Quality attributes and related enzyme activities in peppers during storage: effect of hydrothermal and calcium chloride treatment

Haishan Xu\textsuperscript{a}, Shenghua Ding\textsuperscript{b}, Hui Zhou\textsuperscript{a}, Youjin Yi\textsuperscript{a}, Fangming Deng\textsuperscript{a}, and Rongrong Wang\textsuperscript{a}

\textsuperscript{a}College of Food Science and Technology, Hunan Agricultural University, Changsha, China; \textsuperscript{b}Hunan Agricultural Product Processing Institute, Hunan Academy of Agricultural Sciences, Changsha, China

\section*{ABSTRACT}

The effects of hydrothermal (HT)-calcium chloride (CaCl\textsubscript{2}) treatment on water loss, chlorophylls, L-ascorbic acid, total phenol, antioxidant capacity, malondialdehyde (MDA), peroxidase (POD), polyphenol oxidase (PPO), catalase (CAT), and phenylalanine ammonia lyase (PAL) of peppers were assessed for 32 days of storage at 8°C. The results showed two water populations corresponding to strongly immobilized water and weakly bound water were observed in all the peppers. Comparing with other treatments, HT-CaCl\textsubscript{2} treatment restricted water mobility and maintained higher immobilized water content during storage. HT-CaCl\textsubscript{2} treated peppers showed lower MDA content whereas presented higher chlorophylls, L-ascorbic acid, total phenol content, and stronger antioxidant capacities than those subjected to other treatments. These characteristics indicated HT-CaCl\textsubscript{2} treatment improved storage quality of postharvest peppers. In addition, HT-CaCl\textsubscript{2} treatment retained lower POD, PPO, and PAL activities and higher CAT activity in the peppers during storage than other treatments, respectively. Based on above results, the combination of HT and CaCl\textsubscript{2} treatment showed positive and continuous effects on the quality attributes and related enzyme activities of peppers during storage.

\section*{Introduction}

Pepper (\textit{Capsicum annuum} L.), which belongs to the Solanaceae family, is planted worldwide and covers approximately 1.99 million ha of harvested area and an annual production of 36.1 million tonnes in 2017 according to FAO.\textsuperscript{[1]} Pepper fruit is rich in nutrients and bioactive compounds, including L-ascorbic acid, phenolic compounds, carotenoids, and capsaicin, which exhibit antioxidant activities and anti-inflammatory effects according to Ribes-Moya et al.\textsuperscript{[2]} Hence, pepper fruit is widely accepted by many consumers and sold worldwide. However, given that fresh pepper consists of approximately 90% water, it easily matures and decays, causing quality deterioration and reducing commodity value. Mohebbi et al.\textsuperscript{[3]} found that shrinkage percentage, firmness, and color of bell peppers were very sensitive to storage time, while storage temperature had the most effect on moisture reduction. Chitravathi et al.\textsuperscript{[4]} found that ascorbic acid, total chlorophylls, capsaicin contents, and total antioxidant activity tended to decrease during storage at 7–9°C (RH: 85%-95%). Hence, the physicochemical characteristic and antioxidant capacities of postharvest peppers decrease at some extent during storage, and the preservation technologies must be applied to retain the storage qualities. Current research about methods regulating the storage qualities of peppers focused on physical, chemical, and biological preservation, particularly through heating treatment.\textsuperscript{[5]}

\section*{CONTACT}

Rongrong Wang \textsuperscript{a}sdauwrr@163.com \textsuperscript{a}College of Food Science and Technology, Hunan Agricultural University, No. 1, Nongda Road, Furong District, Changsha, Hunan 410128, China

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coating,[3] salicylic acid, and calcium chloride (CaCl₂) treatment.[6] Thus, novel preservation methods must be constantly improved based on the needs of high efficiency, green, and safe.

Hydrothermal (HT) or CaCl₂ treatment maintains the qualities of fruits and vegetables. Positively maintaining physicochemical quality, hot air or water at 45–65°C was used as HT treatment in the research of Glowacz et al.[7] and Maxin et al.[8] The quality deterioration of fruits and vegetables with HT treatment was less than that of untreated samples, and their texture was better than the control. Ravanfar et al.[9] found that sour cherry immersed at 40°C hot water for 2 min showed improved water holding capacity and defense. Calcium plays a crucial role in reducing water desorption because it prevents or delays the loss of firmness. Calcium may diffuse within the cell wall structure by increasing the amount of endogenous calcium that can combine with pectin and form calcium bridge according to Ngamchuachit et al.[10] Thus, calcium treatment poses a positive and continues effect on the retention of fruits and vegetables quality during storage. Ngamchuachit et al.[10] found that fresh-cut Tommy Atkin mangos treated with 0.136 M CaCl₂ at 10°C for 2.5 min maintained better texture and other qualities than control. Belge et al.[11] found that cherry fruit with 3% CaCl₂ treatment for 2 min showed lower water loss and slower delay than the control. Some advances had recently focused on the combination of HT and CaCl₂ treatment in postharvest fruits and vegetables. Comparing with HT or CaCl₂ treatment alone, a better co-effect is found in HT-CaCl₂ treatment. Ayón-Reyna et al.[12] subjected fresh-cut papaya to HT treatment at 49°C for 25 min with CaCl₂ (1%) and dipping in chitosan (Chit; 1%, 3 min). The result showed that all treatments reduced the deterioration processes, maintained microbial, chemical and physical qualities, and extended the shelf life, but HT-CaCl₂ treatment resulted in the best texture and qualities compared with other treatments. However, studies on the HT-CaCl₂ treatment of peppers are rare, even on treatment using HT or CaCl₂ alone. Hence, the effects of HT-CaCl₂ treatment on the storage qualities of peppers should be studied and used as reference for the development of safe and effective preservation method for peppers.

