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ORIGINAL ARTICLE

Cell wall stabilization and calcium absorption on mango fruit treated with a quarantine hot water treatment combined with calcium salts and stored at chilling temperature

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Abstract

Hot water treatment (HT) induces chilling injury (CI) tolerance in mango, but prolonged exposure to HT causes softening. In this sense, calcium salts stabilize the cell wall. Nevertheless, there is little information on the effect of HT combined with calcium salts (HT-Ca) on calcium absorption and cell wall stability during storage of mango at CI temperature. We evaluated the effect of quarantine HT in combination with calcium chloride ($CaCl_2$), calcium citrate (CaCit), or calcium lactate (CaLac) on calcium absorption, CI tolerance, and cell wall stabilization. HT and HT-$CaCl_2$ had the lowest CI development. HT increased firmness loss and electrolyte leakage, and HT-Ca counteracted this effect. Overall, HT-Ca treatments had a similar effect on the cell wall degrading enzymes. HT-$CaCl_2$ was the best treatment and did not present alterations on the epicuticular wax as observed on HT. HT-$CaCl_2$ is a useful technology to stabilize cell wall and preserve mango during chilling storage.

Practical applications

The addition of calcium salts in an established hot water quarantine procedure for mango exportation represents a viable alternative to counteract the negative effects of this thermal treatment upon cell microstructure, maintaining its positive effect of tolerance to chilling injury. In this sense, mango producers and packers can use a HT-$CaCl_2$ treatment to reduce the presence of chilling injury and extent the fruit shelf life and improve its commercialization. Furthermore, technical and infrastructure changes are not necessary for the packaging chain.

KEYWORDS
calcium, cell wall, chilling injury, hot water, mango

Jordi G. López-Velázquez and Martha E. López-López should be considered joint first authors.

1 | INTRODUCTION

Mango (*Mangifera indica* L.) is a tropical fruit native to southeast Asia and classified as climacteric (Zhang et al., 2012). Worldwide, India is the major mango producer; however, Mexico is the principal exporter, and its main abroad markets are developed countries such as the United States and Canada (FAOSTAT, 2021). Nowadays, refrigeration is essential to achieve mango exportation; however, prolonged mango exposure to temperatures below critical (13°C) induces chilling injury (CI) (Díaz-Corona et al., 2020).

The development of CI is associated with membrane dysfunction, redox imbalance, and lipid peroxidation. Chilling temperatures alter the fluidity of the plasmatic membrane, and, therefore, the functionality of the proteins associated with it (Hodges, 2004). Among these proteins is the electron transport chain. Once these series of enzymatic complexes are damaged, the efficiency of the electron efflux is compromised, and the oxidative stress is triggered originating from the apparitions of numerous reactive oxygen species (ROS) (Demidchik, 2014). ROS compromise the structure of the plasmatic membrane and cell wall, disrupting cell compartmentalization, and inducing the apparition of CI symptoms in mango such as uneven ripening, lenticel darkening, pitting, internal browning, and decay (Zhang et al., 2012).

Hot water immersion (HT) is one of the most practical, economical, and effective technologies to induce CI tolerance in many fruits such as tomato, bell pepper, and mango (López-Velázquez et al., 2020; Salazar-Salas et al., 2017; Vega-Álvarez et al., 2021), showing that the induced tolerance was associated to remarkable changes in genes, proteins, and metabolites. On mango fruit destined for exportation, a HT is applied as a quarantine treatment to keep it free from the fruit fly (46.1°C, 75–90 min) (USDA-APHIS, 2014). The application of a HT under appropriate conditions of time and temperature represents moderate stress that modulates many defense mechanisms to prepare the fruit for biotic and abiotic stress (Salazar-Salas et al., 2017). However, prolonged exposure to HT as quarantine treatment can lead to firmness loss, which shortens the mango shelf life. This phenomenon is closely related to cell wall degrading enzymes such as PME, PG, and β-Gal (Nyanjage et al., 1999). It is reported that CI is also involved in premature softening, accelerating cell wall solubilization, and accentuating the negative effect of HT (Díaz-Corona et al., 2020).

Fruit softening can be reduced by applying calcium salts. The calcium ions generate cross-links between pairs of unesterified homogalacturonan molecules to form calcium pectates. These interactions give stability to the cell wall and increase resistance to fruit softening (Aguayo et al., 2008). Moreover, the application of calcium on fruits has proved to play an important role in many metabolic processes: reduces respiration, regulates the activity of cell wall degrading enzymes, diminish the negative effect of ROS, and it has been associated in horticultural products with an enhancement in chilling tolerance (Hewajulige et al., 2003; Hou et al., 2021; Kittermann et al., 2010; Mirdehghan & Ghotbi, 2014).

The application of a HT combined with different calcium salts (HT-Ca) is effective to counteract CI, maintaining the firmness, and regulating many enzymes in mango fruit (Díaz-Corona et al., 2020; López-López et al., 2018). However, it is still unclear if there is calcium absorption when this treatment is applied on mango and how this absorption is associated with the calcium salt applied, the development of CI symptoms, and the maintenance of the fruit cell structure. Therefore, this paper aimed to analyze the effect of a quarantine hot water treatment in combination with $CaCl_2$, CaCit, or CaLac on calcium content, chilling tolerance, pulp softening, the activity of the cell wall degrading enzymes, and microstructural changes in mango fruit cv. Keitt stored at chilling temperature.

