

### CyTA - Journal of Food



Date: 18 April 2016, At: 02:34

ISSN: 1947-6337 (Print) 1947-6345 (Online) Journal homepage: http://www.tandfonline.com/loi/tcyt20

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**To cite this article:** R. Martínez-Las Heras, J.C. Amigo-Sánchez, A. Heredia, M.L. Castelló & A. Andrés (2016) Influence of preharvest treatments to reduce the seasonality of persimmon production on color, texture and antioxidant properties during storage, CyTA - Journal of Food, 14:2, 333-339, DOI: 10.1080/19476337.2015.1113204

To link to this article: <a href="http://dx.doi.org/10.1080/19476337.2015.1113204">http://dx.doi.org/10.1080/19476337.2015.1113204</a>

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## Influence of preharvest treatments to reduce the seasonality of persimmon production on color, texture and antioxidant properties during storage

# Influencia de los tratamientos precosecha para reducir la estacionalidad de la producción de caqui en el color, la textura y las propiedades antioxidantes durante el almacenamiento

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(Received 29 June 2015; final version received 23 October 2015)

Persimmon production has increased considerably, thanks to techniques for removing astringency whilst maintaining the strong consistency. Currently, the needs of cooperatives are focused on increasing the commercial period. Thus, the aim of this study was to analyze the effect of preharvest treatments (paclobutrazol (PBZ) and Ethephon to accelerate ripening and GA<sub>3</sub> to delay it) on persimmon size, composition, color index (CI), texture and antioxidant properties over 11 days of postharvest storage at 4°C. The results showed that the size of fruits subjected to preharvest treatment was smaller than in untreated fruit. Moreover, CI of the apical zone was higher in samples of standard ripening throughout the first few days of storage. It is also noteworthy that the treated fruits at the beginning of storage reported greater antioxidant properties. Finally, the evolution of the antioxidants has been fitted with a first-order model to predict their kinetic degradation depending on the persimmon harvest period.

Keywords: persimmon; harvest ripening; texture; color index; total phenolic compounds; antioxidant activity; kinetics

La producción de caqui ha aumentado considerablemente gracias a las técnicas para eliminar la astringencia manteniendo su consistencia firme. Actualmente, las cooperativas buscan aumentar su periodo comercial. Por ello, el objetivo de este estudio fue analizar el efecto de los tratamientos precosecha (PBZ y etefón para acelerar la maduración y GA<sub>3</sub> para retrasarla) en el tamaño del caqui, su composición, color (CI), textura y propiedades antioxidantes durante 11 días de almacenamiento postcosecha a 4°C. Los resultados mostraron que el tamaño de los frutos sometidos a tratamientos precosecha fue más pequeño. Además, el CI de la zona apical fue mayor en las muestras con maduración convencional al principio del almacenamiento. Los frutos tratados al inicio del almacenamiento registraron mejores propiedades antioxidantes. Finalmente, la evolución de los antioxidantes se ajustó a un modelo de primer orden para predecir su degradación cinética dependiendo del periodo de recolección del caqui.

Palabras clave: Caqui; Madurez de recolección; Textura; Índice de Color; Compuestos fenólicos totales; Actividad antioxidante; Cinética

### 1. Introduction

The persimmon species (Diospyros kaki Thunb.) is a fruit tree originating from China. In Spain, the species spread for ornamental and wood uses at the end of the nineteenth century. The tree was later used to produce fruit for consumption and became a traditional crop located in Mediterranean gardens or orchards for domestic consumption. However, in the last 15 years, there has been a high increase in production of the cultivar "Rojo Brillante" in the region of Valencia due to the application of techniques to remove the astringency from fruits whilst maintaining their firm texture (Arnal & Del Río, 2003; Ben-Aire & Sonego, 1993; Taira & Matsumoto, 1997). These techniques mainly consist of using CO<sub>2</sub>-rich atmospheres for 24 hours at 20-24°C to insolubilize the tannins in the pulp of persimmon, which are responsible for the astringency. Thus, there are two ways to commercialize this fruit: "Classic," fruits with natural ripening and therefore with soft texture; and "Persimmon," fruits subjected to techniques to remove astringency and keep their firm texture, but with a good taste. This fact has led to a wide increase in the distribution of this product and 70% of this production is now exported to countries like Germany, France, Holland, Czech Republic, Russia and Brazil (IVIA, 2014)

