

Preharvest chlorogenic acid treatments enhance fruit quality and affect the phenolic composition of 'Navel' oranges during cold storage

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ABSTRACT

The storability of sweet oranges is determined by its quality at harvest. Therefore, this study explores the effect of preharvest treatments with chlorogenic acid (CGA) at 5 and 50 mg L⁻¹ on quality traits and phenolics of 'Navel' oranges at harvest and during 60 days of cold storage. The results showed that weight loss and firmness were 14 % and 20 %, respectively, higher in oranges treated with 5 mg L⁻¹ CGA than in the controls. In addition, this treatment reduced the respiration rate at harvest and during cold storage. It was observed that oranges treated with 5 mg L⁻¹ CGA had lower maturity index values due to the fact that the titratable acidity was 12 % higher compared to the controls and those treated with 50 mg L⁻¹ CGA. For the citrus colour index, the results showed the lower values in fruits treated with 5 mg L⁻¹ CGA, which coincided with the accumulation of carotenoids in the flavedo. On average, the total phenolic content was 10 % lower in fruits treated with 5 mg L⁻¹ CGA in the flavedo and juice than in the controls. These differences were mainly due to the accumulation of hesperidin. Furthermore, an increase in naringin and CGA content was also observed in oranges treated with 5 mg L⁻¹ CGA. In conclusion, a concentration of 5 mg L⁻¹ CGA could be an effective strategy for improving the postharvest storability of 'Navel' oranges by regulating the maturation process and phenolic metabolism.

1. Introduction

Sweet oranges (*Citrus sinensis* L.) are one of the most important fruit crops with an annual production of 70 million tonnes in 2023 (FAOSTAT, 2023). These fruits are widely consumed worldwide as fresh product or juice, accounting for about 70 % of total citrus production and consumption due to their external appearance, organoleptic properties and nutritional quality (Ma et al., 2021). In addition, orange fruits are rich in bioactive compounds such as ascorbic acid and phenolics, mainly flavonoids, which together with carotenoids and essential oils play an important role in the health benefits associated with their consumption (Lu et al., 2021). Oranges are non-climacteric fruits, with low respiration and ethylene production during ripening. However, during prolonged storage, oranges are susceptible to physiological, biochemical and pathological disorders, resulting in quality losses during storage and transportation, causing significant economic losses (Ding et al., 2015). Therefore, adopting methods to maintain orange quality and extend its shelf life is crucial. In recent years, several safe and environmentally

friendly strategies such as salicylic acid (Ramezani et al., 2018), methyl jasmonate (Rehman et al., 2018), glycerol (Zhu et al., 2023) or inorganic salts (Serna-Escolano et al., 2023) have been tested in pre-harvest to increase the storability of oranges.

Phenolic compounds are secondary metabolites represented by flavonoids and phenolic acids. These compounds play a crucial role in plants as internal physiological regulators associated with the defence system against pathogens and stress, increasing the antioxidant capacity of the fruit (Ignat et al., 2010). Fewer reports exist on the impact of pure phenolic acids on physiological response and postharvest quality preservation of fruits and vegetables. However, postharvest treatments with trans-cinnamic acid in strawberries have shown higher levels of primary and secondary metabolites and lower polyphenol oxidase activity compared to the controls, which was associated with less incidence of browning (Song et al., 2025). Similarly, tomatoes treated with p-coumaric acid exhibited delayed ripening due to the regulation of maniferic acid biosynthesis and phenylalanine metabolism (Lu et al., 2024).

Chlorogenic acid (CGA, 3-O-caffeoylquinic acid), is an important

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phenolic compound in the hydroxycinnamic acid family (Santana-Galvez et al., 2017). It is one of the most abundant free phenolic acids in a wide range of fresh fruits. Furthermore, CGA is an important signalling molecule and plant activator with multiple physiological functions in plants related to the induction of the resistance response during biotic and abiotic stress. In this sense, CGA is considered an antioxidant due to its ability to donate hydrogen groups that act as free-radical scavengers. Most studies on the application of CGA in fruit crops have focused mainly on postharvest treatments. An 'in vitro' experiment with nectarine tissue discs treated with CGA highlighted its effect in enhancing antioxidant systems (Xi et al., 2017). A previous 'in vitro' study with apple tissue discs, showed that CGA treatment could regulate the NADP-malic enzyme activity, which affected fruit ripening and senescence (Xi et al., 2016). Recently, postharvest treatments of apples with CGA 100 mg l⁻¹ were found to regulate internal energy metabolism and alleviate the effects of physical injury, thereby promoting postharvest healing of the fruit (Shu et al., 2020). In tomatoes, postharvest CGA treatments at 50 mg l⁻¹ effectively delayed weight loss and maintained firmness during cold storage (Ilea et al., 2024). Moreover, postharvest CGA treatments at 25–150 mg l⁻¹ improved the resistance to *Penicillium expansum* in peach by increasing the expression of genes related to the SA signalling pathway (Jiao et al., 2018). Other works using postharvest CGA have shown a direct effect in controlling spore germination and mycelial growth of several pathogenic fungi associated with horticultural crops by reducing the stability of the fungal cell wall (Martínez et al., 2017; Zhang et al., 2020). In preharvest, the use of CGA at 0.5 mmol l⁻¹ reduced the incidence of peel browning by regulating the activity of polyphenol oxidase and peroxidase enzymes in 'Golden Delicious' apples (Wang et al., 2014). Moreover, the exogenous application of 1 mg mL⁻¹ CGA increased the levels of polyphenols, flavonoids and antioxidant compounds in *Solanum melongena*, thereby reducing stress levels and the incidence of pest attacks (Talukder et al., 2025).