2 | MATERIALS AND METHODS

2.1 | Plant material

Mango fruits cv. Keitt were harvested from a commercial plantation near Culiacan, Sinaloa, Mexico. Mature green fruit (400–700 g) were washed and disinfected with sodium hypochlorite (4 mM).

2.2 | Treatment application and storage conditions

Mango fruits were divided into five batches (20 fruits each, 5 fruits per evaluation day) for the application of the following treatments: untreated fruit (control), hot water treatment (HT), HT with calcium chloride (HT-$CaCl_2$), HT with calcium citrate (HT-CaCit), and HT with calcium lactate (HT-CaLac). HT was applied by immersion at 46.1°C in a water bath (1266–02, Cole Parmer). Immersion time depended on the fruit size. Weight bands up to 500 g and 501 to 700 g were immersed for 75 and 90 min, respectively (USDA-APHIS, 2014). All calcium salts concentrations were 0.5% (w/v), as previously described by López-López et al. (2018). Fruits were stored at 0, 10, and 20 days at 5°C to induce CI (Díaz-Corona et al., 2020) plus a ripening period of 7 days at 21°C to accentuate the apparition of symptoms. The application of treatments was carried out independently three times (60 fruits per treatment in total).

2.3 | Calcium absorption

The evaluation of calcium content on mango peel was carried out using the methodology described by Ayón-Reyna et al. (2017). Mango peel was dried in an oven with air circulation at 60°C for 5 h. Three dry samples (0.25 g) from each mango were grounded and digested with sulfuric acid at 450°C. Then, a solution of hydrogen peroxide was added to the mixture and heated at 450°C. The digested samples were diluted with deionized water and the calcium content was evaluated using an atomic absorption spectrophotometer (AVIO 550, Perkin Elmer Inc.). The results were expressed as mg of Ca g^{-1} of the fresh peel.

2.4 | Chilling injury index

The percentage of fruit surface with noticeable CI symptoms (uneven ripening, lenticel darkening, pitting, internal browning, and decay) was fit into a scale from 0 to 5, where 0 = No damage, 1 = ≤ 10% of damage, 2 = 11–25% of damage, 3 = 26–40% of damage, 4 = 41–50% of damage, 5 = ≥ 51% of damage (Díaz-Corona et al., 2020). Results were obtained following the next equation:

$$\text{CII} = \frac{\sum(\text{CI Index})(\text{number of fruit at this level})}{\text{Total fruit number}}$$

2.5 | Pulp softening

2.5.1 | Firmness

Firmness was determined according to López-López et al. (2018). A penetrometer (Chatillon DFE-100, AMETEK Inc.) equipped with a flat tip of 11 mm in diameter was used at a constant penetration rate (50 mm min^{-1} and 5 mm of penetration). Both sides of mango fruit were separated, and the skin of each one was longitudinally removed from the central portion, the penetrometer tip was placed on the surface, and 6 measurements were carried out per fruit. The results are expressed in Newton (N).

2.5.2 | Electrolyte leakage

The evaluation of EL was carried out as described by Díaz-Corona et al. (2020). Pulp discs (7 mm width) were cut with a cork borer. The samples (24 per fruit) were washed three times with deionized water, mixed with 25 ml of mannitol (0.4 M) (FAGALAB Inc.), and incubated at 25°C for 2 h under constant shaking. Then, the conductivity of the solution was measured with a manual conductivity meter (HI98312, Hanna Instruments) before (initial) and after (final) autoclaving the samples at 121°C for 30 min. The percentage of EL was determined as described in the next equation:

$$\text{EL}(\%) = \frac{\text{Initial conductivity}}{\text{Final conductivity}} \times 100$$

2.6 | Cell wall degrading enzymes

2.6.1 | Enzyme extract

Cell wall enzymes were extracted and evaluated as described by Díaz-Corona et al. (2020). Fifteen grams of mango pulp were mixed with a NaCl solution (1 M, 4°C), and homogenized with an Ultra Turrax (T18 basic, IKA Works, Inc.) for 1 min. The homogenate was centrifuged at 17,000*g* for 45 min at 4°C and filtered. The supernatant was saturated at 80% with ammonium sulfate and stirred for 1 h under refrigeration to precipitate the protein, followed by second centrifugation at 17,000*g* for 20 min. The precipitate was re-suspended in distilled water (4°C). For each replicate, three enzyme extracts were prepared, and at least 5 enzymatic measurements were carried out on each. Reagents to perform the enzymatic analysis were provided by FAGALAB and Sigma–Aldrich Inc.

2.6.2 | PME activity

Citrus pectin (0.5% w/v) was mixed with bromothymol blue (0.01% w/v) in potassium phosphate buffer (0.003 M). The mixture pH was adjusted to 7.5 and the reaction started after adding the enzyme extract. The reaction was carried out at 30°C and monitored for 5 min at 620 nm. A standard curve of galacturonic acid (0.5% w/v) was used. The PME activity was expressed as PME units per gram of fresh pulp (U g^{-1}).

2.6.3 | PG activity

The enzymatic extract and a polygalacturonic acid (0.5% w/v) solution were mixed with sodium acetate buffer (pH 4.6), and incubated for 1 h at 37°C. The activity was measured by mixing the incubated extract, sodium tetraborate buffer (pH 9.0), and a solution of 2-cyanoacetamide (1% w/v). This mixture was stirred and heated (100°C) for 10 min and cooled at room temperature. The changes in absorbance were recorded at 278 nm. The blank was prepared without the enzyme extract. The standard curve was constructed with galacturonic acid (0.5% w/v). The enzymatic activity was reported as U g^{-1}.

