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CALCIUM CHLORIDE REGULATES CELLULAR CALCIUM METABOLISM DURING THE POSTHARVEST SENESCENCE OF RIPE PASSION FRUIT

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ABSTRACT

The impact of calcium chloride ($CaCl_2$) treatment and water soaking on the postharvest senescence of the ripened passion fruit was investigated. The physicochemical indexes, including weight loss, color change, membrane permeability, malondialdehyde (MDA) content, total phenolics, flavonoids, ascorbic acid content, peroxidase (POD), polyphenol oxidase (PPO), and distinct forms of calcium of the fruit were determined using the established methods for analysis. The correlation and principal component analyses were also conducted on the experimental data. The results demonstrated that the calcium ions were effective in maintaining the appearance and color of the fruit peel, enhancing its antioxidant capacity, and regulating its nutrient content. This treatment significantly increased the calcium content of the passion fruit peels, extending the fruit's freshness beyond four days. The correlation analysis revealed a positive correlation between the mass loss rate of the passion fruit and the majority of the physicochemical properties examined. The findings indicated that the calcium chloride treatment could effectively maintain fruit storage quality and prolong the shelf life of postharvest passion fruit by several days. It seems reasonable to posit that calcium chloride will become the preferred method for postharvest fruit preservation in the near future.

Keywords: calcium ion, color change, fruit freshness, oxidative damage, *Passiflora edulis*

INTRODUCTION

Passion fruit (*Passiflora edulis* Sims), also referred to as egg fruit, is a member of the Passifloraceae family and the *Passiflora* genus. It is cultivated primarily in Asia, America, Australia, and other tropical regions. The passion fruit cultivation in China is distributed throughout the Guangdong, Guangxi, and Fujian provinces [Guo et al. 2020]. The passion fruit is an egg-shaped fruit with a central cavity filled with multiple minute seeds and aromatic, juicy pulp. In China, it is regarded as the "king of fruit juice" [Niu et al. 2021]. Moreover, passion fruit is a highly sought-after consumer item in southern China and Southeast Asia. The fruit is a rich source of nutrients, including polysaccharides, amino acids, vitamins, minerals, and dietary fiber. Furthermore, it is rich in flavonoids and triterpenoids [dos Reis et al. 2018].

An elevated respiration rate has been demonstrated to result in fruit weight loss, overall deterioration, the formation of wrinkles on the peel, and decay. This deterioration in quality has a detrimental impact on the commercial value of the fruit [Zhu et al. 2022]. It is thus imperative to identify methods of preserving fruit

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freshness that will benefit both consumers and the food industry. Various preservation techniques have been employed to maintain the quality of fruits, including cold storage, irradiation, packaging, and film coating [Nair et al. 2020]. However, some of these methods, such as cold storage and irradiation, have disadvantages, including high costs and the potential for microbial growth [Loaharanu and Ahmed 1991]. Calcium chloride ($CaCl_2$) is a preservative and pickling agent that is frequently employed within the fruit and vegetable industry. It has been demonstrated to inhibit microbial growth on the surface of fruits effectively, minimize ethylene production, and decelerate the fruit ripening process [Martin-Diana et al. 2007, Hou et al. 2021]. The application of $CaCl_2$ liquid to postharvest fruit has been demonstrated to effectively reduce the fruit respiration rate, maintain the fruit quality, and reduce the incidence of postharvest diseases [Zhang et al. 2019a,b]. Moreover, the literature indicates that the $CaCl_2$ treatment significantly reduces the browning index, weight loss, and membrane permeability of jujube, effectively delaying the rate of superoxide production, reducing the excessive accumulation of malondialdehyde (MDA) and hydrogen peroxide in the fruit, and enhancing its antioxidant capacity [Wei and Zhao 2020]. The addition of Ca during storage may influence cellular calcium metabolism and its antioxidant properties.

In view of the evidence presented, it is imperative to undertake a study to elucidate the cellular calcium metabolism of passion fruit treated with $CaCl_2$ stored at room temperature. The objective of this study was to predict the principal physical and biochemical parameters involved in calcium metabolism and the regulation of fruit senescence, as well as develop an environmentally friendly and efficient preservation method to enhance the quality of passion fruit and extend its storage period. In light of these findings, the obtained results could provide theoretical support for the application of $CaCl_2$ treatment in the field of postharvest fruit preservation.

MATERIALS AND METHODS

Sample preparation and treatment

The purple passion fruit (*Passiflora edulis* var. Tainong No. 1) was harvested from the passion fruit plantation of Wanhe Agricultural Development Group Co., Ltd. (Guilin, Guangxi, China). The egg-shaped fruit was selected based on the following criteria: uniform coloration, absence of mechanical damage, and absence of pests. All fruits exhibited a similar degree of maturity, characterized by a purplish-red peel. The fruits were randomly divided into two groups, with 220 fruits in each group. One group served as the control and was subjected to a 5-min. immersion in distilled water, while another group underwent a 5-min. immersion in a 5% $CaCl_2$ solution.

The fruit samples were positioned on a benchtop in a manner that avoided direct exposure to sunlight, with an ambient temperature of 22 °C and a relative humidity of 75 ±2% for 8 days. Ten fruits were selected from each group at two-day intervals to determine the physicochemical and antioxidant parameters. The entire fruit was subjected to analysis of texture and color. The fruit peels were collected and pulverized in liquid nitrogen, then stored at –80 °C before further analysis, which was conducted in three replicates.

Determination of mass loss rate, fruit appearance and color change. The initial mass of the passion fruit was recorded as M_1 (g). The masses of the fruit samples collected on Days 2, 4, 6, and 8 were recorded as M_2 (g). The weight changes were calculated in accordance with the equation reported in the literature [Xie et al. 2022]. The color of the passion fruit peel was determined using a computerized colorimeter (Sanenshi Technology Co., Ltd., Shenzhen, China), and the values of L*, a*, b*, c*, and h angle (h*) were measured and recorded. Each treatment comprised a sample of ten fruits. The scanning was conducted in triplicate on the front, side, and back of the fruit. Additionally, the difference in the peel color (ΔE) was also calculated in accordance with the methodology described previously [Xie et al. 2022].

Determination of membrane permeability. The membrane permeability assay was conducted using the relative conductivity method for passion fruit peels, in accordance with the literature with certain modifications [Ali et al. 2020]. Five tissue discs of uniform thickness and size were obtained from each peel for the analysis. The initial reading of the untreated tissue disc (L_1) was measured using a digital conductivity meter after 60 min, and the reading of the tissue disc that was boiled in a water bath for 20 min

(L_0) was also measured. The relative conductivity was calculated using the following equation:

$$Y\ (\%) = \frac{L_1}{L_0} \times 100$$

Determination of MDA content. The MDA content was determined using the thiobarbituric acid (TBA) colorimetric method [Nie et al. 2020, Xu et al. 2023] with certain modifications. Briefly, 2.0 g of passion fruit peel powder was obtained and combined with a 10 mL solution of 10% precooled trichloroacetic acid (TCA) solution before vortexing for 30s. The absorbance was then measured at 450 nm, 532 nm, and 600 nm using Youke L3 IoT Visible Spectrophotometer (Shanghai Yoke Instrument Co., Ltd., Shanghai, China) to calculate the MDA content.