In this study, postharvest peppers were preserved by HT-CaCl₂ treatment, and the effects of HT, CaCl₂, and HT-CaCl₂ treatments on the qualities and related enzymes of peppers during storage were investigated. Then, water loss; chlorophyll a and b contents; L-ascorbic acid content; total phenol content; antioxidant capacity; malondialdehyde (MDA) content; and the activity of related enzymes, including peroxidase (POD), polyphenol oxidase (PPO), catalase (CAT), and phenylalanine ammonia lyase (PAL), in peppers stored at 8°C for up to 32 days were determined. This experiment will provide theoretical evidence with HT-CaCl₂ treatment regulating the qualities and related enzymes of postharvest peppers during storage.

Materials and methods

Materials

Fresh green peppers cv xiangyan No. 16 were harvested from Yueyang, Hunan province, China, on 26th July 2018. All the peppers reached commercial maturity (about 78 maturity). After harvest, the peppers were delivered to the laboratory immediately. Thereafter, they were washed and drained. Peppers with uniformity of size, color, and weight, free from visible blemishes, disease and/or physical damage were selected as the experiment raw materials. Selected peppers were divided into four groups for treatment and storage.

Treatments

Based on previous research, the pre-experiments were carried out to explore suitable HT temperature (40°C, 45°C, 50°C for 2 min) and CaCl₂ concentration (1.5%, 2.5%, 3.5% for 20 min) during storage of 32 days at 8°C (RH: 90–95%) for peppers. By comparing the changes of appearance
qualities, such as color, firmness, shrinkage, and so on, we selected 45°C/2 min HT and 2.5%/20 min 
CaCl₂ to treat peppers.

HT: the peppers were placed in the 45°C hot water for 2 min under atmospheric pressure; CaCl₂ 
treatment: the peppers were placed in the 2.5% CaCl₂ solution for 20 min at room temperature under 
atmospheric pressure; HT-CaCl₂ treatment: the peppers were placed in the 45°C hot water for 2 min, 
and then placed in the 2.5% CaCl₂ solution for 20 min at room temperature under atmospheric 
pressure; untreated samples was used as control. After the above treatments, all of the peppers were 
drained and cooled into room temperature. Treated samples (approximately 250 g each) were pack-
aged into No.10 sealed bag (34 cm length × 24 cm width) before storage.

Storage condition

Sample storage was conducted at 8°C (RH: 90–95%). All treated and untreated samples were 
prepared in triplicate and stored at above conditions. The samples were considered and analyzed 
at 0 (untreated), 8, 16, 24, and 32 days during storage.

Water relaxation time measurement

Water relaxation time was determined using a low field nuclear magnetic resonance (NMR) 
MesoMR12-150H-I (Shanghai Niumag Corporation, China) with 12.7977 MHz with 
a modification of the method described by Bulut et al.[13] The same pepper in every treatment 
was inserted in the NMR probe equipped with 0.5 T strength magnets at 32°C for different storage 
time. The transverse relaxation time (T₂) was measured using a CPMG pulse sequence. A 90° pulse 
followed by a train of 180° pulses was contained in this sequence to refocus the NMR signal. 
Relaxation curves obtained from the CPMG sequence were analyzed using NMR software.

Chlorophyll a and b contents analysis

Chlorophyll extraction was performed according to the method described by Xie et al.[14] In brief, 
1 g lyophilization of freeze-dried pepper powder was mixed with 10 mL 80% (v/v) cold acetone 
solution, and centrifuged at 5300 × g for 10 min at 4°C. The supernatant was collected and filtered 
through a centrifugal filter before HPLC analysis.

HPLC analysis was performed using a Waters HPLC System (Waters 2695 Separations Module, 
Milford, USA) equipped with a photodiode array detector (Waters 2996, Milford, USA) according to 
the method of Teng and Chen et al.[15] Chlorophylls were separated within 20 min by a C18 column 
(Cosmosil 5C18-AR-II; 250 × 4.6 mm; i.d., 5 µm; Nacalai Tesque Inc., Japan) with a flow rate of 
1.0 mL/min at 30°C, using a solvent system of acetonitrile/methanol/chloroform/n-hexane (75: 12.5:
7.5: 7.5, v/v/v/v) as the isocratic mobile phase. The injection volume was 20 µL. The identification of 
chlorophyll a and b was based on the comparison with peaks of standards according to the 
absorption spectrum at 432 nm. Results were expressed as mg g⁻¹ DW.