2.6.4 | β-Gal activity

The substrate (ρ-nitrophenyl-β-D-galactopyranoside) was mixed with potassium phosphate buffer (pH 6.0). Then, the crude extract was added and incubated at 37°C for 1 h. To stop the reaction, a glycine-NaOH buffer (pH 10.5) was added. The liberation of ρ-nitrophenol by the enzymatic extract was measured on a spectrophotometer at 420 nm. One unit of enzymatic activity (U g^{-1}) was defined as the amount of enzyme required to release 1 nmol of ρ-nitrophenol per minute at 37°C.

2.6.5 | Microstructural analysis

Mango sections of 2 × 2 × 2 cm were taken from control, HT and the best HT-Ca treatment on days 0 and 20. Microstructural analysis was carried out according to Phothiset and Charoenrein (2013). Sections were placed in a fixative solution for 24–48 h and subsequently dehydrated using different ethanol gradients, then transferred three times for 5 h to absolute ethanol-xylene (1:1). Next, the samples were infiltrated twice in histological grade paraffin every 12 h. The paraffin samples were cut (10 μm of thickness) and placed

in a flotation bath with hot water and gelatin. The paraffin was eliminated using xylene, and the samples were hydrated with a gradual series of ethanol (100, 96, 70, and 50%). Staining with safranin in 50% ethanol was carried out for 24 h. The sections were dehydrated in a gradual series of ethanol (50, 70, and 96%). The samples were also stained with fast green and washed with ethanol (96%). To finish the dehydration, samples were washed once with absolute ethanol and three times with xylene. The stained sections were mounted on a slide, protected with a coverslip, and observed under a light microscope (BA210, Motic-microscope). Reagents for optical microscopy were provided by J.T. Baker and Sigma–Aldrich Inc.

2.7 | Statistical analysis

A completely randomized experimental design with three replicates was performed. The multiple analysis of variance was carried out using the Statgraphics Centurion XVI software (Statpoint Technologies, Inc.) and the means were compared using the Fisher's least significant difference (LSD) test ($^*p < .05$).

3 | RESULTS

3.1 | Calcium absorption

Calcium content on mango peel varied among treatments applied (Table 1). After treatment application (day 0), control and HT presented lower calcium content (0.47–0.54 mg g^{-1}) than HT-Ca treated fruit (0.72–0.74 mg g^{-1}), without significant differences among the calcium salts applied. On day 20 and after 7 days at 21°C peel fruit presented a similar behavior.

3.2 | Chilling injury index

After 10 days at 5°C, CI symptoms were visible on mango fruit. HT-$CaCl_2$ and HT-CaCit showed lower CII than control and HT (Figure 1). Fruit treated with HT combined with calcium salts showed lower pitting development and uneven ripening; however, presented lenticel darkening. On day 20, all treatments showed lower CII and statistical differences with control fruit. HT and HT-Ca treated fruit exhibited a higher incidence of pitting than control. On the last evaluation day, all treatments still showed a statistical difference with control fruit, which had a major incidence of necrotic areas (Figure 2). HT and HT-$CaCl_2$ treatments showed the lowest CII after the storage at 21°C, without significant differences between them. HT-$CaCl_2$ presented a statistical difference from the other HT-Ca treatments.

3.3 | Pulp softening

3.3.1 | Firmness

After treatment application, the fruit had a firmness range of 80–92 N (Figure 3a). After 10 days of storage at 5°C, HT and control treatment had an important firmness reduction (~50% loss); whereas, HT-Ca treatments remained with similar firmness values. After 20 days, firmness slightly decreased in all treatments, except for HT-CaLac. On the last evaluation (Day 20 + 7), all treatments reached similar firmness values (<10 N).

3.3.2 | Electrolyte leakage

The fruit had an initial EL of approximately 60% (Figure 3b). Throughout the storage at 5°C, the control treatment had higher EL than the treated fruit. On day 20, HT-$CaCl_2$ treatment had the lowest EL. After 7 days at 21°C control and HT treatments presented higher EL (80–82%) than HT-Ca treatments (62–70%).

3.4 | Cell wall degrading enzymes

Overall, the PME activity was statistically affected by the treatments applied (Figure 4a). The enzyme activity increased throughout the storage in all treatments. On day 10, HT-Ca treatments remained without significant changes. After 20 days, HT and HT-Ca treatments had higher PME activity than control, and after 7 days at 21°C, only HT-$CaCl_2$ and HT-CaCit had higher activity than control fruit. PG activity was affected by the HT and HT-Ca treatments, and the activity fluctuated throughout the storage in all treatments. On day 20, HT had the most notable effect on this enzyme, with an increase of approximately 15% (Figure 4b). It is important to mention that HT-$CaCl_2$ and HT-CaCit had lower PG activity than control on days 20 and 20 + 7. Out of the three enzymes evaluated, β-Gal was

TABLE 1 Calcium content (mg g^{-1}) in mango peel treated with hot water treatment (HT) and hot water treatment combined with calcium chloride ($CaCl_2$), calcium citrate (CaCit), or calcium lactate (CaLac). The fruit was stored for 20 days at 5°C plus 7 days at 21°C.