However, availability of this crop in markets is restricted to October-December due to its short seasonality. One of the major challenges today is to extend the period this fruit is commercially available to avoid price drops during maximum production and hence improve profitability. This can be achieved by using different treatments in preharvest stages such as paclobutrazol (PBZ) and Ethephon in order to accelerate the ripening of fruits and gibberellic acid (GA<sub>3</sub>) to delay it. PBZ has been found to be predominantly effective in the induction of early flowering and thus finding scope for offseason production in different crops such as mango (Upreti et al., 2013), citrus (Martínez-Fuentes et al., 2013), wheat (Kondhare, Kettlewell, Farrell, Hedden, & Monaghan, 2012, 2013, 2014), among others. Ethephon treatment caused a transient increase in ethylene production and inhibited vegetative growth (Tamari, Pappa, Zered, & Borochov, 1998). Moreover, ethylene is known to generally inhibit shoot extension whilst promoting root proliferation (Jaiswal & Sawhney, 2006; Liu, Mukherjee, & Reid, 1990; Pan, Wang, & Tian, 2002) and significantly speeds up senescence (Hodges & Forney, 2000; Philosoph-Hadas, Meir, & Aharoni, 1991). The treatment of orchards with GA3 is one of the most widely used plant growth

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regulators for manipulation of fruit development and ripening (Dagar, Weksler, Friedman, & Lurie, 2012; Facteau, Rowe, & Chectnut, 1985; Southwick & Yeager, 1995; Zilka et al., 1997). It has recently been also found to be useful in reducing flower density, which consequently reduces crop load and increases fruit size in peaches and nectarine (Stern & Ben-Arie, 2009). GA<sub>3</sub> applied at the end of pit hardening led to a delay in fruit ripening and in the development of internal breakdown in stored nectarines (Dagar et al., 2012; Zilka et al., 1997).

The influence of these pretreatments on the quality of persimmon (size, taste, color, texture, etc.) when fruits are in the orchard (preharvest period), at the moment of consumption and during storage (post-harvest period), should be studied in order to verify whether it is really possible to offer a homogenous product during the campaign of this fruit. Besides, the response of bioactive compounds (such as antioxidants) to these plant growth regulators has not been widely studied. Persimmon fruit contains a large amount of polyphenols, including condensed tannins and other phenolic compounds, which have conventionally been used to treat health problems such as coughs, hypertension, dyspnea, paralysis, frostbite, burns and bleeding (Matsuo &Ito, 1978; Mowat, 1990). Gorinstein et al. (2000) proved that a persimmon-supplemented diet significantly lowered total cholesterol, triglycerides in plasma and LDL-cholesterol in rats fed on a high-cholesterol diet. According to many authors (Al-Maiman & Ahmad, 2002; Mirdehghan & Rahemi, 2007; Odriozola-Serrano, Soliva-Fortuny, & Martín-Belloso, 2008; Siriwoharn, Wrolstad, Finn, & Pereira, 2004), fruit chemical, especially phenolic and antioxidant properties, is influenced by cultivar, growing region and degree of fruit ripeness at harvest. The decrease in total phenolic content (TPC) in many fruits has been attributed to the oxidation of polyphenols by polyphenoloxidase during ripening (Amiot, Tacchini, Aubert, & Oleszek, 1995; Kulkarni & Aradhya, 2005; Shwartz et al., 2009). Other possible reasons related to this phenomenon could be the regulation of polyphenol biosynthesis during fruit ripening (a dilution effect as fruit increased in size), as well as the contribution of phenolic compounds to the biosynthesis of the flavylium ring of anthocyanins (Kulkarni & Aradhya, 2005). Recently, Sanchis, Mateos, and Pérez-Gago (2015) have observed that the maturity stage of fresh-cut Rojo Brillante persimmon at harvest had an effect on both fruit firmness and the efficacy of the antioxidants to control enzymatic browning. Thus, the persimmon harvested at the beginning of the season could be processed as a fresh-cut commodity, even after 3 days of storage at 15°C if treated with 0.01 kgL<sup>-1</sup> ascorbic acid or 0.01 kgL<sup>-1</sup> citric acid. However, processing fruits from late season immediately after harvest and being treated with ascorbic acid were recommended.

