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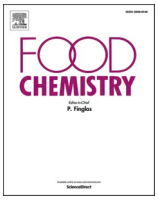

Influence of Preharvest sorbitol and calcium-sorbitol applications on the ripening process and anthocyanin biosynthesis in blood Orange (*Citrus sinensis* cv. Sanguinelli)

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ABSTRACT

Blood oranges are valued for their color and nutritional properties, thriving in Mediterranean climates where temperature variations enhance anthocyanin (ACN) synthesis. Climate change threatens this process. This study evaluated six foliar applications of sorbitol (2 %, 5 %) and sorbitol-Ca (2 % + 0.7 %) from early fruit development to harvest. All treatments enhanced peel and pulp redness, particularly sorbitol-Ca, as confirmed by lower hue angle and higher color index. Treated fruits had higher total soluble solids (TSS), with 11.07 % in 2 % sorbitol-treated fruits versus 9.63 % in controls. Sorbitol-Ca reduced respiration rates (15.63 vs. 21.57 mg CO_2 kg^{-1} h^{-1}) and increased firmness (9.72 vs. 8.89 Nmm^{-1}). Phenolic content, antioxidant activity, and bound calcium levels improved fruit quality. ACN content increased over 20 % and 40 % in sorbitol- and sorbitol-Ca-treated fruits, mainly due to Cyanidin derivatives. Sorbitol-based treatments offer a strategy to enhance blood orange resilience to climate change, improving functional and commercial value.

1. Introduction

Blood oranges (*Citrus sinensis* L. Osbeck) are a distinctive variety of citrus fruit characterized by their dark red color in both the pulp and peel due to the presence of anthocyanins (ACNs) (Fig. 1). These oranges are valued not only for their striking visual appeal but also for their enhanced nutritional properties, including high antioxidant activity and elevated (poly) phenol content. Blood oranges contain significantly more anthocyanins than other sweet orange varieties, contributing to their superior antioxidant capacity. They also have high levels of flavonoids, such as hesperidin and naringin, and vitamin C, which further enhance their nutritional value (Habibi et al., 2020). These qualities make blood oranges highly desirable for various applications, particularly in the juice industry (Scordino et al., 2015).

Blood oranges are primarily grown in Mediterranean climates, where environmental factors, such as cold temperatures and fluctuations in day-night temperature play a crucial role in promoting the synthesis and accumulation of anthocyanins (Habibi et al., 2023). These climatic conditions activate the Ruby transcription factor, along with key enzymes like phenylalanine ammonia-lyase (PAL) and chalcone isomerase (CHI), which are critical for anthocyanin biosynthesis (Chen et al., 2022; Lo Piero, 2015). Moreover, the accumulation of ACNs in blood oranges serves as a protective mechanism against both abiotic and biotic stresses, shielding the plant from radiation damage and reactive oxygen species (Cheaib et al., 2023).

In addition to anthocyanins, blood oranges also contain significant levels of flavonoids and hydroxycinnamic acids, such as p-coumaric acid, which contribute to their antioxidant potential (Legua et al., 2022). Blood oranges also have higher β-carotene content compared to other orange varieties, making them an important source of bioactive compounds in the human diet (Cebadera-Miranda et al., 2020; Legua et al., 2022). The accumulation of these bioactive compounds during fruit development depends on various factors, including plant species (Choo et al., 2022; Tan et al., 2023), cultivar, maturity stage, rootstock, climatic conditions, and cultural practices related to blood oranges (Forner-Giner et al., 2023; Habibi et al., 2023; Legua et al., 2022).

The anthocyanins in blood oranges, including cyanidin 3-O-glucoside (C3G), cyanidin 3-(6-Malonyl)-glucoside (C3MG), delphinidin 3-

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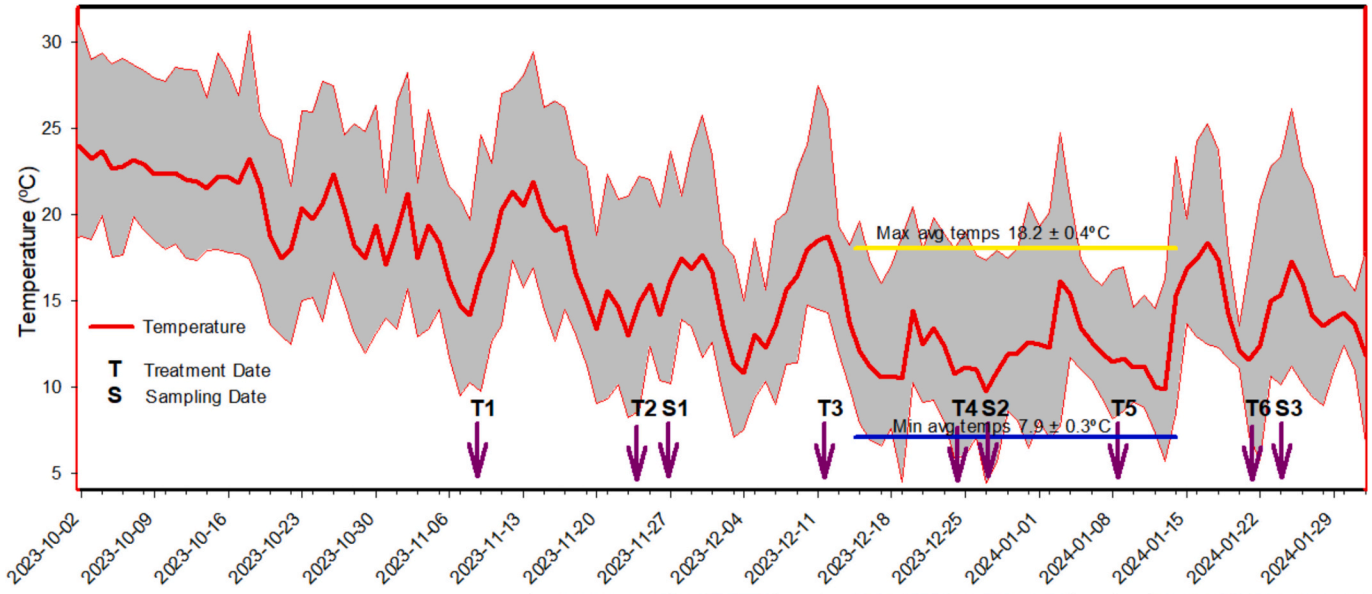
O-glucoside (D3G), cyanidin 3-(6-Dimalonyl)-glucoside (C3MGlu), and peonidin 3-(6-Malonyl)-glucoside (P3MG), make up more than 80 % of the total anthocyanins in blood orange juices, with delphinidin 3-O-glucoside (D3G) being the most abundant (Fabroni et al., 2016; Lee, 2002). The anthocyanin content varies across different cultivars, with the highest concentrations found in the 'Moro' cultivar, followed by 'Sanguinello', and the lowest in 'Tarocco' (Legua et al., 2022).

Endogenous polyols, such as mannitol and sorbitol, play essential roles in plant stress defense mechanisms, enhancing tolerance to salt, drought, and temperature stress (Conde et al., 2015; Conde, Chaves, & Gerós, 2011; Conde, Silva, et al., 2011). These compounds also act as scavengers of reactive oxygen species (ROS) and function as carbon sources and osmoprotectants (Pleyerová et al., 2022; Conde, Silva, et al., 2011). In this context, researchers have applied polyols as preharvest treatments to mitigate stress in crops such as wheat (Khaliq et al., 2023), maize (Li et al., 2023), and grapes (Conde et al., 2015). These preharvest treatments have proven effective in enhancing the accumulation of anthocyanins, stilbenes, and other bioactive compounds in various crops, including grapes. For instance, sorbitol and mannitol treatments increased the concentration of anthocyanins and other phenolic compounds in *Vitis vinifera* (Conde et al., 2024). Polyols activate key enzymes, such as PAL, and transcription factors, such as VviPAL1 and stilbene synthase 1 (VviSTS1), which are involved in the biosynthesis of phenolic compounds and anthocyanins. Furthermore, polyols affect the activity of UDP-glucose 3-O-glucosyltransferase (UFGT) and the expression of VviUFGT1, which play a vital role in anthocyanin accumulation (Conde et al., 2024).

Calcium plays a crucial role in fruit quality by stabilizing cell walls and membranes and preventing physiological disorders (Serrano et al., 2004). Calcium deficiencies can lead to various fruit quality issues, including albedo breakdown in oranges and chilling injury in mandarins (Kalatippi et al., 2024; Pham et al., 2012; D'Aquino et al., 2004). Preharvest calcium treatments have been desmostrated to enhance anthocyanin accumulation in other fruits, such as grapes, by activating transcription factors and key enzymes in the anthocyanin biosynthetic pathway (Zhu et al., 2019).