L-ascorbic acid content measurement

L-ascorbic acid was determined using a method reported by Valdenegro et al.[16] with some modific-
tions. Peppers (20.0 ± 5.0 g) were smashed by shredding machine. All pulverized peppers were placed 
in a 100 mL beaker, and 50 mL of cold (4°C) 2.5% meta-phosphoric acid (Acros Organics, UK) was 
added. The processed peppers were immediately centrifuged at 5300 × g for 20 min at 4°C. 
Supernatants were filtered using Sep Pak filters (Phenomenex, UK), and 1.5 mL was collected in 
100 mL volumetric flask. Samples were analyzed using an Agilent 1100.

HPLC (Agilent, UK) with a Luna 5 µm NH₂ 100 A column (250 mm × 4.6 mm) (Phenomenex, 
UK) at a flow rate of 0.5 mL min⁻¹ with a pressure in the range of 70–80 bars at 30°C, using a solvent
system of n-hexane/acetonitrile/methanol/chloroform/(75: 7.5: 12.5: 7.5, v/v/v/v) as the isocratic mobile phase. The identification of L-ascorbic acid was based on the comparison with peaks of standards according to the absorption spectrum at 254 nm. Results were expressed as mg 100 g⁻¹ FW.

**Total phenol content measurement**

Total phenol content was measured using Folin–Ciocalteu method according to Deng et al.\(^{[17]}\) Approximately 0.5 g freeze-dried pepper powder was extracted using 80% methanol. After 30 min, 0.4 mL of extract was mixed with 2 mL of Folin-Ciocalteu reagent, then 3 mL of Na₂CO₃ (10%) was added to the mixture and incubated at room temperature for 60 min. The absorbance was measured at 760 nm. GAE was used as standard, and the result was expressed as µg GAE eq mg⁻¹ DW.

**Antioxidant capacities analysis**

**DPPH radical scavenging capacity**

DPPH radical scavenging capacity was determined using a method according to Li et al.\(^{[18]}\) with some modification. DPPH solution (0.0552 g) was prepared using 100 mL of methanol solution. Approximately 0.5 g of freeze-dried pepper powder was extracted with 80% methanol. Approximately 0.4 mL of pepper extract was mixed with 3.5 mL of DPPH solution and was incubated at room temperature for 20 min. The absorbance was measured at 517 nm. Trolox was the standard substance, and the result was expressed as mg Trolox eq g⁻¹ DW.

**ABTS⁺ radical scavenging capacity**

ABTS⁺ radical scavenging capacity was measured with a method reported by Lin et al.\(^{[19]}\) Approximately 0.5 g of freeze-dried pepper powder was extracted using 80% methanol. The reaction solution was mixed with 0.4 mL of pepper extract and 3.6 mL ABTS radical cation solution. After reacting at 25°C for 30 min, the reaction solution was immediately measured at 735 nm. Trolox was used for the calibration curves, and the result was expressed as mg Trolox eq g⁻¹ DW.

**FRAP**

FRAP was performed using the method described by Anand et al.\(^{[20]}\) with some modification. Approximately 0.5 g of freeze-dried pepper powder was extracted using 80% methanol. The radical FRAP solution contained 2.5 mL of a 10 mM TPTZ solution in 40 mM HCl, 2.5 mL of 20 mM FeCl₃, and 25 mL of acetate buffer (0.3 M, pH 3.6). The reaction solution consisted of 0.08 mL of the radical FRAP solution and 0.1 mL of pepper extract. The mixture was measured at 593 nm after the reaction in water bath at 37°C for 5 min. Trolox was used as the standard, and the result was expressed as mg Trolox eq g⁻¹ DW.

**MDA content measurement**

MDA content was determined with a modified method described by Liu et al.\(^{[21]}\) In brief, 30 g of fresh peppers were homogenized in 50 mL of 100 g L⁻¹ trichloroacetic acid solution and centrifuged at 5300 × g for 30 min at 4°C. The supernatant was then placed in a volumetric flask. The reaction mixture absorbance was measured at 450, 532, and 600 nm. The results were expressed as mmol 100 g⁻¹ FW.

**POD and PPO enzyme activities analysis**

The solution of POD and PPO was extracted using the method described by Terefe et al.\(^{[22]}\) with some modifications. POD and PPO were extracted from peppers (30 g) with extraction solution
(50 mL) consisting of 0.1 M sodium acetate buffer solution (pH 5.5) and 20 g L\(^{-1}\) crospolyvinylpyrrolidone (PVPP). The mixture was homogenized for 2 min at 1200 \(\times\) g and then strewed at 4°C for 1 h. The mixture was then centrifuged at 4°C for 30 min at 5300 \(\times\) g (Avanti J-26 XP, Beckman Coulter Inc., USA). The supernatant of POD (200 \(\mu\)L) was mixed with 0.05 M guaiacol in 0.05 M sodium acetate buffer solution at pH 5.5 (10 mL). In the blank, the sample was replaced with the extraction solution. The absorbance of the reaction mixture at 20°C-22°C was measured at 470 nm every 30 s for 7 min (Agilent 8453 Spectrophotometer, Agilent Technologies, Wald-bronn, Germany). The supernatant of PPO (200 \(\mu\)L) was mixed with 0.01 M catechol in 0.05 M sodium acetate buffer solution at pH 5.5 (8 mL) and then placed in a water-bath at 37°C for 10 min. The blank was the same as the POD. The absorbance of the reaction mixture at 20°C-22°C was measured at 420 nm every 5 s for 100 s (Agilent 8453 Spectrophotometer, Agilent Technologies, Wald-bronn, Germany). Enzyme activity was determined in the linearrange (the first 3 min for POD and the first 30 s for PPO) and expressed as U g\(^{-1}\) FW.