Evaluation day	Control	HT	HT-$CaCl_2$	HT-CaCit	HT-CaLac
0	0.479 ± 0.049^{d}	0.549 ± 0.045^{cd}	0.722 ± 0.068^{ab}	0.733 ± 0.045^{ab}	0.746 ± 0.034^{ab}
20	0.554 ± 0.020^{cd}	0.599 ± 0.042^{c}	0.771 ± 0.082^{a}	0.813 ± 0.077^{a}	0.721 ± 0.080^{ab}
20 + 7	0.536 ± 0.063^{cd}	0.646 ± 0.078^{bc}	0.741 ± 0.008^{ab}	0.778 ± 0.074^{a}	0.758 ± 0.07^{ab}

Notes: Different letters indicate significant differences according to the Fisher's least significant difference (LSD) test. Values correspond to means ± standard deviation of data for three replicates (LSD = 0.116).

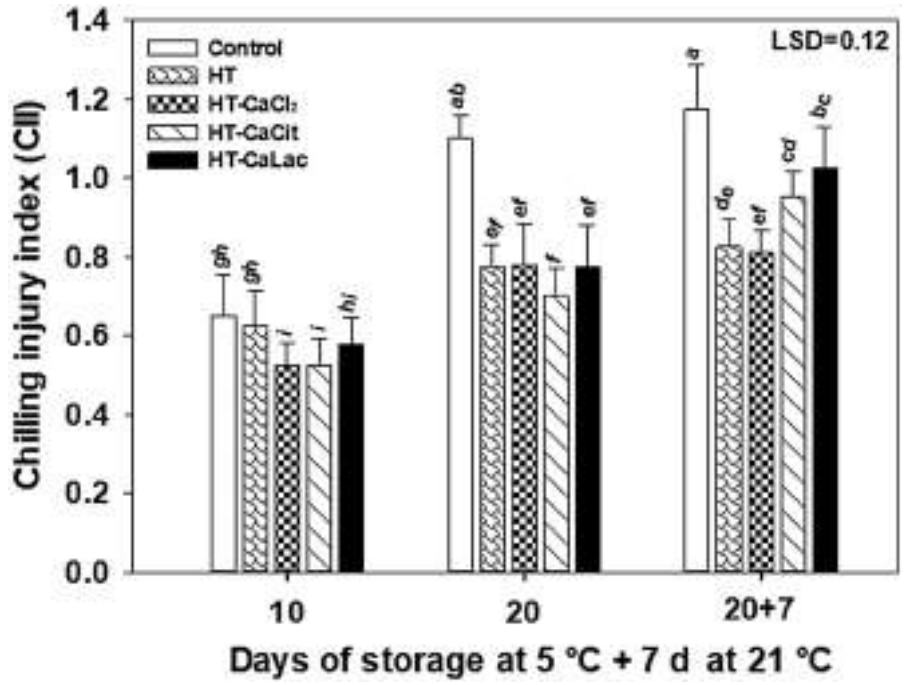

FIGURE 1 Chilling injury index of mango fruit treated with a hot water treatment (HT) and hot water treatment combined with calcium chloride (HT-$CaCl_2$), calcium citrate (HT-CaCit), or calcium lactate (HT-CaLac) and stored for 20 days at 5°C plus 7 days at 21°C. Vertical bars on columns represent the standard deviation of the means of three replicates. Different letters indicate significant differences according to the Fisher's least significance difference (LSD) test.

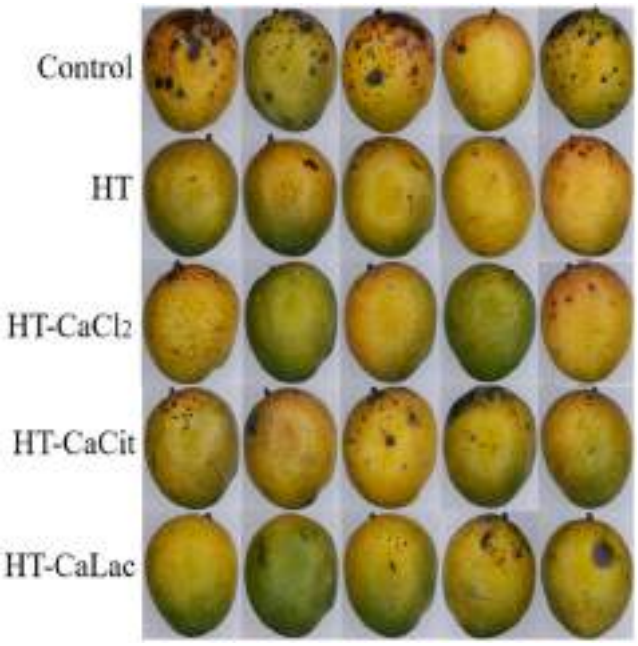

FIGURE 2 Representative images of mango fruit treated with a hot water treatment (HT) and hot water treatment combined with calcium chloride (HT-$CaCl_2$), calcium citrate (HT-CaCit), or calcium lactate (HT-CaLac) and stored for 20 days at 5°C plus 7 days at 21°C.

the most affected. Control and HT had an increase throughout the storage (5 and 21°C) of approximately 123 and 128%, respectively (Figure 4c). On day 10, HT showed the highest activity. On day 20, all HT-Ca treatments had lower activity than control and HT fruit. After the ripening period (7 days at 21°C), important differences were observed among treatments. Control fruit had the highest activity (2.74 U g^{-1}), followed by the HT fruit (2.54 U g^{-1}) and the HT-Ca treatments (0.55–0.98 U g^{-1}).