Polyols also interact with calcium, forming stable complexes that improve calcium uptake in plants (Will et al., 2011). These polyol-calcium complexes can be absorbed through the foliage and transported to various plant organs, enhancing calcium accumulation in fruits and other tissues (Li et al., 2020; Liu et al., 2021). Studies have shown that polyol-calcium complexes increase fruit quality and crop yield in a variety of species, including mango, apple, and tomato (Kim et al., 2014; Singh et al., 2013; Zhang et al., 2013). These treatments have also been associated with improved disease resistance (Meng et al., 2018) and higher concentrations of bioactive compounds, such as



Treatment Date	Sampling Date
T1 2023-11-10	
T2 2023-11-24	S1 2023-11-27
T3 2023-12-11	
T4 2023-12-26	S2 2023-12-29
T5 2024-01-09	
T6 2024-01-22	S3 2024-01-25

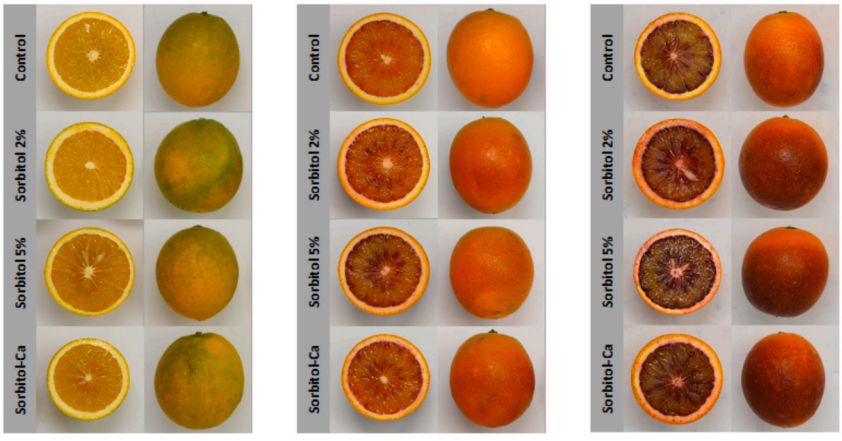


Fig. 1. Ranges of daily maximum and minimum temperatures (gray area on the chart bounded by red lines) during the experiment, recorded by the IVIA weather station located in Almoradí - Alicante (Spain) from October 2, 2023, to February 4, 2024. The dates of treatment application (T) and orange sampling (S) are indicated. The external and internal appearance of the oranges on each of the sampling days, S1, S2, and S3, is shown in pictures. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

ascorbic acid, lycopene and β-carotene (Kim et al., 2014; Singh et al., 2013; Zhang et al., 2013).

In this regard, climate change is expected to alter temperature dynamics in Mediterranean climate regions, potentially reducing the cold exposure and day-night temperature variations required for anthocyanin synthesis in blood oranges. This reduction in cold exposure may compromise anthocyanin production, impacting both the commercial quality and nutritional benefits of the fruit. Given the increasing risk of losing the distinctive pigmentation and associated health benefits of blood oranges, agronomic strategies are needed to counteract these climatic changes. This study presents an innovative solution through the pre-harvest application of sorbitol and sorbitol-Ca, offering a viable alternative to mitigate these adverse effects, enhance anthocyanin synthesis and accumulation during fruit maturation on the tree, and evaluate their impact on fruit quality, antioxidant activity, and mineral accumulation.

2. Materials and methods

2.1. Preparation of planting material

Blood oranges (*Citrus sinensis* L. Osbeck) of the 'Sanguinelli' cultivar, grafted on *Citrus macrophylla* rootstock, were grown on a commercial estate operated by 'Las Moreras Fruit & Veggies S.L.' situated in Alicante, Spain (38°05′04.1" N 0°46'39.9" W), characterized by Mediterranean weather conditions. Weather data for the experimental period were collected by the Almoradí weather station (IVIA, 2024), located just 5 km from the field site in Almoradí (38°1′53.15" N 0°46'28.34" W, elevation 58 m) (Fig. 1). The orchard, with 11-year-old trees, was arranged in a 5 × 4 m planting grid.

2.2. Treatment application

2.2.1. Foliar application of sorbitol and sorbitol-calcium

The experimental setup involved applying food-grade 2 % and 5 % sorbitol solutions (Barcelonesa Group SAU, Spain), a blend of food-grade sorbitol and calcium nitrate at 2 % and 0.7 %, respectively (sorbitol-ca) (Calcium Nitrate, Fertiberia SA, Spain), and a control using plain tap water. A non-ionic surfactant, Polyglycol alkyl 20 % *w/v* (Sipcam Iberia SL, Spain), was added to all solutions, including the control. Treatments were applied using a manual foliar sprayer.

The initial treatment was applied before onset of orange color change on November 10, 2023, with subsequent treatments administered every 15 days until January 10, 2024, resulting in a total of six applications. Each treatment was administered to 3 groups of 3 trees (9 trees per treatment), with untreated buffer trees separating each group. Five border trees were excluded from the experiment.

2.2.2. Sampling methods and periods

After every two treatments, 4 fruits were harvested from both the north and south sides of each tree (24 fruits per group/treatment). The first sampling (S1 - November 27th) occurred when control fruits still had about 50 % green skin – 240 days after full bloom (DAFB). The second sampling (S2 - December 29th) took place when control fruits were fully orange without ACNs – 270 DAFB. The third sampling (S3 - January 25th) was conducted when control fruits exhibited 15–20 % red skin coloration – 300 DAFB.

Additionally, 10 leaves were collected from each tree for mineral content analysis. Fruits and leaves were transported to the laboratory within two hours in refrigerated conditions. In the laboratory, all fruits were weighed and measured for equatorial diameter (Table S1), and defective fruits were excluded. Ten fruits per group/treatment (three replicates of 10 fruits each) were selected for further analysis (whole fruit, juice, flavedo, albedo, or drained pulp).

2.3. Firmness and color

The firmness of the oranges was measured using a TX-XT2i Texture Analyzer (Stable Microsystems, Godalming, UK) following Lorente-Mento et al. (2021). A 10 cm diameter flat probe was employed to apply force, deforming the equatorial region of each orange by 5 %. The results were calculated as the force per unit distance (N mm^{-1}).

External color was determined using hue angle (h) and citrus color index (CCI), while internal color was assessed using the h and a/b ratios. Measurements were performed with a CR200 colorimeter (Konica Minolta, Japan). The formulas used were:

$$h = \arctan(b/a) \quad (1)$$

$$CCI = 1000*a/(L*b) \quad (2)$$

2.4. Fruit respiration rate

To measure the respiration rate of oranges, a closed static system was employed. Prior to this measurement, the fruits were conditioned at 20 °C for 3 h. Each treatment utilized three airtight 5-l containers, with each container holding 10 oranges for one hour. Gas samples (1 mL) were taken from the headspace of each container for CO_2 analysis using gas chromatography. The analysis was conducted with a Shimadzu CG-14B gas chromatograph, equipped with a thermal conductivity detector and a 2-m by 1/8-in. CHROMOSORB 102 80/100 column, following the method outlined by Valverde et al. (2005). The results were reported in mg CO_2 kg^{-1} h^{-1}.

2.5. Total soluble solids (TSS), titratable acidity (TA), and maturation index (MI)

TSS was measured using a digital refractometer (Atago PR-101) and expressed as % g per 100 g of fresh juice. TA was measured by titration with 0.1 N NaOH using an automatic titrator (Metrohm 785 DMP Titrino) and expressed as g citric acid equivalents per 100 mL of juice.

2.6. Organic acids and sugars content

Orange juice was centrifuged at 10,000 xg for 10 min to obtain a clear liquid, which was then filtered through a 0.45 μm Millipore filter. This filtered liquid was subsequently analyzed using an HPLC system (Hewlett-Packard HPLC-1100) for determining individual sugars and organic acids. The chromatographic separation involved a constant flow of 0.1 % phosphoric acid at 0.5 mL per minute through a Supelco column (Supelcogel Ce610H, Supelco Park, Bellefonte, PA, USA). Organic acids were detected by their absorbance at 210 nm, while sugars were measured using a refractive index detector. The results were reported in grams per 100 mL of fresh weight (FW), and quantification was performed using standard curves made with pure sugars and organic acids from Sigma (Poole, UK).

2.7. Total phenolic content (TPC), hydrophilic and lipophilic antioxidant activity

To quantify total phenolic compounds, 10 mL of juice from S1, S2, and S3 samples or 10 g of flavedo, albedo, and pulp from S3 samples were mixed with a water solution (2:8) containing 2 mM NaF in a 1:3 ratio. The mixture was centrifuged at 10,000 xg for 15 min, and the resulting supernatant was analyzed in duplicate using the Folin-Ciocalteu reagent to determine the total phenolic content, following the method of García-Pastor et al. (2020). The results were expressed as mg of gallic acid equivalents 100 mL^{-1} of juice or mg of gallic acid equivalents 100 g^{-1} of fresh weight (FW).

For the evaluation of total antioxidant activity (TAA), 5 mL of juice or 5 g of flavedo, albedo, or pulp tissue from S3 samples were combined

with 10 mL of 50 mM phosphate buffer (pH 7.8) and 5 mL of ethyl acetate. After centrifuging at 10,000 x g for 15 min at 4 °C, the upper and lower layers were separated to assess lipophilic (L-TAA) and hydrophilic (H-TAA) antioxidant activities, respectively. Each extract was tested in duplicate using a reaction mixture that included ABTS (2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt), horseradish peroxidase, and hydrogen peroxide as the oxidant. The generation of ABTS+ radicals was measured at 730 nm, and the decrease in absorbance after the addition of the sample extract indicated the TAA, calculated based on a Trolox calibration curve (0 to 20 nmol) provided by Sigma Aldrich (Madrid, Spain). The findings were expressed as milligrams of Trolox equivalent (TE) 100 g^{-1} of fresh weight.