**CAT enzyme activity analysis**

CAT activity was determined according to method of Wang et al.\(^{[23]}\) with the following modifications. The mixture was homogenized in extraction solution (50 mL) consisting of 0.05 M phosphate buffer (pH 7.5) containing 5 mM dithiothreitol and 20 g/L PVPP. The mixture was then stirred for 20 min at 4°C, and centrifuged for 20 min at 5300 \(\times\) g at 4°C. The reaction mixture contained 1.5 mL of 0.05 mM H\(_2\)O\(_2\) in 0.05 M phosphate buffer. The reaction started by the addition of 0.2 mL of enzyme extract. CAT activity was monitored at 240 nm for 5 min at room temperature by using a UV-vis spectrophotometer. CAT specific activity was reported as U g\(^{-1}\) FW.

**PAL enzyme activity analysis**

PAL activity was determined using a modified method by Chen et al.\(^{[24]}\). The peppers (30 g) were homogenized on ice with 50 mL of 100 mM sodium borate buffer (pH 8.8) containing 2 mM EDTA, 40 g L\(^{-1}\) PVPP, and 5 mM b-mercaptoethanol. The mixture was centrifuged for 30 min at 5300 \(\times\) g at 4°C. The reaction mixture consisted of 0.5 ml of 20 mM L-phenylalanine, 0.9 mL of the crude extract, and 3 mL of 50 mM sodium borate buffer at pH 8.8. The mixture was incubated for 60 min at 37°C, and the reaction was stopped using 0.2 mL of 6 M HCl. PAL activity was monitored at 290 nm for 3 min at room temperature with a UV-vis spectrophotometer. PAL activity was expressed as U g\(^{-1}\) FW.

**Statistical analysis**

All the treatments were performed in triplicate. ANOVA was performed using the software Microcal Origin 8.0 (Microcal Software, Inc., Northampton, USA). The results were expressed or plotted as the mean value ± standard deviation.

**Results and discussion**

**Appearance quality**

The effects of HT-CaCl\(_2\) treatment on appearance quality are illustrated in Figure 1. Commercial postharvest quality is important to practical use of peppers. As shown in Figure 1, appearance quality of treated peppers was better maintained than that of untreated ones during storage. The shrinkage of all peppers increased with storage, especially for untreated ones. The upper and stem parts of untreated peppers had a notable shrinkage after 24 days. However, shrinkage rate of treated peppers was less than untreated peppers. For HT-CaCl\(_2\) treated peppers, they looked fuller than other three
groups. Above results indicated that HT-CaCl₂ treatment could effectively inhibit water loss and senescence of peppers during storage, further retained better appearance quality.

**Water relaxation time**

The water Carr–Purcell–Meiboom–Gill (CPMG) signals and relaxation time of all treatments are illustrated in Figure 2. According to Figure 2 (A₁, B₁, C₁, D₁), the decay time showed a decreasing trend with storage time, which was related to water changes during storage. By comparing untreated and treated peppers, it was clear that untreated peppers decayed the fastest and the decayed time was less than 1000 ms. Whereas HT-CaCl₂ treated peppers showed the longest decay time, more than 1000 ms. However, only from the CPMG decay curves, the water states were difficult to distinguish. To describe the water stages during storage, T₂ spectra were obtained by the multi-exponential fitting of CPMG decay curves using an NMR analysis software. T₂ represents the mobility of water and can be assigned to four water population, including T₂₁, T₂₂, T₂₃, and T₂₄. T₂₁, T₂₂, T₂₃, and T₂₄ refer to the transverse relaxation time of bound water, weakly bound water, immobilized water, and free water, respectively. As shown in Figure 2 (A₂, B₂, C₂, D₂), two peaks in untreated and treated peppers were observed. The highest peak, from 100 ms to 1000 ms, represented immobilized water (T₂₃), and the lowest peak, from 0 ms to 10 ms, represented bound water (T₂₁). The higher value of T₂₃ showed higher water mobility at some extent. From Figure 2 (A₂, B₂, C₂, D₂), T₂₃ values increased in the early storage and then decreased in the late storage. The result indicated that some of the immobilized water in peppers gradually transformed into other water forms during storage. However, T₂₁ values were minimally changed. From the result of Wang et al. [²⁶], bound water was firmly associated with other components in peppers, such as protein, and complex structure was stable. Comparing untreated, HT-treated, and CaCl₂-treated samples, the T₂₃ value was similar with each other. This result demonstrated that the effects of HT or CaCl₂ treatment on restricting water mobility were not evident. However, the T₂₃ value in Figure 2D₂ was approximately 1.5 times higher than that in Figure 2A₂. This result indicated that HT-CaCl₂ treatment could effectively restrict water mobility and maintain high immobilized water content. This finding was
Figure 2. (1) CPMG relaxation decay curves; (2) $T_2$ relaxation time of the peppers treated with CaCl$_2$, HT, and HT-CaCl$_2$ for 32 days at 8°C. $A_1$, $B_1$, $C_1$, $D_1$: CPMG relaxation decay curves; $A_2$, $B_2$, $C_2$, $D_2$: $T_2$ relaxation time; $A_1$, $A_2$: Control; $B_1$, $B_2$: CaCl$_2$; $C_1$, $C_2$: HT; $D_1$, $D_2$: HT-CaCl$_2$. 
because Ca\(^{2+}\) could form clathrate hydrates with water by chemical bonding at a certain temperature and restrict water mobility.\(^4\)

