



Article

Controlled Atmosphere Storage and Sorbitol Dipping Minimize Chilling Injuries in 'Palmer' Mangoes

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Abstract: Our previous studies have shown that 'Palmer' mangoes immersed in solutions containing 2.5% sorbitol and stored under a controlled atmosphere (CA) at 8 °C for 30 days had fewer symptoms of a chilling injury. However, there is no information regarding the effectiveness of sorbitol treatment in other atmospheres and/or in combination with lower temperatures. Thus, the objective of this study was to assess the impact of dipping 'Palmer' mangoes in 0.1% and 2.5% (*w/v*) sorbitol solutions and storing the fruit under a CA without atmosphere modification (21 kPa O₂ + 0.03 kPa CO₂) at 8 °C/95% relative humidity (RH) or with 5 kPa O₂ + 5 kPa CO₂ at 4 °C/95% RH for 28 days. The fruits were evaluated periodically for chilling injuries, quality, and oxidative metabolism. A chilling injury (CI) was correlated with increased fresh weight loss (FWL) and changes in the color of the epicarp (L_{peel} , h°_{peel} , and C_{peel}) and mesocarp (L^{*}_{pulp}). Lipid peroxidation (LP_{pulp} and LP_{peel}) and the hydrogen peroxide content ($H_2O_{2\text{peel}}$ and $H_2O_{2\text{pulp}}$) were associated with the development of a CI, particularly after being transferred to ambient. The treatment with 2.5% sorbitol was more effective in minimizing the chilling injury symptoms and did not compromise the fruit quality, especially when it was stored at 4 °C in association with a CA containing 5 kPa O₂ + 5 kPa CO₂. This treatment reduced lipid peroxidation and increased the activities of the superoxide dismutase (SOD) and ascorbate peroxidase (APX) enzymes in the epicarp and mesocarp, providing greater cold tolerance. The use of 2.5% sorbitol has been identified as the most efficacious approach for mitigating the adverse impacts of chilling injuries, preserving the fruit quality, and enhancing oxidative metabolism, even at lower temperatures. Thus, this treatment represents a viable alternative for managing chilling injuries in mangoes.

Keywords: *Mangifera indica* L.; polyols; oxidative metabolism; SOD; APX



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1. Introduction

As observed in other horticultural products, cold storage is the primary post-harvest technology used to extend the shelf life of mangoes because low temperatures reduce their metabolic activity [1]. Mangoes stored at temperatures between 8 and 13 °C have a post-harvest shelf life of up to 21–30 days, depending on the cultivar and fruit maturity [2,3]. However, prolonged storage at temperatures below 13 °C can lead to the development of a physiological disorder known as a chilling injury (CI), resulting in qualitative and quantitative losses [4,5].

The symptoms of a CI in mangoes are most evident in the epicarp and are characterized by the presence of dark, sunken spots resembling burns, which can hinder the marketing

of fresh fruit, as this defect may render the product unacceptable according to market standards. The mesocarp is also affected, as the fruit may exhibit irregular ripening [5,6].

The mechanisms related to the development of CIs in plants are related to changes in the lipid bilayer of the plasma membrane, i.e., transitioning from a more flexible fluid state to a rigid gel phase due to exposure to low temperatures. This change in rigidity leads to the loss of functions and the rupture of cell membranes, resulting in cell death [7]. Several studies have reported changes in the rigidity of cell membranes in mangoes with CIs [8–11].

Exposure to low temperatures also induces the overproduction of reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), superoxide radicals ($\text{O}_2^{\bullet-}$), and hydroxyl radicals ($\bullet\text{OH}$), that react with various molecules, leading to lipid membrane peroxidation [12]. In mangoes, ROS overproduction has been correlated with a higher incidence of CIs [5,13–15] and the manifestation of their various symptoms [16–19].

Sorbitol ($\text{C}_6\text{H}_{14}\text{O}_6$) is a water-soluble polyol that naturally occurs in various fruits [20,21] and plays an important role in the osmotic adjustment of the cytoplasm under stress conditions, potentially stabilizing membranes [22]. It can increase cold stress tolerance [23] by binding to water and lowering the dielectric constant, even at temperatures above freezing [24]. Sorbitol acts as an osmoprotective agent that has been linked to CI tolerance [25], contributing to membrane stabilization and reducing structural damage [26]. It can also act as an antioxidant, affecting the activity of enzymes, such as superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT), as well as scavenging ROS [27].

In this context, Sanches et al. [15] have reported that ‘Palmer’ mangoes stored at 8 °C for 30 days, when treated with 2.5% sorbitol, showed reduced H_2O_2 accumulation and polyphenol oxidase (PPO) activity, higher membrane integrity (malondialdehyde—MDA), and increased activity of the enzymes SOD, CAT, and ascorbate peroxidase (APX), both in the epicarp and the mesocarp compared to those in standard storage or storage after treatment with the polyols propylene glycol and glycerol. Consequently, sorbitol immersion has been considered a feasible approach to alleviating CI during refrigerated storage.

Under standard cold quarantine treatment conditions (1 °C for 14 days), immersion in a solution containing 0.1% sorbitol was the most effective in mitigating CIs. This effect was associated with lower MDA and H_2O_2 concentrations and PPO activity levels, as well as higher SOD, CAT, and APX enzyme activity and ascorbic acid levels, especially in the pericarp [28]. The combination of immersion of ‘Palmer’ mangoes in solutions containing 2.5% sorbitol with controlled atmosphere (CA) storage reduced CIs through the activation of non-enzymatic (ascorbic acid and total phenolic compounds) and enzymatic (SOD, CAT, and APX) mechanisms in fruits kept at 8 °C for 30 days [28]. Despite these results, there is no information available regarding the effectiveness of sorbitol treatment in other storage atmospheres and/or in combination with lower temperatures.

Thus, the objective of this study was to evaluate the effect of dipping ‘Palmer’ mangoes in 0.1 and 2.5% (*w/v*) sorbitol solutions and storing them under a CA with ambient atmospheric conditions (21 kPa O_2 + 0.03 kPa CO_2) at 8 °C/95% relative humidity (RH) or under 5 kPa O_2 + 5 kPa CO_2 at 4 °C/95% RH for 28 days. The development of chilling injuries, quality modification, and oxidative metabolism were evaluated periodically.

2. Materials and Methods

2.1. Plant Material

‘Palmer’ mangoes were obtained from commercial orchards located in Taquaritinga (21°25′57.72″ latitude south, 48°32′50.46″ longitude west), São Paulo, Brazil (Experiment I), and Belém do São Francisco (8°32′1.89″ latitude south, 38°58′53.97″ longitude west), Pernambuco, Brazil (Experiment II). The fruits were harvested at physiological maturity and selected manually, and then classified according to size, soundness, the absence of mechanical damage, and pest and disease lesions. Fruit physiological maturity was evaluated based on the dry matter (DM) content of 20 fruits from each experiment. The DM content was $13.1\% \pm 1\%$ and $13.0\% \pm 1\%$ in the fruits from Experiments I and II, respectively.

2.2. Experiment I: Without Gas Modification (Air)

After selection, the mangoes were washed with soap, rinsed with running water, and dried. Following that, they were dipped in one of the following solutions at 5 °C for 60 min: i. distilled water (control), ii. 0.1% (*w/v*) food-grade sorbitol, or iii. 2.5% (*w/v*) food-grade sorbitol (Sigma-Aldrich, St. Louis, MO, USA) [15]. Subsequently, the mangoes were stored at 8.0 ± 1.0 °C and $95 \pm 0.5\%$ RH in a controlled atmosphere chamber (Venezia PCM 1000 model; Fruit Control Equipment, Milan, Italy), with ambient atmosphere conditions maintained ($21 \text{ kPa O}_2 + 0.03 \text{ kPa CO}_2$) for 28 days. The fruits were evaluated every 7 days. During each evaluation, the chilling injury was immediately evaluated, and some of the fruits were moved to ambient conditions ($\sim 24 \pm 2.0$ °C and $75 \pm 2.0\%$ RH) to evaluate them for signs of chilling injuries whenever the fruit became ripe (from 5 to 10 days). This study was set according to a completely randomized design (CRD) in a factorial arrangement of 3 (treatments: control, 0.1% sorbitol, and 2.5% sorbitol) \times 5 (storage periods: 0, 7, 14, 21, and 28 days) with three replicates.

2.3. Experiment II: CA—Modification of Atmosphere Gases

The fruits for this experiment were washed, rinsed with water, and dried as in the other study. Following this, they were i. dipped in distilled water and stored in CA with $21 \text{ kPa O}_2 + 0.03 \text{ kPa CO}_2$ (negative control), ii. dipped in distilled water and stored in CA with $5 \text{ kPa O}_2 + 5 \text{ kPa CO}_2$ (positive control), iii. dipped in 0.1% (*w/v*) sorbitol and stored in CA with $5 \text{ kPa O}_2 + 5 \text{ kPa CO}_2$, or iv. dipped in 2.5% (*w/v*) sorbitol and stored in CA with $5 \text{ kPa O}_2 + 5 \text{ kPa CO}_2$. The fruits were stored at 4.0 ± 1.0 °C and $95 \pm 0.5\%$ RH in a controlled atmosphere chamber (Venezia PCM 1000 model; Fruit Control Equipment, Milan, Italy), and control of the oxygen (O_2), carbon dioxide (CO_2), and ethylene (C_2H_4) levels was performed using SWINGLOS[®] software, GAC 5000 (Fruit Control Equipment, Milan, Italy). These conditions were maintained for 28 days, and evaluations were conducted every 7 days. At each evaluation, some of the fruits were moved to ambient conditions ($\sim 24 \pm 2.0$ °C and $75 \pm 2.0\%$ RH) to be evaluated for signs of chilling injuries whenever the fruit became ripe (from 4 to 7 days). This experiment was set according to a completely randomized design (CRD) in a factorial arrangement of 4 (treatments: negative control, positive control, 0.1% sorbitol, and 2.5% sorbitol) \times 5 (storage periods: 0, 7, 14, 21, and 28 days) with three replications.

2.4. Evaluations

In both experiments, the fruits were evaluated using the same method.

2.4.1. Chilling Injury Development

To evaluate chilling injury (CI) development, the fruits had their pericarp lesions visually rated according to the process of Miguel et al. [29] with modifications. Fruits with no visible symptoms ($\text{CI} = 0\%$) were rated as 1, mild symptoms ($\text{CI} = 0\text{--}25\%$) as 2, moderate symptoms ($\text{CI} = 25\text{--}50\%$) as 3, and severe symptoms ($\text{CI} \geq 50\%$) as 4.

2.4.2. Fresh Weight Loss

Fresh weight loss (FWL) was determined by weighing the mangoes on an analytical balance (AS 2000 model; Mars, Brazil), and the accumulated weight loss was calculated according to the method of Sanches et al. [15] for each experiment and each evaluation day. FWL is expressed as a percentage (%).

2.4.3. Firmness

Fruit firmness was measured in the mid portion between the stem end and the remains of stigma and style of each mango without the epicarp (skin). Firmness was measured using a penetrometer (Effegi Fruit Tester, Forlì, Italy) with an 8 mm tip, and the results are expressed in Newtons (N), as has been previously described by Watkins and Harman [30].

2.4.4. Color

The epicarp (skin) and mesocarp (flesh) colors were measured in the mid portion between the stem end and the remains of the stigma and style. The skin color was also measured on opposite sides of each fruit on the blush and green areas of the protruding side. A reflectometer (CR-400; Minolta, Osaka, Japan) was used to obtain the L^* , a^* , and b^* values, and chromaticity (C^*) and hue angle (h°) were calculated according to the method of McGuire [31].

2.4.5. Physicochemical Evaluation

The soluble solid content (SSC) of the fruits was measured using a digital refractometer (Alpha; Atago Co., Ltd., Tokyo, Japan), and the results are expressed as percentages [32]. Titratable acidity (TA) was measured via titration using 0.1% phenolphthalein as an indicator, and the results are expressed in g kg^{-1} citric acid [31]. The ratio (SSC/TA) was calculated according to the method of the AOAC [31], and the pH was determined using a pH meter (Orion 3 Star; Thermo Scientific, Waltham, MA, USA).

