature neonates. Clinical Science, 84, 407–412.
- Moon, D. G., Mizutani, F., Rutto, K. L., & Bhusal, R. C. (2001). Fruit quality and inflorescence formation as affected by mechanical injury inflicted on secondary scaffolds in Satsuma mandarin. Bulletin of the Experimental Farm, Faculty of Agriculture, Ehime University, 23, 1–5.
- Mougheith, M. J., El-Ashram, M., Amerhom, G., & Madbouly, W. (1977). Effect of different rates of irrigation on Navel oranges trees. II. Yield and fruit quality. Annual of Agricultural Science of Moshtohor, 8, 119–127.
- Mukai, H., Takagi, T., Teshima, Y., & Suzuki, T. (1996). Sugar contents in parts of fruit of Satsuma mandarin tree under water stress in autumn. Journal of the Japanese Society for Horticultural Science, 65, 479–485.
- Nagata, M., & Yamashita, I. (1992). Simple method for simultaneous determination of chlorophyll and carotenoids in tomato fruit. Journal Japanese Society of Food Science and Technology, 39, 925–928.
- Pennisi, L. (1985). Utilizzazione industriale. In: Trattato di agrumicoltura. Edagricole Ed. pp. 481–542.
- Pérez-Pérez, J. G., Romero, P., Navarro, J. M., & Botía, P. (2008). Response of sweet orange cv 'lane late' to deficit irrigation in two rootstocks. II: Flowering, fruit growth, yield and fruit quality. Irrigation Science, 26, 519–529.
- Roby, G., Harbertson, J. F., Adams, D. A., Matthews, M., & Matthews, M. (2004). Berry size and vine water deficits as factors in winegrape composition: Anthocyanins and tannins. Australian Journal of Grape and Wine Research, 10, 100–107.
- Rodrigo, M. J., Marcos, J. F., & Zacarías, L. (2004). Biochemical and molecular analysis of carotenoid biosynthesis in flavedo of orange (Citrus sinensis L.) during fruit development and maturation. Journal of Agricultural and Food Chemistry, 52, 6274–6731.
- Romero, P., Navarro, J. M., Pérez-Pérez, J. G., García-Sánchez, F., Gómez-Gómez, A., Martínez, V., et al. (2006). Effects of deficit irrigation on water relations, vegetative development, yield, fruit quality and mineral nutrition of clemenules mandarin on two rootstocks. Tree Physiology, 26, 1537–1548.
- Ronen, G., Cohen, M., Zamir, D., & Hirschberg, J. (1999). Regulation of carotenoid biosynthesis during tomato fruit development: Expression of the gene for lycopene epsilon-cyclase is down-regulated during ripening and is elevated in the mutant delta. The Plant Journal, 17, 341–351.
- Schölander, P. F., Hammel, H. T., Bradstreet, E. D., & Hemmingsen, E. A. (1965). Sap pressure in vascular plants. Science, 148, 339–346.
- Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphotungstic–phosphomolybdic acid reagents. American Journal of Enology and Viticulture, 16, 144–157.
- Tovar, M. J., Romero, M. P., Girona, J., & Motilva, M. J. (2002). L-Phenylalanine ammonia lyase activity and concentration of phenolics in developing olive (Olea europaea L. cv. Arbequina) fruit grown under different irrigation regimes. Journal of Science of Food Agriculture, 82, 892–898.
- Wagner, M., Laborem, G., Marín, C., Medina, G., & Rangel, L. (2002). Efecto de diferentes patrones de cítricas e intervalos de riego sobre la calidad y producción de la naranja 'Valencia'. Bioagro, 14, 71–76.
- Yakushiji, H., Morinaga, K., & Nonami, H. (1998). Sugar accumulation and partitioning in Satsuma mandarin tree tissues and fruit in response to drought stress. Journal of the American Society for Horticultural Science, 123, 719–726.
- Yakushiji, H., Nonami, H., Fukuyama, T., Ono, S., Takagi, N., & Hashimoto, Y. (1996). Sugar accumulation enhanced by osmoregulation in Satsuma mandarin fruit. Journal of the American Society for Horticultural Science, 121, 466–472.