Considering the role of CGA as a biological signalling molecule, as well as the importance of developing safe and environmentally friendly treatments. In this sense, CGA can be employed to prevent orange quality losses during cold storage. Furthermore, to the best of our knowledge, no research has been conducted on using this treatment to improve 'Navel' orange quality. Therefore, the present study aims to evaluate the effect of preharvest treatments with CGA at 5 and 50 mg l⁻¹ on quality parameters of 'Navel' oranges, as well as on endogenous phenolic compounds, at harvest and during postharvest storage.

2. Materials and methods

2.1. Plant material and experimental design

The experiments for the 2023–2024 season were conducted on a commercial plot situated in Librilla (Murcia, Spain). This location experiences a Mediterranean climate, with an average annual temperature of 17 °C and daily rainfall of 15.7 mm, and utilizes standard orange growing practices. Tree blocks of five 'Navel' orange trees grafted on *Citrus macrophylla*, twelve years old, were randomly selected for each treatment, 5 mg l⁻¹ CGA, 50 mg l⁻¹ CGA and control. These CGA concentrations were selected according to unpublished previous results. Orange trees were treated with 5 litres of the solution per tree by foliar spraying, using a mechanical system to try to wet the entire canopy and fruit on the trees. These treatments were administered monthly, beginning in December 2023 and concluding five days prior harvest in March 2024. All CGA treatments (Biosynth, Staad, Switzerland) were prepared in distilled water with 0.1 % Tween 20 as surfactant. Control trees were treated with 0.1 % Tween 20 dissolved in an aqueous solution. Oranges were harvested once they achieved the commercial maturity index mandated by the market as defined by Lado et al. (2014). Subsequently, the harvested fruit was transported to the laboratory within two hours. There, five homogeneous lots, each consisting of 15 oranges (comprising

three replicates of five fruits), were chosen based on their consistent size, colour, and absence of physical damage. These fruits were then stored for 60 days under controlled conditions of 8 °C and 85 % relative humidity. Fortnightly analyses were performed on a randomly selected lot from each treatment group.

2.2. Fruit quality evaluation

Weight loss (WL) was quantified by individually weighing oranges before and after cold storage, then expressing the results as a percentage (%). Firmness measurements were taken using a TX-XT2i plus texture analyser (Stable Microsystems, Godalming, UK) fitted with a steel plate. A 5 % deformation force relative to the fruit's diameter was applied once per experimental unit, with results reported in N mm⁻¹. For respiration rate (RR), five oranges were sealed in 4.6 L plastic containers for one hour. Afterward, a 1 mL sample from the container's headspace was extracted and injected into a gas chromatograph (Shimadzu 14B-GC, Kyoto, Japan) coupled to a thermal conductivity detector. The results were expressed as CO₂ kg⁻¹ h⁻¹ (Martínez-Esplá et al., 2018). Orange colour was determined on five fruits per replicate using a CRC 400 colorimeter (Minolta Camera Co., Tokyo, Japan), taking three measurements around the equatorial surface. The Hunter Lab values obtained were subsequently used to calculate the citrus colour index (CCI) using Eq. (1):

$$CCI = 1000 \times \frac{a}{L \times b} \quad (1)$$

Total soluble solids (TSS) were measured in duplicate using a digital refractometer (Hanna Instruments, Rhode Island, USA) on juice from five oranges per replicate. Titratable acidity (TA) was determined by titrating a 1 mL juice sample in 25 mL of distilled water with 0.1 mM NaOH until a pH of 8.1 was reached, using an OMNIS automatic titrator (OMNIS, Metrohm AG, Herisau, Switzerland). TSS and TA results were expressed in g per 100 g⁻¹ or g citric acid equivalents per 100 g⁻¹, respectively. The Maturity Index (MI) for these oranges was calculated as the absolute ratio of TSS to TA.