2.5. Oxidative Metabolism

2.5.1. Lipid Peroxidation

For this analysis, samples of peel and pulp (0.5 g) were homogenized with 2.5 mL of a solution containing 0.1% (*w/v*) trichloroacetic acid (TCA) and 20% (*w/v*) polyvinylpyrrolidone (PVP). After centrifugation (ST16-R; Thermo Scientific, Waltham, MA, USA) at $12,298 \times g$ and 4°C for 15 min, 250 μL of the supernatant was mixed with 1 mL 20% TCA (*w/v*) and 0.5% thiobarbituric acid (TBA) and incubated in a water bath at 95°C for 30 min. After this period, the samples were cooled in ice for 10 min and centrifuged at $12,298 \times g$ for 15 min at 4°C . Lipid peroxidation was calculated according to Gratao et al. [33] and is expressed in μmol of malondialdehyde (MDA) per kg of fresh weight.

2.5.2. Hydrogen Peroxide (H_2O_2)

The hydrogen peroxide (H_2O_2) content was determined in 1.0 g peel and pulp samples according to the process of Alexieva et al. [34]. The samples were homogenized in 0.1% (*w/v*) trichloroacetic acid at 4°C and centrifuged at $12,298 \times g$ for 20 min at 4°C . Following this, the supernatant (200 μL) was mixed with 200 μL of 100 mM potassium phosphate buffer (pH 7.5) and 800 μL of 1 M potassium iodide (KI). The samples were incubated in ice for 1 h in the dark. The hydrogen peroxide content was measured at 390 nm and expressed in $\text{mol H}_2\text{O}_2$ per kg of fresh weight.

2.5.3. Superoxide Dismutase (SOD) and Ascorbate Peroxidase (APX) Extraction

These two enzymes were extracted according to the process of Yang et al. [35]. The peel and pulp samples (1.0 g) were homogenized in 100 mM potassium phosphate buffer (pH 7.0), 1 mM ethylenediaminetetraacetic acid (EDTA), and 20% (*w/v*) polyvinylpolypyrrolidone (PVP). The homogenates were filtered using a fine nylon mesh, centrifuged at $12,298 \times g$ for 25 min, and immediately frozen at -18°C . The protein levels of the homogenates were determined [36].

2.5.4. Superoxide Dismutase (SOD) Activity

SOD (SOD, EC 1.15.1.1) activity was measured using the method described by Giannopolitis and Ries [37]. The enzymatic extract (50 μL) was mixed with 1.0 mL of 50 mM sodium phosphate buffer (pH 7.8), 19.5 mM methionine, 150 μL of NBT, and 300 μL of riboflavin. The incubation took place under light, and after 15 min, the absorbance was measured at 560 nm (Beckman spectrophotometer, DU-640; GMI: Mequon, WI, USA). The SOD activity is defined and expressed in $\text{U min}^{-1} \text{kg}^{-1} 10^6 \text{ protein}$ [38].

2.5.5. Ascorbate Peroxidase (APX) Activity

APX (APX, EC 1.11.1.1) activity was measured according to the process of Nakano and Asada [39]. The enzymatic extract (50 μ L) was mixed with 800 μ L of 50 mM potassium phosphate buffer (pH 7.0), 0.1 mM EDTA, 100 μ L of H₂O₂ (0.03 M), and 50 μ L of ascorbic acid (0.015 M). Ascorbic acid oxidation was measured at 290 nm (Beckman spectrophotometer, DU-640; USA), and the APX results are expressed in mol H₂O₂ min^{−1} kg^{−1} protein.

2.5.6. Polyphenol Oxidase (PPO) Extraction and Activity Measurements

To extract the polyphenol oxidase (PPO) enzyme (EC 1.14.18.1), 1.0 g of peel and pulp was homogenized with 50 mM potassium phosphate buffer (pH 7.0) and 1% (*w/v*) polyvinylpyrrolidone (PVP) at 4 °C. The homogenate was centrifuged (12,298 \times *g* for 10 min at 4 °C), and the supernatant was immediately frozen [40]. PPO activity was measured by mixing 100 μ L of enzymatic extract with 1.85 mL of 100 mM potassium phosphate buffer (pH 6.0). Catechol (100 mM) was used as a substrate, and after 30 min of incubation in a water bath (30 °C for 30 min), the reactions were terminated (800 μ L of 2 N perchloric acid) and the absorbance measured at 395 nm (Beckman spectrophotometer, DU-640; USA). The activity is expressed in U min^{−1} kg^{−1} 10⁶ protein [41].

2.6. Statistical Analysis

2.6.1. Univariate

Analysis of variance (ANOVA) was performed on the data. R software version 3.0 (R Core Team, 2020; Auckland, New Zealand) was employed to obtain the means and compare them using Tukey's test at 0.05%.

2.6.2. Multivariate

Multivariate analysis was performed to reduce the variables capable of interpretation, summarizing much of the variability among them. Numerical matrices were constructed using chilling injury (CI) symptoms, physicochemical variables, and oxidative metabolism in the peel and pulp of the fruits from the different storage periods in Experiment I (0, 0 + 10, 7, 7 + 5, 14, 14 + 6, 21, 21 + 5, 28, 28 + 5 days) and Experiment II (0, 0 + 7, 7, 7 + 6, 14, 14 + 4, 21, 21 + 4, 28, and 28 + 4 days). Principal component analysis (PCA) was performed by extracting the principal components via correlation matrix using R software (R Core Team, 2020; Auckland, New Zealand), and the first two principal components (PC1 and PC2) were used.

3. Results

The results of univariate analysis can be observed in the Supplementary Materials. Due to the large number of variables evaluated, we chose to focus on the results from multivariate analysis. Thus, the score plots and loading plots (biplots) of the PCA were obtained through correlation matrices based on the analyzed variables (Tables 1 and 2). The factor loadings used in both the experiments were based on the first two principal components (PC1 and PC2), correlating chilling injuries with the other analyzed variables.

Table 1. Variable codes for quality attributes used in the principal component analysis of Experiments I and II.

| Variables | Codes |
|------------------------|--------------------|
| Firmness | Firmness |
| pH | pH |
| Soluble solids content | SSC |
| Titrateable acidity | TA |
| Ratio SSC/TA | SSC/TA |
| Fresh weight loss | FWL |
| Chilling injury | CI |
| Luminosity peel | L* _{peel} |

Table 1. *Cont.*

| Variables | Codes |
|-------------------|--------------------------------|
| Luminosity pulp | L [*] _{pulp} |
| Hue angle peel | h ^o _{peel} |
| Hue angle pulp | h ^o _{pulp} |
| Chromaticity peel | C [*] _{peel} |
| Chromaticity pulp | C [*] _{pulp} |

Table 2. Variable codes for oxidative metabolism variables used in the principal component analysis of Experiments I and II.

| Variables | Codes |
|---------------------------|---|
| Lipid peroxidation peel | LP _{peel} |
| Lipid peroxidation pulp | LP _{pulp} |
| Hydrogen peroxide peel | H ₂ O ₂ _{peel} |
| Hydrogen peroxide pulp | H ₂ O ₂ _{pulp} |
| Superoxide dismutase peel | SOD _{peel} |
| Superoxide dismutase pulp | SOD _{pulp} |
| Ascorbate peroxidase peel | APX _{peel} |
| Ascorbate peroxidase pulp | APX _{pulp} |
| Polyphenol oxidase peel | PPO _{peel} |
| Polyphenol oxidase pulp | PPO _{pulp} |

3.1. Experiment I: Without Gas Modification (Air)

3.1.1. Chilling Injury and Physicochemical Variables

During cold storage, chilling injury (CI) development was not affected by the sorbitol treatments (Table 3). At the end of cold storage, the mangoes showed mild-to-moderate CI symptoms (Figure 1). On the other hand, after being transferred to ambient conditions, the 2.5% sorbitol treatment reduced CI development (Table 4) and the mangoes showed a better visual quality (Figures S1 and S2). If the score 3 (moderate symptoms—CI = 25–50%) is used as a shelf-life threshold, mangoes from the control treatment had a shelf-life of 21 + 5 days and 28 + 5 days when treated with 0.1 and 2.5% sorbitol (Figure 2).

Table 3. Effect of dipping ‘Palmer’ mangoes (Experiment I) in sorbitol (0.1 and 2.5%) and storage at 8.0 ± 1.0 °C and $95 \pm 0.5\%$ RH without atmosphere modification (21 kPa O₂ + 0.03 kPa CO₂) for 28 days on parameters fresh weight loss (FWL), chilling injury (CI), soluble solids content (SSC), titratable acidity (TA), ratio (SSC/TA), and pH.

| Main Effect | FWL (%) | CI (1–4) | SSC (%) | TA (g kg ^{−1}) | SSC/TA | pH |
|-----------------------|----------------------|--------------------|--------------------|--------------------------|--------------------|--------------------|
| Treatments (A) | | | | | | |
| Control | 0.86 ^a | 1.30 ^a | 6.49 ^a | 0.280 ^a | 24.39 ^a | 3.83 ^a |
| Sorbitol 0.1% | 0.86 ^a | 1.15 ^a | 6.39 ^a | 0.276 ^a | 25.91 ^a | 3.79 ^a |
| Sorbitol 2.5% | 0.72 ^b | 1.10 ^a | 6.47 ^a | 0.254 ^a | 23.70 ^a | 3.88 ^a |
| <i>F test</i> | 7.63 ^{**} | 1.86 ^{ns} | 0.44 ^{ns} | 2.91 ^{ns} | 1.96 ^{ns} | 1.54 ^{ns} |
| Days (B) | | | | | | |
| 0 | 0.00 ^e | 1.00 ^c | 6.38 ^{ab} | 0.255 ^{bc} | 25.07 ^b | 3.84 ^a |
| 7 | 0.46 ^d | 1.11 ^{bc} | 6.21 ^b | 0.267 ^{abc} | 23.74 ^b | 3.87 ^a |
| 14 | 0.82 ^c | 1.11 ^{bc} | 6.38 ^{ab} | 0.305 ^a | 21.70 ^b | 3.75 ^a |
| 21 | 1.23 ^b | 1.25 ^{ab} | 6.54 ^{ab} | 0.292 ^{ab} | 23.22 ^b | 3.80 ^a |
| 28 | 1.60 ^a | 1.50 ^a | 6.73 ^a | 0.231 ^c | 29.60 ^a | 3.93 ^a |
| <i>F test</i> | 220.93 ^{**} | 4.07 ^{**} | 3.59 [*] | 7.74 ^{**} | 8.33 ^{**} | 2.00 ^{ns} |
| Interaction | | | | | | |
| <i>F test</i> | 0.75 ^{ns} | 1.50 ^{ns} | 0.50 ^{ns} | 3.12 ^{**} | 2.86 [*] | 0.70 ^{ns} |

Means followed by the same letter within each column do not differ statistically from each other using Tukey’s test. Non-significant interaction (^{ns}), significant interaction at $p < 0.05$ (*), and significant interaction at $p < 0.01$ (**).