Determination of total phenolics, flavonoids, and ascorbic acid content. The total phenolic content (TPC) was determined in accordance with the method described previously by Nam et al. [2019]. An amount of 2.0 g of passion fruit peel powder was weighed and extracted with 10 mL of 60% ethanol for a period of 2 h. Following this, the solution was centrifuged at 1000 rpm for 15 min. The resulting supernatant was utilized to determine the TPC. Briefly, 0.5 mL of the supernatant was transferred to a 25-mL test tube, followed by the addition of 1.3 mL of distilled water, 0.2 mL of Folin-Ciocalteu reagent, and 2.0 mL of a 7% sodium bicarbonate solution. The absorbance was determined at a wavelength of 760 nm. The TPC was expressed as milligrams (mg) of gallic acid equivalent per gram (g) of sample.

The flavonoid content was determined using the aluminum ion colorimetric method with certain modifications [Li et al. 2015]. In brief, 0.5 mL of the supernatant was added to a 25-mL test tube, followed by the addition of 2 mL of deionized water and 0.15 mL of a 5% sodium nitrite solution. Subsequently, 0.15 mL of a5 % aluminum nitrate solution was added after a 5-min. incubation period at room temperature. This was followed by the addition of 1 mL of a 1 M sodium hydroxide solution after a further 5 min. Subsequently, the mixture was diluted with 70% ethanol, after which the absorbance was measured at 415 nm. The flavonoid content was expressed as mg of quercetin acid equivalent per g of extract.

The ascorbic acid content was determined by mixing 2.0 g passion fruit peel powder with 50 g L^{-1} trichloroacetic acid (TCA) solution and incubating the mixture at room temperature for 10 min. Following homogenization, 1 mL of the extract was added to 1.0 mL of a mixture comprising 50 g L^{-1} TCA solution and 0.5 mL of 0.4% phosphoric acid-ethanol solution. The absorbance was determined at a wavelength of 534 nm.

Determination of peroxidase (POD) and polyphenol oxidase (PPO) activities. The POD and PPO enzymes were extracted from the fruit peel powder via a process of mixing the 2.0 g peel powder with 10 mL of a 0.1 M extraction buffer solution of pH 5.5 (containing 4% polyvinylpyrrolidone, 1% Triton, and 1 mM polyethylene glycol). Following homogenization, centrifugation was performed, and the resulting supernatant was analyzed for POD and PPO activities. The POD activity was determined in accordance with the methodology described by Zhang et al. [2022]. The PPO activity was determined in accordance with the methodology outlined by Xu et al. [2022].

Determination of different forms of calcium. The selected forms of calcium present in the passion fruit peels following the $CaCl_2$ treatment were extracted using the stepwise extraction method, as described by Dong et al. [2004], with certain modifications. The water-soluble calcium, calcium from calcified pectin, calcium phosphate, and calcium oxalate were extracted using deionized water, a 1 M NaCl solution, a 2% acetic acid (HAC) solution, and a 5% HCl solution, respectively. A quantity of 5.0 g passion fruit peel powder was added to a 30-mL extraction solvent. The sample solution was left to stand in a water bath at a constant temperature of 30 °C for 18 h, after which it was centrifuged for 10 min. The supernatant was then transferred to a 100-mL volumetric flask, and the aforementioned procedure was repeated three times. Subsequently, the corresponding extract was diluted to 100 mL.

Statistical analysis. All experiments were repeated three times, and the results were averaged to obtain a mean value. The relative error was calculated. SPSS version 25 software was used to analyze the data based on the one-way analysis of variance, correlation, and principal component analysis. Origin 8.5 software was used to plot images and graphs. A *P*-value of less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Changes in fruit appearance, mass loss rate, and color attributes

The physiological metabolism of postharvest passion fruit results in water loss and fruit shrinkage. As illustrated in Fig. 1A, the fruit samples treated with $CaCl_2$ exhibited a superior fruit appearance relative to the control group. By the fourth day of storage, the control group exhibited noticeable wrinkling and shrinkage of the fruit surface. The fruit treated with $CaCl_2$ exhibited a smooth peel surface on the fourth day of storage. The surface of the control fruit peels exhibited an uneven and cracked texture. Additionally, browning of the peel was observed.

On the eighth day of the study, the fruit samples in the treatment group exhibited a slightly superior appearance in comparison to the control group. The rationale behind this phenomenon is that Ca^{2+} transporters regulate the endogenous Ca level, which in turn maintains membrane integrity [Sangsoy et al. 2024]. The findings of this study are in line with the existing literature, which indicates that grapes treated with $CaCl_2$ have an extended shelf life compared to the control samples [Fu et al. 2021]. The findings of the current study demonstrated that the mass loss rates of the fruit samples exhibited a continued increase with the extension of storage time. At the end of the study (Day 8), the $CaCl_2$ treatment exhibited a statistically significant reduction in the mass loss rate of passion fruit in comparison to the control group ($P < 0.05$). This indicated that the treatment could effectively retard the loss of moisture in passion fruit. Consequently, the Ca ion was observed to play a crucial role in maintaining the surface structure of the fruit peel.

A previous study demonstrated that a 2% $CaCl_2$ treatment of the red passion fruit stored at a temperature of 20 °C exhibited a significantly lower mass loss rate in comparison to the control fruit sample [Xu et al. 2023]. In contrast, Venâncio et al. [2013] demonstrated that the yellow passion fruit treated with 2% $CaCl_2$ and stored at a temperature of 21 ±2 °C did not exhibit any significant changes in the fruit's fresh weight. Given the inferior efficacy of the 2% $CaCl_2$ treatment in maintaining the mass of the passion fruit, a higher $CaCl_2$ concentration is required to achieve a more pronounced preservation effect. In this study, a 5% $CaCl_2$ treatment was employed, and the results were found to be favorable.