**Chlorophyll a and b contents**

The effects of HT-CaCl\(_2\) treatment on chlorophyll a and b contents are presented in Figure 3. Chlorophyll a and b contents in fresh peppers were 0.41 and 0.32 mg g\(^{-1}\) DW, respectively. Chlorophyll a and b contents of all the peppers first decreased and then increased during storage. Since chlorophylls are sensitive to heat\(^{27}\), their decrease in the early storage period was probably due to respiration heat increase, which accelerated chlorophyll degradation. In the late storage, chlorophyll a and b contents increase could be ascribed to the accumulation of light harvest complex II (LHCII) under stress. Sato et al.\(^{28}\) found that both chlorophyll a and b were related to LHCII, especially for chlorophyll b. When preceding the degradation of LHCII, the chlorophylls in LHCII could be degraded. Horton et al.\(^{29}\) showed that variable amounts of LHCII could not be degraded under stress, but organized PSII into large super-complexes to capture light. Based on above researches, we speculated that LHCII could not be properly degraded and caused chlorophylls accumulation in the late storage, especially for chlorophyll b. Further explore needs to be focused on the changes of LHCII in peppers during storage. According to Figure 3, the chlorophyll contents in CaCl\(_2\)-treated peppers were the most comparing other treatments during storage. He et al.\(^{30}\) also found that CaCl\(_2\) treatment reduced the hypoxic damage of cucumber leaves and enhanced the stability of chlorophylls. However, HT and HT-CaCl\(_2\) treatments were related to temperature, and chlorophylls are sensitive to temperature, which accelerated chlorophylls degradation in some extent. Moreover, an interesting phenomenon was found, that is, the content of chlorophyll b was more than chlorophyll a in peppers after 24 days. This result was related to the accumulation of LHCII under stress. Nick et al.\(^{31}\) showed that a chlorophyll b-less mutant did not accumulate LHCII, and chlorophyll b metabolism was closely related to LHCII content. Meanwhile, sato et al.\(^{28}\) showed that chlorophyll cycle existed in land plant, which was believed to facilitate the regulation of the chlorophyll composition throughout developmental stages. However, rüdiger et al.\(^{32}\) found that chlorophyll cycle did not function as a complete cycle at one and the same time, and its function was believed to supply either chlorophyll a or chlorophyll b according to the particular physiological need. It was possible that there was a conversion between chlorophyll a and b in the late storage, and it resulted in the increase of chlorophyll b content in peppers. Above speculation needs to be further studied, basing on the changes of LHCII and enzymes activities related to chlorophyll cycle in pepper during storage.

![Figure 3. Chlorophyll a and b contents of the peppers treated with CaCl\(_2\), HT, and HT-CaCl\(_2\) for 32 days at 8°C. Each value is the mean of three replications, and vertical bar represents the standard error of the means (n = 3).](attachment:image)
**L-ascorbic acid content**

Changes in L-ascorbic acid content of peppers with different pretreatments during storage are illustrated in Figure 4. The initial content of L-ascorbic acid was 195.60 mg 100 g$^{-1}$ FW. From Figure 4, L-ascorbic acid content of all samples showed a decrease trend with storage time and was evident in the first 16 days. Change in L-ascorbic acid content was probably related to external environment and internal factors. Gu et al.\cite{27} found that L-ascorbic acid degradation was probably caused by genetics, temperature, light, water content, and so on. Herbig et al.\cite{33} found that high PPO activity accelerated the degradation of L-ascorbic acid during early storage. The result about the variation of PPO activity further confirmed this view. Compared with the control, peppers with HT or CaCl$_2$ treatment decreased slower, especially in the late storage. Kumar et al.\cite{34} explored the effects of different temperatures at different times for L-ascorbic acid content in citrus fruit and found that citrus heated at 50℃ for 90 s delayed the decrease of L-ascorbic acid content. Hussain et al.\cite{35} also found that the total L-ascorbic acid content in apple with CaCl$_2$ treatment was higher than that in the control. The above results showed that HT or CaCl$_2$ treatment could improve the stability of L-ascorbic acid. As shown in Figure 4, when combining HT and CaCl$_2$ treatment, the decreasing rate was less and had a significant ($P<.05$) difference comparing with other groups, especially for untreated peppers. On the 16, 24 and 32 days, L-ascorbic acid content in HT-CaCl$_2$ treated peppers could, respectively, reach 169.62, 162.97, and 156.84 mg 100 g$^{-1}$ FW. Especially on the last day, L-ascorbic acid content in HT-CaCl$_2$ treated peppers was still 1.29 times than that in untreated peppers. Meanwhile, because of antioxidant capacities of L-ascorbic acid, HT-CaCl$_2$ treated peppers had a higher antioxidant capacity than other groups. The later conclusions for total phenol and antioxidant capacities of the peppers further confirmed this view.