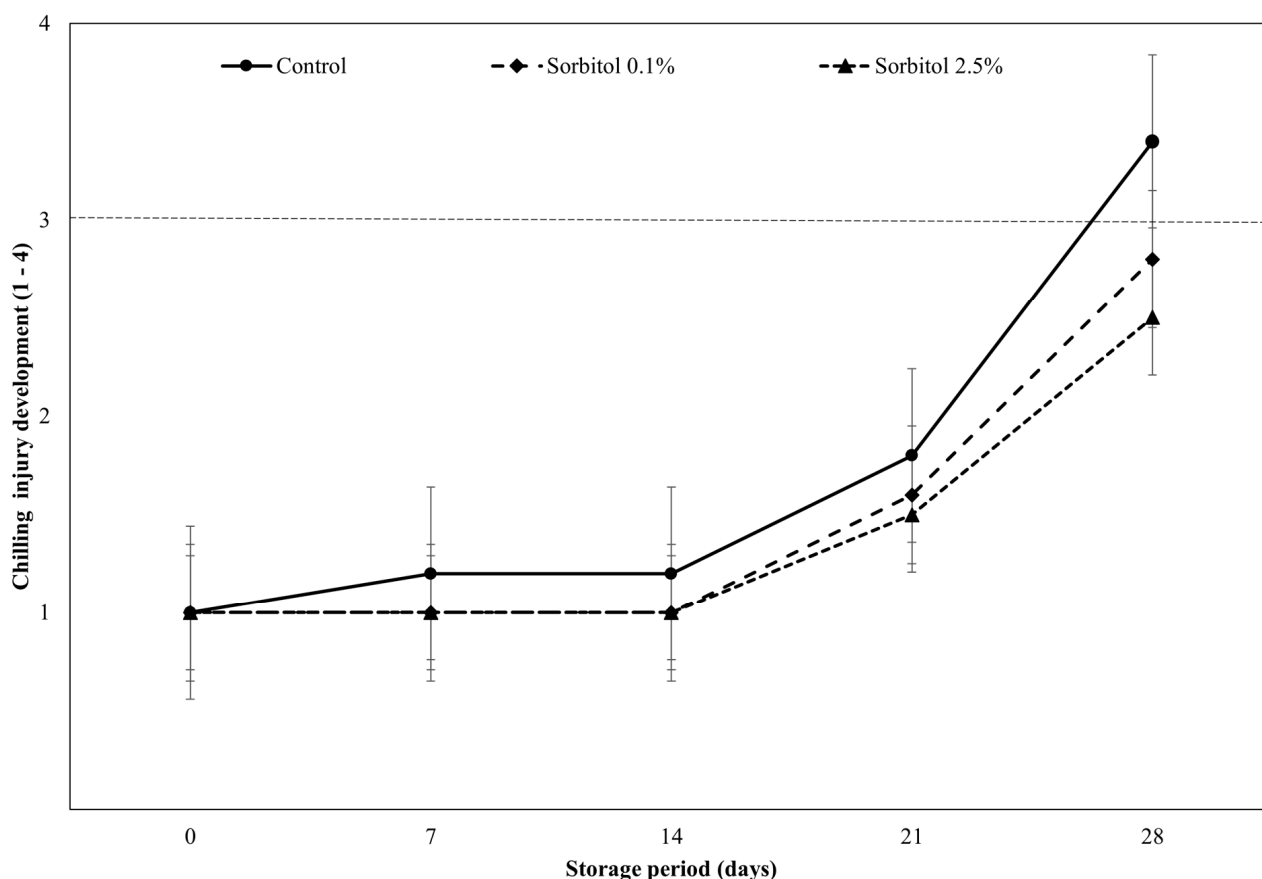


Figure 1. Interaction between treatments and storage period for chilling injury (CI) in ‘Palmer’ mangoes (Experiment I) stored at 8.0 ± 1.0 °C and $95 \pm 0.5\%$ RH without atmosphere modification ($21 \text{ kPa O}_2 + 0.03 \text{ kPa CO}_2$) for 28 days, and then transferred to ambient conditions (24 ± 2.0 °C and $75 \pm 2.0\%$ RH).

Table 4. Effect of dipping ‘Palmer’ mangoes (Experiment I) in sorbitol (0.1 and 2.5%) and storage at 8.0 ± 1.0 °C and $95 \pm 0.5\%$ RH without atmosphere modification ($21 \text{ kPa O}_2 + 0.03 \text{ kPa CO}_2$) for 28 days on the parameters of chilling injury (CI), soluble solids content (SSC), titratable acidity (TA), SSC/TA ratio, and pH after being transferred to ambient conditions (24 ± 2.0 °C and $75 \pm 2.0\%$ RH) from 5 to 10 days.

| Main Effects | CI (1–4) | SSC (%) | TA (g kg^{-1}) | SSC/TA | pH |
|-----------------------|---------------------|---------------------|---------------------------|---------------------|--------------------|
| Treatments (A) | | | | | |
| Control | 1.80 ^a | 14.13 ^a | 0.252 ^a | 60.53 ^b | 4.00 ^a |
| Sorbitol 0.1% | 1.70 ^a | 14.05 ^a | 0.209 ^b | 73.21 ^a | 4.04 ^a |
| Sorbitol 2.5% | 1.65 ^a | 14.29 ^a | 0.234 ^{ab} | 66.64 ^{ab} | 4.06 ^a |
| Test F | 0.30 ^{ns} | 0.27 ^{ns} | 4.52 [*] | 7.77 ^{**} | 0.38 ^{ns} |
| Days (B) | | | | | |
| 0 + 10 | 1.25 ^b | 15.19 ^a | 0.245 ^{ab} | 62.56 ^a | 3.94 ^{ab} |
| 7 + 5 | 1.33 ^b | 13.98 ^{ab} | 0.197 ^b | 70.46 ^a | 4.19 ^a |
| 14 + 6 | 1.75 ^b | 14.06 ^{ab} | 0.209 ^b | 67.30 ^a | 4.12 ^a |
| 21 + 5 | 1.41 ^b | 14.35 ^{ab} | 0.214 ^b | 67.05 ^a | 4.09 ^{ab} |
| 28 + 5 | 2.83 ^a | 13.19 ^b | 0.292 ^a | 45.17 ^b | 3.83 ^b |
| Test F | 12.95 ^{**} | 5.44 ^{**} | 8.40 ^{**} | 3.17 ^{ns} | 4.49 ^{**} |
| Interaction | | | | | |
| Test F | 2.46 ^{**} | 1.69 ^{ns} | 3.77 ^{**} | 3.81 ^{**} | 1.5 ^{ns} |

Means followed by the same letter within each column do not differ statistically from each other using Tukey’s test. Non-significant interaction (^{ns}), significant interaction at $p < 0.05$ (*), and significant interaction at $p < 0.01$ (**).

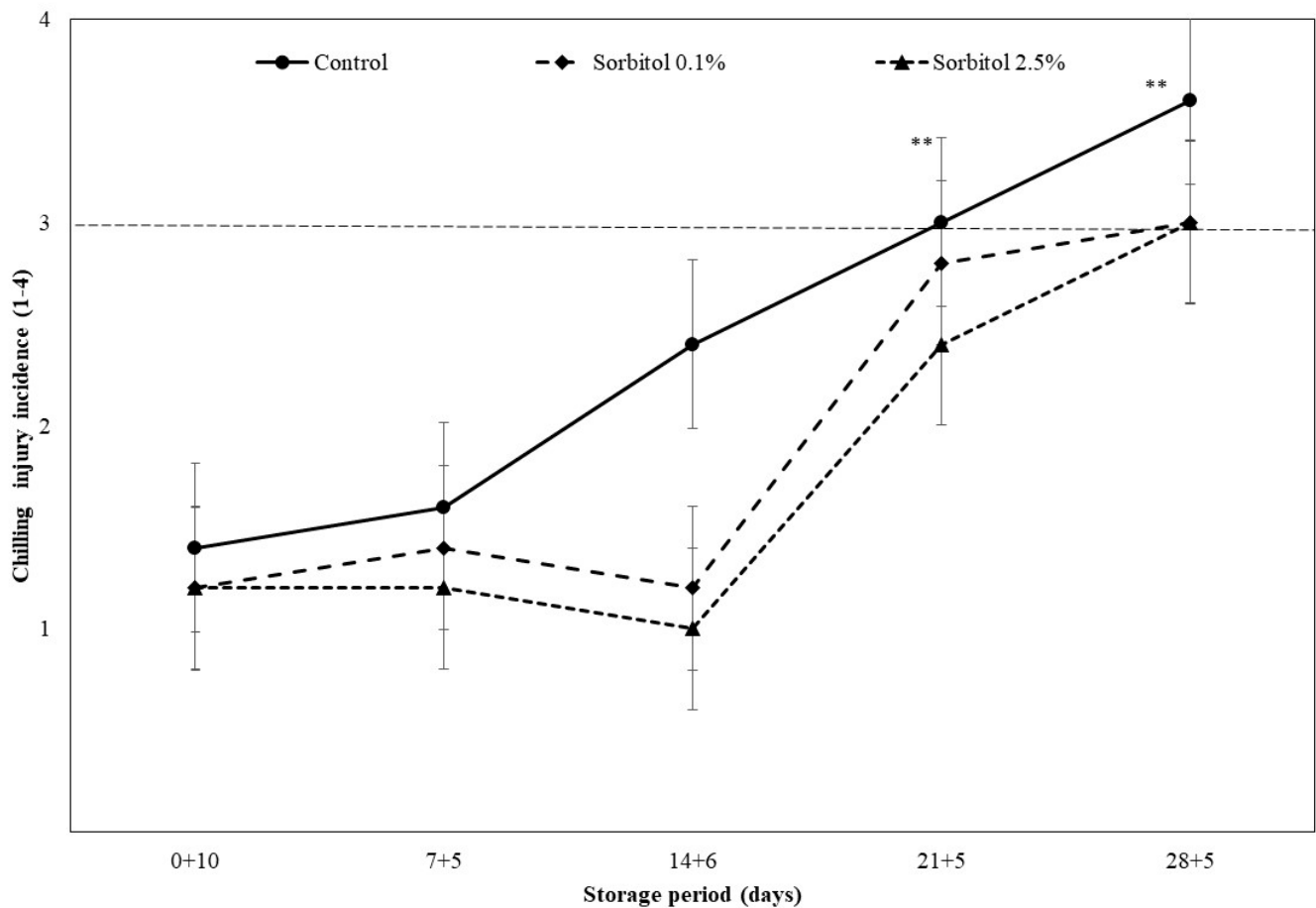


Figure 2. Interaction between treatments and storage period for (Experiment I) stored at 8.0 ± 1.0 °C and $95 \pm 0.5\%$ RH without atmosphere modification ($21 \text{ kPa O}_2 + 0.03 \text{ kPa CO}_2$) for 28 days, and then transferred to ambient conditions (24 ± 2.0 °C and $75 \pm 2.0\%$ RH) from 5 to 10 days. Means followed by double asterisks (**) are significantly different using Tukey's test at $p < 0.01$.

The variable related to chilling injury lesions (CI vector) was positioned in the lower positive quadrant of PC1, grouping with fresh weight loss (FWL), the soluble solid content (SSC), and pulp luminosity (L_{pulp}), as shown in Figure 3A. CIs do not correlate with firmness, peel and pulp chromaticity (C^*_{peel} and C^*_{pulp}), the pulp hue angle (h°_{pulp}), the ratio (SSC/TA), or the pH. The data from the treatments evaluated on the last storage days (21 and 28 days) tended to be closer to the CI vector, while those from the control treatment, followed by immersion in 0.1% sorbitol and the 2.5% sorbitol treatment, were distanced more from this vector (Figure 3A).

Upon moving the fruits to ambient conditions, the CI vector was positioned in the upper-left quadrant of PC2, grouping with pulp luminosity (L_{pulp}), titratable acidity (TA), and firmness, and was not associated with the SSC levels, pH, SSC/TA, peel chromaticity (C^*_{peel}), or peel hue angle (h°_{peel}), as shown in Figure 3A. At the beginning of the storage period, CIs were associated with the control treatment and with the 0.1% and 2.5% sorbitol treatments at the end of storage (21 and 28 days) (Figure 3B).