The color attributes of the passion fruit samples are shown in Fig. 2. The findings indicated that extended storage of passion fruit resulted in a reduction in L* and b* values for the fruit peel (Fig. 2A and Fig. 2C). However, these values exhibited an increase after four days of storage. From Day 4 onwards, there was a slight increase in the L* and b* values for the

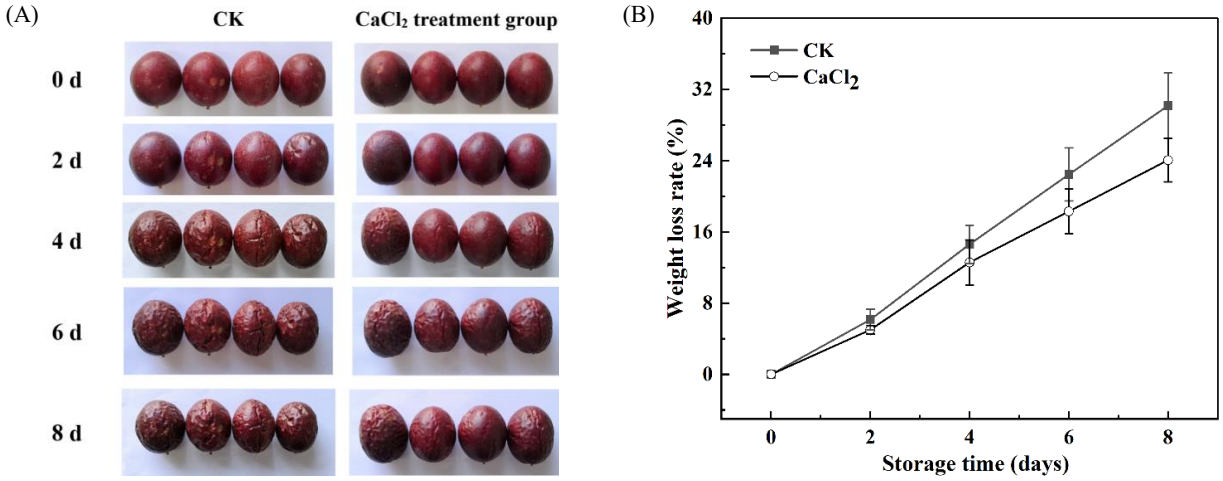

Fig. 1. Appearance of passion fruit (A) and mass loss rates (B) during postharvest storage. The letter "d" is used to denote the day of storage; CK – control group

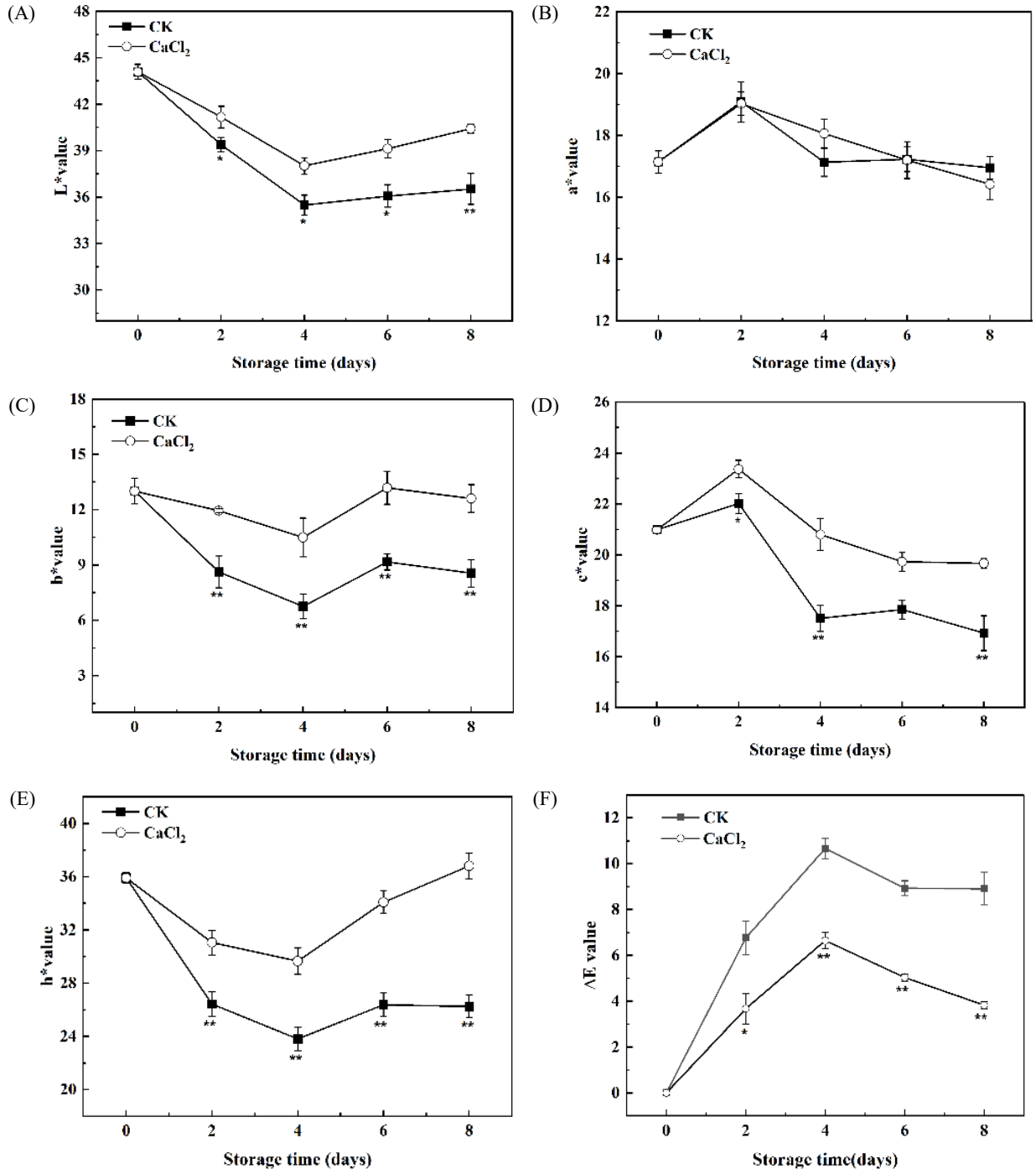

Fig. 2. Effect of $CaCl_2$ treatment on peel color (A) L*, (B) a*, (C) b*, (D) c*, (E) h*, and (F) ΔE values of the passion fruit peels. CK – control group

fruit peel. These findings are consistent with those of a previous study by Sati and Qubbaj [2021], which found that $CaCl_2$-treated tomatoes exhibited significantly higher L* and b* values than their non-treated counterparts. The $CaCl_2$-treated passion fruit exhibited a statistically significant increase in L* and b* values ($P < 0.05$). The b* values of the Ca^{2+}-treated fruit peel stored for six and eight days were not found to differ significantly from the baseline ($P > 0.05$). The treatment was unable to affect the color lightness (L* value) of the fruit peel but was successful in maintaining its yellow hue (b* value). In accordance with the findings of this study, the literature indicates that the L* and b* values of the mulberries treated with 1% $CaCl_2$ exhibited a downward trend, yet both remained higher than those of the control group [Oz and Ulukanli 2014].

The a* values of the fruit peel remained unaltered by $CaCl_2$ treatment. No statistically significant differences were observed in the a* values between the treatment and control groups (Fig. 2B). Additionally, the data indicated a modest elevation in the a* value on the second day of the storage. The results demonstrated that the ripening of passion fruit was expedited on the second day of storage at 22 °C, accompanied by a slight enhancement in the redness of the peel during the ripening process. Similarly, the c* values of the fruit peels in both treatment and control groups exhibited a notable increase on Day 2, followed by a pronounced decline until Day 4 of the storage period ($P < 0.05$). The c* values of the fruit peels in these groups exhibited no significant change from Day 4 to Day 8 (Fig. 2D). However, the $CaCl_2$ treatment group exhibited significantly higher c* values than the control group ($P < 0.01$), particularly on Days 4 and 8 of the storage period. In a previous study, no significant difference in the a* value of red pitaya was observed between the $CaCl_2$-treated and control groups, although a significant difference was noted in the c* value [Suttanew et al. 2022].