3.5 | Microstructural analysis

Considering firmness, EL, and enzymatic analyses, all three HT-Ca treatments had similar positive results; however, HT-$CaCl_2$ was selected as the best treatment based on its lower development of CI symptoms. Therefore, control, HT and HT-$CaCl_2$ were chosen for optical microscopy evaluation. The microstructural analysis made it possible to observe: cell walls (CW), vascular bundles (VB), vesicles (V), and the epicuticular wax (EW) (Figure 5). On day 0, peel and pulp sections from HT and HT-$CaCl_2$ presented a similar undamaged microstructure to untreated fruit. After 20 days at 5°C, HT treated fruit had damage on CW, VB, and the EW; whereas, control mangoes exhibited only damaged CW and VB. On treatment with HT-$CaCl_2$, no damage was observed on any of these structures.

4 | DISCUSSION

The increase in calcium content in the HT-Ca treatments indicates that the three calcium salts applied were absorbed through the apoplast, and deposited on the fruit peel (Hemmaty et al., 2007),

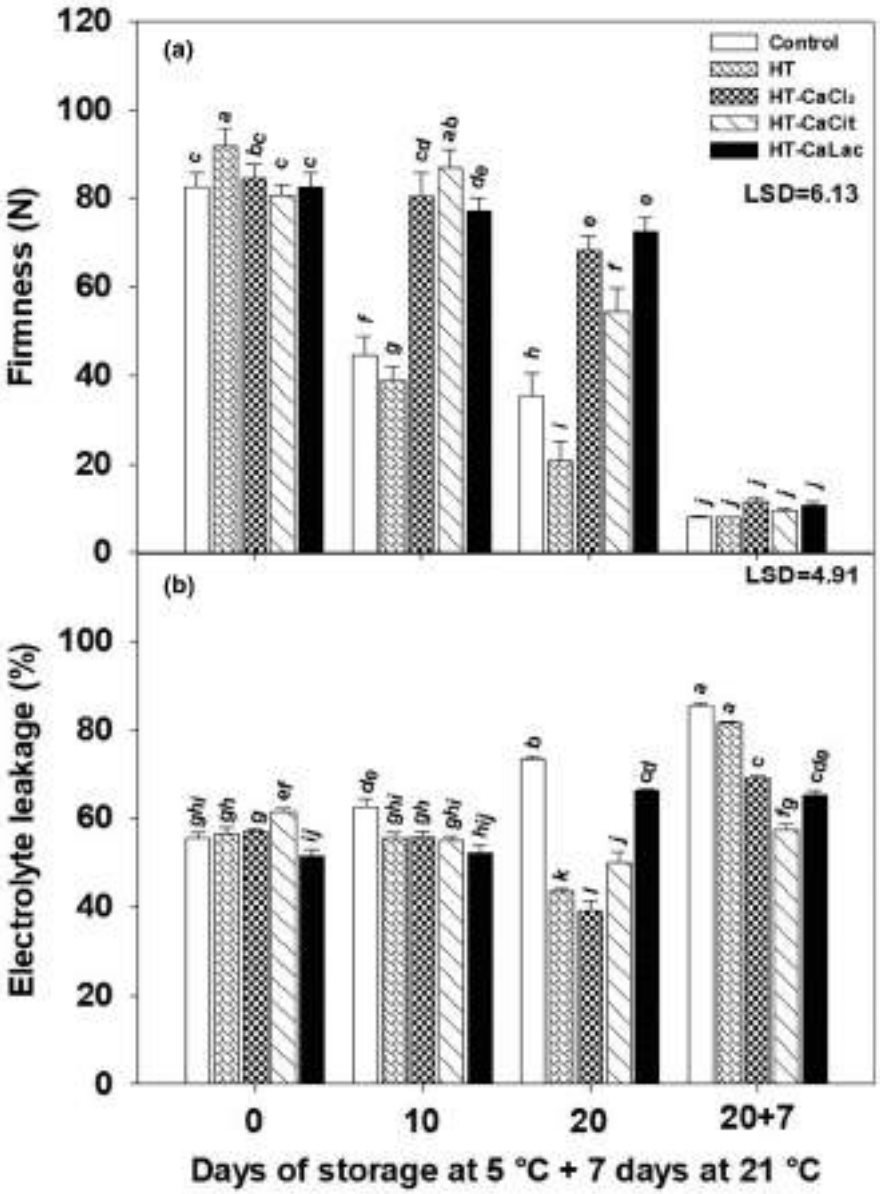

FIGURE 3 Firmness (a) and electrolyte leakage (b) in mango fruit treated with a hot water treatment (HT) and hot water treatment combined with calcium chloride (HT-$CaCl_2$), calcium citrate (HT-CaCit), or calcium lactate (HT-CaLac) and stored for 20 days at 5°C plus 7 days at 21°C. vertical bars on columns represent the standard deviation of the means of three replicates. Different letters indicate significant differences according to the Fisher's least significance difference (LSD) test.

suggesting that the solubility differences in the salts employed did not interfere with the ion absorption in mango fruit. Similar to our results, the application of HT-$CaCl_2$ in papaya fruit increased its calcium content (Ayón-Reyna et al., 2017). The authors discussed that the combination of HT and calcium salts alters the cell membrane and increases the infiltration of minerals and interaction with pectin. Additionally, García-Serrano et al. (2020) and Lovera et al. (2014) applied different calcium salts on black olives and fresh-cut papaya, respectively. These authors found higher calcium content on treated fruit, indicating that calcium intake can occur without the implementation of hot water. It is important to mention that, as observed in our study all calcium salts had a similar effect on calcium absorption. On the contrary, the application of HT-$CaCl_2$ on kiwi did not allow the incorporation of calcium in the tissue (Beirão-da-Costa et al., 2008), indicating that this treatment combination may not be useful for all fruit, and maybe delimited by the peel fruit characteristics.