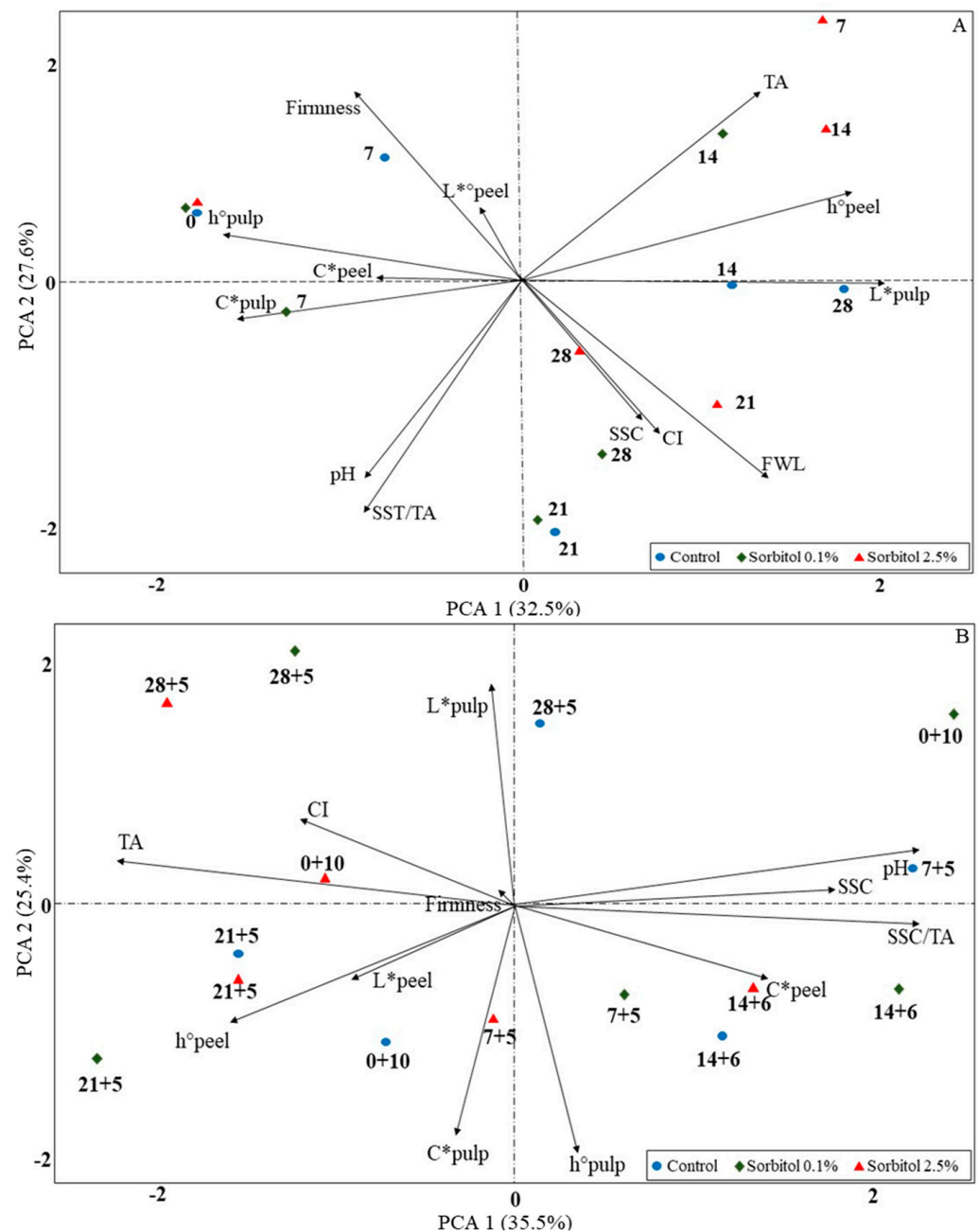


Figure 3. Biplot of the first principal component (PC1) and the second principal component (PC2) of 'Palmer' mango samples (Experiment I) stored at 8.0 ± 1.0 °C and $95 \pm 0.5\%$ RH without atmosphere modification ($21 \text{ kPa O}_2 + 0.03 \text{ kPa CO}_2$) for 28 days (A) and transferred to ambient conditions (24 ± 2.0 °C and $75 \pm 2.0\%$ RH) from 5 to 10 days (B). Chilling injury (CI), fresh weight loss (FWL), luminosity (L^*), hue angle (h°), chromaticity (C^*), firmness, soluble solids content (SSC), titratable acidity (TA), ratio (SSC/TA), and pH. Treatments include control, 0.1% sorbitol, and 2.5% sorbitol.

3.1.2. Cold Damage and Oxidative Metabolism

The sorbitol treatments affected the oxidative metabolism during cold storage (Table 5) and after moving to ambient conditions (Table 6). The CI vector correlated with the hydrogen peroxide content in the peel ($\text{H}_2\text{O}_{2\text{peel}}$), especially for the samples evaluated at the end of storage (21 and 28 days). The 0.1% and 2.5% sorbitol treatments were distanced from the CI vector, associating with the vectors of the superoxide dismutase enzyme in the peel and pulp (SOD_{peel} and SOD_{pulp}) and the ascorbate peroxidase in the peel (APX_{peel}). On the other hand, the control treatment correlated with the vectors related to the hydrogen

peroxide content in the pulp ($\text{H}_2\text{O}_{2\text{pulp}}$) and the PPO activity in the peel (PPO_{peel}), as shown in Figure 4A.

Table 5. Effect of dipping ‘Palmer’ mangoes (Experiment I) in sorbitol (0.1 and 2.5%) and storage at 8.0 ± 1.0 °C and $95 \pm 0.5\%$ RH without atmosphere modification ($21 \text{ kPa O}_2 + 0.03 \text{ kPa CO}_2$) for 28 days on the parameters of lipid peroxidation (LP), hydrogen peroxide content (H_2O_2), and the activity of the enzymes superoxide dismutase (SOD), ascorbate peroxidase (APX), and polyphenol oxidase (PPO).

| Main Effects | LP Peel | LP Pulp | H_2O_2 Peel | H_2O_2 Pulp | SOD Peel | SOD Pulp | APX Peel | APX Pulp | PPO Peel | PPO Pulp |
|-----------------------|--------------------|--------------------|-----------------------------|-----------------------------|----------------------|----------------------|---------------------|----------------------|----------------------|----------------------|
| Treatments (A) | | | | | | | | | | |
| Control | 2.72 ^a | 2.28 ^a | 76.87 ^a | 57.59 ^a | 129.06 ^a | 204.12 ^a | 64.65 ^c | 123.60 ^b | 133.13 ^a | 180.13 ^a |
| Sorbitol 0.1% | 2.76 ^a | 2.28 ^a | 75.04 ^a | 51.68 ^b | 120.66 ^{ab} | 218.50 ^a | 83.82 ^b | 127.87 ^{ab} | 107.08 ^b | 161.14 ^b |
| Sorbitol 2.5% | 2.51 ^a | 2.05 ^b | 70.57 ^a | 48.52 ^b | 129.06 ^a | 227.54 ^a | 96.21 ^a | 134.76 ^a | 100.53 ^b | 119.17 ^c |
| Test F | 2.27 ^{ns} | 6.27 ^{**} | 2.66 ^{ns} | 9.41 ^{**} | 4.63 [*] | 2.80 ^{ns} | 70.62 ^{**} | 4.90 [*] | 51.85 ^{**} | 56.95 ^{**} |
| Days (B) | | | | | | | | | | |
| 0 | 3.06 ^a | 2.34 ^a | 8.80 ^d | 91.94 ^b | 105.24 ^a | 101.46 ^d | 41.19 ^d | 227.41 ^a | 184.02 ^a | 81.63 ^c |
| 7 | 2.33 ^b | 2.21 ^a | 53.14 ^c | 100.93 ^a | 106.24 ^a | 203.85 ^c | 96.77 ^{ab} | 97.37 ^c | 90.32 ^{cd} | 151.36 ^b |
| 14 | 2.56 ^b | 1.87 ^b | 89.08 ^b | 14.94 ^d | 125.34 ^a | 296.07 ^b | 102.15 ^a | 84.06 ^{cd} | 107.83 ^b | 307.77 ^a |
| 21 | 2.78 ^{ab} | 2.27 ^a | 108.91 ^a | 37.63 ^c | 130.58 ^a | 362.51 ^a | 77.83 ^c | 154.71 ^b | 102.46 ^{bc} | 133.20 ^b |
| 28 | 2.59 ^b | 2.33 ^a | 110.86 ^a | 17.48 ^d | 105.24 ^a | 119.71 ^d | 89.85 ^b | 80.18 ^d | 83.27 ^d | 93.45 ^c |
| Test F | 5.67 ^{**} | 8.20 ^{**} | 284.82 ^{**} | 450.63 ^{**} | 2.55 ^{ns} | 152.09 ^{**} | 99.21 ^{**} | 364.92 ^{**} | 172.00 ^{**} | 289.64 ^{**} |
| Interaction | | | | | | | | | | |
| Test F | 2.04 ^{ns} | 1.62 ^{ns} | 0.99 ^{ns} | 1.40 ^{ns} | 0.94 ^{ns} | 0.37 ^{ns} | 14.67 ^{**} | 1.32 ^{ns} | 5.59 ^{**} | 24.47 ^{**} |

Means followed by the same letter within each column do not differ statistically from each other by Tukey’s test. Non-significant interaction (^{ns}), significant interaction at $p < 0.05$ (*), and significant interaction at $p < 0.01$ (**).

Table 6. Effect of dipping ‘Palmer’ mangoes (Experiment I) in sorbitol (0.1 and 2.5%) and storage at 8.0 ± 1.0 °C and $95 \pm 0.5\%$ RH without atmosphere modification ($21 \text{ kPa O}_2 + 0.03 \text{ kPa CO}_2$) for 28 days on the parameters of lipid peroxidation (LP), hydrogen peroxide content (H_2O_2), and the activity of the enzymes superoxide dismutase (SOD), ascorbate peroxidase (APX), and polyphenol oxidase (PPO) after transferring the mangoes to ambient conditions (24 ± 2.0 °C and $75 \pm 2.0\%$ RH) from 5 to 10 days.

| Main Effects | LP Peel | LP Pulp | H_2O_2 Peel | H_2O_2 Pulp | SOD Peel | SOD Pulp | APX Peel | APX Pulp | PPO Peel | PPO Pulp |
|-----------------------|--------------------|--------------------|-----------------------------|-----------------------------|----------------------|----------------------|----------------------|----------------------|---------------------|---------------------|
| Treatments (A) | | | | | | | | | | |
| Control | 4.93 ^a | 4.23 ^a | 104.03 ^a | 41.43 ^a | 89.19 ^b | 181.85 ^c | 80.55 ^c | 276.41 ^c | 212.56 ^a | 269.28 ^a |
| Sorbitol 0.1% | 4.57 ^{ab} | 4.32 ^a | 99.59 ^{ab} | 37.36 ^{ab} | 92.29 ^b | 192.95 ^b | 90.17 ^b | 320.59 ^b | 183.17 ^b | 233.92 ^b |
| Sorbitol 2.5% | 4.21 ^b | 4.01 ^a | 95.55 ^b | 35.71 ^b | 113.96 ^a | 202.52 ^a | 110.69 ^a | 353.81 ^a | 170.95 ^c | 197.12 ^c |
| Test F | 4.88 [*] | 1.79 ^{ns} | 7.29 ^{**} | 5.15 [*] | 45.45 ^{**} | 15.43 ^{**} | 159.49 ^{**} | 254.76 ^{**} | 52.74 ^{**} | 37.95 ^{**} |
| Days (B) | | | | | | | | | | |
| 0 + 10 | 4.64 ^{ab} | 4.08 ^{ab} | 89.85 ^c | 63.87 ^a | 57.91 ^d | 257.26 ^a | 90.37 ^b | 374.25 ^a | 189.69 ^b | 150.12 ^c |
| 7 + 5 | 5.10 ^a | 3.61 ^b | 108.41 ^a | 25.25 ^c | 162.49 ^a | 119.01 ^c | 111.85 ^b | 311.69 ^c | 236.11 ^a | 284.72 ^a |
| 14 + 6 | 4.67 ^{ab} | 4.56 ^a | 90.78 ^c | 23.75 ^c | 77.87 ^c | 120.19 ^c | 87.39 ^c | 263.10 ^e | 203.12 ^b | 300.59 ^a |
| 21 + 5 | 4.27 ^{ab} | 4.51 ^a | 99.11 ^b | 40.92 ^b | 122.27 ^b | 200.14 ^b | 129.06 ^a | 345.08 ^b | 154.53 ^c | 208.38 ^b |
| 28 + 5 | 4.18 ^b | 4.16 ^{ab} | 110.47 ^a | 37.01 ^b | 71.91 ^c | 265.26 ^a | 63.68 ^d | 290.73 ^d | 161.53 ^c | 223.38 ^b |
| Test F | 3.11 [*] | 6.14 ^{**} | 22.41 ^{**} | 92.83 ^{**} | 265.85 ^{**} | 436.84 ^{**} | 260.14 ^{**} | 194.87 ^{**} | 76.03 ^{**} | 64.76 ^{**} |
| Interaction | | | | | | | | | | |
| Test F | 1.65 ^{ns} | 0.33 ^{ns} | 0.12 ^{ns} | 0.39 ^{ns} | 15.52 ^{**} | 0.81 ^{ns} | 14.59 ^{**} | 48.50 ^{**} | 12.07 ^{**} | 2.05 ^{ns} |

Means followed by the same letter within each column do not differ statistically from each other by Tukey’s test. Non-significant interaction (^{ns}), significant interaction at $p < 0.05$ (*), and significant interaction at $p < 0.01$ (**).