**Total phenol content**

Total phenol is important antioxidant components in fruits and vegetables.\cite{36} The effect of HT-CaCl$_2$ treatment on total phenol content is presented in Figure 5A. The initial content of total phenol was 0.57 µg GAE eq mg$^{-1}$ DW. As shown in Figure 5A, total phenol content in all samples decreased with the storage time. The decrease of total phenol content was related to PPO activity. Deng

![Figure 4](image-url)

*Figure 4. L-ascorbic acid content of the peppers treated with CaCl$_2$, HT, and HT-CaCl$_2$ for 32 days at 8℃. Each value is the mean of three replications, and vertical bar represents the standard error of the means ($n=3$).*
et al.\textsuperscript{[17]} found that faster water loss and higher PPO activity in litchi pericarp could accelerate the oxidation of phenolics during storage. The maximum decrease of total phenol was shown in the early 8 days but slowed in the late storage. The result was because high PPO activity accelerated total phenol degradation in the early storage. HT-CaCl\textsubscript{2} treatment presented the highest total phenol content in all the treatments, and there was a significant ($P < .05$) difference between untreated and HT-CaCl\textsubscript{2} treated peppers in the first 16 days. On the eighth day, total phenol content with HT-CaCl\textsubscript{2} treatment was up to 0.54 µg GAE eq mg\textsuperscript{−1} DW and was 1.22 times higher than that of the control. According to above result, it showed that HT-CaCl\textsubscript{2} treatment could positively affect the maintenance of total phenol content. This result had been proven in previous studies. Ayón-Reyna et al.\textsuperscript{[12]} found that fresh-cut papaya with HT (49°C, 25 min) containing CaCl\textsubscript{2} (1%) followed by dipping in Chit (1%) could positively inhibit the reduction of total phenol. Aghdam et al.\textsuperscript{[37]} used 40, 60, and 80 mM CaCl\textsubscript{2} to treat cherry and found that all the treatments could maintain high total phenol, especially for 80 mM CaCl\textsubscript{2} treatment.

\begin{figure}
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\includegraphics[width=\textwidth]{figure5}
\caption{Total phenol content and antioxidant capacities of the peppers treated with CaCl\textsubscript{2}, HT, and HT-CaCl\textsubscript{2} for 32 days at 8°C. Each value is the mean of three replications, and vertical bar represents the standard error of the means ($n = 3$).}
\end{figure}
Antioxidant capacities

The antioxidant capacities of fruits and vegetables are affected by different antioxidant components.\cite{38} The effect of HT-CaCl$_2$ treatment on antioxidant capacities is presented in Figure 5B–D. In Figure 5B, there were decrease trend for DPPH, ABTS$^+$ and FRAP radical scavenging capacity during storage, but the variation was different. For the DPPH radical scavenging capacity, the antioxidant capacities of untreated peppers decreased from 86.84 mg Trolox eq g$^{-1}$ DW to 72.21 mg Trolox eq g$^{-1}$ DW. After treatment, the decrease in the DPPH radical scavenging capacity was inhibited, especially for HT-CaCl$_2$ treatment. On the final day, DPPH radical scavenging capacity in HT-CaCl$_2$ treated peppers maintained the highest values, up to 79.00 mg Trolox eq g$^{-1}$ DW, and had a significant ($P<.05$) difference comparing untreated peppers. The decrease of DPPH radical scavenging capacity could be ascribed to the loss of antioxidants, such as total phenol and L-ascorbic acid. Wojdylo et al.\cite{39} had shown that positive correlations were observed between DPPH radical scavenging capacity and total phenol or L-ascorbic acid. Above results indicated that HT-CaCl$_2$ treatment could positively maintain DPPH radical scavenging capacity. ABTS$^+$ and FRAP radical scavenging capacities also exhibited similar trends. In Figure 5C,D, more difference was found between HT-CaCl$_2$ treated peppers and others in the early storage. On the eighth day, ABTS$^+$ and FRAP radical scavenging capacities in HT-CaCl$_2$ treatment were 1.34 and 1.05 times higher than that of the control, respectively. Antunes et al.\cite{40} found that kiwifruit treated with ascorbic acid or 2% CaCl$_2$ positively affected DPPH and ABTS$^+$ radical scavenging capacities maintenance mainly in the first 4 days, but CaCl$_2$ treatment exhibited a continuous effect until the last day. Viña et al.\cite{41} also found that the antioxidant capacity of pre-cut celery could be better retained with 50°C for 90 s than that of untreated ones. Above results suggested that HT-CaCl$_2$ treatment was available to maintain antioxidant capacities.