2.8. Extraction, identification and quantification of ACNs

2.8.1. Sample preparation for ACNs analysis

The extraction of ACNs was performed by applying the method previously reported in the literature Habibi et al. (2020), with a few modifications. Juice samples (5 mL) were mixed with 10 mL of methanol/formic acid/water (79:1:20, *v*/v/v), before being centrifuged at 10,500 ×*g* for 10 min. After centrifugation, the supernatant was filtered through a 0.45 μm syringe filter prior to analysis by HPLC-DAD-ESI/MSn and RP-HPLC-DAD for the identification and quantification, respectively.

2.8.2. Identification of ACNs via HPLC-DAD-ESI/MS^n

The identification of ACNs was performed following the method previously reported by Gonçalves et al. (2021), with few modifications. Briefly, juice samples (20 μL) were analyzed in triplicate, and the chromatographic separation was performed using 1 % formic acid (solvent A) and acetonitrile (solvent B). The solvent system started with 15 % of B, and reached 30 % of B at 20 min, 40 % at 30 min, 60 % at 35 min, and 90 % at 40 min. After 4 min in these last conditions (44 min; 90 % of B), it returned to initial conditions, allowing 10 min for column stabilization. The flow rate was maintained at 0.9 mL/min throughout the entire run. The scan acquisition for mass spectra ranged from *m/z* 100 to 1000, and mass spectrometry data were acquired in positive ionization mode. The equipment and the rest of MS parameters did not change from the Gonçalves et al. (2021) method. ACNs were tentatively identified based on their elution order, retention times, and ultraviolet-visible and mass spectral characteristics, compared to authentic standards analyzed under the same conditions, the research group experience and data available in the literature (Cebadera-Miranda et al., 2019).

2.8.3. Quantification of ACNs via RT-HPLC-DAD

Regarding the quantification of the ACNs previously identified, the method described by Agulló et al. (2021) was followed with few modifications. Briefly, juice samples (20 μL) were analyzed in triplicate, and the chromatographic separation was performed using 5 % formic acid (solvent A) and methanol (solvent B). The same linear gradient and flow rate described in the previous section was used. The equipment and the rest of chromatographic parameters did not change from the Agulló et al. (2021) method. ACNs were quantified by comparison with authentic standards of analytical grade as cyanidin 3-*O*-glucoside ($y = 67.9\times - 0.6333$ $R^2 = 0.9997$) and delphinidin 3-*O*-glucoside ($y = 83.3\times - 0.4333$ $R^2 = 0.9999$) at 520 nm. The standards of the ACNs cyanidin 3-*O*-glucoside chloride and delphinidin 3-*O*-glucoside chloride were obtained from Sigma-Aldrich (Steinheim, Germany). The concentration of ACNs was expressed as mg L^{-1} of juice.

2.9. Minerals content

Mineral content was analyzed following Lorente-Mento et al. (2023). This involved processing two samples from each replicate, with each sample consisting of 0.2 g of dehydrated albedo and leaf tissues. These tissues were collected from a mix of 10 fruit tissues or 20 leaves per replicate. Initially, the leaves were washed with deionized water. Both types of tissue were then dried at 60 °C for at least 48 h, ground into a fine powder, and subjected to digestion using a microwave digester (CEM Mars One) with a 1 % HNO_3 solution. Post-digestion, the samples were diluted to a final volume of 50 mL with distilled water. Aliquots from these solutions were analyzed for macro and microelements using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Shimadzu ICP-MS-2030), with quantification performed against standard curves for elements including Ca, Cu, Fe, K, Mg, Mn, Na, and P.

Bound calcium was determined using the method of Guirao et al. (2024). This procedure involved homogenizing 20 g of fruit tissue and boiling it in 95 % ethanol for 20 min. The mixture was then centrifuged at 11,000 *g* for 10 min at 4 °C, with the supernatant discarded. The remaining residue was subjected to several cycles of centrifugation with 80 % ethanol (11,000 g for 10 min at 4 °C) until the solution cleared. The final residue was washed with acetone and centrifuged once more (11,000 *g* for 10 min at 4 °C), after which the acetone was evaporated by drying the pellet at 60 °C. This pellet was stored until Ca^{2+} analysis was carried out as described for mineral content. Results were expressed as mg 100 g^{-1} of dry weight.

2.10. Statistical analysis

To analyze the effects of treatments, time, and their interactions, a two-way ANOVA was performed. Tukey's test was applied to pinpoint significant differences between treatments and across various time points. The impact of treatments on hydrosoluble and liposoluble antioxidant activities, as well as on total phenolic content in the peel at stage S3, was assessed using a Student's *t*-test. Statistical significance was defined as $p < 0.05$. Results are presented as mean values ± standard error (SE) from three independent replicates. All statistical computations were executed using IBM SPSS Statistics for Windows, version 21.0 (IBM Corp., USA).

3. Results and discussion

3.1. Effect of sorbitol and sorbitol-calcium on fruit growth, quality, and respiration rate

During the final stages of fruit maturation, the control oranges from stage S1 to the end of the harvest had the same weight and size. However, the oranges treated with 2 % sorbitol, 5 % sorbitol, and sorbitol-Ca in stages S2 and S3 reached a significantly larger diameter than those at maturity stage S1 and the control fruits at stages S2 and S3 (Table S1).

Up to stage S1, the albedo still had a green-orange coloration, and the pulp was completely orange. However, the fruits at stage S2, visibly (Fig. 1), increased their red color both internally and externally until stage S3 when they reached their maximum coloration. According to the weather conditions (Fig. 1), the fruits were exposed from December 13, 2023, to January 14, 2024, to average minimum temperatures of 7.9 ± 0.3 °C and average maximum temperatures of 18.2 ± 0.4 °C, this being the period with the greatest temperature difference between night and day (10.3 ± 0.5 °C).

Nevertheless, the fruits treated with 2 % and 5 % sorbitol and sorbitol-Ca always showed a greater red coloration in both the albedo and pulp in stages S2 and S3 (Fig. 1). This is confirmed by the lower hue angle values ($p < 0.01$) in the albedo and pulp of treated fruits at stage S3 (Fig. 2A and C), and by the higher color index ICC and a/b values ($p < 0.01$) in the albedo and pulp of treated oranges in stages S2 and S3 (Fig. 2B and D). However, the fruits treated with sorbitol-Ca at stage S3 consistently exhibited a significantly lower ICC in the flavedo (51.30 ± 2.25) compared to the ICC values of the 2 % sorbitol (57.13 ± 2.85), 5 % sorbitol (55.19 ± 1.61), and control (64.72 ± 1.96); as well as a higher a/b ratio in the pulp (0.83 ± 0.07), compared to the a/b values of the 2 % sorbitol (0.61 ± 0.07), 5 % sorbitol (0.71 ± 0.04), and control (0.46