HT application alone or in combination with calcium salts reduced the incidence of CI symptoms. In previous reports, HT application on mango induced a strong CI tolerance, and this effect was correlated to the activation of the enzymatic and non-enzymatic antioxidant system (Díaz-Corona et al., 2020; López-López et al., 2018). Moreover, heat shock proteins accumulate on the tissue and help proteins to fold properly under stress. Moreover, metabolites such as osmolytes and phenolics appear to stabilize the plasmatic membrane and avoid structural damage (Vega-Álvarez et al., 2020). Regarding calcium, this ion itself affects chilling tolerance since it can act as

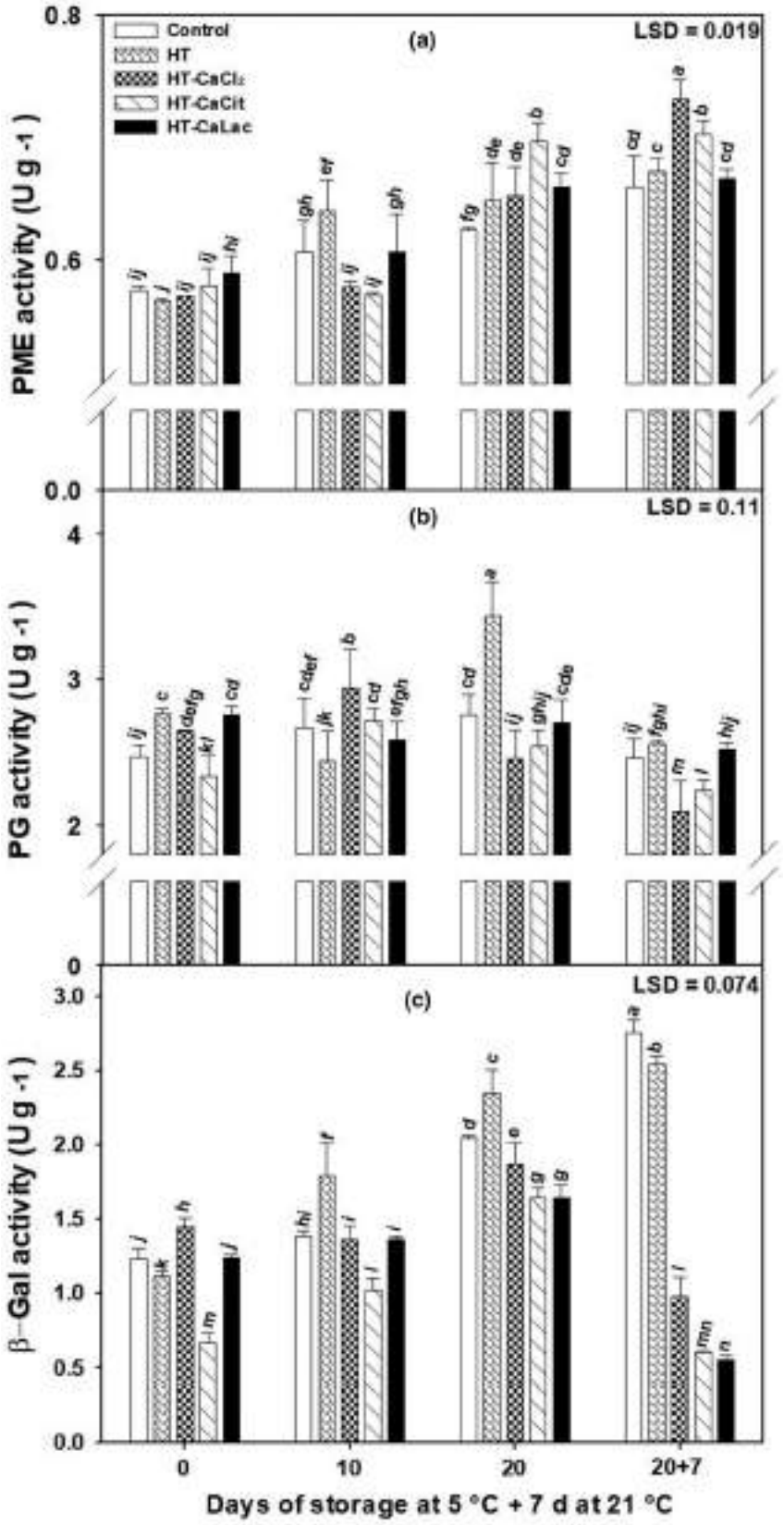

FIGURE 4 Pectin methylesterase (a), polygalacturonase (b), and β-galactosidase (c) activities in mango fruit treated with a hot water treatment (HT) and hot water treatment combined with calcium chloride (HT-$CaCl_2$), calcium citrate (HT-CaCit), or calcium lactate (HT-CaLac) and stored for 20 days at 5°C plus 7 days at 21°C. vertical bars on columns represent the standard deviation of the means of three replicates. Different letters indicate significant differences according to the Fisher's least significance difference (LSD) test.