2.3. Total carotenoids

Total carotenoids were extracted by homogenizing 2 g of flavedo in 5 mL of ethyl acetate. Then, the extracts were centrifuged at 10,000 × g for 12 min at 4 °C. Total carotenoid content (TCC) was directly determined by analysing the supernatant's absorbance at 450 nm using a spectrophotometer as previously reported Serna-Escolano et al. (2023). The results were expressed as mg β-carotene equivalent per 100 g⁻¹ of fresh weight (FW).

2.4. Total and individual phenolics

Total phenolics were extracted from flavedo by homogenizing 2 g of tissue with 15 mL of a water:methanol solution (2:8) supplemented with 2 mmol l⁻¹ NaF to inhibit polyphenol oxidase activity. The homogenate was then centrifuged at 10,000 × g for 10 min at 4 °C, and the supernatant was diluted 1:5. Meanwhile, the juice was centrifuged, and 25 µL was used for total phenolic content (TPC) quantification with Folin-Ciocalteu reagent according to Serna-Escolano et al. (2019). The results were expressed as mg of gallic acid equivalent per 100 g⁻¹ of FW.

For individual phenolic quantification, 1 mL of the flavedo extraction supernatant and 1 mL of centrifuged juice were filtered through a 0.45 µm filter. A 20 µL aliquot was then injected into an HPLC Hewlett Packard 1100 system (Agilent, California, United States) to quantify the flavonones, hesperidin and narirutin, and the phenolic compound, CGA, in duplicate in each sample, using analytical standards for quantification of each bioactive compound as reported by Gironés-Vilaplana et al. (2013). The results (mean ± SE) were expressed as mg 100 g⁻¹ FW.

2.4. Statistical analysis

The data were expressed as the mean $\pm$ SE of three random replicates. For this assay, a Two-way Analysis of Variance (ANOVA) was conducted. Subsequently, Tukey's HSD multiple range test was applied to assess the level of significance ($p < 0.05$) in order to determine significant differences between treatments and storage time. All statistical analyses were performed using SPSS v. 20 for Windows (IBM, Chicago, USA).

3. Results

Weight loss increased during cold storage in all treatments, and during the first 45 days of cold storage no significant ($p > 0.05$) differences were observed between treatments. However, after 60 days of cold storage, the results showed a decrease in WL of 14 % in fruits treated with 5 mg l⁻¹ CGA compared to controls (5.15 ± 0.15 %), with no significant ($p > 0.05$) differences with those treated with 50 mg l⁻¹ CGA (Fig. 1A). At harvest, the highest respiration rate was observed in fruits treated with 50 mg l⁻¹ CGA followed by fruits treated with 5 mg l⁻¹ CGA and found the lowest respiration rate in control fruits as shown in Fig. 1B. After 15 days of cold storage, fruits treated with 50 mg l⁻¹ CGA maintained the highest respiration rate, while the lowest values were found in fruits treated with 5 mg l⁻¹ CGA and controls, with no significant ($p > 0.05$) differences between them. After 45 days of cold storage, fruits treated with 50 mg l⁻¹ CGA and controls showed similar respiration rate values, with the lowest results in those treated with 5 mg l⁻¹ CGA. These differences were maintained until the end of the experiment (Fig. 1B). Firmness decreased in all treatments during cold storage. However, after 60 days of cold storage, the results showed that the fruits treated with 5 mg l⁻¹ CGA maintained the higher firmness values (5.55 ± 0.17 N mm⁻¹) compared to the controls and those treated with 50 mg l⁻¹ CGA, with a decrease ca. 20 % on average in both treatments (Fig. 1C).

Regarding TSS an increase of ca. 10 % was observed in oranges treated with 50 mg l⁻¹ CGA compared to the control batches ($8.50 \pm 0.08^\circ$ Brix) and those treated with 5 mg l⁻¹ CGA (8.57 ± 0.06) at harvest. In addition, TSS increased in all treatments during 60 days cold storage, maintaining the differences observed at the beginning of the experiment (Fig. 2A). In contrast, TA decreased during the cold storage in all

treatments. The results at harvest showed the highest values of TA in fruits treated with 5 mg l⁻¹ CGA (1.41 ± 0.02 g 100 mL⁻¹) followed by those treated with 50 mg l⁻¹ CGA (1.32 ± 0.04 g 100 mL⁻¹) and the controls (1.26 ± 0.04 g 100 mL⁻¹). These significant ($p < 0.05$) differences were maintained during cold storage (Fig. 2B). Therefore, when assessing the MI, fruits treated with 50 mg l⁻¹ CGA and the controls showed higher values than those treated with 5 mg l⁻¹ CGA at harvest and during the cold storage (Fig. 2C). In this regard, the results showed that the preharvest application of 5 mg l⁻¹ CGA was effective in significantly ($p < 0.05$) delaying MI during storage compared to the control and 50 mg l⁻¹ CGA batches. The highest concentration of CGA and the control fruit generally showed no significant ($p > 0.05$) differences between them throughout the experiment.