The h* values of the fruit peels exhibited a downward and upward trend (Fig. 2E). The hue angles of the peel samples exhibited a marked decline until Day 4 period, followed by a gradual increase until Day 8. The treatment group exhibited an increase in the h* until Day 8. The value was slightly higher than the baseline value. In the control group, a slight increase in the hue angle, with the increment not exceeding the value, was reported for Day 4 ($P > 0.05$). In contrast, the literature indicates that no statistically significant differences were observed in the h* values ($P > 0.05$) of red pitaya peels between the treatment and control groups, with the exception of the fruit treated with 1.5% $CaCl_2$, as reported by Suttanew et al. [2022].

The findings indicated that extended storage of the passion fruit resulted in accelerated ripening and decay, leading to an increase in the ΔE value of the control group (Fig. 2F). The total color difference was particularly notable for the samples exhibiting decay, with the value reaching its peak on Day 4 of the storage period. The $CaCl_2$ treatment resulted in a statistically significant reduction in the ΔE value ($P < 0.01$). The findings revealed that passion fruit treated with $CaCl_2$ exhibited a markedly reduced total color difference in the peel when compared to the control fruit sample.

The literature reveals contradictory findings regarding the color values determined for the postharvest preservation of various fruit samples treated with different chemicals, including $CaCl_2$, melatonin, and 1-methylcyclopropene (1-MCP). Some studies reported no significant changes in the color parameters, whereas others demonstrated a significantly different color between the treatment and control groups. A previous study of passion fruit treated with 2% $CaCl_2$ for 5 min revealed a significant difference in the L* value between the treatment and control groups [Xu et al. 2023]. Furthermore, the impact of $CaCl_2$ on the postharvest preservation of other fruit varieties has been documented in the scientific literature. However, the most recent study did not determine the color parameters of the studied fruits. As reported by Venâncio et al. [2013], the color variation of the yellow passion fruit peel treated with $CaCl_2$ did not undergo any notable changes during a 16-day storage period at 21 °C. Moreover, Suttanew et al. [2022] demonstrated that the color values of the 0.5–2.0% $CaCl_2$-treated red pitaya remained unchanged, with the exception of one reported instance.

The previous literature has documented the use of multiple chemical agents in the postharvest preservation of passion fruit. The application of 1-MCP in the postharvest preservation of passion fruit has been demonstrated to result in superior maintenance of the peel color of the fruit [Dutra et al. 2018]. Another study reported the use of melatonin (0.1–0.4 M) for the post-

harvest preservation of yellow (golden) passion fruit. The findings revealed that there were no notable alterations in the color parameters between the treatment and control groups, with the exception of the b* value for the control group. The reported b* value of the control group at the conclusion of the study (Day 8) was significantly lower than all treatment groups [Cai et al. 2024].

A higher b* value indicates a greater yellow hue of the yellow passion fruit [Xu et al. 2023], whereas a high a* value denotes a superior red hue [Xie et al. 2022]. Therefore, the treatment was effective in maintaining the yellow hue of the yellow passion fruit. However, the findings of the current study indicated that notable discrepancies in the color parameters were observed between the treatment and control groups at the end of the study, with the exception of the a* value. Furthermore, da Silva and Vieites [2021] indicated that the sweet passion fruit (*P. alata* Dryander) treated with 1% $CaCl_2$ was the most effective method for postponing alterations in peel color.

Physicochemical properties of passion fruit samples

During the storage period, the membrane permeability of the passion fruit peels in the $CaCl_2$ treatment and control groups exhibited an upward trend (Fig. 3A). The initial decrease in membrane permeability of the fruit peels in the $CaCl_2$-treated and control groups was observed on Day 2 of the storage period. The respective values of membrane permeability were 23.74% and 18.15%. The membrane permeability of the peel samples exhibited a gradual increase until the end of the study (Day 8). The increase exceeded 50%. The $CaCl_2$ treatment resulted in a notable reduction in membrane permeability of passion fruit peels in comparison to the control samples. The findings suggest that the $CaCl_2$ treatment may mitigate the physical damage to passion fruit peels resulting from elevated respiration rates under RT conditions. These findings are also in line with a previous study that demonstrated the efficacy of 4% $CaCl_2$ in preserving the cell membrane integrity of Chinese winter jujube fruit subjected to chilling stress [Wei and Zhao 2020]. Furthermore, Zhang et al. [2019a] demonstrated that a 2% $CaCl_2$ treatment had an inhibitory effect on the elevation of MDA in the peel tissues of pears stored at 20 °C for 150 days.

MDA is the final product of lipid peroxidation, and it is used as an indicator of oxidative damage to fruit cells [Valenzuela et al. 2017]. The MDA content of the $CaCl_2$ treatment and control groups exhibited fluctuations during the storage period, with both groups reaching maximum values on the sixth day. On the second day of the storage period, the protective effect of $CaCl_2$ in the fruit peels was not statistically significant ($P > 0.05$). The reduction in MDA content was markedly less pronounced in the treatment group than in the control group. The MDA content of the untreated fruit samples remained constant after the sixth day of storage. It is possible that the Ca formed a defensive layer on the passion fruit peel, thereby reducing cellular oxidative damage and decreasing the accumulation of MDA in the fruit peel [Wang et al. 2023].

The phenolic compounds represent a significant nutrient in passion fruit, as evidenced by Fonseca et al. [2022]. As illustrated in Fig. 3C, the TPC of the fruit peels in the $CaCl_2$ treatment and control groups exhibited an upward trend. However, the TPC of the non-treated fruit peels demonstrated a slight decline on the second day of storage, followed by a gradual increase during the ripening process. The TPC of the fruit peels in the $CaCl_2$ treatment group also exhibited a significant increase on Day 4 ($P < 0.01$), and it remained constant thereafter. Furthermore, no statistically significant difference was observed in the TPC of the fruit peels between the treatment and control groups on Day 8 ($P > 0.05$). The elevated TPC suggests that the $CaCl_2$ treatment may enhance the antioxidant profile of the fruit, thereby mitigating cellular oxidative stress [Madani et al. 2016].

As illustrated in Figs. 3C and D, the TPC and flavonoid content of the fruit peels exhibit a comparable trend, depicted by S-shaped curves. A notable discrepancy was observed in the flavonoid content of the fruit peels between the $CaCl_2$ treatment and control groups on Day 4 ($P < 0.05$). Furthermore, the data indicated that the $CaCl_2$ treatment did not result in a statistically significant alteration in the flavonoid content of the fruit peels. This finding is corroborated by the data obtained from peel color change, which revealed no alteration in the peel redness observed during the eight days of storage, with the exception of Day 4.