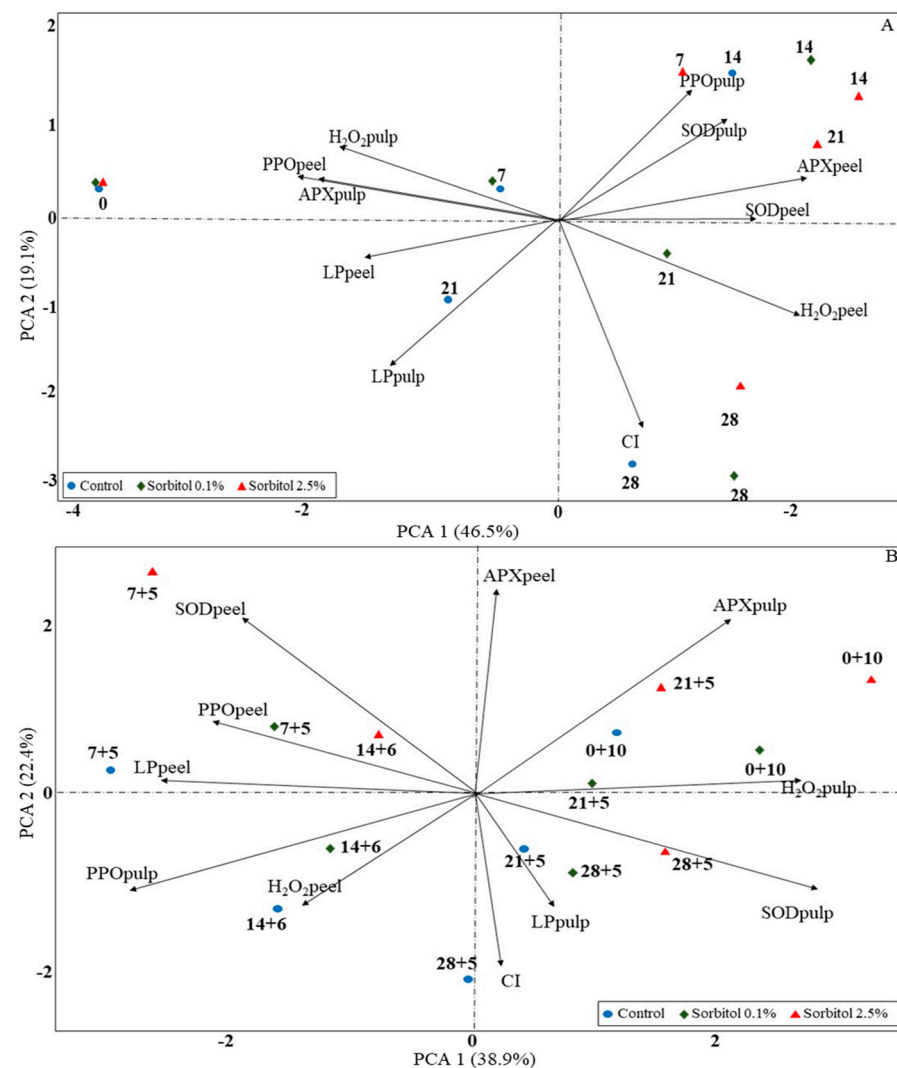


Figure 4. Biplot of the first principal component (PC1) and the second principal component (PC2) of ‘Palmer’ mango samples (Experiment I) stored at 8.0 ± 1.0 °C and $95 \pm 0.5\%$ RH without atmosphere modification ($21 \text{ kPa O}_2 + 0.03 \text{ kPa CO}_2$) for 28 days (A) and transferred to ambient conditions (24 ± 2.0 °C and $75 \pm 2.0\%$ RH) from 5 to 10 days (B). Lipid peroxidation (LP), hydrogen peroxide (H_2O_2), superoxide dismutase (SOD), ascorbate peroxidase (APX), and polyphenol oxidase (PPO). Treatments: control, 0.1% sorbitol, and 2.5% sorbitol.

Upon being transferred to ambient conditions, the vector related to chilling injuries (Cis) was positioned in the lower positive quadrant of PC1, grouping with the vectors of lipid peroxidation (LP_{pulp}) and superoxide dismutase (SOD_{pulp}), and even more with the data from the control treatment on evaluation days 21 + 5 and 28 + 5, which were closer to the CI vector (Figure 4B).

3.2. Experiment II: CA—Modification of Atmospheric Gases

3.2.1. Cold Damage and Physicochemical Variables

The association of sorbitol treatments and CA resulted in fewer chilling injuries (CIs) developing (Table 7) and a better fruit quality during cold storage compared to the control treatment (Figure 5). However, the CI symptoms were more severe in this condition with respect to Experiment I (Tables 3 and 4). Upon moving the fruits to ambient conditions, the 0.1% and 2.5% sorbitol treatments reduced CI development (Table 8), and the mangoes showed a better visual quality (Figures S3 and S4). Again, if score 3 (moderate symptoms—CI = 25–50%) is used as the shelf-life threshold, the only treatment that reached this level

of CI was the control and CA treatments (Figure 6). Overall, the shelf-life was 21 ± 4 and 28 ± 4 days for all the treatments (Figure 6).

Table 7. Effect of dipping ‘Palmer’ mangoes (Experiment II) in sorbitol (0.1 and 2.5%) and storage at 4.0 ± 1.0 °C and $95 \pm 0.5\%$ RH under controlled atmosphere with modified gases (5 kPa O₂ + 5 kPa CO₂) for 28 days on the parameters of fresh weight loss (FWL), chilling injury (CI), soluble solids content (SSC), titratable acidity (TA), the ratio (SSC/TA), and pH.

| Main Effect | FWL (%) | CI (1–4) | SSC (%) | TA (g kg ^{−1}) | SSC/TA | pH |
|-----------------------|----------------------|----------------------|---------------------|--------------------------|---------------------|--------------------|
| Treatments (A) | | | | | | |
| Control | 0.86 ^a | 1.70 ^a | 8.91 ^a | 0.435 ^{ab} | 20.65 ^a | 3.63 ^a |
| CA | 0.77 ^a | 1.60 ^{ab} | 8.74 ^a | 0.427 ^{ab} | 20.88 ^{ab} | 3.70 ^a |
| Sorbitol 0.1% + CA | 0.76 ^a | 1.40 ^b | 8.38 ^a | 0.476 ^a | 18.37 ^b | 3.72 ^a |
| Sorbitol 2.5% + CA | 0.73 ^a | 1.35 ^b | 8.66 ^a | 0.418 ^b | 21.16 ^{ab} | 3.71 ^a |
| Test F | 1.43 ^{ns} | 5.24 ^{**} | 2.43 ^{ns} | 3.15 [*] | 3.06 [*] | 1.14 ^{ns} |
| Days (B) | | | | | | |
| 0 | 0.00 ^e | 1.00 ^c | 6.90 ^d | 0.340 ^c | 21.70 ^{ab} | 3.81 ^a |
| 7 | 0.39 ^d | 1.00 ^c | 7.88 ^c | 0.521 ^a | 15.37 ^c | 3.57 ^b |
| 14 | 0.76 ^c | 1.00 ^c | 8.42 ^c | 0.452 ^b | 18.81 ^{bc} | 3.66 ^{ab} |
| 21 | 1.12 ^b | 1.68 ^b | 9.74 ^b | 0.439 ^b | 22.41 ^{ab} | 3.73 ^{ab} |
| 28 | 1.62 ^a | 2.87 ^a | 10.42 ^a | 0.441 ^b | 24.28 ^a | 3.68 ^{ab} |
| Test F | 149.57 ^{**} | 102.72 ^{**} | 80.90 ^{**} | 15.60 ^{**} | 13.84 ^{**} | 4.23 ^{**} |
| Interaction | | | | | | |
| Test F | 0.82 ^{ns} | 2.64 ^{**} | 2.85 ^{**} | 1.78 ^{ns} | 0.85 ^{ns} | 0.43 ^{ns} |

Means followed by the same letter within each column do not differ statistically from each other using Tukey’s test. Non-significant interaction (^{ns}), significant interaction at $p < 0.05$ (*), and significant interaction at $p < 0.01$ (**).

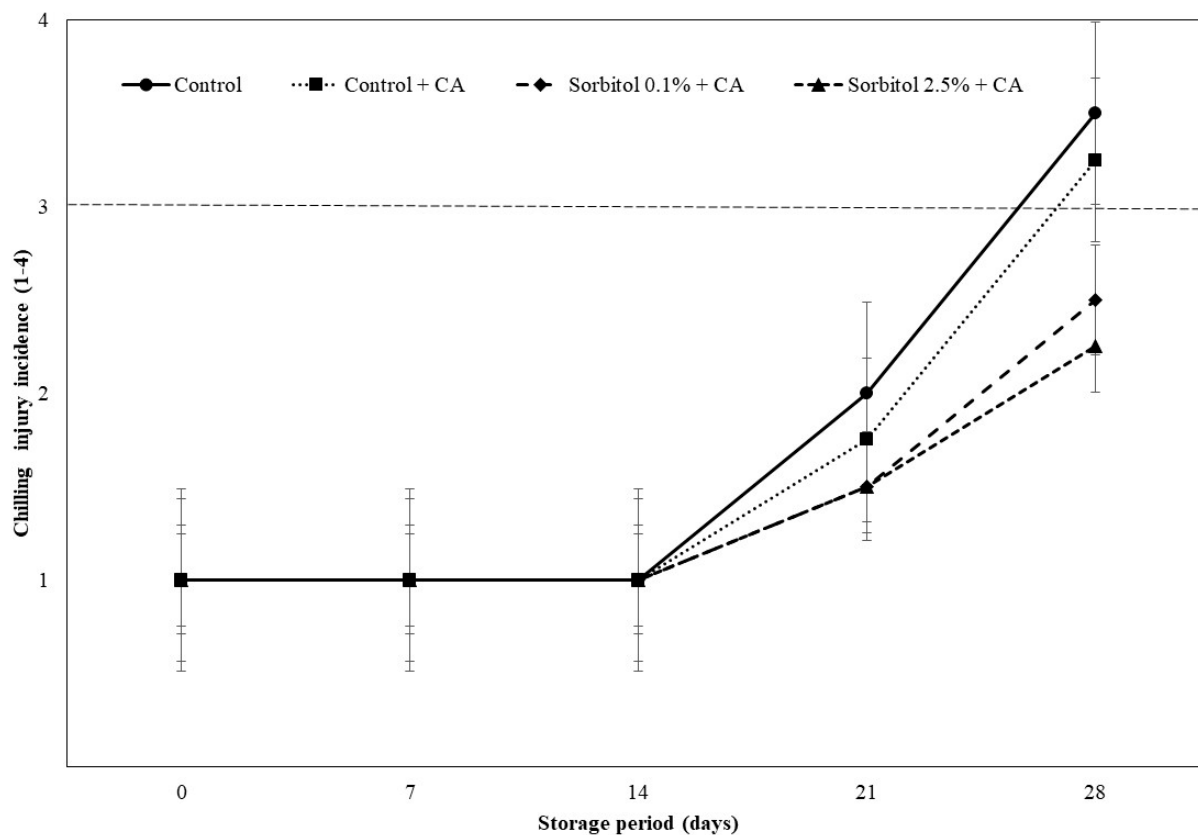


Figure 5. Interaction between treatments and storage period for chilling injury (CI) in ‘Palmer’ mangoes (Experiment II) stored at 4.0 ± 1.0 °C and $95 \pm 0.5\%$ RH under controlled atmosphere with gas modification (5 kPa O₂ + 5 kPa CO₂) for 28 days, and then transferred to ambient conditions (24 ± 2.0 °C and $75 \pm 2.0\%$ RH).