For orange fruit colour at harvest, the results showed the highest CCI values in fruits treated with 50 mg l⁻¹ CGA followed by the controls and those treated with 5 mg l⁻¹ CGA. These differences were generally maintained during storage as shown in Fig. 3A. Thus, after 45 days of cold storage the results showed the higher increase in CCI values in the control batches followed by fruits treated with 50 mg l⁻¹ CGA and those treated with 5 mg l⁻¹ CGA. These differences were significant ($p < 0.05$) and maintained until the end of the experiment (Fig. 3A). Furthermore, the results observed for the TCC in flavedo were consistent with CCI. At harvest, the TCC in fruits treated with 50 mg l⁻¹ CGA were ca. 19 % and 42 % higher than in the controls (10.41 ± 0.46 mg 100 g⁻¹) and those treated with 5 mg l⁻¹ CGA (7.21 ± 0.46 mg 100 g⁻¹), respectively, and these differences were maintained during the cold storage (Fig. 3B). In fact, 5 mg l⁻¹ CGA showed a significantly ($p < 0.05$) delayed pattern compared to the rest of the fruit batches evaluated.

The results at harvest showed the highest TPC values in the control fruits (412.26 ± 6.23 mg 100 g⁻¹) followed by those treated with 50 mg l⁻¹ CGA (386.17 ± 8.31 mg 100 g⁻¹) and 5 mg l⁻¹ CGA (376.73 ± 9.83 mg 100 g⁻¹), with no significant ($p > 0.05$) differences between CGA treatments. This pattern was maintained during storage (Fig. 4A). In fact, after 30 days of cold storage, the TPC in the flavedo of fruits treated with 5 mg l⁻¹ CGA was significantly ($p < 0.05$) delayed compared with the control batch and those batches treated with 50 mg l⁻¹ CGA. Therefore, after 60 days of cold storage, the TPC in the flavedo of the control and fruits treated with 50 mg l⁻¹ CGA was ca. 18 % and 10 % higher, respectively, than in those treated with 5 mg l⁻¹ CGA (400.89 ± 8.31 mg

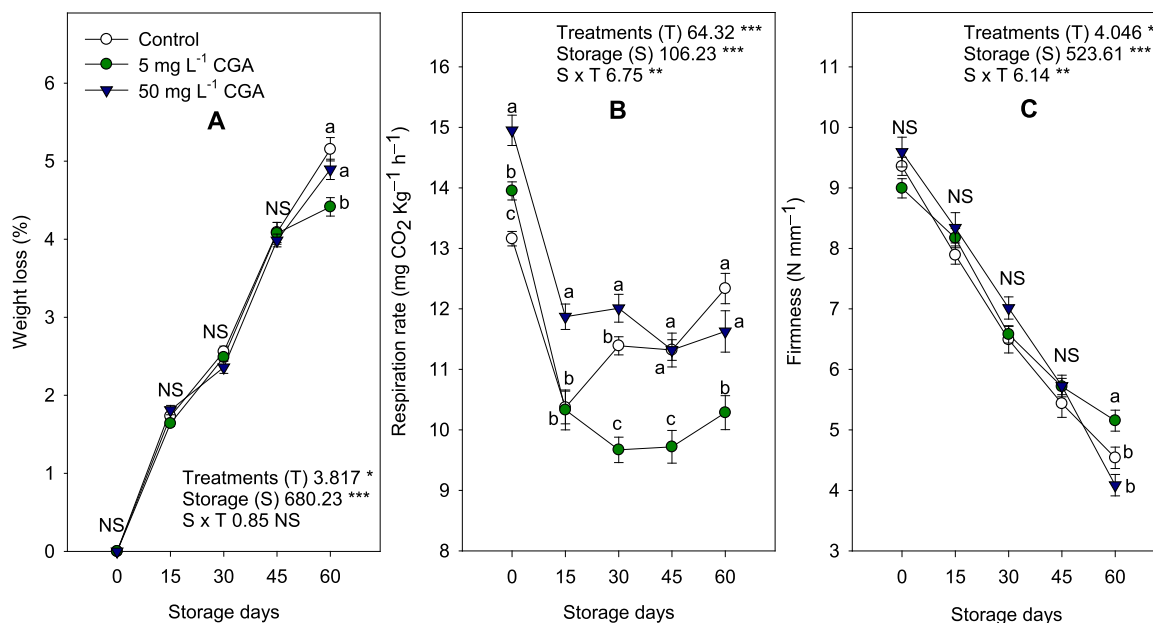


Fig. 1. Effect of preharvest treatments with chlorogenic acid (CGA) at 5 and 50 mg l⁻¹ on (A) weight loss, (B) respiration rate and (C) firmness of Navel oranges stored during 60 days at 8 °C. Significant differences are presented with the F-value and asterisks (* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$). When no significant differences were detected, 'NS' is used. Different letters indicate significant differences according to Tukey's test at the 95 % confidence level.