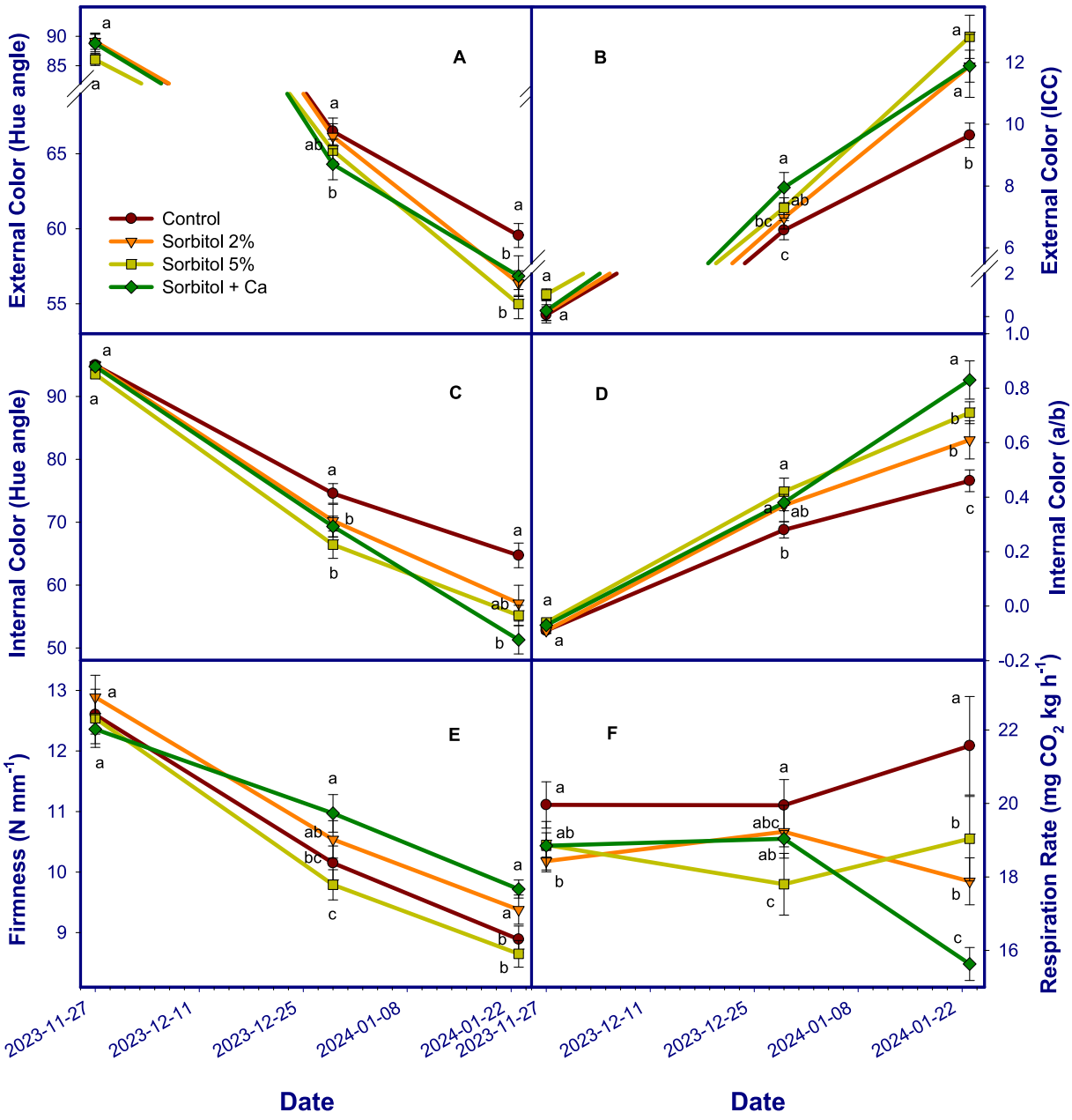


Fig. 2. External color: Hue angle (A) and ICC (B); Internal color: Hue angle (C) and a/b (D); Firmness (N mm^{-1}) (E) and Respiration Rate (mg CO_2 kg^{-1} h^{-1}) (F) of Blood Oranges cv 'Sanguinelli' treated with sorbitol 2 % (orange), sorbitol 5 % (yellow), Sorbitol-Ca (green) and control (red) during ripening and sampled at S1 (240 DAFB), S2 (270 DAFB) and S3 (300 DAFB). Results are presented as the mean ± SE from ten samples per replicate and treatment. Significant differences between treatments on the same sampling day are marked by different lowercase letters ($p < 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

± 0.04) treatments.

Skin color is a key quality trait in blood oranges, reflecting ripeness and anthocyanin content. Consumers associate deeper red hues with higher antioxidant levels and better quality. Given its importance, our study evaluated how sorbitol-Ca treatments influenced pigmentation, showing enhanced red coloration, likely due to improved nutrient transport and metabolic regulation. The appearance of red coloration in both the skin and pulp of blood oranges is primarily dependent on climatic conditions during their maturation. Specifically, it has been reported that the synthesis of ACNs in blood oranges requires low nighttime temperatures, moderate daytime temperatures, and a significant difference between day and night temperatures (Habibi et al., 2023). In this context, although S1 fruits reached commercial size and weight typical for this orange variety once the development process is completed, they still did not exhibit the expected color changes for this orange variety (Table S1) by the end of the development process (Tarancón et al., 2022). However, the red coloration of the skin and pulp of the oranges became visible in the S2 and S3 maturation stages (Fig. 1), coinciding with the period of greatest temperature differences between day and night throughout the growing cycle. Recent studies (Chen et al., 2022; Lo Piero, 2015) show that the Ruby transcription factor functions as a master regulator in citrus fruits, activating structural genes such as CHS (chalcone synthase), DFR (dihydroflavonol 4-reductase), and UFGT (UDP-glucose flavonoid glucosyltransferase), which contribute to anthocyanin biosynthesis. Additionally, key enzymes like PAL and CHI increase their activity under thermal stress, promoting the accumulation of these compounds in the fruit peel.

The firmness of all fruits significantly decreased progressively from maturity stage S1 to S3 (Fig. 2E). Fruits at stage S3 treated with sorbitol-Ca and 2 % sorbitol showed significantly higher firmness (9.72 ± 0.15 and 9.38 ± 0.24 N mm^{-1}) compared to the control and 5 % sorbitol-treated fruits (8.89 ± 0.22 and 8.65 ± 0.22 N mm^{-1}). The higher firmness in sorbitol-Ca treated fruits is likely due to the role of calcium in cell wall strengthening and maintaining membrane integrity, which reduces enzymatic softening (Huai et al., 2022).

The respiration rate of the fruits in maturity stages S1 and S2 ranged between 18.4 and 19.9 mg CO_2 kg^{-1} h^{-1} (Fig. 2F). In these maturity stages, the treated fruits always showed the lowest respiration rate. However, at stage S3, the fruits treated with sorbitol-Ca had a significantly lower respiration rate (15.63 ± 0.45 mg CO_2 kg^{-1} h^{-1}, $p < 0.001$) compared to the fruits treated with 2 % sorbitol (17.88 ± 0.64 mg CO_2 kg^{-1} h^{-1}), 5 % sorbitol (19.04 ± 1.15 mg CO_2 kg^{-1} h^{-1}), and the controls (21.57 ± 1.34 mg CO_2 kg^{-1} h^{-1}). A lower respiration rate is associated with delayed senescence and extended fruit shelf life, indicating that sorbitol-Ca may contribute to preserving fruit quality postharvest. Previous studies have shown that pre-harvest treatments with sorbitol, calcium, or calcium chelated with sorbitol can modify fruit metabolism, in alignment with the results obtained in this work (Fig. 2). These treatments reduce respiration rates and improve quality parameters such as firmness, soluble solids content, and acidity by influencing metabolic pathways related to organic acid retention and cell wall integrity. This has been demonstrated in different fruit species, including 'Doña María' table grapes, where pre-harvest sorbitol and calcium applications delayed ripening and maintained higher firmness and acidity levels (Guirao et al., 2024), as well as in peppers and mangoes, where calcium-based treatments improved postharvest quality and reduced physiological disorders (Navarro-León et al., 2022; Sankar et al., 2013). Additionally, these treatments have been shown to enhance anthocyanin accumulation and red coloration in 'Crimson Seedless' table grapes (Soliman et al., 2023), further supporting their role in modulating fruit metabolism.

3.2. TSS, sugar content, and organic acids

The TSS content of treated fruits significantly increased across all three maturity stages, remaining similar between stages S1 and S2 (Fig. 3A). However, the control fruits had the lowest TSS content ($p < 0.001$), ranging from 9.77 to 9.63 % across all maturity stages. In stage S3, fruits treated with 2 % sorbitol had the highest TSS (11.07 ± 0.06 %, $p < 0.01$) compared to those treated with 5 % sorbitol and sorbitol-Ca (10.59 ± 0.09 and 10.63 ± 0.07 %, respectively).

Sucrose, glucose, and fructose were the predominant sugars identified and quantified, showing a similar trend to TSS (Fig. 3B, C, D). Treated fruits consistently had higher sugar concentrations ($p < 0.001$) compared to controls. Sucrose was the most abundant sugar (2.2–2.6 g 100 mL^{-1}), followed by glucose (1.6–1.9 g 100 mL^{-1}) and fructose (1.3^{-1}.6 g 100 mL^{-1}).

For sucrose (Fig. 3B), control fruits at stage S3 had 2.28 ± 0.06 g/100 mL, while fruits treated with 2 % sorbitol, 5 % sorbitol, and sorbitol-Ca had significantly higher levels of 2.61 ± 0.15, 2.51 ± 0.13, and 2.54 ± 0.07 g 100 mL^{-1}, respectively. For glucose (Fig. 3C), control fruits at