MDA content

MDA is one of the end products of membrane lipid oxidation and is a crucial indicator of membrane damage according to Yan et al.\cite{42} If an increased amount of MDA is produced in fruits and vegetables, cell will be severely damaged, and cause fruits and vegetables easily decay. As shown in Figure 6, MDA content in fresh peppers was 0.05 mmol 100 g$^{-1}$ FW. MDA content increased with storage time for all the peppers, which could be due to membrane lipid oxidation. Comparing with untreated and treated peppers, MDA content in untreated peppers was the highest during storage. Significant ($P<.05$) differences were shown between untreated and treated peppers on the eighth and sixteenth days. MDA content of the untreated peppers, respectively, increased into 0.15 and 0.18 mmol 100 g$^{-1}$ FW at 8 and 16 days, more than treated peppers. For all treatments, HT-CaCl$_2$ treatment could effectively inhibit the increase of MDA during storage, followed by HT or CaCl$_2$ treatment, and the effect was more obvious in the first 16 days. Similar results were found in other researches. Wu et al.\cite{43} also found that calcium, chlorine dioxide, and heat treatment could reduce respiration production and MDA content of apricots stored at 20°C for 10 days. HT-CaCl$_2$ treatment could positively inactivate oxidase enzymes and increase Ca$^{2+}$ content in membrane, further sustaining membrane stability. Hence, HT-CaCl$_2$ treatment was an effect of pretreatment for inhibiting the increase of MDA content in peppers and extending its shelf life.

Related enzyme activities

POD activity: POD is a physiological index of the ripening and senescence of fruits and vegetables. The effect of HT-CaCl$_2$ treatment on POD activity in peppers during storage is shown in Figure 7A. POD activity decreased with storage time in HT, CaCl$_2$, and HT-CaCl$_2$-treated peppers. This phenomenon was probably caused by the decrease of the physiochemical metabolism in peppers. POD activity of untreated peppers showed a decreasing fluctuation trend. As shown in Figure 7A, POD activity in untreated peppers showed a higher value compared with treated peppers during
storage. Especially on the 16th day, POD in control reached 1.16 U g\(^{-1}\) FW and was 1.63 times higher than in HT-CaCl\(_2\) treatment. However, Higher POD could cause numerous adverse physiological reactions. Liu et al.\(^{44}\) had proved POD catalyzed various oxidation reactions involving CAT and caused enzymatic browning with PPO. Kim et al.\(^{45}\) also showed that PPO and POD were crucial for the oxidation of phenolic compounds and resulted in enzymatic browning. Liu et al.\(^{44}\) also found that POD was related to anti-browning of fresh-cut potato slices during storage. When other physiochemical qualities were considered, POD played a key role in regulating enzymatic browning compared with oxidation resistance in our research. Hence, according to above results, increased POD activity accelerated oxidation reactions and caused enzymatic browning.\(^{46}\) As shown in Figure 7A, HT, CaCl\(_2\), HT-CaCl\(_2\) treatments could decrease POD activity during storage, especially for HT-CaCl\(_2\) treatment. This observation corresponded well with the result of Marszałek et al.\(^{47}\), who found that the POD activity of strawberries treated with 50°C hot water for 15 min was obviously inhibited than that of untreated ones. Reichel et al.\(^{48}\) also found that CaCl\(_2\) treatment could decrease the POD activity of litchi pericarp and showed improved cooperating effect with other chemical agent, such as cysteine.

PPO activity: PPO is associated with the deposition of phenolic compounds into plant cell walls during resistance interactions.\(^{49}\) The effect of HT-CaCl\(_2\) treatment on POD activity in peppers during storage is shown in Figure 7B. It was evident that PPO activity showed a decreasing trend in untreated and treated peppers, and reached a minimum value on the final day. Comparing four groups, PPO activity in untreated peppers was the highest, and the HT-CaCl\(_2\) treated peppers were the lowest during storage. From Figure 7B, there were significant (\(P < .05\)) differences between untreated and HT-CaCl\(_2\) treated peppers in the first 16 days. Especially on the eighth day, PPO activity of HT-CaCl\(_2\) treated peppers only up to 45% of that in untreated peppers. Above results showed that POD activities were effectively inhibited by HT-CaCl\(_2\) treatment. Some previous researches have proved HT or CaCl\(_2\) treatment inhibited PPO activity. Vámos-Vigyázó et al.\(^{50}\) found that fruits and vegetables shortly exposed to temperatures from 70°C to 90°C showed lower activity of PPO than that of untreated ones. Youryon et al.\(^{51}\) also showed that 48 h 2% calcium gluconate treatment effectively retarded the PPO activity of pineapple. However, high PPO activity could cause adverse reaction. Bajwa et al.\(^{52}\) found that PPO was involved in enzymatic browning of fruits and vegetables, which could catalyze phenolic
substances and various oxidation reactions involving CAT. Mrad et al.\textsuperscript{[53]} found that browning in pears was mainly caused by increasing PPO activity. Hence, High PPO activity was associated with L-ascorbic acid and chlorophyll degradation. According to above results, HT-CaCl\textsubscript{2} treatment could decrease PPO activity and maintain some qualities.