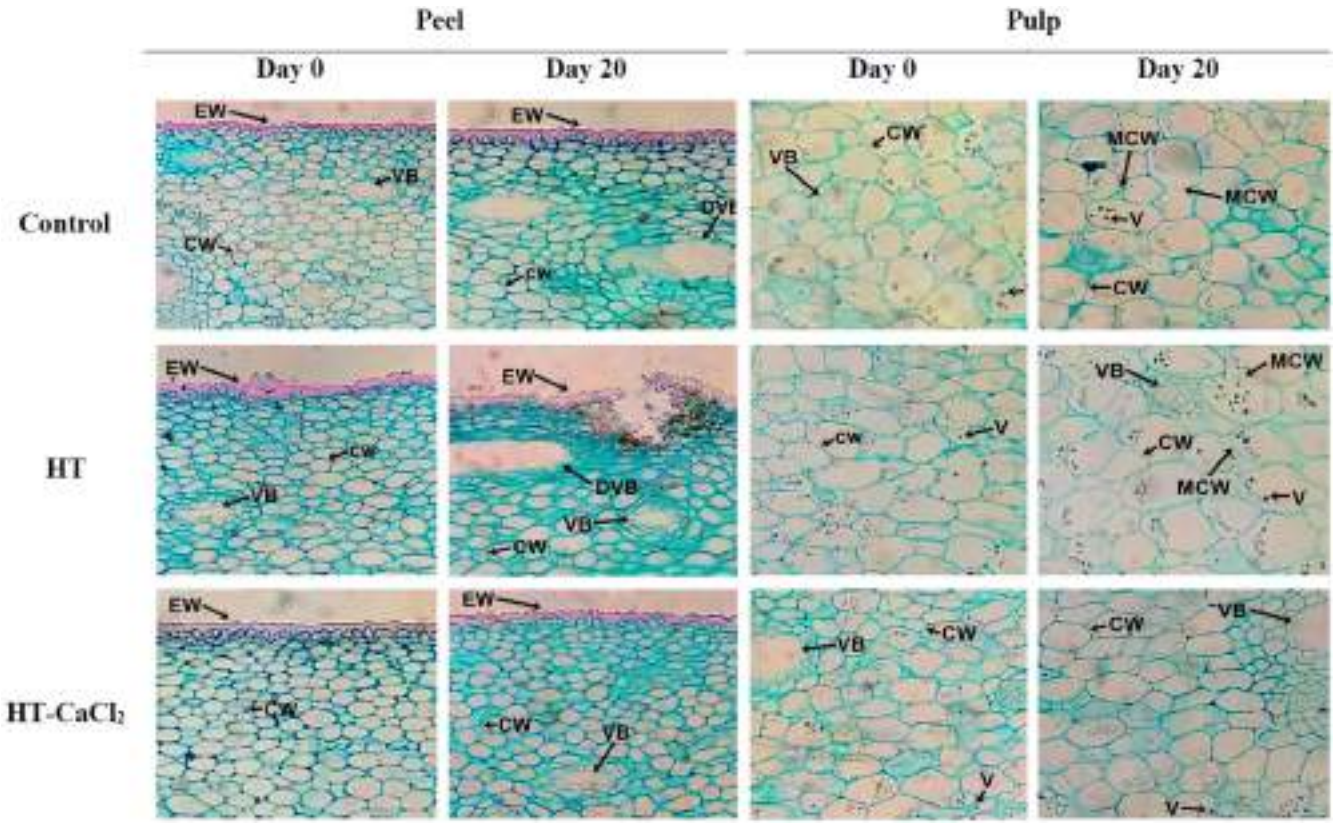

FIGURE 5 Representative light micrographs of transversal section of control and treated (HT and HT-$CaCl_2$) mangoes before storage and after 20 days at 5°C. Images were taken on a light microscope at 10×. CW, cell wall; DVB, deformed vascular bundle; EW, epicuticular wax; MCW, missing cell wall; V, vesicle; VB, vascular bundle.

a cofactor for the antioxidant system enzymes (Jin et al., 2009). Furthermore, the presence of the observed calcium ions favors their interaction with cell wall polymers, delaying cell disruption (Aguayo et al., 2008). Regardless of the calcium absorption, in this work, the addition of calcium did not offer an additional effect on the development of symptoms, as HT and HT-$CaCl_2$ had both the lowest symptom incidence.

In mango fruit, cell wall degradation is associated with increased pectin solubilization, leakage of intracellular content, and progressive firmness loss (Zhang et al., 2012). HT-Ca and its induction in calcium absorption had a remarkable effect on mango firmness, diminishing the negative repercussions of HT. According to Ngamchuachit et al. (2014), higher calcium concentration resulted in better firmness values in mango. Ayón-Reyna et al. (2017) and Hemmaty et al. (2007) explained this in terms of the PME activation, which liberates the carboxyl groups on the polymer to interact with calcium, favoring its incorporation. On the last evaluation day, no statistical differences were observed among treatments, and the firmness values obtained were associated with commercial ripening. This indicates that the treatment deaccelerated firmness loss; however, this process was not completely inhibited. Additionally, as previously discussed HT may not be interfering with calcium absorption since it has been reported that calcium itself increases firmness values on horticultural products (García-Serrano et al., 2020; Lovera et al., 2014).