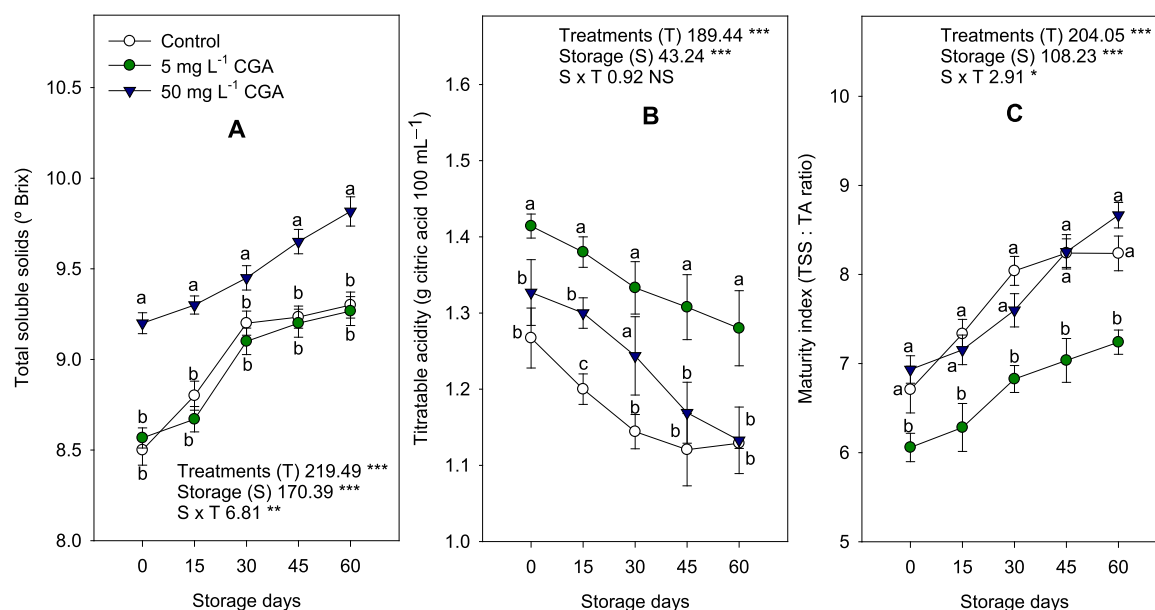


Fig. 2. Effect of preharvest treatments with chlorogenic acid (CGA) at 5 and 50 mg L⁻¹ on (A) total soluble solids, (B) titratable acidity and (C) maturity index of Navel oranges stored during 60 days at 8 °C. Significant differences are presented with the F-value and asterisks (* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$). Different letters indicate significant differences according to Tukey's test at the 95 % confidence level.

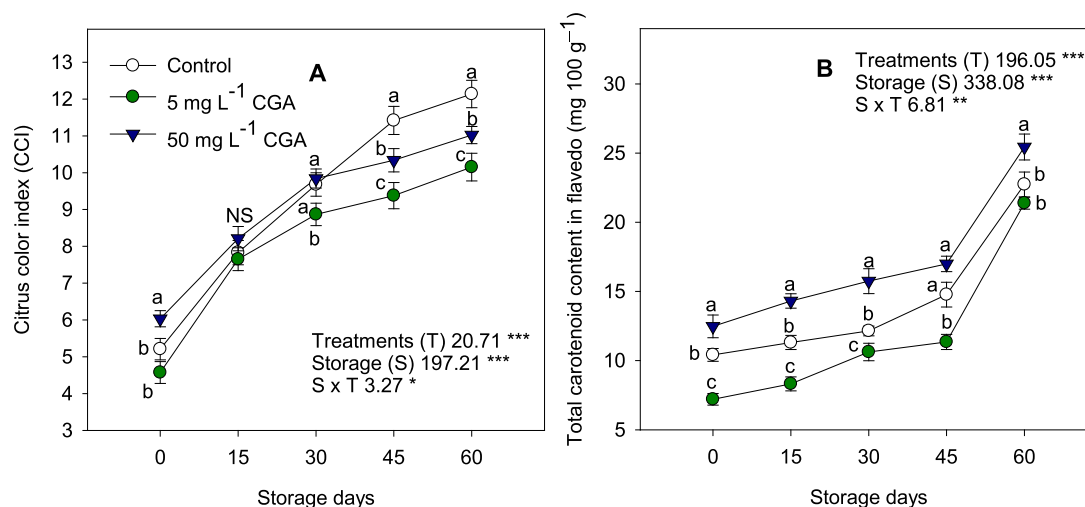


Fig. 3. Effect of preharvest treatments with chlorogenic acid (CGA) at 5 and 50 mg L⁻¹ on (A) citrus colour index and (B) total carotenoid content in flavedo of Navel oranges stored during 60 days at 8 °C. Significant differences are presented with the F-value and asterisks (* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$). When no significant differences were detected, 'NS' is used. Different letters indicate significant differences according to Tukey's test at the 95 % confidence level.