The scientific literature indicates that the exogenous melatonin treatment may influence flavonoid

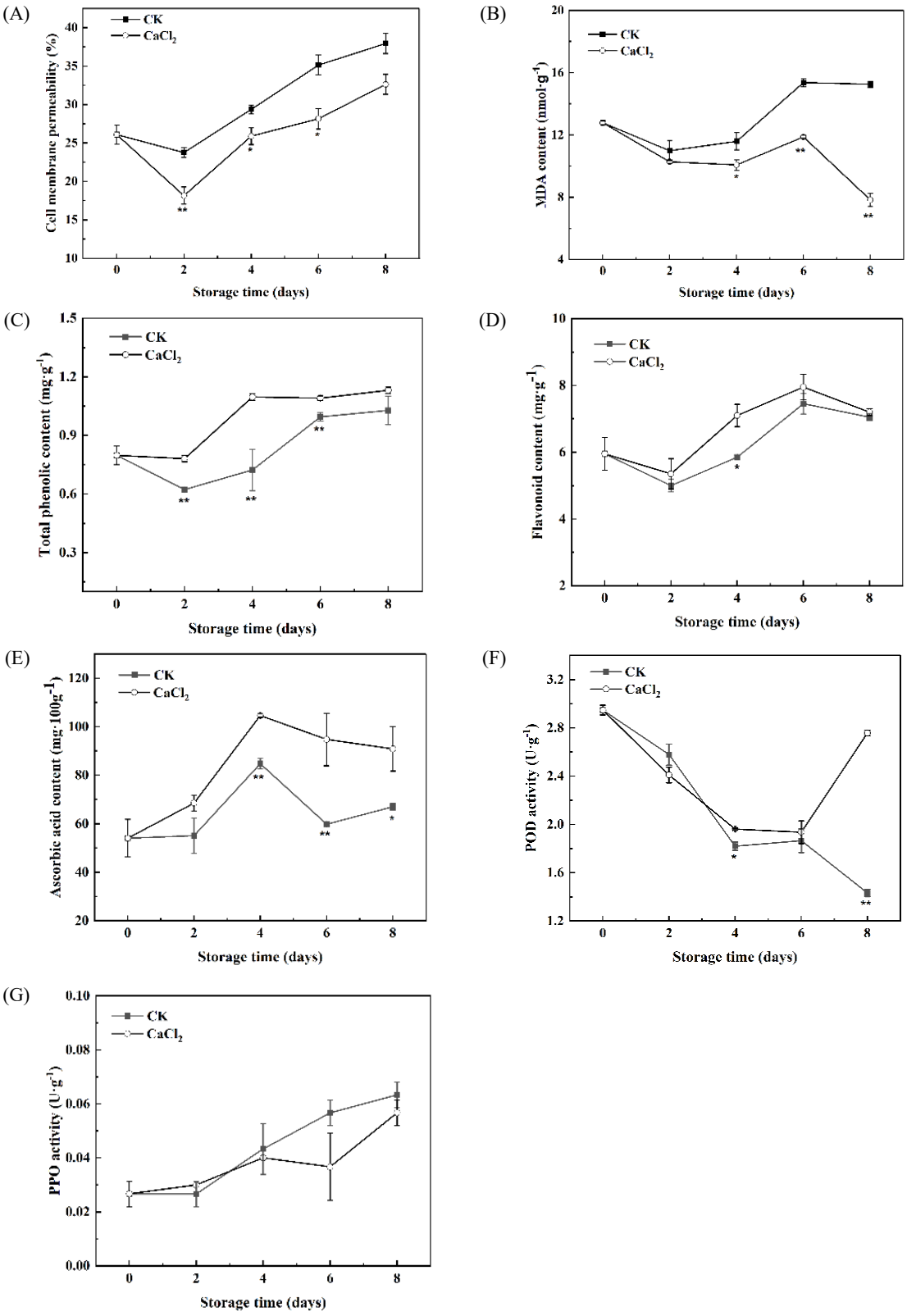

Fig. 3. Effect of $CaCl_2$ treatment on (A) cell membrane permeability, (B) MDA, (C) total phenolic content, (D) flavonoid content, (E) ascorbic acid content, (F) POD activity, and (G) PPO activity of the passion fruit peels. CK – control group

biosynthesis in the fruit during storage, thereby enhancing the accumulation of anthocyanin in the fruit [Xu et al. 2014]. The rapid reduction of total flavonoid content in the ripening fruit may be attributed to the accumulation of anthocyanin in the fruit during ripening, whereby flavonoids are converted into anthocyanins [Pervaiz et al. 2017]. The total flavonoid content in melatonin-treated leaves was found to be higher than that observed in the control.

The ascorbic acid content of the fruit peels in the $CaCl_2$ treatment and control groups exhibited a gradual increase from Day 0 to Day 4, reaching a maximum level (Fig. 3E). The control samples exhibited a decline in ascorbic acid content from Day 4 to Day 6, followed by a slight increase. In contrast, the ascorbic acid content of the fruit peels in the $CaCl_2$ treatment group exhibited a decline from Day 4 to Day 8. Nevertheless, the ascorbic acid content in the treatment group was markedly higher than that of the control group from Day 4 to Day 8 of the storage period ($P < 0.05$). The diminished ascorbic acid concentration observed in the control group may be attributed to the rapid oxidation of this compound in the ripe fruit, as previously documented by Markus et al. [1999]. Ascorbic acid plays a pivotal role in regulating fruit ripening and associated color changes. The acid functions as a catalyst and cofactor in the biosynthesis of fruit color pigments [Arabia et al. 2024]. The compound plays a role in cellular metabolism and the senescence of the fruit, exerting regulatory effects on ethylene production and abscisic acid (ABA) signaling. Furthermore, ABA has been demonstrated to facilitate the synthesis of ethylene in fruit tissue [Kou et al. 2021].

Enzyme activities of passion fruit samples

The POD activity of the fruit peels in the treatment and control groups exhibited a gradual decline until Day 4 of the storage period (Fig. 3F). The $CaCl_2$ treatment resulted in an enhancement of POD activity from Day 6 to Day 8. However, the enzyme activity in the control group exhibited a continuous decline until Day 8 of the storage period. On the last day of the storage period, the fruit peels treated with $CaCl_2$ exhibited a markedly elevated POD activity in comparison with the control sample ($P < 0.01$). The findings suggest that the $CaCl_2$ treatment effectively preserves the POD activity in the passion fruit peels. As documented in the literature, alterations in POD activity in fruit are indicative of senescence or quality deterioration [González-Gordo et al. 2023]. POD is capable of eliminating surplus ROS in postharvest fruit, thereby preserving its robust antioxidant capacity.