The aim of this study was to assess the effect of preharvest treatments (PBZ and Ethephon to accelerate and GA<sub>3</sub> to delay ripening) on the size, soluble solids, moisture content, color, texture and antioxidant properties (TPC and antioxidant activity (AA)) with a view to extending the commercial availability season of "Rojo Brillante" persimmon.

### 2. Materials and methods

### 2.1. Plant materials and preharvest treatments

Persimmon (Diospyros kaki Thunb. cv. Rojo Brillante) trees were grown in different orchards in Alginet (Spain). Trees were divided in three groups; the first group, labeled as "natural," was used as a reference group because the trees were not treated and the fruit was harvested in mid-November; the second group of trees was treated with GA<sub>3</sub> (CEKU-GIB, 1.6%) to delay fruit ripening and the fruit (labeled as "delayed") was harvested in mid-December; the third group of trees was treated with PBZ (Cultar) and Ethephon (Fruitel® 48% Bayer) to accelerate the ripening process and the fruit (labeled as "accelerated") was harvested in mid-September.

### 2.2. Postharvest treatment

After harvest, fruits were stored at room temperature for 24 h before applying deastringency treatment, which was carried out in closed chambers with 95% CO<sub>2</sub> for 24 h at 20°C and 90% of relative humidity (Arnal & Del Río, 2003).

### 2.3. Experimental design

After this treatment to remove astringency, 40 fruits from each group were stored at 4°C and the evolution of moisture content, color, texture and antioxidant properties was evaluated after 1, 3, 5, 7, 9 and 11 days.

### 2.4. Analytical methods

Moisture content was determined using the 934.06 method (AOAC, 2000) for sugar-rich fruits.

### 2.4.1 Soluble solids content

Soluble solids content was determined using a Carl Zeiss Abbe Atago refractometer model 89,553 with thermostat set to 20°C using a solution of polyethyleneglycol at 5% (w/w) (PEG 6000, Panreac) to precipitate the tannins still present in the juice (Sugiura, Kataoka, & Tomana, 1983).

### 2.4.2 Diameter

Diameter was determined using a universal fruit calliper (TR 53,307 with a range 25–95 mm).

### 2.4.3 TPC and AA

Samples were spectrophotometrically analyzed, employing a modified version of the Folin-Ciocalteu method (Sakanaka, Tachibana, & Okada, 2005), in order to determine the TPC. The TPC was extracted with methanol (3 grams of crushed fruit/5 mL of methanol) and then continuously stirred at 200 rpm for 1 hour (horizontal shaker COMECTA WY-100). The test tubes were centrifuged for 10 minutes at 10,000 rpm (Medifriger BL-S, P-Selecta). 0.5 mL of distilled water and 0.125 mL of the supernatant of the extract were added to a cuvette followed by the addition of 0.125 mL of Folin-Ciocalteu reagent. The mixture was shaken and 1.25 mL of a 7% sodium carbonate solution and 1 mL of distilled water were added after 6 min. The color was left to develop for 90 min and the absorbance was measured at 760 nm using a spectrophotometer (JASCO V-630). The measurement was compared to a standard curve of gallic acid solutions and expressed as mg of gallic acid equivalents per 100 grams of persimmon (mg GAE/ 100 g of fruit). A blank was prepared in the same way but without any sample.