Table 8. Effect of dipping ‘Palmer’ mangoes (Experiment II) in sorbitol (0.1 and 2.5%) and storage at 4.0 ± 1.0 °C and $95 \pm 0.5\%$ RH under controlled atmosphere with modified gases ($5 \text{ kPa O}_2 + 5 \text{ kPa CO}_2$) for 28 days on the parameters of chilling injury (CI), soluble solids content (SSC), titratable acidity (TA), ratio (SSC/TA), and pH after being transferred to ambient conditions (24 ± 2.0 °C and $75 \pm 2.0\%$ RH) from 4 to 7 days.

| Main Effects | CI (1–4) | SSC (%) | TA (g kg^{-1}) | SSC/TA | pH |
|-----------------------|---------------------|---------------------|---------------------------|---------------------|--------------------|
| Treatments (A) | | | | | |
| Control | 2.10 ^a | 11.86 ^a | 0.351 ^a | 34.07 ^a | 3.99 ^a |
| CA | 1.90 ^{ab} | 11.75 ^a | 0.336 ^{ab} | 35.28 ^a | 4.06 ^a |
| Sorbitol 0.1% + CA | 1.55 ^b | 11.50 ^a | 0.330 ^b | 34.87 ^a | 4.08 ^a |
| Sorbitol 2.5% + CA | 1.50 ^b | 10.61 ^b | 0.350 ^a | 30.52 ^b | 4.13 ^a |
| Test F | 5.41 ^{**} | 6.63 ^{**} | 4.96 ^{**} | 7.65 ^{**} | 1.29 ^{ns} |
| Days (B) | | | | | |
| 0 + 7 | 1.12 ^c | 11.40 ^{ab} | 0.338 ^{bc} | 33.85 ^b | 4.02 ^a |
| 7 + 6 | 1.12 ^c | 12.21 ^a | 0.324 ^c | 37.81 ^a | 4.19 ^a |
| 14 + 4 | 1.56 ^c | 11.41 ^{ab} | 0.327 ^c | 35.04 ^{ab} | 4.02 ^a |
| 21 + 4 | 2.18 ^b | 10.83 ^b | 0.368 ^a | 29.44 ^c | 4.07 ^a |
| 28 + 4 | 2.81 ^a | 11.30 ^{ab} | 0.353 ^{ab} | 32.30 ^{bc} | 4.03 ^a |
| Test F | 28.09 ^{**} | 4.11 ^{**} | 12.77 ^{**} | 12.59 ^{**} | 1.72 ^{ns} |
| Interaction | | | | | |
| Test F | 0.86 ^{ns} | 2.68 ^{**} | 3.74 ^{**} | 1.75 ^{ns} | 1.34 ^{ns} |

Means followed by the same letter within each column do not differ statistically from each other using Tukey's test. Non-significant interaction (^{ns}), and significant interaction at $p < 0.01$ (**).

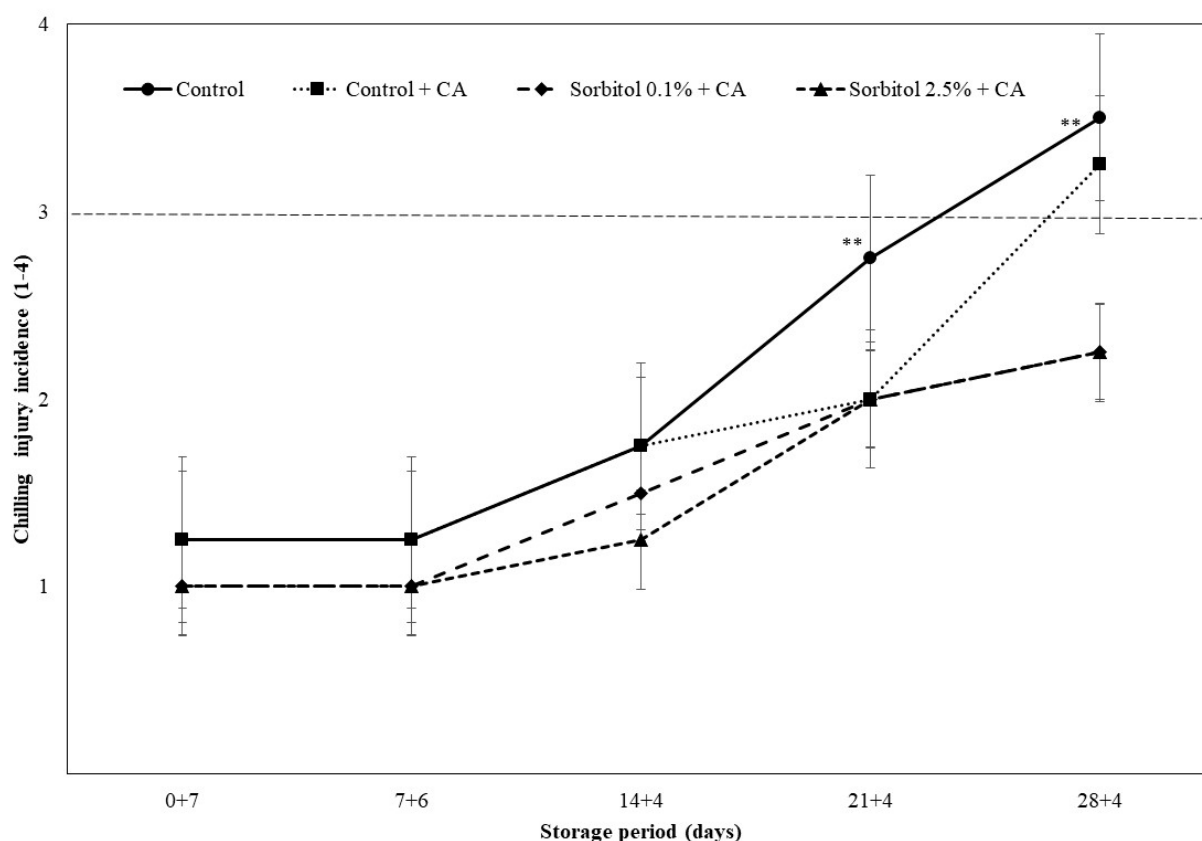


Figure 6. Interaction between treatments and storage period for chilling injury (CI) in ‘Palmer’ mangoes (Experiment II) stored at 4.0 ± 1.0 °C and $95 \pm 0.5\%$ RH under controlled atmosphere with gas modification ($5 \text{ kPa O}_2 + 5 \text{ kPa CO}_2$) for 28 days, and then transferred to ambient conditions (24 ± 2.0 °C and $75 \pm 2.0\%$ RH) from 4 to 7 days. Means followed by double asterisks (**) are significantly different using Tukey's test at $p < 0.01$.

The CI vector was in the lower-right quadrant of PC1 and correlated with the FWL, SSC, C^*_{peel} , SSC/TA, and hue angle (h°_{peel}) vectors, grouping with the vectors of the control and CA treatments at both 21 and 28 days of storage (Figure 7A). The vectors of the variables of firmness, L_{pulp} , pH, h°_{pulp} , and C^*_{pulp} were in PC2 and did not correlate with CIs. In the ambient conditions, the CI vector positioned itself in the upper quadrant of PC1 and correlated with L_{peel} , L_{pulp} , h°_{peel} , and C^*_{peel} , with the vectors of the control and CA treatments more grouped with this vector, especially on the last evaluation days (14 + 4, 21 + 4, and 28 + 4). On the other hand, the data from the 2.5% sorbitol treatment grouped in the lower quadrant of PC1 (Figure 7B).

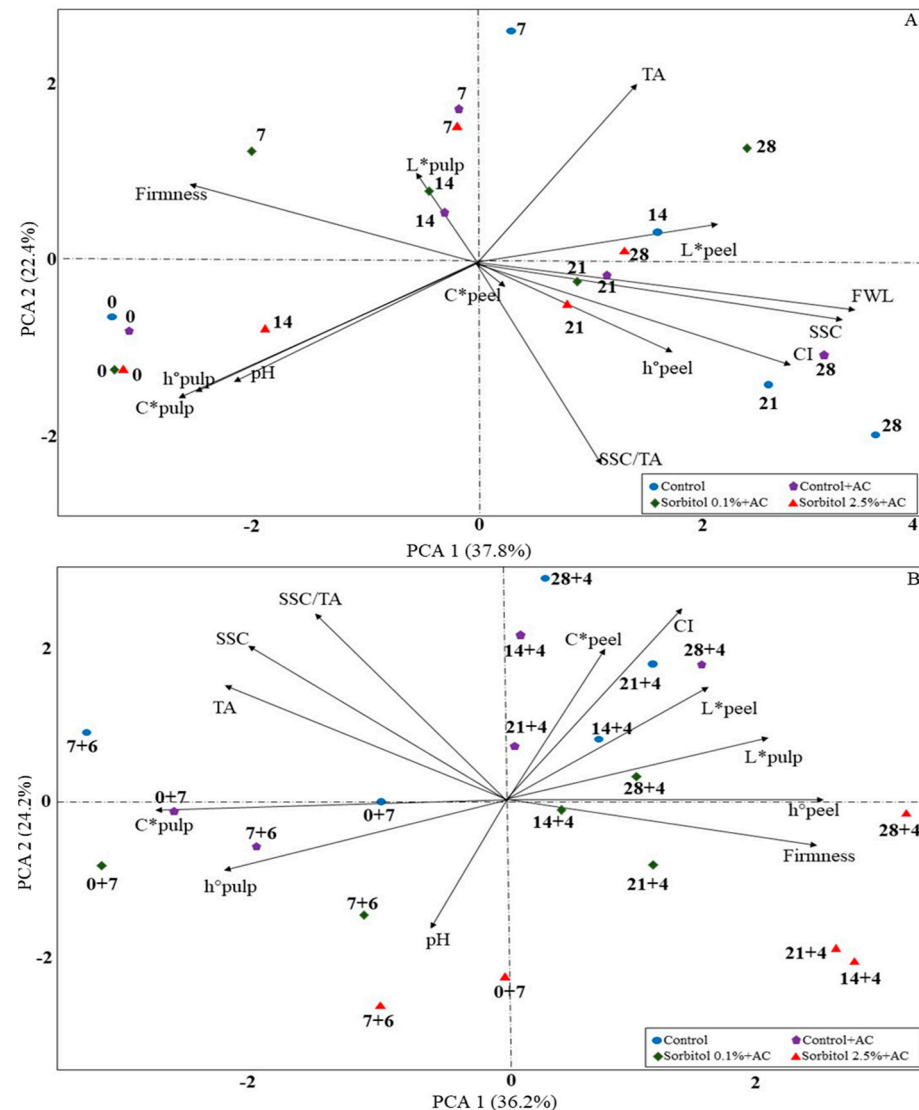


Figure 7. Biplot of the first principal component (PC1) and the second principal component (PC2) of 'Palmer' mango samples (Experiment II) stored at 4 ± 1.0 °C and $95 \pm 0.5\%$ RH under controlled atmosphere ($5 \text{ kPa O}_2 + 5 \text{ kPa CO}_2$) for 28 days (A) and transferred to ambient conditions (24 ± 2.0 °C and $75 \pm 2.0\%$ RH) from 4 to 7 days (B). Chilling injury (CI), fresh weight loss (FWL), luminosity (L^*), hue angle (h°), chromaticity (C^*), firmness, soluble solids content (SSC), titratable acidity (TA), ratio (SSC/TA), and pH. Treatments: negative control, positive CA, 0.1% sorbitol + CA, and 2.5% sorbitol + CA.