Another enzyme that causes enzymatic browning in fruits is PPO. It has been demonstrated that this enzyme can catalyze the oxidation of phenolic substances into quinone compounds, which are then further polymerized to form brown or black polymers, resulting in tissue browning [Nath et al. 2022]. As illustrated in Fig. 3G, the PPO activity of fruit peels in the $CaCl_2$ treatment and control groups exhibited a continuous increase during the eight-day storage period. The postharvest storage of passion fruit in the control group demonstrated a notable increase in PPO activity, reaching approximately 1.4 fold the initial level. In contrast, the $CaCl_2$ treatment resulted in a relatively modest elevation of PPO activity, reaching only 1.1 fold the initial level. The treatment resulted in a delay in PPO activity in the fruit peels during the eight days of postharvest storage, thereby inhibiting the oxidative browning of the fruit peels [Xie et al. 2022].

Calcium content in passion fruit samples

The diverse forms of calcium content present in the fruit peels of $CaCl_2$ treatment and control groups are illustrated in Fig. 4. Fluctuating trends for the four Ca forms were observed. The water-soluble calcium and calcium oxalate (Figs. 4A and D) in the experimental samples demonstrated a decline in concentration over the course of the eight-day storage period. Conversely, the concentration of calcified pectin in the fruit peels extracted using strong alkali exhibited an upward trend (Fig. 4B). The $CaCl_2$ treatment resulted in a notable increase in the concentrations of these Ca forms on Day 8 of the study ($P < 0.01$), with the exception of calcium oxalate, which exhibited no statistically significant change ($P > 0.05$). The calcium oxalate content of the fruit peels in the treatment group was significantly lower than that of the control group from Day 4 to Day 8 of the storage period ($P < 0.01$).

A previous study demonstrated that the application of $CaCl_2$ solution at varying concentrations to papayas resulted in a notable enhancement in the Ca content of the fruit pulp and peel, with the exception of concentra-

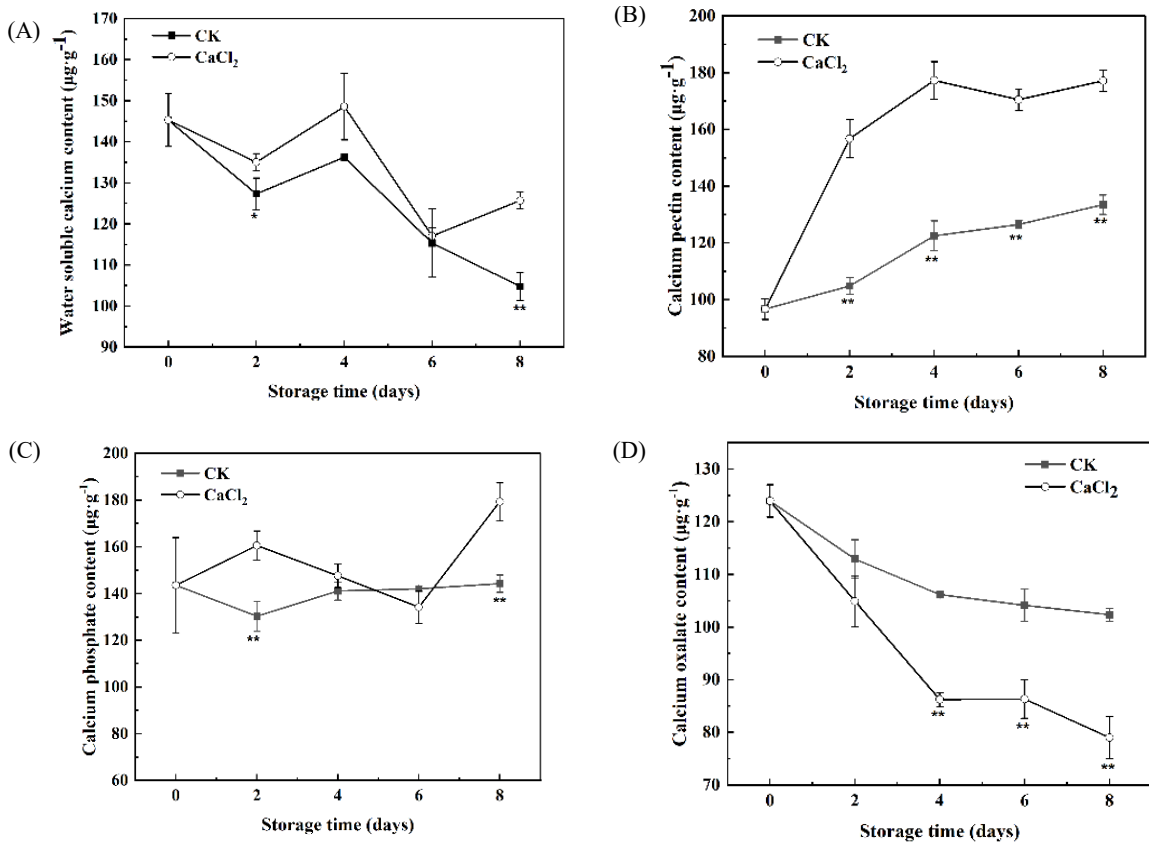

Fig. 4. Effect of $CaCl_2$ treatment on (A) water-soluble calcium, (B) calcified pectin, (C) calcium phosphate, and (D) calcium oxalate content of the passion fruit peels

tions below 1% [Madani et al. 2016]. Calcified pectin, which is one of the primary forms of calcium bound to the cell wall of the fruit peel, has been demonstrated to enhance the peel's hardness and maintain its firm texture [Chen et al. 2023]. As illustrated in Fig. 4B, the calcified pectin content of the fruit peels in the $CaCl_2$ treatment and control groups demonstrated an upward trajectory. To the best of the authors' knowledge, no previous study has determined the Ca content of the calcified pectin in passion fruit peel. The Ca content of calcified pectin in the pear peel treated with 2% $CaCl_2$, as reported in the literature [Kou et al. 2015], and the passion fruit peels of this study exhibit a comparable trend. The concentration of calcified pectin in the fruit samples treated with $CaCl_2$ from both studies demonstrated an upward trend.

The calcium phosphate content of the fruit peel in the $CaCl_2$ treatment and control groups exhibited fluctuations over the eight-day storage period (Fig. 4C). No statistically significant differences were observed in the calcium phosphate content between the treatment and control groups during the storage period, with the exception of Day 2 and Day 8 ($P > 0.05$). The $CaCl_2$ treatment resulted in a statistically significant increase in the calcium phosphate content of the passion fruit peels on Day 2 and Day 8 ($P < 0.01$). The $CaCl_2$ treatment was observed to effectively increase the calcium phosphate content of the fruit peels, in addition to the water-soluble calcium and calcified pectin content, on Day 8 of the storage period. A 24.32% increase in the calcium phosphate content was observed in the $CaCl_2$ treatment group in comparison to the control group.