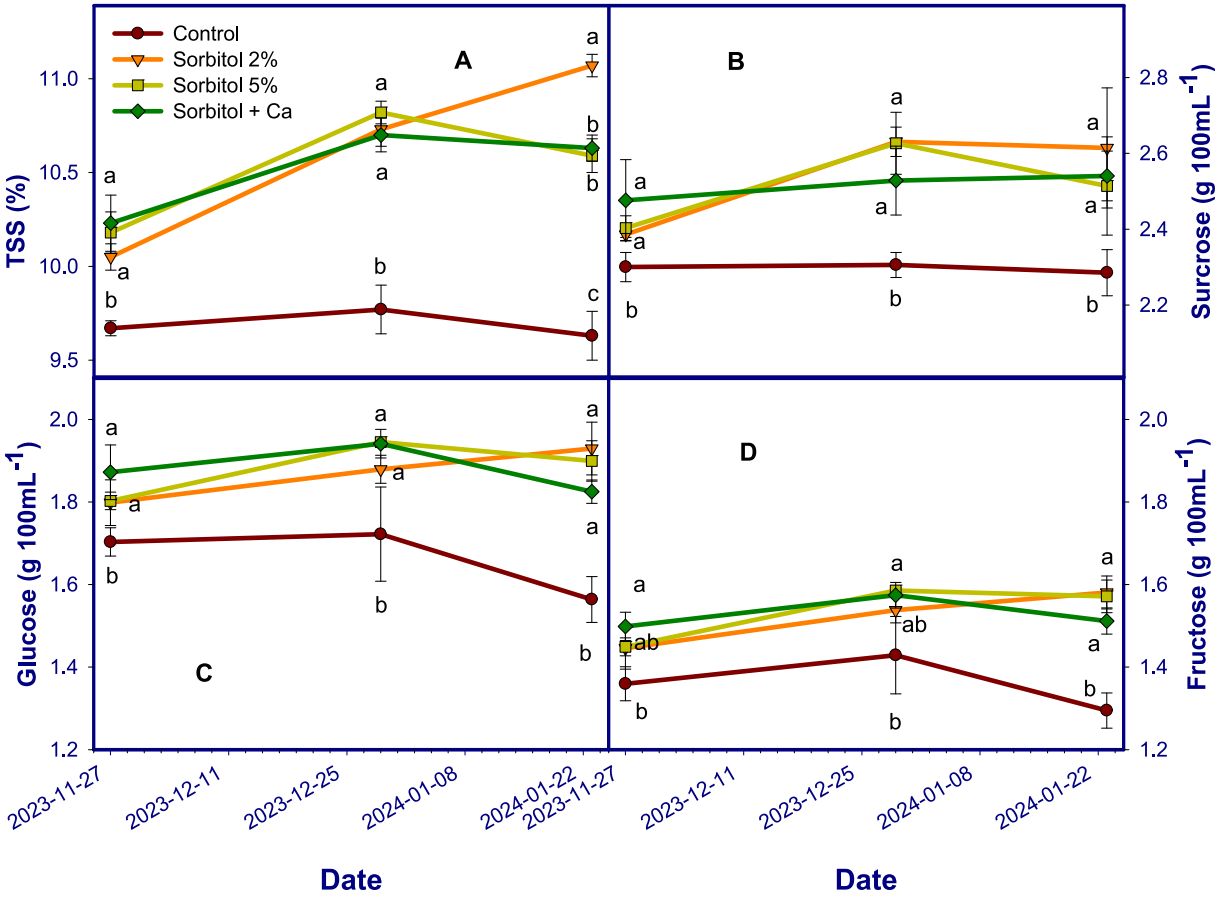


Fig. 3. TSS (%) (A), Sucrose (g 100 mL^{-1}) (B), Glucose (g 100 mL^{-1}) (C) and Fructose (g 100 mL^{-1}) (D) in juice of Blood Oranges cv 'Sanguinelli' treated with sorbitol 2 % (orange), sorbitol 5 % (yellow), Sorbitol-Ca (green) and control (red) during ripening and sampled at S1 (240 DAFB), S2 (270 DAFB) and S3 (300 DAFB). Results are presented as the mean ± SE from ten samples per replicate and treatment. Significant differences between treatments on the same sampling day are marked by different lowercase letters ($p < 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

stage S3 had 1.56 ± 0.06 g 100 mL^{-1}, whereas treated fruits reached 1.92 ± 0.06, 1.90 ± 0.05, and 1.82 ± 0.02 g 100 mL^{-1} for 2 % sorbitol, 5 % sorbitol, and sorbitol-Ca, respectively. Similarly, for fructose (Fig. 3D), control fruits at stage S3 had 1.30 ± 0.04 g 100 mL^{-1}, while treated fruits had significantly higher levels of 1.58 ± 0.04, 1.57 ± 0.04, and 1.51 ± 0.03 g 100 mL^{-1} for 2 % sorbitol, 5 % sorbitol, and sorbitol-Ca, respectively.

Pre-harvest applications of sorbitol alone or in combination with sorbitol-chelated calcium have generally been effective in increasing TSS in various fruits. For instance, such treatments have been reported to enhance TSS levels in mangoes (Talang et al., 2016), tomatoes (Kim et al., 2014), and wine grapes (Ma et al., 2022). Additionally, they have been shown to elevate sugar concentrations, including glucose and fructose, in table grapes (Guirao et al., 2024). In the case of oranges treated with sorbitol or sorbitol-Ca, a similar response was observed (Fig. 3). Grapes, like blood oranges, are non-climacteric fruits rich in anthocyanins, which synthesis is highly dependent on environmental and agronomic factors. Previous studies (Ma et al., 2022) have demonstrated that sorbitol treatments can improve anthocyanin stability in grapes by regulating sugar metabolism and the expression of genes related to flavonoid biosynthesis. Conde et al. (2015) discovered a sorbitol transporter (VvPLT1) in the grape mesocarp cells, which is crucial for the uptake of sugar alcohols. This transport mechanism is particularly beneficial under stressful conditions when sorbitol dehydrogenases rapidly convert sugar alcohols into reducing sugars. Moreover, pre-harvest applications of polyols to grapes have been found to enhance the activity of sorbitol dehydrogenase enzymes (Conde et al., 2024).

TA, regardless of treatments, decreased significantly ($p < 0.001$) in all fruits during on-tree maturation, with TA levels following the order S1 > S2 > S3 (Fig. 4A). However, fruits treated with sorbitol-Ca

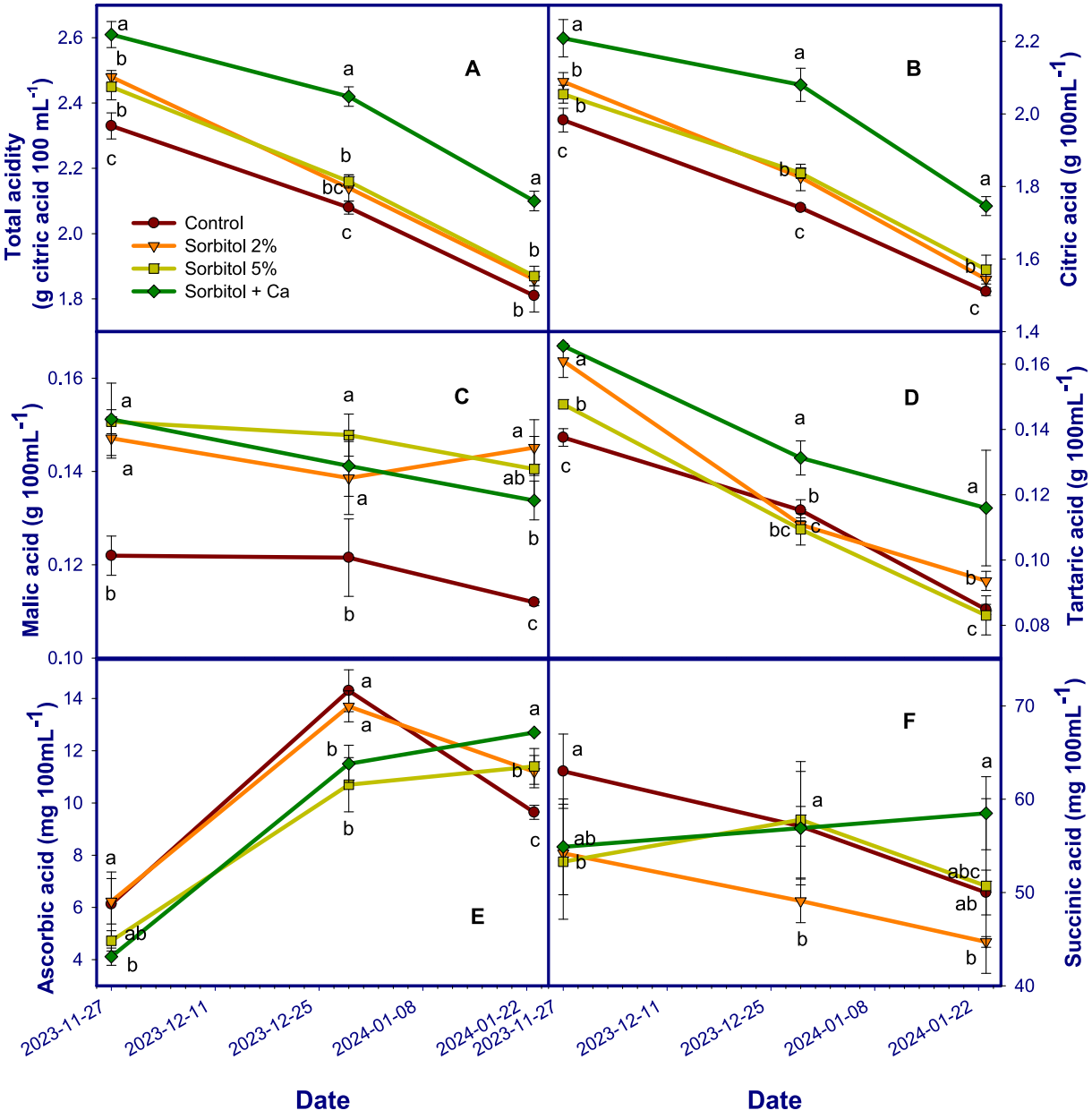


Fig. 4. TA (eq g Citric acid100 mL^{-1}) (A), Citric acid (g 100 mL^{-1}) (B), Malic acid (g 100 mL^{-1}) (C), Tartaric acid (g 100 mL^{-1}) (D), Ascorbic acid (mg 100 mL^{-1}) (E) and Succinic acid (mg 100 mL^{-1}) (F) in juice of Blood Oranges cv 'Sanguinelli' treated with sorbitol 2 % (orange), sorbitol 5 % (yellow), Sorbitol-Ca (green) and control (red) during ripening and sampled at S1 (240 DAFB), S2 (270 DAFB) and S3 (300 DAFB). Results are presented as the mean ± SE from ten samples per replicate and treatment. Significant differences between treatments on the same sampling day are marked by different lowercase letters ($p < 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

consistently showed significantly higher TA than those treated with 2 % sorbitol, 5 % sorbitol, and the control across all maturity stages.

Regarding quantified organic acids, citric acid was the most abundant, representing over 90 % of the total acids at all maturity stages, with a concentration exceeding 1.5 g 100 mL^{-1} (Fig. 4B). Malic acid concentrations ranged between 0.12 and 0.15 g 100 mL^{-1} (Fig. 4C), tartaric acid between 0.08 and 0.16 g 100 mL^{-1} (Fig. 4D), and both succinic and ascorbic acids were minor, with concentrations below 70 mg 100 mL^{-1} at all maturity stages (Fig. 4E-F). The evolution of citric and tartaric acids paralleled TA, following S1 > S2 > S3. However, the concentrations of malic and succinic acids did not change among maturity stages.