100 g⁻¹) (Fig. 4A). For TPC in juice, the results were similar, with higher values in the control batches and fruits treated with 50 mg L⁻¹ CGA than in those treated with 5 mg L⁻¹ CGA from harvest to 45 days of cold storage. However, after 60 days of cold storage no significant ($p > 0.05$) differences between treatments were found as shown in Fig. 4B.

The results of the effect of preharvest treatments with CGA on the individual flavanones and phenolics of flavedo and juice were shown in Table 1. The main flavanones found in the flavedo and juice of oranges were hesperidin and narirutin, while CGA was the main phenolic compound identified. The results at harvest in flavedo showed that the control fruits had the highest hesperidin content with an average increase of ca. 12 % compared to those treated with CGA. However, the levels of narirutin and CGA were higher in fruits treated with 5 mg L⁻¹ CGA than in the controls and those treated with 50 mg L⁻¹ CGA. After 60 days of cold storage, the results in flavedo showed an increase in the hesperidin content of ca. 41 % and 20 % in controls and fruits treated

with 50 mg L⁻¹ CGA, respectively. This effect was not observed in fruits treated with 5 mg L⁻¹ CGA, as the hesperidin content was maintained at the same level as the harvest results. Chlorogenic acid reached the highest levels in fruits treated with 5 mg L⁻¹ CGA, while the lowest levels were found in those treated with 50 mg L⁻¹ CGA and in the controls. Narirutin content was not detected in any sample after 60 days of cold storage. For juice, results at harvest showed that hesperidin and narirutin levels were higher in control fruits than those treated with CGA. On the other hand, CGA was only detected in fruits treated with CGA, with no significant ($p > 0.05$) differences between them. After 60 days of cold storage, an increase in hesperidin, narirutin and CGA content was observed in fruits from all treatments. The hesperidin content was similar in all samples, while the highest levels of narirutin were found in the controls and the lowest in the fruits treated with CGA. With regard to CGA levels, the highest values were still found in samples treated in preharvest with CGA.

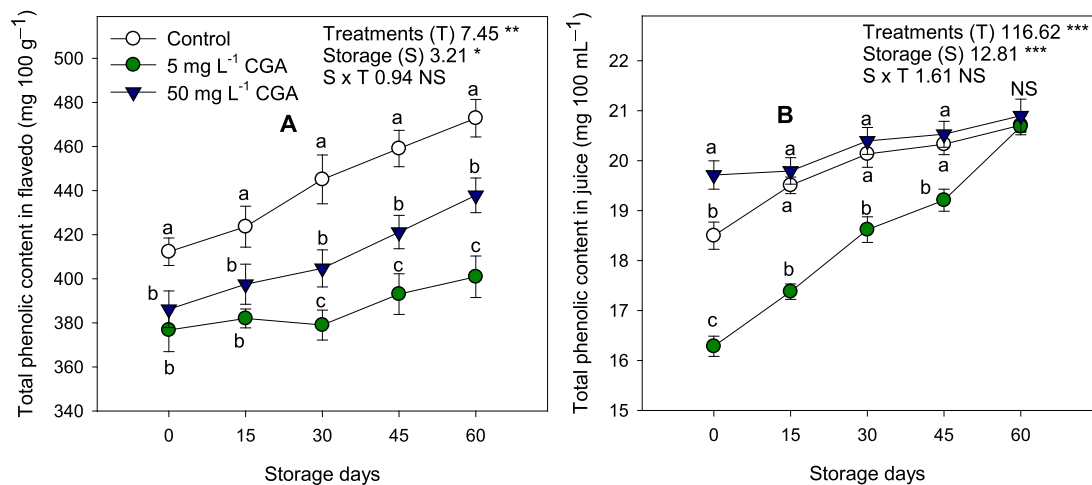


Fig. 4. Effect of preharvest treatments with chlorogenic acid (CGA) at 5 and 50 mg L⁻¹ on total phenolic content in flavedo (A) and juice (B) of Navel oranges stored during 60 days at 8 °C. Significant differences are presented with the F-value and asterisks (* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$). When no significant differences were detected, 'NS' is used. Different letters indicate significant differences according to Tukey's test at the 95 % confidence level.

Table 1
Effect of chlorogenic acid preharvest treatments on hesperidin, narirutin and chlorogenic acid of flavedo (mg 100 g⁻¹) and juice (mg 100 mL⁻¹) at 0 and 60 days of storage.