As illustrated in Fig. 4D, the calcium oxalate content of the fruit peels in the $CaCl_2$ treatment and control groups exhibited a decline from 123.95 μg g^{-1} to 102.34 and 78.99 μg g^{-1}, respectively. The findings suggest that the $CaCl_2$ treatment may be effective in reducing oxalate accumulation in the peel of postharvest passion fruit. The $CaCl_2$ treatment group, stored for 4–8 days, exhibited a significantly lower calcium oxalate content than the control group. However, the literature indicates that the 2% $CaCl_2$ treatment effectively increased the calcium oxalate (HCl-soluble Ca) content in the pear fruit peels [Kou et al. 2015]. Furthermore, the scientific literature indicates that fruit firmness and softening are attributed to the distinct forms of Ca present in the fruit peel [Deng 2008]. In light of these findings, it can be concluded that different types of endogenous Ca play a role in maintaining fruit freshness, including the regulation of cell wall metabolism and fruit hardness.

Correlation and principal component analysis

A correlation analysis of the physiological indexes of the postharvest passion fruit is presented in Table 1. The mass loss rate of the passion fruit exhibited a significant and positive correlation with ΔE, membrane permeability, total phenol content, flavonoid content, PPO activity, and calcified pectin content. However, the mass loss rate of the fruit sample was significantly and negatively correlated with the POD activity, water-soluble Ca, and Ca oxalate content. The findings indicate that the reduction in the water-soluble Ca and Ca oxalate levels has a direct impact on the weight of the passion fruit peel. Given that these forms of Ca are soluble in water, the loss of moisture in the fruit peel results in a reduction in the retention of water-soluble Ca in the peel. Similarly, the fruit stored from Day 0 to Day 6 exhibited a reduction in POD activity, which could be attributed to the low endogenous water activity.

Conversely, the water-soluble calcium content of the passion fruit peels was significantly and positively correlated with the POD activity and significantly and negatively correlated with mass loss rate, ΔE, membrane permeability, flavonoid content, PPO activity, and MDA content. The correlation analysis revealed a number of significant correlations between different physiological indexes of the passion fruit treated with $CaCl_2$ that may reflect the storage quality of the Ca-treated passion fruit. The majority of the parameters determined were not directly related to the water-soluble calcium in the fruit peels, with the exception of the POD and PPO activities. The primary reason is that free Ca plays a role in regulating the activity of endogenous enzymes. As documented in the literature, alterations in the intracellular Ca^{2+} distribution may influence endogenous PPO activity, whereas the POD enzyme responds to cellular oxidative stress [Sangsoy et al. 2024]. The markedly elevated POD activity observed on Day 8 of the study may be attributed to the high antioxidant levels present in the passion fruit peels, which appear to have suppressed the production of MDA in the peels.

Principal component analysis (PCA) is employed to ascertain the relationship between the Ca-treated and control groups, with the resulting data visualized in plots [Langsrud and Næs 2003]. Three principal components with eigenvalues exceeding 1 were identified (see Table 2). The contribution rates of these three principal components were found to be 47.08%, 24.57%, and 9.99%, respectively. The cumulative variance contribution rate was 81.64%. This represented the majority of the information present within each principal component. As illustrated in Fig. 5A, the factor loading diagrams indicate that the absolute values of the mass loss rate, PPO activity, and flavonoid content from the Y-axis are notably high. These variables contributed the most to the first principal component. The MDA content exhibited the greatest absolute value on the X-axis. It contributed the most to the second principal component. The absolute value of ΔE from the PC3 axis (9.99%) (X-axis) exhibited the greatest discrepancy. It contributed the most to the third principal component. The load value of ΔE exhibited minimal discrepancy between the second and third components when the load matrix was combined. This suggests that ΔE exerts a synergistic effect on the second and third principal components.

The PCA effectively differentiated the biochemical and physicochemical indicators of the Ca-treated and control groups. The factor loading diagrams indicate that the biochemical and physicochemical parameters, which were clustered at the same quartile, exhibited a similar trend in the preservation of passion fruit by the $CaCl_2$ treatment. The mass loss rate, membrane permeability, flavonoid, PPO, and ΔE value were the

Table 1. Correlation analysis between various physiological indicators

Physiological index	Mass loss rate	ΔE	MP	MDA	Total phenolics	Flavonoid	Ascorbic acid	POD	PPO	Water soluble Ca	Calcified pectin	Ca phosphate	Ca oxalate
Mass loss rate	1.000												
ΔE	0.641**	1.000											
MP	0.793**	0.456**	1.000										
MDA	0.187	0.241	0.474**	1.000									
Total phenolics	0.624**	0.100	0.509**	–0.041	1.000								
Flavonoid	0.704**	0.246	0.630**	0.135	0.831**	1.000							
Ascorbic acid	0.379*	0.270	0.096	–0.505**	0.607**	0.497**	1.000						
POD	–0.704**	–0.844**	–0.498**	–0.424**	–0.357*	–0.456**	–0.365*	1.000					
PPO	0.854**	0.534**	0.767**	0.219	0.517**	0.580**	0.178	–0.568**	1.000				
Water soluble Ca	–0.746**	–0.487**	–0.639**	–0.404*	–0.299	–0.432**	0.064	0.544**	–0.630**	1.000			
Calcified pectin	0.482**	0.187	0.040	–0.515**	0.729**	0.507**	0.823**	–0.333*	0.336*	–0.159	1.000		
Ca phosphate	0.170	–0.195	0.058	–0.443**	0.337*	0.135	0.306*	0.211	0.217	0.113	0.430**	1.000	
Ca oxalate	–0.634**	–0.330*	–0.266	0.462**	–0.734**	–0.588**	–0.800**	0.381*	–0.502**	0.312*	–0.921**	–0.293	1.000

* a statistically significant difference between the two treatment groups ($P < 0.05$); ** the difference between the two treatment groups is statistically significant at $P < 0.01$; Ca – calcium; MDA – malondialdehyde; MP – membrane permeability; POD – peroxidase; PPO – polyphenol oxidase

primary attributes responsible for the differentiation between the Ca-treated and control groups. The key attributes contributing the most to the $CaCl_2$ treatment were ascorbic acid, TPC, calcified pectin, and Ca phosphate. In contrast, water-soluble Ca and POD contributed the least. The calcium oxalate and MDA content were the sole key attributes contributing to the control group.

As illustrated in the factor score plots (Fig. 5B), the scores of the physicochemical parameters of the fruit peels in the $CaCl_2$ treatment and control groups are markedly disparate from the point of Day 0. On Day 8, the control group contributed the most to PC1, while the treatment group contributed the most to PC2 on the same day. Moreover, the scores of the treatment group also deviated from the control group score. Furthermore, the quality of the passion fruit began to undergo a transformation from Day 4 onwards. The fourth day contributed the most to PC3, and the increase in ΔE demonstrated the most significant changes. The score plots clearly distinguished the selected biochemical and physicochemical properties of passion fruit peels between the $CaCl_2$ treatment and control groups. The differences in the bio-physicochemical indicators related to the Ca metabolism in the fruit peels between the two distinct groups are noteworthy.