Treated fruits, regardless of the treatment, consistently showed significantly higher concentrations of citric and malic acids compared to controls at all three maturity stages (Fig. 4B-C). In any case, citric and tartaric acid concentrations were always significantly higher in sorbitol-Ca treated fruits compared to the other fruits (Fig. 4B-D). Ascorbic acid increased significantly during ripening in all fruits (Fig. 4E). In control and 2 % sorbitol-treated fruits, ascorbic acid peaked at S2 (~14 mg 100 mL^{-1}). However, in 5 % sorbitol and sorbitol-Ca treated fruits, it continued to increase until S3, where it reached its highest concentration. Succinic acid levels did not significantly change during ripening (Fig. 4F). Treated fruits showed lower succinic acid accumulation, particularly those with 2 % sorbitol.

Our findings align with previous research on the role of preharvest sorbitol applications in improving fruit quality and anthocyanin accumulation. Studies on table grapes and other fruit crops have demonstrated that sorbitol enhances metabolic stability by modulating organic acid metabolism and secondary metabolite pathways (Guirao et al., 2024; Soliman et al., 2023; Navarro-León et al., 2022). Specifically, sorbitol has been linked to the stabilization of malic acid concentration, preventing excessive acid degradation during ripening (Guirao et al., 2024).

3.3. *Effect of treatments on total phenolic content and antioxidant activity*

The total phenolic content in the juice of all oranges, regardless of the treatment, increased significantly ($p < 0.01$) during the ripening process (Fig. 5A). Juices from sorbitol-Ca treated fruits consistently showed higher phenolic concentrations across all maturity stages than those treated with 5 % sorbitol, 2 % sorbitol, and control. The total (poly)phenol content at stage S3 was 122.89 ± 2.50, 127.55 ± 1.10, 138.34 ± 3.24, and 143.34 ± 1.52 mg gallic acid equivalents (GAE) per 100 mL in the juices of control, 2 % sorbitol, 5 % sorbitol, and sorbitol-Ca treated fruits, respectively.

Similarly, the total (poly)phenol content of the three tissue fractions analyzed at stage S3 was highest in the flavedo (ranging between 896.92 and 990.68 mg GAE 100 mL^{-1}), followed by the albedo (ranging between 385.11 and 425.50 mg GAE 100 mL^{-1}), and the pulp (ranging between 233.00 and 268.71 mg GAE 100 mL^{-1}) (Fig. 5B). Control fruits consistently showed significantly lower (poly)phenol concentrations, while fractions from sorbitol-Ca treated fruits had the highest concentrations: 990.68 ± 24.91, 416.85 ± 6.13, and 268.71 ± 4.55 mg GAE 100 mL^{-1} in the flavedo, albedo, and pulp, respectively.

Water-soluble antioxidant activity was significantly higher than lipid-soluble activity in all four analyzed fractions: juice, flavedo, albedo, and pulp (Fig. 6). Among the antioxidant activities analyzed, total, water-soluble, and lipid-soluble, the flavedo consistently showed the highest antioxidant activity, followed by the albedo, pulp, and juice. The highest water-soluble antioxidant activity was always found in the tissues of sorbitol-Ca treated fruits. In contrast, the control fruit tissues had the lowest water-soluble antioxidant activity. However, lipid-soluble antioxidant activity in the four tissue fractions varied depending on the treatments applied. Lipid-soluble antioxidant activity in the juice and albedo of sorbitol-Ca treated fruits was significantly higher, while there were no significant differences in the flavedo, and the pulp had the lowest lipid-soluble antioxidant activity.

According to these results, it is known that polyols exhibit antioxidant properties, especially in plants under stress conditions. Under adverse conditions, such as water scarcity or excessive salinity, plants may experience metabolic imbalances leading to the accumulation of ROS. Polyols help neutralize these ROS, reducing oxidative stress and protecting plant cells (Conde et al., 2024). Additionally, higher levels of activity in key antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) have been documented, reflecting a more enhanced antioxidant defense mechanism. These enzymes play a fundamental role in neutralizing ROS, and thus protecting cells from oxidative damage (Sanches et al., 2021). By protecting plant tissues from potential damage, treatments with polyols on blood oranges could enhance the observed antioxidant activity, allowing plants and their fruits to maintain their viability and functionality even under stress conditions. In this regard, Guirao et al. (2024) found that grapes treated with sorbitol-Ca from the 'Doña María' variety showed higher

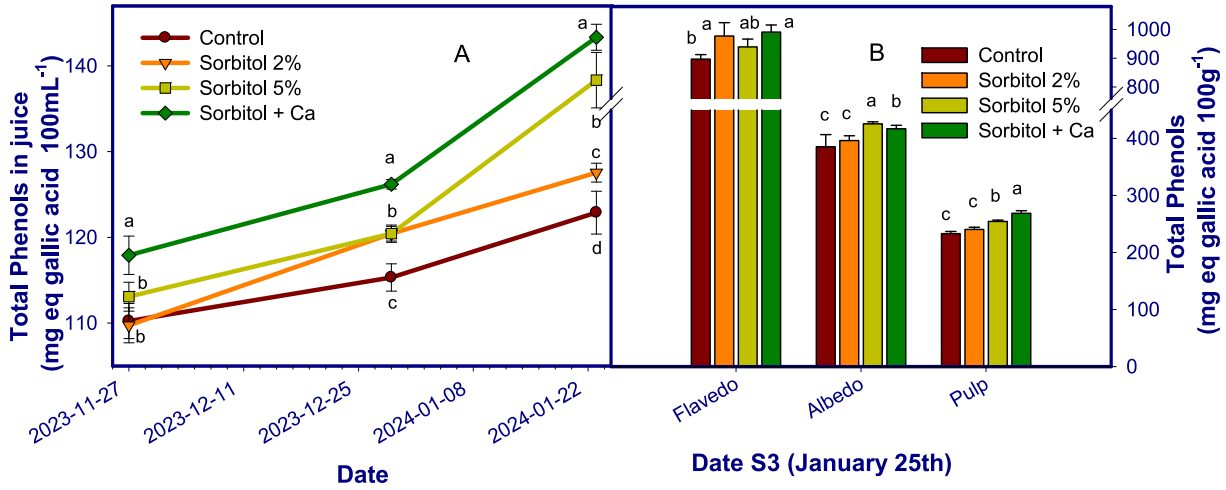


Fig. 5. Total Phenols in juice (A) and Total Phenols in flavedo, Albedo and pulp (B) (mg eq Gallic acid 100 g^{-1}) in Blood Oranges cv 'Sanguinelli' treated with sorbitol 2 % (orange), sorbitol 5 % (yellow), Sorbitol-Ca (green) and control (red) during ripening and sampled at S1 (240 DAFB), S2 (270 DAFB) and S3 (300 DAFB). Results are presented as the mean ± SE from ten samples per replicate and treatment. Significant differences between treatments on the same sampling day are marked by different lowercase letters ($p < 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

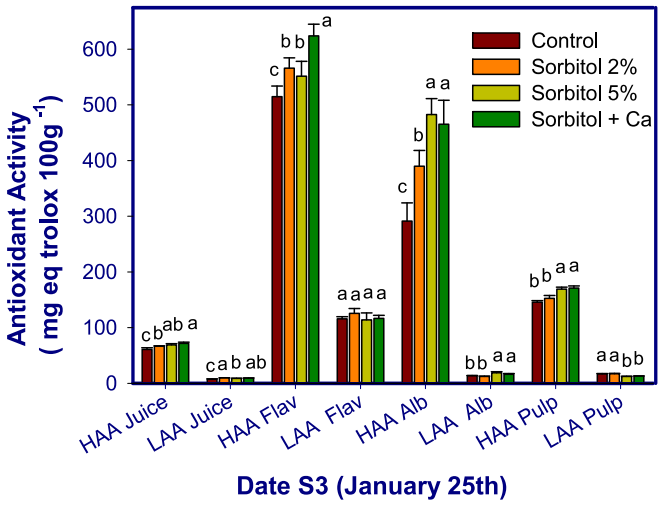


Fig. 6. Hidrophylic Antioxidant Activity (HAA) (mg eq trolox 100 g^{-1}) (C) and Lipophylic Antioxidant Activity (LAA) (mg eq trolox 100 g^{-1}) in juice, flavedo, albedo and pulp of Blood Oranges cv 'Sanguinelli' treated with sorbitol 2 % (orange), sorbitol 5 % (yellow), Sorbitol-Ca (green) and control (red) during ripening and sampled at S1 (240 DAFB), S2 (270 DAFB) and S3 (300 DAFB). Results are presented as the mean ± SE from ten samples per replicate and treatment. Significant differences between treatments on the same sampling day are marked by different lowercase letters ($p < 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

antioxidant activity (both hydrophilic and lipophilic) compared to control grapes.