CAT activity: CAT is related to oxidation resistance. According to Gao et al.\textsuperscript{[54]}, high CAT activity is effective to remove ROS, especially O\textsuperscript{2-} produced by metabolism. Otherwise, too much O\textsuperscript{2-} could accelerate the membrane lipid oxidation and produce mass MDA. The test result of CAT activity is illustrated in Figure 7C. A sharp increasing trend for untreated and treated peppers in the first 24 days and thereafter decreased. Huang et al.\textsuperscript{[55]} proved high metabolism of postharvest fruits and vegetables increased the production of ROS in the early storage, and the accumulation of ROS further caused senescence. Hence, when CAT activity increased, O\textsuperscript{2-} accumulated in peppers could easily be removed. Meanwhile, High CAT activity was beneficial for maintaining the oxidation resistance and delayed senescence of peppers in the early storage. The similar variation trend was found in the experiment of Wu et al.\textsuperscript{[43]}, CAT activity of apricots slightly increased in the first 6 days and then decreased. After HT or CaCl\textsubscript{2} treatment, CAT activity showed similar level but was higher than that in untreated peppers. Raseetha et al.\textsuperscript{[56]} showed that broccoli heated at 70°C for 10 min showed similar increasing trend for CAT activity. For CaCl\textsubscript{2} treatment, Wu et al.\textsuperscript{[43]} found that CAT activity in apricots with 0.5% CaCl\textsubscript{2} treatment at 20°C for 5 min increased and was higher than control. It was obvious shown that when treating with the combination of HT and CaCl\textsubscript{2}, peppers showed the highest CAT level in untreated and treated peppers during storage, ranging from 0.29 U g\textsuperscript{-1} FW to 2.73 U g\textsuperscript{-1} FW. On the 24th day, a significant (P < .05) difference was found between HT-CaCl\textsubscript{2} treatment and other three groups, especially for untreated peppers. The CAT activity in HT-CaCl\textsubscript{2} treated peppers reached up to 2.73 U g\textsuperscript{-1} FW on the 24th day, but the untreated peppers were only 1.5 U g\textsuperscript{-1} FW. Hence, HT-CaCl\textsubscript{2} treatment was effective for maintaining CAT activity and improving resistance.

PAL activity: Figure 7D shows the effect of HT-CaCl\textsubscript{2} treatment on PAL activity in peppers during storage. For the control, CaCl\textsubscript{2}, and HT-CaCl\textsubscript{2} treatment, PAL activity decreased drastically in the first 8 days and increased from the eighth day to 16th day, thereafter decreased to the lowest level. However, PAL activity decreased with the storage time for HT treated peppers. Wulfkuehler et al.\textsuperscript{[57]} speculated that the reduced PAL activities in lettuce were correlated with delayed browning at cut edges, and perceived by sensory evaluation and stereo microscopy. Khan et al.\textsuperscript{[58]} also found that CaCl\textsubscript{2} treatment exhibited a beneficial effect on inhibiting PAL activity. However, in this experiment, the effect of PAL activity on quality was not clear, so further research needs to be carried out. In Figure 7D, PAL activity in untreated peppers was higher than that in treated peppers during storage. On the 16th day, there was a significant (P < .05) difference between untreated and treated peppers. PAL activity was 2.44, 3.38, 2.10 times than CaCl\textsubscript{2}, HT and HT-CaCl\textsubscript{2} treatments, respectively. Hence, HT, CaCl\textsubscript{2} or HT-CaCl\textsubscript{2} treatment had a better effect on inhibiting PAL activity.

**Conclusion**

Based on the results of our investigation, HT-CaCl\textsubscript{2} treatment exhibited the most positive and continuous effect for quality attributes and related enzyme activities of peppers during storage in all the treatments. Two water populations corresponding to strongly immobilized water and weakly bound water were observed in all the peppers. The immobilized water content first increased considerably and then decreased with storage time. However, the bound water content was stable during storage. Compared with other treatments, HT-CaCl\textsubscript{2} treatment could restrict water mobility and maintain higher immobilized water content during storage. HT-CaCl\textsubscript{2}-treated peppers exhibited lower MDA content, higher chlorophyll, L-ascorbic acid, and total phenol content, and antioxidant capacities than those of other treatments. For the related enzymes, HT-CaCl\textsubscript{2} treatment retained low POD, PPO, and PAL activity and high CAT activity of peppers during storage. For the further...
verification of our results, the next research will focus on the effects of HT-CaCl$_2$ treatment on physiological metabolism, including respiration, ethylene production, and so on.

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