3.2.2. Cold Damage and Oxidative Metabolism

The sorbitol treatments affected the oxidative metabolism during cold storage (Table 9) and after the move to ambient conditions (Table 10). The CI vector was positioned in the upper-right quadrant of PC1, correlating with L_{pulp} and grouping with the data from the

negative control treatment on all the evaluated days as well as the CA and 0.1% sorbitol treatments on day 28 (Figure 8A). On the other hand, the vectors related to the activity of the ascorbate peroxidase enzyme (APX_{peel} and APX_{pulp}) and superoxide dismutase (SOD_{pulp}) were located on the opposite side of CI, highlighting the 0.1% and 2.5% sorbitol treatments (7 and 14 days), which grouped with the vectors of these enzymes (Figure 8A).

Table 9. Effect of dipping ‘Palmer’ mangoes (Experiment II) in sorbitol (0.1 and 2.5%) and storage at 4.0 ± 1.0 °C and $95 \pm 0.5\%$ RH under controlled atmosphere with modified gases ($5 \text{ kPa O}_2 + 5 \text{ kPa CO}_2$) for 28 days on the parameters of lipid peroxidation (LP), hydrogen peroxide content (H_2O_2), and the activity of the enzymes superoxide dismutase (SOD), ascorbate peroxidase (APX), and polyphenol oxidase (PPO).

| Main Effects | LP Peel | LP Pulp | H_2O_2 Peel | H_2O_2 Pulp | SOD Peel | SOD Pulp | APX Peel | APX Pulp | PPO Peel | PPO Pulp |
|-----------------------|---------------------|--------------------|-----------------------------|-----------------------------|-----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|
| Treatments (A) | | | | | | | | | | |
| Control | 2.92 ^a | 2.27 ^a | 58.45 ^a | 68.71 ^a | 168.40 ^d | 363.31 ^c | 86.98 ^c | 193.83 ^c | 84.94 ^a | 149.18 ^a |
| CA | 2.76 ^a | 1.97 ^a | 56.05 ^{ab} | 53.46 ^b | 182.48 ^c | 378.48 ^c | 99.39 ^b | 232.09 ^b | 73.16 ^b | 131.22 ^b |
| Sorbitol 0.1% + CA | 2.76 ^a | 1.91 ^a | 52.08 ^b | 48.18 ^b | 200.17 ^b | 403.71 ^b | 102.88 ^b | 238.93 ^b | 67.59 ^c | 100.98 ^c |
| Sorbitol 2.5% + CA | 2.72 ^a | 1.88 ^a | 50.21 ^b | 38.46 ^c | 207.86 ^a | 424.14 ^a | 112.19 ^a | 267.53 ^a | 61.38 ^d | 91.34 ^d |
| Test F | 0.53 ^{ns} | 2.74 ^{ns} | 5.24 ^{**} | 54.63 ^{**} | 176.66 ^{**} | 27.05 ^{**} | 24.05 ^{**} | 157.39 ^{**} | 50.98 ^{**} | 275.54 ^{**} |
| Days (B) | | | | | | | | | | |
| 0 | 2.10 ^c | 1.82 ^b | 69.99 ^a | 11.85 ^c | 178.66 ^b | 646.47 ^a | 160.55 ^a | 2131.24 ^b | 134.80 ^a | 127.04 ^b |
| 7 | 2.45 ^{bc} | 1.99 ^{ab} | 46.25 ^c | 54.71 ^b | 148.81 ^d | 420.82 ^b | 79.08 ^d | 268.81 ^a | 39.30 ^d | 119.52 ^c |
| 14 | 2.66 ^b | 2.43 ^a | 46.77 ^c | 59.99 ^b | 112.11 ^e | 320.52 ^c | 124.69 ^b | 261.82 ^a | 50.98 ^c | 142.11 ^a |
| 21 | 3.42 ^a | 1.76 ^b | 46.36 ^c | 80.53 ^a | 349.38 ^a | 333.08 ^c | 102.28 ^c | 216.51 ^c | 47.32 ^c | 94.19 ^e |
| 28 | 3.33 ^a | 2.05 ^{ab} | 61.56 ^b | 53.94 ^b | 159.67 ^c | 241.17 ^d | 35.20 ^e | 186.10 ^d | 86.43 ^b | 108.05 ^d |
| Test F | 18.12 ^{**} | 4.71 ^{**} | 36.18 ^{**} | 170.95 ^{**} | 3837.48 ^{**} | 722.86 ^{**} | 394.41 ^{**} | 156.76 ^{**} | 637.60 ^{**} | 102.51 ^{**} |
| Interaction | | | | | | | | | | |
| Test F | 0.59 ^{ns} | 0.64 ^{ns} | 1.22 ^{ns} | 6.59 ^{**} | 65.60 ^{**} | 6.12 ^{**} | 4.11 ^{**} | 29.51 ^{**} | 13.01 ^{**} | 30.02 ^{**} |

Means followed by the same letter within each column do not differ statistically from each other using Tukey's test. Non-significant interaction (^{ns}), and significant interaction at $p < 0.01$ (**).

Table 10. Effect of dipping ‘Palmer’ mangoes (Experiment II) in sorbitol (0.1 and 2.5%) and storage at 4.0 ± 1.0 °C and $95 \pm 0.5\%$ RH under controlled atmosphere with modified gases ($5 \text{ kPa O}_2 + 5 \text{ kPa CO}_2$) for 28 days on the parameters of lipid peroxidation (LP), hydrogen peroxide content (H_2O_2), and the activity of the enzymes superoxide dismutase (SOD), ascorbate peroxidase (APX), and polyphenol oxidase (PPO) after being transferred to the environment (24 ± 2.0 °C and $75 \pm 2.0\%$ RH) from 4 to 7 days.

| Main Effects | LP Peel | LP Pulp | H_2O_2 Peel | H_2O_2 Pulp | SOD Peel | SOD Pulp | APX Peel | APX Pulp | PPO Peel | PPO Pulp |
|-----------------------|---------------------|--------------------|-----------------------------|-----------------------------|-----------------------|----------------------|----------------------|----------------------|-----------------------|-----------------------|
| Treatments (A) | | | | | | | | | | |
| Control | 4.11 ^a | 3.41 ^a | 82.29 ^a | 53.85 ^a | 104.39 ^d | 125.64 ^d | 56.16 ^c | 162.15 ^d | 190.58 ^a | 119.27 ^a |
| CA | 4.01 ^a | 3.32 ^a | 71.72 ^b | 50.94 ^a | 131.48 ^c | 131.77 ^c | 65.07 ^b | 174.05 ^c | 167.62 ^b | 104.32 ^b |
| Sorbitol 0.1% + CA | 3.81 ^a | 3.30 ^a | 66.37 ^c | 42.66 ^b | 145.41 ^b | 141.46 ^b | 67.27 ^b | 181.72 ^b | 160.28 ^c | 99.93 ^b |
| Sorbitol 2.5% + CA | 3.75 ^a | 2.95 ^a | 58.92 ^c | 36.86 ^b | 161.92 ^a | 155.30 ^a | 76.92 ^a | 190.04 ^a | 143.45 ^d | 90.31 ^c |
| Test F | 1.04 ^{ns} | 1.84 ^{ns} | 32.23 ^{**} | 18.74 ^{**} | 241.88 ^{**} | 86.97 ^{**} | 45.45 ^{**} | 44.85 ^{**} | 149.68 ^{**} | 50.56 ^{**} |
| Days (B) | | | | | | | | | | |
| 0 + 7 | 3.43 ^b | 3.15 ^{ab} | 71.87 ^{ab} | 47.60 ^b | 69.92 ^e | 202.64 ^a | 67.60 ^c | 185.59 ^b | 140.00 ^d | 57.69 ^d |
| 7 + 6 | 3.58 ^b | 3.80 ^a | 71.01 ^{ab} | 27.92 ^c | 95.68 ^c | 103.92 ^d | 86.84 ^a | 166.61 ^c | 180.65 ^c | 33.91 ^e |
| 14 + 4 | 4.42 ^a | 2.90 ^b | 77.66 ^a | 59.88 ^a | 241.43 ^a | 147.17 ^b | 75.51 ^b | 147.69 ^d | 82.92 ^e | 111.31 ^c |
| 21 + 4 | 3.42 ^b | 2.90 ^b | 62.66 ^c | 64.25 ^a | 185.57 ^b | 103.12 ^d | 51.55 ^d | 178.61 ^c | 232.32 ^a | 128.54 ^b |
| 28 + 4 | 4.74 ^a | 3.48 ^{ab} | 65.93 ^b | 27.90 ^c | 86.39 ^d | 135.87 ^c | 50.33 ^d | 206.15 ^a | 191.52 ^b | 185.91 ^a |
| Test F | 11.35 ^{**} | 5.37 ^{**} | 8.91 ^{**} | 67.64 ^{**} | 1796.24 ^{**} | 690.96 ^{**} | 121.81 ^{**} | 120.85 ^{**} | 1005.68 ^{**} | 1003.14 ^{**} |
| Interaction | | | | | | | | | | |
| Test F | 0.14 ^{ns} | 0.2 ^{ns} | 1.04 ^{ns} | 2.45 [*] | 63.20 ^{**} | 9.55 ^{**} | 3.38 ^{**} | 3.95 ^{**} | 26.77 ^{**} | 3.45 ^{**} |

Means followed by the same letter within each column do not differ statistically from each other using Tukey's test. Non-significant interaction (^{ns}), significant interaction at $p < 0.05$ (*), and significant interaction at $p < 0.01$ (**).

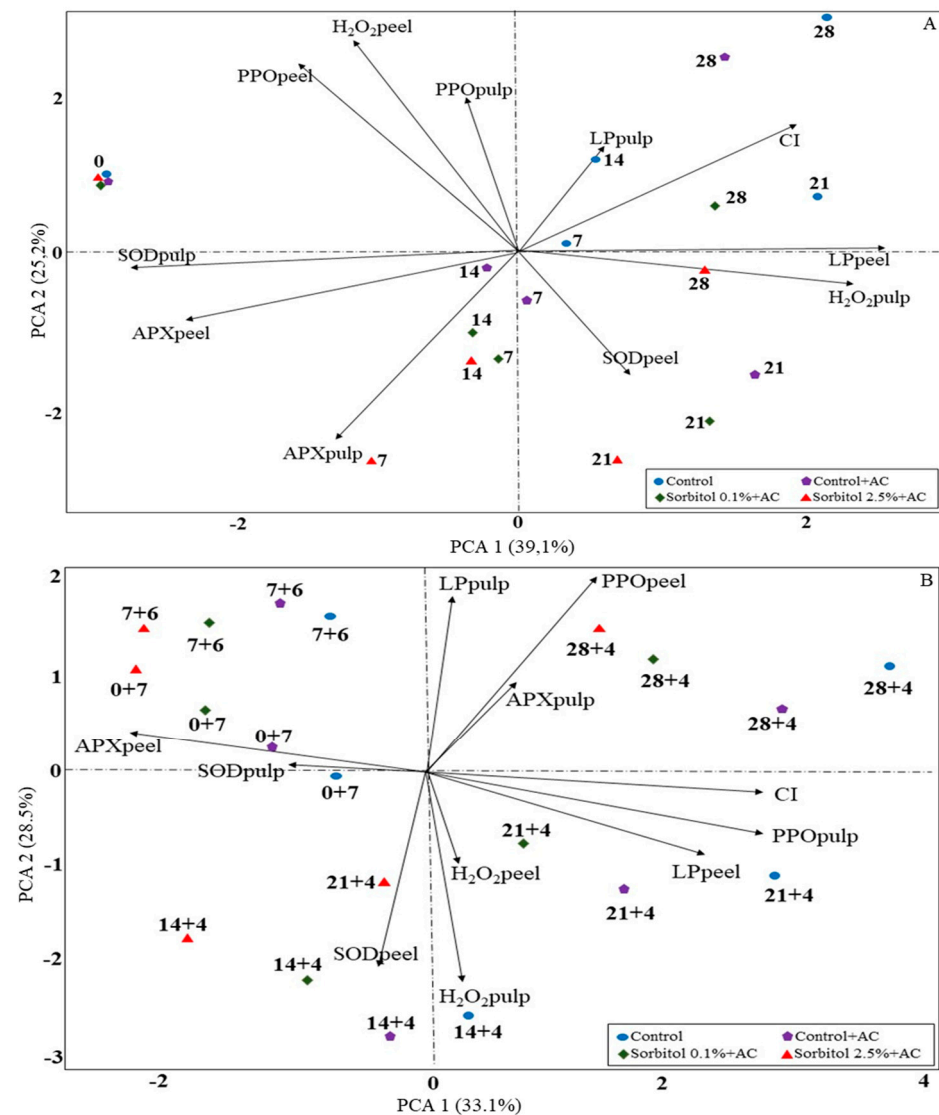


Figure 8. Biplot of the first principal component (PC1) and the second principal component (PC2) of 'Palmer' mango samples (Experiment II) stored at 4 ± 1.0 °C and $95 \pm 0.5\%$ RH under controlled atmosphere ($5 \text{ kPa O}_2 + 5 \text{ kPa CO}_2$) for 28 days (A) and transferred to ambient conditions (24 ± 2.0 °C and $75 \pm 2.0\%$ RH) from 4 to 7 days (B). Lipid peroxidation (LP), hydrogen peroxide (H_2O_2), superoxide dismutase (SOD), ascorbate peroxidase (APX), and polyphenol oxidase (PPO). Treatments: negative control, CA, 0.1% sorbitol + CA, and 2.5% sorbitol + CA.