The AA of the persimmon fruit was measured on the basis of the scavenging activities of the Stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical as described by Shahidi, Liyana-Pathirana, and Wall (2006) with some modifications. According to this method, the intensity of the purple color of DPPH solution decreases in the presence of an antioxidant, and this absorbance change is measured spectrophotometrically at 515 nm.

Three grams of the crushed sample was diluted in 5 mL of methanol and stirred for 5 minutes. The test tubes with the samplemethanol mixture were then centrifuged at 10,000 rpm for 10 minutes (Medifriger BL-S, P-Selecta). 0.1 mL of the supernatant was added to 3.9 mL of a methanol solution of DPPH (80:20; methanol:water) (0.025 mg/mL). The solution was shaken and after 30 min the absorbance of the sample was measured at 515 nm using methanol as a blank. The differences in absorbance of the samples with respect to the blank in that time were compared with a standard Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) curve and expressed as mg of Trolox equivalents per 100 grams of persimmon (TE/100 g of fruit).

### 2.5. Texture measurements

Flesh firmness was determined with a Texturometer TA.XTplus Texture Analyser by Stable using a 10 mm flat plunger at a test speed of 2 mm/s. The plunger penetration depth was 20 mm. Six persimmon fruits from each ripening stage and day of storage were used to analyze texture. Fruits were cut in halves longitudinally and the flat side was placed on the platform of the texture analyzer to keep the sample fixed. The parameters recorded in the compression test were the force required by the plunger to pass through the sample, expressed in Newtons (N).

### 2.6. Color measurements

Skin color was evaluated using a Minolta Colorimeter (Model CM-3600d) spectrophotometer with D65 illuminant, 10° observer and SCE mode. L\*, a\*, b\* Hunter parameters were measured in two zones (equatorial and apical) and results were expressed as a skin color index (CI) (Equation (1)), as described by Jiménez-Cuesta, Cuquerella, and Martínez-Jávega (1981).

$$CI = \frac{1000 \cdot a}{L \cdot b} \tag{1}$$

### 2.7. Statistical analysis

At least three replicates were carried out for each determination. Results are expressed as mean values  $\pm$  standard deviation. Data were subjected to analyses of variance (ANOVA), and multiple comparisons between means were determined using the LSD test

 $(P \le 0.05)$  using the Statgraphics Plus 5.1 software application (Manugistics, Inc., Rockville, MD, USA).

### 3. Results and discussion

Preharvest treatments to control the ripening process can result in changes in fruit quality and storage capacity. The influence of the applied preharvest treatments on the moisture content, Brix and equatorial diameter, was analyzed just after harvest and the LSD averages given by the ANOVA analysis (number of observations = 72) are shown in Table 1.

Fruits harvested from the trees pretreated to delay ripening had a lower moisture content and higher soluble solids (°Brix) at the time of harvest than those ripened naturally or those subjected to accelerated ripening. Since small fruit size is one of the limiting factors in marketing persimmon fruit, and many other species, the equatorial diameter was measured. The size of the harvested fruits (equatorial diameter) was affected by both preharvest treatments; the naturally ripened fruits were larger than those harvested from the treated trees.

The evolution of texture, color and antioxidant properties of fruits ripened under different conditions (natural, delayed and accelerated) during the postharvest period after removing astringency was analyzed.

In terms of texture, the maximum force  $(F_{max})$  was the parameter selected to discuss the results (Figure 1). The  $F_{max}$  recorded in the reference samples ("natural") remained nearly constant throughout the testing period, while a decrease is observed in the fruits treated to accelerate or delay ripening. The drop in the maximum force required for samples that underwent delayed ripening is observed after the 7-day control period in spite of the initially higher values, reaching similar values to those recorded in the accelerated maturation samples.