3.4. Role of sorbitol and sorbitol-ca on ACNs accumulation

According to the identification of ACNs, in the juices of oranges at stages S1, S2, and S3, performed by HPLC-DAD-MS, nine ACNs were detected: Delphinidin 3-O-glucoside (D3G), Cyanidin-3-O-sophoroside (C3S), Cyanidin-3-O-glucoside (C3G), Delphinidin 3-(6-malonyl) glucoside (D3MG), Cyanidin 3-(6″-malonylgalactoside) (C3Mgal), Cyanidin 3-(6″-malonylglucoside) (C3Mglu), Cyanidin 3-(6-dioxalyl) glucoside (C3DG), Peonidin 3-(6″-malonyl) glucoside (P3MG), and Cyanidin malonyl-(dioxalyl)-hexoside (CMDH) (Fig. 7). Regardless of the treatments applied, each ACN significantly accumulated during maturation, reaching the highest concentrations at maturity stage S3. At stage S1, only C3G and D3MG were identified at concentrations below 0.8 and 0.4 mg 100 mL^{-1}, respectively. However, at maturity stage S3, C3Mglu, C3G, and D3MG were the predominant ACNs, each representing approximately 45 %, 25 %, and 9 % of the total identified ACNs, respectively (Fig. 7C, D and F).

Juices from fruits treated with 2 % sorbitol, 5 % sorbitol, and sorbitol-Ca significantly increased ($p < 0.001$) ACNs concentrations at stages S2 and S3. In stage S2, the total ACNs concentration was 19.9 %, 31.7 %, and 23.5 % higher compared to the control for the 2 % sorbitol, 5 % sorbitol, and sorbitol-Ca treatments, respectively. At stage S3, these increases were 40.3 %, 40.0 %, and 41.1 %, respectively, in the juices of fruits treated with 2 % sorbitol, 5 % sorbitol and sorbitol-Ca (Fig. 7J).

C3Mglu and C3G were the ACNs that accumulated the most in the juices of sorbitol-Ca treated fruits, reaching 10.25 ± 0.89 and 5.72 ± 0.57 mg 100 mL^{-1}, respectively, while in the control fruits, the concentrations were 7.23 ± 1.86 and 3.70 ± 0.12 mg 100 mL^{-1}, respectively (Fig. 7C and F).

In this regard, Conde et al. (2024) demonstrated that vineyards of the Touriga Nacional variety, treated with 2 mM mannitol and 2 mM sorbitol solutions, positively regulated the biosynthesis of ACNs, as well as phenylpropanoids and stilbenes. These authors found that both phenylalanine ammonia-lyase (PAL) and UDP-glucose: flavonoid 3-*O*-glucosyltransferase (UFGT) enzymes, stilbene synthase (VviSTS1) transcripts, and the expression of VviPAL1 and VviUFGT1, were significantly increased. Among the ACNs, mainly cyanidin-3-O-glucoside and malvidin-3-*O*-(6-p-coumaroyl)-glucoside, showed the highest increases. Additionally, Zhu et al. (2019) and Ju et al. (2020) found that calcium treatments regulate ACNs accumulation in 'Manicure Finger' grapes by enhancing the interaction between calmodulin and UDP-glucose: flavonoid 3-O-glucosyltransferase (UFGT). This process activated transcription factors for the genes PAL, 4CL, CHS, CHI, F3H, DFR, LDOX, UFGT, and mybA1, all of which are directly involved in ACN production. According to the results obtained, the ACN content in oranges increased in response to the sorbitol dosage applied, as well as with the application of calcium chelated with sorbitol, with C3Mglu and C3G being the ACNs that accumulated the most, particularly with sorbitol-Ca treatments (Fig. 7).

Some studies have been conducted to investigate the sugar-sensing mechanism involved in the ACNs biosynthesis pathway (Vitrac et al., 2000). Specifically, Zheng et al. (2009) demonstrated that sugars induce the accumulation of ACNs and flavanone 3-hydroxylase (F3H) in Cabernet Sauvignon grapes. Flavanone 3-hydroxylase (F3H) is a key enzyme in the biosynthesis of flavonoids, regulating the accumulation of flavonols and ACNs in blood oranges (Ma et al., 2023).

In this context, the increase of sugars in 'Sanguinelli' oranges (sucrose, glucose, and fructose) could contribute to increasing the ACNs content in the fruits. Yu, Wang, et al. (2020) suggested that the increase in fructose and glucose levels in blood oranges during ripening might significantly contribute to the synthesis of ACNs. They observed a notable positive correlation between the ACNs levels and the amounts of fructose and glucose, with correlation coefficients of 0.810 and 0.799, respectively.

In addition to increasing ACNs content, polyols have been found to enhance other secondary metabolites that are part of the synthesis of phenolic compounds, consequently boosting their antioxidant activity (Fig. 5–6). In the case of vines treated with mannitol and/or sorbitol, or sorbitol-Ca, the grapes showed an increase in total (poly)phenol content (Ma et al., 2022). Specifically, Conde et al. (2024) observed a greater accumulation of ferulic acid, *E*-resveratrol, E-piceatannol, piceid, pallidol, E-ε-viniferin, and myricetin-hexoside.

3.5. Mineral accumulation and balance in leaves and fruits

Total mineral content varied among the different tissues analyzed. Leaves had a significantly higher concentration (7926.31–9078.49 mg 100 g^{-1}) than the albedo (1592.03–1769.10 mg 100 g^{-1}) and pulp (1507.17–1677.91 mg 100 g^{-1}) of the fruits (Fig. S1). The predominant mineral in leaves was Ca, followed by K, Mg, P, and Na, representing 85 %, 3.1 %, 1.4 %, and 1.2 % of the total minerals in controls, respectively (Fig. S1 and Table S2). However, in the albedo and pulp, K was the predominant mineral, followed by Ca, P, Mg, and Na, representing 49.5 %, 43.1 %, 3.9 %, 2.8 %, and 0.6 % of the total minerals in the albedo, and 54.8 %, 30.4 %, 5.0 %, 8.1 %, and 1.5 % in the pulp of control fruits, respectively. In all tissues, the concentration of Cu, Fe, and Mn was below 18 and 1.5 mg 100 g^{-1}, representing less than 1 % and 0.1 % of the total minerals in the leaves and fruit tissues (Table S2).

Treatments with 2 %, 5 % sorbitol, and sorbitol-Ca altered the mineral profile of the leaves, albedo, and pulp. Total and bound calcium concentrations significantly increased in all tissues (leaves, albedo, and pulp) with the treatments. Total calcium in control leaves was 6827.28 ± 126.28 mg 100 g^{-1}, increasing to 7579.76 ± 116.42, 7656.36 ± 124.35, and 8099.85 ± 276.15 mg 100 g^{-1} with 2 %, 5 % sorbitol, and sorbitol-Ca treatments, respectively, representing increases of 11.1 %, 12.1 %, and 18.6 %. Similarly, bound calcium increased from 6370.50 ± 152.38 mg 100 g^{-1} to 7239.52 ± 229.07, 7363.29 ± 165.67, and 7691.10 ± 163.04 mg 100 g^{-1} with 2 %, 5 % sorbitol, and sorbitol-Ca treatments, respectively, representing increases of 11.3 %, 11.5 % and 12.0 %.