When the fruits were transferred to ambient conditions, the vector of chilling injury (CI) was positioned in the lower-right quadrant of PC1, correlating with the activity of the PPO enzyme in the pulp (PPO_{pulp}), LP_{peel}, H_2O_2 peel, and H_2O_2 pulp and grouping with the data from the negative control and CA treatments on days 21 + 4 and 28 + 4, which presented more pronounced chilling injury lesions at the end of storage compared to the 2.5% sorbitol treatment (Figure 8B).

4. Discussion

4.1. Cold Damage and Physicochemical Variables

In both the experiments, the incidence of chilling injuries (CIs) correlated with fresh weight loss (FWL), especially in the control treatments on the last evaluation days. The same was reported by Sanches et al. [15], who stored mangoes at 8 °C. However, those authors reported that a 75% RH cold room provided a fresh weight loss of 18%, and even under

those conditions, immersion in solutions containing polyols (propylene glycol, glycerol, and sorbitol) significantly reduced the fresh weight loss to 17% [15]. This may suggest an effect of the modified atmosphere even at reduced sorbitol concentrations (0.1%).

To verify the effectiveness of sorbitol immersion by isolating the control of fresh weight loss on chilling injury development, we stored the fruit at 8.0 ± 1.0 °C and $95 \pm 0.5\%$ RH without gas modification ($21 \text{ kPa O}_2 + 0.03 \text{ kPa CO}_2$) for 28 days in CA chambers. This minimized the fresh weight loss (0.78–0.89%), and the sorbitol treatments had no significant effect. The same occurred during the storage of the fruits at 4.0 ± 1.0 °C and $95 \pm 0.5\%$ RH under a CA containing $5 \text{ kPa O}_2 + 5 \text{ kPa CO}_2$ for 28 days, i.e., the fresh weight loss was reduced to 0.65–0.88%. Thus, the effect of the immersion in the sorbitol-containing solutions was not related to the reduction in fresh weight loss, as there was no significant effect compared to the control treatments, which showed more severe chilling injury symptoms regardless of the CA conditions.

The worsening of the chilling injury symptoms when the fruits were moved to ambient conditions was evidenced by the darkening of the epicarp, which is usually observed after removal from cold storage and fruits become ripe [42]. For this reason, the chilling injuries correlated with variables such as L_{peel} , h°_{peel} , C^*_{peel} , and h°_{pulp} (Tables S1–S4). However, the chilling injuries were not restricted to damage to the epicarp and led to irregular ripening, affecting the color parameters of the pulp, the SSC, the TA, and the ratio. The discoloration of the epicarp (peel) and irregular ripening are commonly observed in mangoes stored at low temperatures [43].

Sanches et al. [44] have reported that a combination of immersion in solutions containing 2.5% sorbitol with storage in CA ($5 \text{ kPa O}_2 + 5 \text{ kPa CO}_2$) has delayed mango ripening and controlled chilling injury development. We observed that the mangoes subjected to the 0.1% and 2.5% sorbitol treatments were firmer even after being transferred to the ambient conditions, which may have affected the ripening process. In both of our experiments, dipping the fruit in 2.5% sorbitol reduced chilling injury incidence, and the fruits remained firmer. Thus, maintaining firmness may be related to the control of chilling injuries. Salazar-Salas et al. [45] observed that ‘Keitt’ mangoes dipped in hot water (HWT) showed a lower incidence of chilling injuries and had better firmness. Thus, firmness may be indicative of maintaining the structure of the cell wall and plasma membrane and be associated with reduced chilling injuries.

4.2. Cold Damage and Oxidative Metabolism

Chilling injury development was related to the accumulation of malondialdehyde (MDA) (LP_{pulp} and LP_{peel}), which is considered a marker of lipid membrane peroxidation [46]. In PCA, it was observed that chilling injury correlated with hydrogen peroxide content ($H_2O_{2\text{peel}}$), LP_{pulp} , LP_{pulp} , LP_{peel} , $H_2O_{2\text{peel}}$, $H_2O_{2\text{pulp}}$, and LP_{peel} , especially in the control treatment on the last evaluation days (21, 21 + 4, 28, and 28 + 4 days), as represented by increases in the MDA and H_2O_2 contents.

Dipping the mangoes in sorbitol (0.1% and 2.5%) resulted in a higher activity level of superoxide dismutase (SOD), which is considered the first enzyme to be activated in the elimination of ROS via O_2 dismutation [13]. Thus, it can be inferred that the sorbitol influenced the control of oxidative stress by increasing the SOD activity level in the fruit, similar to other polyols [47].

Other enzymes, such as APX, CAT, and POD, are also considered important in initiating plant defenses against oxidative stress. In this sense, higher activity levels of APX enzymes can reduce hydrogen peroxide accumulation, which was observed in the fruit treated with the sorbitol (0.1% and 2.5%) compared to the $H_2O_{2\text{peel}}$ and $H_2O_{2\text{pulp}}$ vectors of the control fruit, which showed significant increases in H_2O_2 and more severe chilling injury symptoms. Pomegranates (*Punica granatum* L.) with chilling injury symptoms had a higher accumulation of H_2O_2 , which was lower in the fruits treated with arginine, also resulting in increased activity levels of the SOD, CAT, and APX enzymes [48]. Thus, the activation of the enzymatic defense system (SOD, APX, and CAT) is fundamental for

reducing H_2O_2 accumulation in plants under stress conditions [49] and, consequently, minimizes chilling injuries in fruits stored at low temperatures, which was possible with the immersion in 2.5% sorbitol.

The most visible symptom of chilling injuries in mangoes is the presence of dark, sunken spots, similar to burns, associated with a higher polyphenol oxidase enzyme (PPO) activity level [50]. In both the experiments, the PPO activity correlated with the highest incidence of chilling injuries, especially in the control treatment of the experiment without gas modification (21 kPa O_2 + 0.03 kPa CO_2) after the fruits were transferred to the ambient conditions in the last days of storage (21 + 4, 28 + 4). However, the treatments with sorbitol, especially the 2.5% treatment, significantly reduced the PPO activity. This effect was evident when the CA (5 kPa O_2 + 5 kPa CO_2) and 2.5% sorbitol treatment were combined, even at lower temperatures (4.0 °C). This demonstrates a synergistic effect between CA and sorbitol treatment in activating oxidative metabolism to control chilling injuries. However, this combination only minimizes the chilling injuries, and further studies should be conducted to explain which mechanisms are involved combining sorbitol and CA.

5. Conclusions

The immersion of ‘Palmer’ mangoes in sorbitol-containing solutions demonstrated efficacy in mitigating chilling injuries under varied storage conditions, which has not been extensively explored in the existing literature, such as differing gas compositions (21 kPa O_2 + 0.03 kPa CO_2 and 5 kPa O_2 + 5 kPa CO_2) and temperatures (8 °C and 4 °C). When the mangoes were stored under controlled atmosphere (CA) conditions with 95% RH, it was possible to mitigate the impact of fresh weight loss and chilling injury symptoms, enabling the preservation of fruit quality for up to 21 + 5 days at 8 °C and to 21 + 4 days at 4 °C.

The effectiveness of controlling chilling injury was attributed to the attenuation of lipid peroxidation in the cell membranes and the activation of the antioxidant enzymes SOD and APX in the epicarp and mesocarp, particularly in the mangoes treated with sorbitol. Notably, the application of 2.5% sorbitol emerged as the most effective approach to mitigating chilling injuries, sustaining the fruit quality, and enhancing oxidative metabolism even at lower temperatures. Thus, this treatment option has established itself as a viable strategy for chilling injury management in mangoes.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/horticulturae10040354/s1>, Figure S1: Epicarp chilling injury (CI) development in ‘Palmer’ mangoes (Experiment I) stored at 8.0 ± 1.0 °C and $95 \pm 0.5\%$ RH without atmosphere modification (21 kPa O_2 + 0.03 kPa CO_2) for 28 days and then transferred to ambient conditions (24 ± 2.0 °C and $75 \pm 2.0\%$ RH) for up to 10 to 5 days; Figure S2: Mesocarp chilling injury (CI) development in ‘Palmer’ mangoes (Experiment I) stored at 8.0 ± 1.0 °C and $95 \pm 0.5\%$ RH without atmosphere modification (21 kPa O_2 + 0.03 kPa CO_2) for 28 days and then transferred to ambient conditions (24 ± 2.0 °C and $75 \pm 2.0\%$ RH) for up to 10 to 5 days; Figure S3: Epicarp chilling injury (CI) development in ‘Palmer’ mangoes (Experiment II) stored at 4.0 ± 1.0 °C and $95 \pm 0.5\%$ RH under controlled atmosphere with gas modification (5 kPa O_2 + 5 kPa CO_2) for 28 days and then transferred to ambient conditions (24 ± 2.0 °C and $75 \pm 2.0\%$ RH) for up to 7 to 4 days; Figure S4: Mesocarp chilling injury (CI) development in ‘Palmer’ mangoes (Experiment II) stored at 4.0 ± 1.0 °C and $95 \pm 0.5\%$ RH under controlled atmosphere with gas modification (5 kPa O_2 + 5 kPa CO_2) for 28 days and then transferred to ambient conditions (24 ± 2.0 °C and $75 \pm 2.0\%$ RH) for up to 7 to 4 days. Table S1: Effect of dipping ‘Palmer’ mangoes (Experiment I) in sorbitol (0.1 and 2.5%) and storage at 8.0 ± 1.0 °C and $95 \pm 0.5\%$ RH without atmosphere modification (21 kPa O_2 + 0.03 kPa CO_2) for 28 days on firmness, luminosity, hue angle, and chromaticity parameters; Table S2: Effect of dipping ‘Palmer’ mangoes (Experiment I) in sorbitol (0.1 and 2.5%) and storage at 8.0 ± 1.0 °C and $95 \pm 0.5\%$ RH without atmosphere modification (21 kPa O_2 + 0.03 kPa CO_2) for 28 days on the parameters of firmness, luminosity, hue angle, and chromaticity after transfer to ambient conditions (24 ± 2.0 °C and $75 \pm 2.0\%$ RH) for up to 10 to 7 days. Table S3: Effect of dipping ‘Palmer’ mangoes (Experiment II) in sorbitol (0.1 and 2.5%) and storage at 4.0 ± 1.0 °C and $95 \pm 0.5\%$ RH under

controlled atmosphere with modification of gases (5 kPa O₂ + 5 kPa CO₂) for 28 days on firmness, luminosity, hue angle, and chromaticity. Table S4: Effect of dipping ‘Palmer’ mangoes (Experiment II) in sorbitol (0.1 and 2.5%) and storage at 4.0 ± 1.0 °C and 95 ± 0.5% RH under controlled atmosphere with modified gases (5 kPa O₂ + 5 kPa CO₂) for 28 days on the parameters of firmness, luminosity, hue angle, and chromaticity after transfer to ambient conditions (24 ± 2.0 °C and 75 ± 2.0% RH) for up to 7 to 4 days.

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