As was expected, CI was higher in the apical than in the equatorial zone, since fruit begins to change color from the bottom, as can be observed in Figure 2. Furthermore, changes in CI throughout storage time were not significant in the equatorial zone. However, the apical zone of the naturally ripened persimmons reported higher CI values during the first week of storage, reaching similar values to the other fruits after that time. Consequently, significant differences between samples with different preharvest treatments were found from the statistical analysis (Figure 3). Lower CI values were reported for fruits treated to delay or accelerate ripening than for fruits that had not been pretreated probably due to the retarded effect of GA2 and Ethephon in the development of new carotenoids. Nevertheless, initial harvest time color shown by preharvest treated and untreated samples resulted in CI values that were within the range considered as being commercial for this cultivar (Novillo, Salvador, Magalhaes, & Besada, 2014). Since this

Table 1. Average values for moisture content, <sup>o</sup>Brix and equatorial diameter of naturally ripened fruits (natural), preharvest treated to delay ripening (delayed) and to accelerate ripening (accelerated).

Tabla 1. Valores medios del contenido en humedad, "Brix y diámetro ecuatorial de frutos de maduración natural o tratados para acelerar o atrasar la maduración.

Ripening type	Moisture Content (%)	°Brix	Equatorial Diameter (mm)	
Delayed	$80.67 \pm 0.13$ (a)	$15.2 \pm 0.4$ (a)	$75.2 \pm 1.7$ (a)	
Natural	$82.12 \pm 0.09$ (b)	$11.1 \pm 0.3$ (b)	$82.6 \pm 1.2$ (b)	
Accelerated	$82.87 \pm 0.07$ (c)	$10.9 \pm 0.2$ (b)	$74.2 \pm 0.9$ (a)	

Note: Equal letters in parentheses indicate homogenous groups. Letras iguales en paréntesis indican grupos homogéneos.

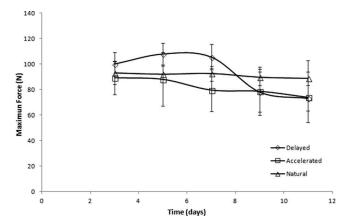


Figure 1. Evolution of the Maximum Force (N) from texture test with storage time after astringency removal post-harvest treatment.

Figura 1. Evolución de la fuerza máxima (N) del ensayo de textura en función del tiempo de almacenamiento después del tratamiento postcosecha para la eliminación de la astringencia.

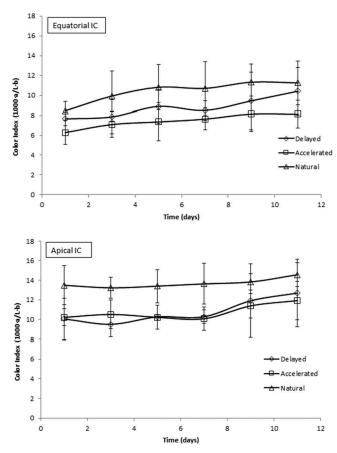


Figure 2. Evolution of the Color Index (CI) with storage time after astringency removal post-harvest treatment.

Figura 2. Evolución del índice de color (CI) con el tiempo de almacenamiento después del tratamiento postcosecha para la eliminación de la astringencia.

cultivar is marketed when orange, all treatments showed a commercially acceptable color, even samples from the accelerated treatment, which had the lowest CI values at harvest.

The total polyphenols content and the AA were also measured throughout the storage time and the evolution is shown in Figure 4.

The degradation kinetics of persimmon fruit phenolic compounds and AA was analyzed by fitting a first-order kinetic

model (Equations 2 and 3) to the experimental data (Gonçalves, Pinheiro, Abreu, Brandao, & Silva, 2010; Martins, Van Boekel, & Jongen, 2000).

$$P = P_0 - k_P t \tag{2}$$

$$AA = AA_0 - k_{AA}t \tag{3}$$

# Means and 95,0 Percent L SD Intervals 12 11 10 9 Accelerated Natural Delayed Maduration

Figure 3. LSD interval results of Color Index (CI) as a function of the moment of harvest (accelerated, natural or delayed).

Figura 3. Intervalos LSD de los resultados del índice de color (CI) en función del momento de la recolección (acelerada, natural o atrasada).