Table 2. Percentage of principal component variance and cumulative contribution rate

Component	Initial eigenvalues			Extraction sums of squared loadings		
	Total	% of variance	Cumulative %	Total	% of variance	Cumulative %
1	6.120	47.080	47.080	6.120	47.080	47.080
2	3.194	24.571	71.651	3.194	24.571	71.651
3	1.299	9.989	81.640	1.299	9.989	81.640
4	0.858	6.601	88.241			
5	0.559	4.301	92.542			
6	0.363	2.795	95.337			
7	0.236	1.812	97.149			
8	0.162	1.248	98.397			
9	0.093	0.719	99.115			
10	0.056	0.433	99.548			
11	0.035	0.273	99.821			
12	0.014	0.110	99.931			
13	0.009	0.069	100.000			

Comprehensive evaluation score

The PCA yielded three principal component variables, designated PCA1, PCA2, and PCA3. The analysis was conducted based on the selected 13 bio-physicochemical indicators. Fig. 5C presents a comprehensive evaluation score of the postharvest storage quality of passion fruit. The comprehensive evaluation scores of the fruit peels in the $CaCl_2$ treatment and control groups demonstrated an upward trend. Although the scores of the control group exhibited a slight decline on the second day of storage, they subsequently demonstrated a gradual increase. The score of the $CaCl_2$ treatment group was found to be significantly higher than that of the control group ($P < 0.01$). Furthermore, the discrepancies in these bio-physicochemical indicators can be more effectively discerned through the use of comprehensive evaluation scores. The scores indicated that the $CaCl_2$ treatment of passion fruit exhibited markedly superior preservation characteristics in comparison to the control fruit sample. The 5% $CaCl_2$ treatment was observed to have a more pronounced effect on the alteration of ascorbic acid, total phenolics, calcified pectin, Ca phosphate, water-soluble Ca, and POD activity in the fruit peel sample in comparison to the control sample. The findings suggest that the $CaCl_2$ treatment delayed the onset of senescence in passion fruit and exerted a beneficial influence on the maintenance of storage quality in postharvest passion fruit.

CONCLUSION

$CaCl_2$ treatment represents an efficacious and environmentally friendly approach to the postharvest preservation of passion fruit, effectively extending its shelf life by several days. The findings indicated that the Ca ion regulated cellular metabolism in the passion fruit peels undergoing the senescence process. The treatment group exhibited superior efficacy compared to the control group, as evidenced by a reduction in mass loss, diminished peel membrane permeability, augmented antioxidative status, and sustained peel brightness and hue. The 5% $CaCl_2$ treatment resulted in a superior appearance and prolonged freshness of passion fruit at room temperature of 22 °C for up to eight days. The correlation analysis indicated that the Ca^{2+} treatment was associated with the regulation of specific biochemical and physicochemical properties

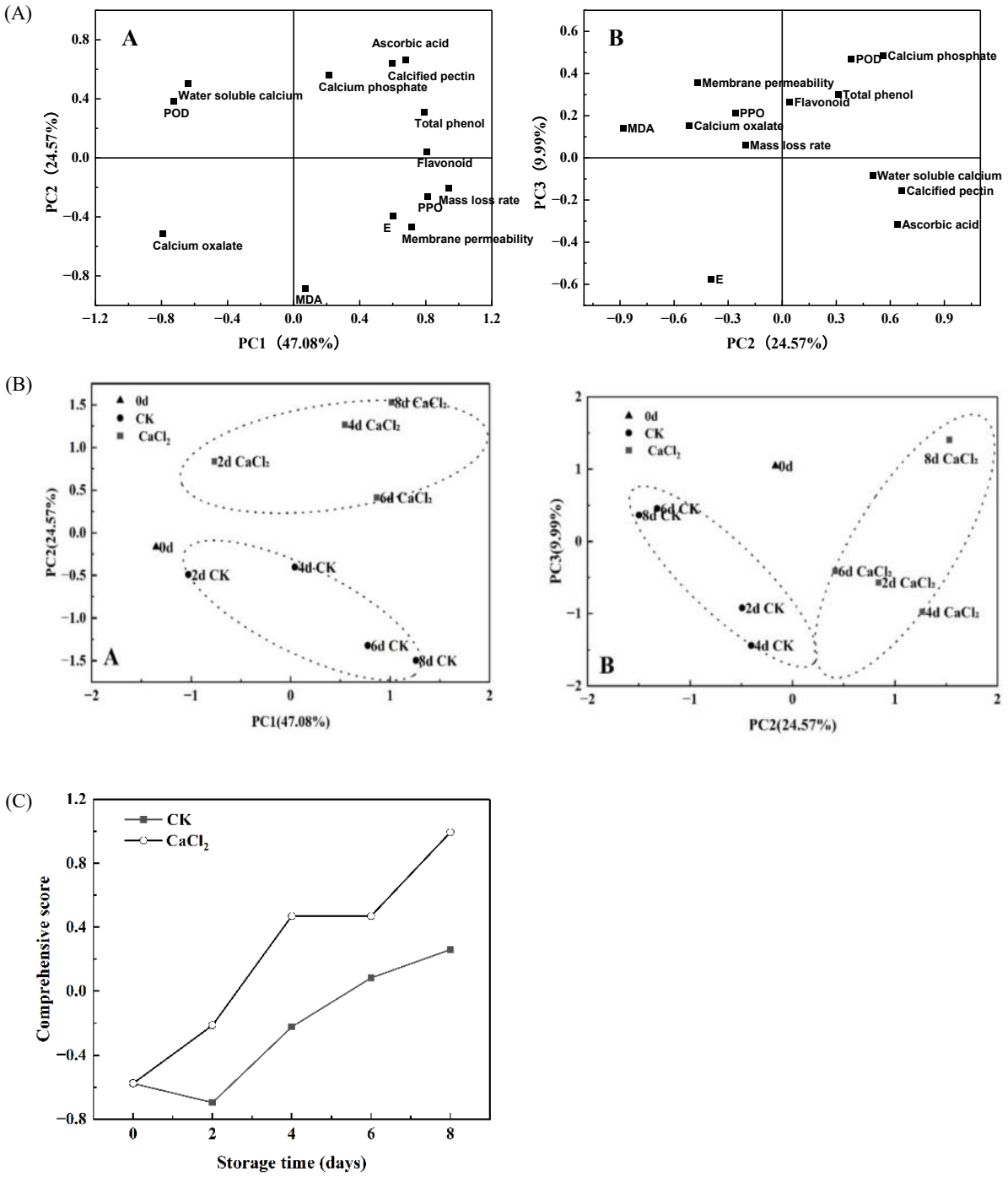

Fig. 5. Factor load diagrams (A), score plots (B), and comprehensive evaluation scores (C) of the selected biochemical and physicochemical properties of passion fruit peels

related to the Ca metabolism in passion fruit peels during the process of fruit senescence. The findings of this study provide a theoretical foundation for the postharvest preservation of passion fruit through the application of $CaCl_2$.

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