Treatment	Storage time	Hesperidin	Flavedo Narirutin	Chlorogenic acid	Hesperidin	Juice Narirutin	Chlorogenic acid
Control	0	242.98 ± 7.15	1.67 ± 0.12	16.45 ± 0.47	10.26 ± 0.26	1.60 ± 0.07	n.d.
5 mg L ⁻¹ CGA		214.90 ± 3.27	2.82 ± 0.11	25.73 ± 0.39	7.63 ± 0.41	0.89 ± 0.05	0.16 ± 0.01
50 mg L ⁻¹ CGA		219.03 ± 7.89	1.60 ± 0.12	19.32 ± 0.38	9.39 ± 0.13	0.73 ± 0.05	0.15 ± 0.01
Control	60	342.29 ± 20.48	n.d.	19.73 ± 0.40	12.21 ± 0.48	1.62 ± 0.06	0.19 ± 0.02
5 mg L ⁻¹ CGA		215.88 ± 7.39	n.d.	23.03 ± 0.13	12.01 ± 0.18	1.21 ± 0.08	0.40 ± 0.01
50 mg L ⁻¹ CGA		263.08 ± 10.17	n.d.	22.34 ± 0.51	12.59 ± 0.41	1.39 ± 0.07	0.33 ± 0.02
ANOVA				F- value			
Treatment (T)		*** 27.849	*** 34.102	*** 128.336	*** 11.706	*** 49.214	*** 105.922
Storage (S)		*** 31.250	*** 405.992	* 4.170	*** 128.091	*** 40.632	*** 374.986
S x T		*** 10.932	*** 42.137	*** 31.316	** 6.187	*** 12.654	** 6.486
Tukey test							
Control		A	B	C	A	A	B
5 mg L ⁻¹ CGA		C	A	A	B	B	A
50 mg L ⁻¹ CGA		B	B	B	A	B	A

Significant differences are presented with F-value, and asterisks denote significant differences (* $p < 0.1$, ** $p < 0.05$ and *** $p < 0.01$). When no significant differences were found, 'NS' was used. Different letters indicate significant differences between treatments according to Tukey's multiple range test at the 95 % confidence level.

4. Discussion

Currently, there is a new commercial trend in the orange industry to replace traditional treatments with others with a lower environmental impact to improve fruit quality. Previous results in fruits such as nectarines (Xi et al., 2017), apples (Shu et al., 2020) and tomatoes (Ilea et al., 2024) showed that postharvest treatments with CGA could delay fruit quality losses during cold storage. However, there is limited research on the effect of CGA applied preharvest on fruit quality. In the present study, it was observed that orange fruits treated with 5 mg L⁻¹ CGA reduced respiration rate, which could be responsible for lower weight loss and higher firmness (Fig. 1A, B, C). These results were consistent with previous findings in pears (Zhang et al., 2022) or litchi fruit (Bai et al., 2022). These differences in weight loss and firmness could be attributed to increased cell stability and reduced hydrolysis of cell wall components in CGA-treated fruits (Shu et al., 2020). The results showed that fruits treated with 5 mg L⁻¹ CGA delayed their ripening process on the tree compared to those treated with 50 mg L⁻¹ CGA and controls (Fig. 2C). This effect was mainly related to the increase in titratable acidity in fruits treated with 5 mg L⁻¹ CGA (Fig. 2B), which could be due to an increase in photosynthetic rate that promotes organic acid accumulation during fruit development on the tree (Yuan et al., 2024). Furthermore, these differences were maintained during cold

storage due to a reduction in the hydrolysis of organic acids, possibly related to a lower respiration rate. These results were similar to previous results obtained in lichi fruit treated with an apple extract rich in CGA, where the main organic acids were highly accumulated compared to controls. This effect was related to suppression of oxidative stress and inhibition of the aerobic respiration (Su et al., 2019). Thus, in nectarines treated with 25 mg L⁻¹ CGA after harvest, the ripening process was delayed by reducing TSS accumulation and consumption of the main organic acids (Xi et al., 2017). Chlorogenic acid CGA treatments delayed apple ripening and senescence by maintaining high levels of organic acids and regulating the activity of NADP-malic enzyme (Xi et al., 2016). Moreover, previous results published by Shu et al. (2020) showed that CGA applied at postharvest improved the energy balance of apples through better management of energy status and activity of enzymes involved in energy metabolism. Colour, expressed as CCI in oranges, is commonly used as an indicator of fruit maturity in the orange industry to determine the correct harvest date and the need for degreening (Jiménez-Cuesta et al., 1981). In this study, fruits treated with 5 mg L⁻¹ CGA showed lower CCI values than those treated with 50 mg L⁻¹ CGA and the control fruit (Fig. 3A). These results could be related to the accumulation of carotenoids in the fruit peel, considering that the lowest levels of total carotenoids were observed in fruits treated with 5 mg L⁻¹ CGA, as shown in Fig. 3B The relationship between carotenoid

accumulation and the expression of carotenoid biosynthesis genes during orange ripening has been extensively studied, concluding that as fruit ripening progresses, there is a concomitant overexpression of genes related to carotenoid biosynthesis, leading to a massive accumulation of carotenoids in flavedo and juice sacs (Kato et al., 2004).