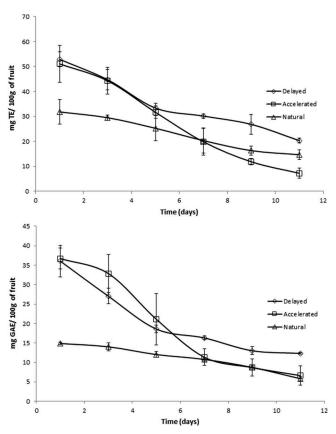


Figure 4. Evolution of the TPC (mg GAE/100 g of fruit) and AA (mg TE/100 g of fruit) with storage time after astringency removal post-harvest treatment.

Figura 4. Evolución del contenido en fenoles totales (mg Ácido Gálico Equivalentes/100 g de fruta) y actividad antioxidante (mg Trolox Equivalentes /100 g de fruta) con el tiempo de almacenamiento después del tratamiento postcosecha para la eliminación de la astringencia.

Here, P and AA are the natural logarithm of phenol content and the AA respectively, the subindex 0 indicates the initial value of the parameter, t is the stored time and  $k_P$  and  $k_{AA}$  are the model parameters for phenols and AA, respectively (Table 2). Thus, the slopes ( $k_P$  and  $k_{AA}$ ) indicate the speed of

degradation, whereas the y-intercepts (P and AA) represent the initial concentration of the antioxidant. This fitting was used based on the results obtained by Jaiswal & Abu-Ghannam (2013) who reported that the first-order reaction model showed a good fit for the degradation of polyphenols and the antioxidant

Table 2. Parameters of first-order kinetic model for TPC and AA adjustments.

Tabla 2. Parámetros del ajuste del modelo cinético de primer orden para el contenido en fenoles totales y actividad antioxidante.

	$P_0$	$k_{P}$	$R^2$	$AA_0$	$K_{AA}$	$R^2$
Natural Accelerated Delayed	$\begin{array}{c} 15.2 \pm 0.5 \\ 36.7 \pm 0.7 \\ 36 \pm 4 \end{array}$	$\begin{array}{l} -0.095 \pm 0.004 \\ -0.192 \pm 0.002 \\ -0.11 \pm 0.01 \end{array}$	$\begin{array}{c} 0.90 \pm 0.02 \\ 0.96 \pm 0.02 \\ 0.94 \pm 0.04 \end{array}$	$32 \pm 2$ $51 \pm 4$ $52 \pm 4$	$\begin{array}{l} -0.082\pm0.008 \\ -0.20\pm0.02 \\ -0.09\pm0.01 \end{array}$	$0.95 \pm 0.01$ $0.96 \pm 0.02$ $0.95 \pm 0.06$

capacity of York cabbage after microwave processing. It is observed that the preharvest treated fruits initially reported greater polyphenol concentration and therefore greater AA than the untreated samples, at least during the first days of storage; however the rate of degradation of phenolic compounds as well as AA is greater in the case of the pretreated samples (Figure 4). Samples treated to accelerate ripening reached similar values to the untreated fruit at the end of the storage period, while fruits treated to retard ripening showed higher values of polyphenols and AA after 11 days of storage (Figure 4). This trend is also reflected in the values obtained for the model parameters (Table 2) in terms of the initial values of polyphenols ( $P_0$ ) and antioxidant activity ( $AA_0$ ) along with the rate of degradation ( $P_0$ ) and  $P_0$ 0 and  $P_0$ 1.

### 4. Conclusions

Preharvest treatments, both to accelerate or to retard ripening, applied to persimmon trees to extend the harvesting period mainly imply a reduction in the size of fruits and an increase in the antioxidant properties at the beginning of the storage period. Furthermore, first-order kinetic models have been fitted to predict the phenolic content and AA versus time depending on the moment of harvest of fruits (accelerated, natural or delayed ripening).

### Acknowledgment

The authors would like to thank the COAGRI Cooperative in Alginet (Valencia) for providing the persimmon samples and the cooperation by their staff to perform this study.

### Disclosure statement

No potential conflict of interest was reported by the authors.

### **Funding**

The authors thank the Universitat Politècnica de València for the PhD scholarship of the author Ruth Martínez Las Heras.

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