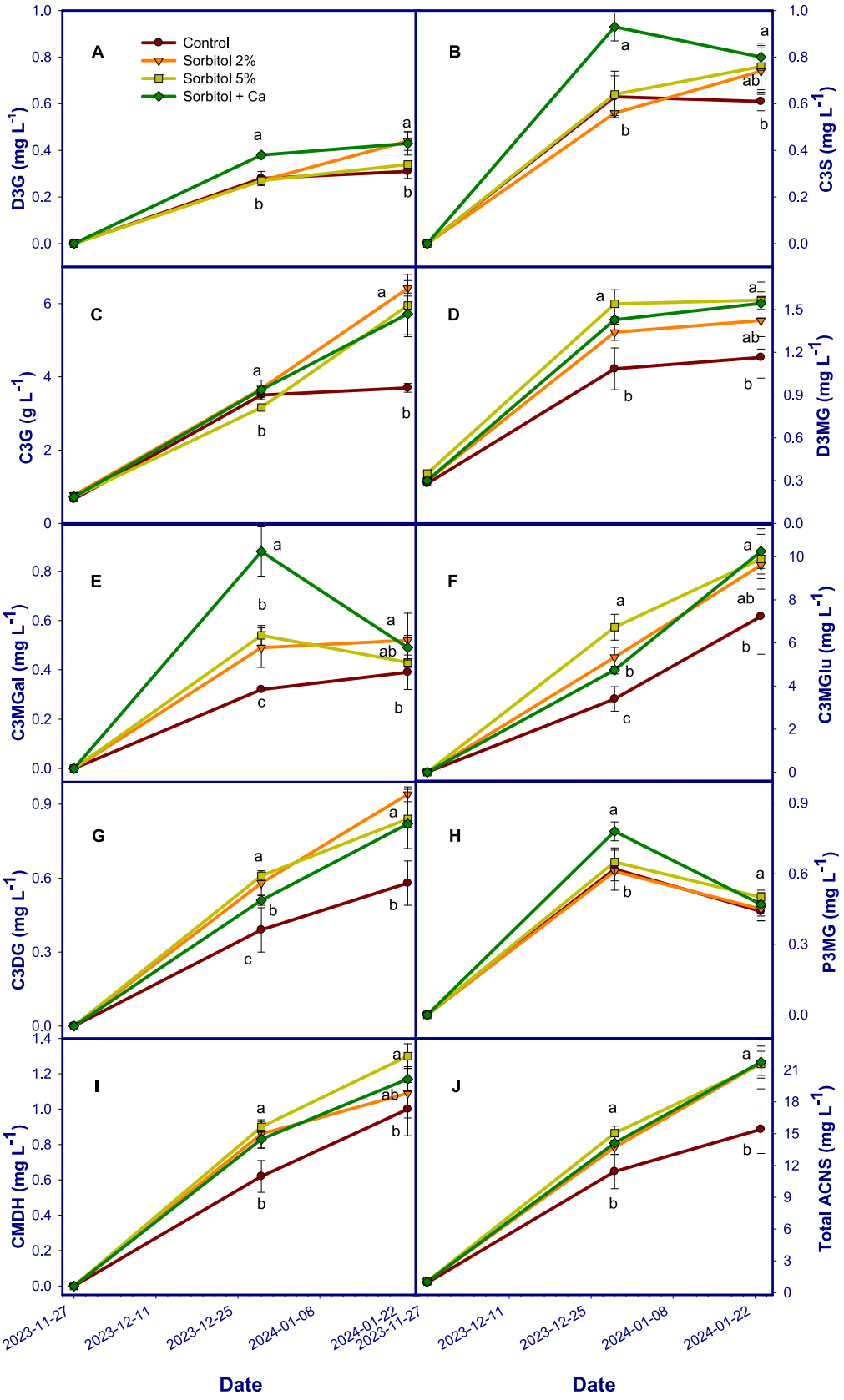


Fig. 7. Anthocyanin (ACN) concentration: Delphinidin 3-O-glucoside (D3G) (A); Cyanidin 3-O-sophoroside (C3S) (B); Cyanidin 3-O-glucoside (C3G) (C); Delphinidin 3-(6-malonyl) glucoside (D3MG) (D); Cyanidin 3-(6″-malonyl) galactoside (C3MGal) (E); Cyanidin 3-(6″-malonyl) glucoside (C3MGlu) (F); Cyanidin 3-(6-dioxalyl) glucoside (C3DG) (G); Peonidin 3-(6′-malonyl) glucoside (P3MG) (H); Cyanidin malonyl-(dioxalyl)-hexoside (CMDH) (I); and total Anthocyanins (J) in treated orange juice at sampling dates S1, S2, and S3. Data are expressed as mg L^{-1} of juice and represent the means ± SE of three replicates. According to Tukey's *t*-test ($p < 0.05$), different letters between different treatments and the same date indicate significant differences in anthocyanin (ACN) concentrations.

In the albedo of fruits treated with 2 %, 5 % sorbitol, and sorbitol-Ca, total calcium increased significantly by 3.7 %, 15.3 %, and 27.6 % compared to the control (715.18 ± 9.82 mg 100 g^{-1}). In the pulp of treated fruits, total calcium increased by 2.0 %, 24.1 %, and 37.7 % compared to the control (482.43 ± 64.8 mg 100 g^{-1}). Similarly, bound calcium in treated fruits increased by 6.4 %, 9.9 %, and 18.9 % in the albedo and by 10.6 %, 34.7 %, and 45.9 % in the pulp, compared to the control albedo (688.67 ± 24.80 mg 100 g^{-1}) and pulp (435.09 ± 12.78 mg 100 g^{-1}).

However, the K content significantly decreased in leaves treated with 2 %, 5 % sorbitol, and sorbitol-Ca. Consequently, K/Ca, K/Mg, and (K + Mg)/Ca ratios significantly decreased in all treated leaves compared to the control, with sorbitol-Ca treated leaves showing the lowest ratios ($p < 0.001$). In fruits, K content was only significantly lower in the albedo of treated fruits compared to the control. Nevertheless, K/Ca, K/Mg, and (K + Mg)/Ca ratios significantly decreased in the albedo of treated fruits compared to the control, while K/Ca and (K + Mg)/Ca ratios in the pulp significantly decreased with the treatments (Table S2).

Furthermore, Cu, Fe, and Mg microelement concentrations significantly increased in treated leaves. Mg concentration increased by 31.0 %, 43.4 %, and 39.0 % in leaves treated with 2 %, 5 % sorbitol, and sorbitol-Ca, respectively, compared to control leaves (243.47 ± 13.56 mg 100 g^{-1}). However, in the albedo, Fe was the only microelement that significantly increased with treatments, while Mg concentration increased in both the albedo and pulp of fruits treated with 5 % sorbitol.

It has been demonstrated that calcium ions play a crucial role in the development and ripening of citrus fruits. Ca^{2+} interacts with cell wall components and is essential for maintaining structural stability, synthesizing Ca^{2+}-pectin in tissues, regulating enzymatic activity involved in metabolism, and preventing the breakdown of pectin, cellulose, and hemicellulose in the peel and pulp of citrus fruits (Juan & Jiezhong, 2017). Calcium deficiencies in oranges are a determining factor in the occurrence of Albedo breakdown (Treeby & Storey, 2002) and fruit softening. Efforts have been made to correct calcium deficiencies in fruits through exogenous treatments. However, calcium transport through the phloem in plants and its accumulation in fruits is challenging (Cronje et al., 2011), making it more effective to apply calcium early to mid-season when fruits are immature, rather than later in the season (Treeby & Storey, 2002). In any case, treatments with sorbitol-chelated calcium improve its transport and accumulation in grapes (Ma et al., 2022). Guirao et al. (2024) demonstrated that sorbitol-chelated calcium was able to be transported from the leaves to the bagged bunches of the cv. 'Doña María' table grape. This calcium, which accumulated in both leaves and grapes in the form of total and bound calcium, was similar to the accumulation observed in the leaves, albedo, and pulp of 'Sanguinelli' oranges in our study (Tables S2-S3).

On the other hand, in leaves treated with sorbitol or sorbitol-Ca, Mg content increased (Fig. S1). Magnesium is vital for photosynthetic activity and leaf health in fruit trees. It plays a key role in chlorophyll formation, carbon fixation during photosynthesis, protein synthesis, and the regulation of reactive oxygen species (ROS), all of which influence plant development and production quality. Additionally, magnesium is involved in the transport of carbohydrates from source (leaves) to sink organs (fruit) (Farhat et al., 2016). A lack of magnesium can significantly reduce biomass, alter nutrient distribution in plant organs, and decrease photosynthetic efficiency, ultimately affecting fruit yield and quality. In fact, magnesium is a component of chlorophyll molecules, and there is a positive correlation between magnesium content and chlorophyll in leaves (Cronje et al., 2011). Ma et al. (2022) demonstrated that sorbitol-Ca treatments increased chlorophyll content and the photosynthetic rate in grapevine leaves. Since magnesium is a key component of chlorophyll molecules and plays a crucial role in photosynthesis (Cronje et al., 2011), the higher Mg content observed in sorbitol-Ca treated leaves (Fig. S1) suggests that these treatments could contribute to increased chlorophyll accumulation and enhanced photosynthetic activity.

The K/Ca ratio is typically used as an indicator of calcium mobility through the phloem, and K and Mg have also been considered as antagonistic elements to Ca (Cronje et al., 2011). Several studies have linked varying K/Mg/Ca ratios to fruit quality and the development of physiological issues, such as bitter pit in apples (Miqueloto et al., 2014), rind breakdown in mandarins (Cronje et al., 2011), shattering in table grapes (Guirao et al., 2024), or the development of rots like apple bitter rot (Everett et al., 2016). In all mineral analyses conducted on the leaves, albedo, and pulp of the oranges, the K/Ca, Mg/Ca, and (K + Mg)/Ca ratios in trees treated with sorbitol or sorbitol-Ca were lower than in the controls (Tables S2-S3). This suggests that blood oranges treated with sorbitol-Ca can more easily mobilize calcium through the phloem. As a result, they would be more resistant to physiological disorders and rot incidence compared to the control fruits.

4. Conclusion

This study demonstrates that preharvest foliar application of sorbitol and sorbitol-ca enhances the physicochemical properties and bioactive compound content of 'Sanguinelli' blood oranges. Treated fruits exhibited higher TSS levels, improved firmness, and greater antioxidant activity, indicating a positive impact on fruit quality. Additionally, anthocyanin accumulation significantly increased, reinforcing the role of these treatments in color development.

A key strength of this study is its practical agronomic approach, which could be easily integrated into current agricultural practices. The findings provide valuable insights into how preharvest treatments influence postharvest fruit quality. Future research should evaluate storage stability, sensory attributes, and large-scale feasibility.

Enhancing functional compound accumulation through sorbitol-Ca treatments may improve dietary intake of health-promoting phytochemicals, which are linked to reduced risk of chronic diseases. Moreover, optimizing anthocyanin synthesis in blood oranges via agronomic practices could ensure consistent production in varying environmental conditions, expanding market opportunities.

Additionally, sorbitol may facilitate calcium transport and accumulation in leaves and fruits, mainly as pectates, which could contribute to pathogen attacks and physiological disorders. These results suggest that sorbitol-based treatments can effectively improve blood orange quality and shelf life. Further studies should explore the biochemical mechanisms behind these benefits and their application in other fruits.

CRediT authorship contribution statement

A. Guirao: Writing – review & editing, Software, Methodology, Investigation, Formal analysis. **D. Martínez-Romero:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Data curation, Conceptualization. **A. Solana-Guilabert:** Investigation, Formal analysis. **V. Agulló:** Writing – original draft, Validation, Methodology, Investigation, Conceptualization. **H.M. Díaz-Mula:** Writing – original draft, Software, Methodology, Investigation, Conceptualization. **J.M. Valverde:** Writing – review & editing, Validation, Supervision, Project administration, Funding acquisition, Data curation, Conceptualization.

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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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2025.144105.

Data availability

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

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