Plants produce a wide range of secondary metabolites, such as phenolics, flavonoids or tannins, which can scavenge free radicals or limit their formation (Quideau et al., 2011). In addition, phenolics not only play an important role in the response to plant stress, but also contribute to the flavour and aroma of the fruit (Tomás-Barberán & Espín, 2001). In this study, the results showed the lowest TPC in flavedo and juice of fruits treated with 5 mg l⁻¹ CGA at harvest and during cold storage (Fig. 4A, B). Therefore, these results suggest that the delay in MI observed in fruits treated with 5 mg l⁻¹ CGA at harvest and during cold storage affects the accumulation of phenolics. Thus, the lower CGA dose regulates the physiological response of oranges during maturation and senescence by maintaining basal cell activity for a longer period than the 50 mg l⁻¹ CGA treatment and the controls. In this sense, results published in 'Washington Navel' oranges confirmed the accumulation of phenolic compounds through its maturation process on the tree (Multari et al., 2020). Furthermore, previous results obtained in sweet orange flavedo and juice supported that the TPC and total flavonoid content of ripe oranges were significantly higher than those obtained in unripe oranges (Omoba et al., 2015; Zhang et al., 2022). In this sense, Ladaniya and Mahalle (2011) found that the TPC in flavedo increased up to 210 days after flowering and then decreased. This effect was attributed in Persian Lime to the activity of the enzyme *p*-coumarate-3-hydroxylase, which reduces its activity while the maturity process advances and increases the accumulation of flavonoids (Ledesma-Escobar et al., 2018). On the other hand, the increase in TPC observed during cold storage in all treatments could be attributed to phenylalanine ammonium lyase activity, which has been associated with tolerance to cold storage conditions (Siboza et al., 2014). Hesperidin was the predominant flavonoid determining the TPC trend in flavedo and juice, and the second most important was narirutin, in agreement with what has been previously reported for orange flavedo and juice (Chen et al., 2015; De Ancos et al., 2017). Both flavanones showed different trends during cold storage, with hesperidin levels increasing in flavedo and juice, and narirutin levels decreasing in flavedo and increasing in juice. The lowest levels of hesperidin at harvest and during cold storage in flavedo and juice were observed in fruits treated with 5 mg l⁻¹ CGA (Table 1). These results showed a positive relationship within the maturity stage of the fruit and the accumulation of hesperidin in flavedo and juice, as the highest hesperidin concentration was found in the most mature fruits. On the other hand, narirutin content in flavedo and juice seems to have a more complex regulation pattern influenced by the maturity of the fruit and the stimulation produced by the preharvest applications of 5 mg l⁻¹ of CGA. The results were in agreement with those published by Zhang et al. (2022) where observed variations in hesperidin and narirutin content in oranges harvested at different maturity stages. Chlorogenic acid is one of the main phenolics present in citrus fruits. The highest concentrations in flavedo and juice were found in fruits treated with CGA, independent of the concentration (Table 1). Therefore, the results could indicate that the maturity stage of the fruit is not as important as the stimulation produced by the preharvest treatment with CGA for its endogenous accumulation. This is the first time that this effect has been described in oranges treated with CGA at preharvest. However, previous results using postharvest treatments with CGA have also reported an increase in endogenous CGA levels associated with a delay in the senescence process (Ilea et al., 2024; Su et al., 2019; Xi et al., 2017; Zhang et al., 2022).

5. Conclusion

The present study was the first to determine the effects of preharvest CGA treatments on the quality of 'Navel' oranges, as well as on phenolic compounds at harvest and during storage. It was shown that the

application of 5 mg l⁻¹ CGA delayed the fruit maturation process on the tree, maintaining high titratable acidity and low respiration rate at harvest and during cold storage. For TPC, the lowest values in flavedo were found in fruit treated with 5 mg l⁻¹ CGA, which was consistent with a lower accumulation of hesperidin. In addition, an increase in narirutin in flavedo and endogenous CGA in flavedo and juice was observed in oranges from this treatment. Therefore, this study provides a scientific basis for the development of an effective preharvest treatment with 5 mg l⁻¹ CGA to improve 'Navel' oranges storability. Future research could explore the cost-benefit of 5 mg l⁻¹ CGA applications for the industry implementation.

Ethical statement

As an author I confirm that this research do not involve any animal or human study.

CRediT authorship contribution statement

Viviana Torres-Vincent: Writing – original draft, Investigation, Formal analysis, Data curation. **Pedro J. Zapata:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Fabian Guillén:** Writing – review & editing, Project administration, Funding acquisition. **Vicente Serna-Escorano:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Formal analysis, Data curation, Conceptualization. **María J. Giménez:** Writing – review & editing, Visualization, Validation, Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

No data was used for the research described in the article.

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