Regarding the EL, this parameter reflects the integrity of the cell boundaries (cell wall and plasmatic membrane). The increase in EL is expected to be associated with a major incidence of CI; however, HT presented high EL. This indicates that even though HT helped to reduce the apparition of CI symptoms on the peel, this treatment did not favor the retention of pulp cell structure as expressed in terms of firmness and EL. In contrast, HT-Ca treatments achieved positive results on both chilling injury incidence and pulp softening. Many HT-Ca treatments have been applied to mango fruit with positive results on pulp structure. Ngamchuachit et al. (2014) employed Tommy Atkins and Kent mangoes and observed lower EL at the highest calcium concentration (0.4%). Díaz-Corona et al. (2020) and López-López et al. (2018) reported that Keitt mango treated with TH-CaLac presented lower EL and firmness loss. Bagheri et al. (2015) reported that HT-$CaCl_2$ is more efficient than the individual treatments to maintain the cell wall integrity. The synergetic effect of these treatments results in a reduction in the oxidative process and maintenance of phospholipids and proteins in the cell network.

On the enzymatic analysis, HT and HT-Ca presented a small but significant effect on PME activation. The activation of this enzyme is associated with cell wall stabilization, as observed on HT-Ca treated

fruit with higher firmness values. This is explained as more deesterified residues are available, and more calcium ions interact with them forming calcium pectates. In previous reports was observed that the PME activity was higher on tomato and mango treated with HT (Anthon et al., 2005; Zhang et al., 2012). Díaz-Corona et al. (2020) and Ranjbar et al. (2018) employed a HT-Ca on mango and apple, respectively, and these authors found higher PME activity on treated fruit.

The variations in PG activity have been previously reported on HT and HT-Ca treated fruit. Zhang et al. (2012) observed higher PG activity on mango treated with HT, resulting in higher content of solubilized pectin. In contrast, Chuni et al. (2010) and Muengkaew et al. (2018) found that HT-Ca application on pitahaya and mango minimized PG activity. This behavior may be caused by calcium, which interferes with PG binding to the unesterified pectin chains (Díaz-Corona et al., 2020).

As observed in control and HT-treated mango, as the fruit ripens β-elimination becomes more common, given that higher pH incentives this reaction catalyzed by β-Gal (McFeeters & Fleming, 1991). Similar to our results, Salazar-Salas et al. (2022) reported that mango cv. Keith treated with HT produces an overexpression of proteins related to cell wall metabolism such as β-Gal. On the contrary, the decrease in this enzyme in HT-Ca treated fruit could be due to the fact that there was a blockage in the branches of the pectin skeleton due to the presence of calcium. Suppression of β-Gal activity in early ripening significantly reduces fruit softening, given that β-Gal removes the non-reducing terminal β-D-galactosyl residue from β-D-galactoside, increasing the access for other enzymes (Paniagua et al., 2015). Similar to our results, Díaz-Corona et al. (2020), Shafiee et al. (2010), and Silveira-Gómez et al. (2011) found that the application of HT-Ca on mango, strawberry, and melon, respectively, leads to a decrease in β-Gal activity.

In our study, greater firmness values in HT-$CaCl_2$ were correlated with a higher compartmentalized cell structure observed by optical microscopy. Hewajulige et al. (2003) reported that calcium plays a key role in cell–cell adhesion, avoiding the formation of intercellular spaces and deformed structures as observed in control and HT treatments. Furthermore, as the fruit ripens, the stability of the cell wall depends more on the amount of calcium and its capacity to produce calcium pectates (Díaz-Corona et al., 2020). Ayón-Reyna et al. (2017) reported that HT changes the epicuticular wax on papaya fruit without any negative effect; however, we observed on mango that this treatment damaged the outer fruit layer.

Similar to our results, Shahkoomahally and Ramezanian (2015) observed major cell integrity on kiwi treated with HT-$CaCl_2$, showing a better effect than the individual treatments, which was associated with an early inactivation of cell wall enzymes. It is important to mention that this is the first study to establish an association between calcium absorption and cell wall stability on mango fruit stored at chilling temperatures.

5 | CONCLUSIONS

HT alone induced CI tolerance. Among HT-Ca treatments, HT-$CaCl_2$ had the best effect to retard the incidence of CI symptoms; however, HT provoked accelerated softening, which was counteracted with the application of calcium. All calcium salts applied were absorbed on the fruit peel, and this contributed to cell wall stabilization. All three HT-Ca treatments applied had similar positive effects on firmness, EL, and regulation of cell wall enzymes; however, HT-$CaCl_2$ had the best effect on CII and was selected as the best treatment. On the microstructural level, this treatment showed lower damage than HT fruit. Therefore, HT-$CaCl_2$ can be a practical strategy to diminish CI on mango and counteract the negative effects of HT.

AUTHOR CONTRIBUTIONS

Jordi G. López-Velázquez: Data curation; investigation; writing – original draft. **Martha E. López López:** Conceptualization; methodology; writing – review and editing. **Andrés Rubio Trías:** Data curation; investigation; writing – original draft. **Lidia E. Ayón-Reyna:** Conceptualization; formal analysis; methodology; writing – review and editing. **Denisse A. Díaz-Corona:** Formal analysis; methodology; resources. **Guadalupe I. Olivas Orozco:** Formal analysis; methodology; resources. **Javier Molina-Corral:** Formal analysis; methodology; resources. **Misael O. Vega-García:** Conceptualization; supervision; writing – review and editing.

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CONFLICT OF INTEREST

The authors declared that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

Data available on request from the authors.

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