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Investigating on the inhibition of high-maturity mango storage diseases by antimicrobial Peptide PNMGL2 through induction and suppression of reactive oxygen metabolism

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Abstract

High-maturity mangoes are highly susceptible to decay during postharvest storage, posing significant challenges for preservation. This study investigated the effects of the antimicrobial peptide PNMGL2 on reactive oxygen species (ROS) metabolism and postharvest disease incidence in mangoes. Mangoes were treated with 0.25 mg/L, 0.50 mg/L, and 0.75 mg/L PNMGL2 in combination with a 2 g/100 mL chitosan coating. Results demonstrated that the combination of chitosan and PNMGL2 significantly reduced anthracnose incidence compared to the untreated control (CK group). On the 12th day of storage, mangoes treated with 0.50 mg/L PNMGL2-chitosan exhibited higher firmness and titratable acid content than other groups. Furthermore, malondialdehyde (MDA), hydrogen peroxide (H_2O_2), and superoxide anion levels decreased by 23.76%, 17.02%, and 40.14%, respectively, while the activities of catalase (CAT), peroxidase (POD), glutathione peroxidase (GPX), and glutathione (GSH) increased by 18.73%, 35.42%, 52.58%, and 68.63%, respectively. The CH-0.50P treatment effectively mitigated ROS accumulation and delayed the decline in key antioxidant enzyme activities (SOD, CAT, APX, POD). This treatment not only preserved the quality and nutritional value of mangoes but also significantly prolonged their shelf life, providing valuable insights for the development of advanced mango preservation technologies.

Keywords: antagonistic preservation; mango anthracnose; storage; reactive oxygen species; glutathione pathway

Introduction

With the advancement of consumer preferences, there is a growing demand for high-quality fruits among consumers. "Tree-ripened" or "already-ripened" fruits are particularly favored due to their naturally mature flavor and enhanced nutritional value. Data indicates that in 2024, 36% of Chinese consumers intend to increase their expenditure on fresh food and health products, with fresh produce ranking first in spending priorities (Wang *et al.*, 2024). This reflects a growing consumer preference for natural and healthy food options, which is accelerating

the expansion of the tree-ripened fruit market. Tree-ripened fruits represent a specific category of agricultural products that are harvested at a defined maturity level. These fruits are typically picked when they reach at least 80% maturity, corresponding to the later stages of fruit development, during which key quality indicators—such as the sugar-acid ratio and aroma compounds—attain their optimal levels (Rodriguez *et al*., 2025). This preharvest ripening characteristic allows the fruit to maximally retain its unique flavor compounds and nutritional components; however, it also results in higher susceptibility of fully mature fruits to senescence-related physiological disorders and microbial-induced decay compared to less mature ones. Research indicates that near-fully mature fruits, owing to elevated cell wall-degrading enzyme activity and intensified respiratory metabolism, encounter significant challenges in postharvest storage and preservation during the logistics process (Shankar *et al*., 2024). The specific manifestation is that shelf life is markedly reduced. For example, kiwifruit can only be preserved for 7–14 days postharvest, while strawberries have a storage limit of less than 48 hours at room temperature (Chang *et al*., 2023). Sensitivity to mechanical damage increases, leading to common issues such as wilting, berry drop, and pathogen infection during grape storage, with decay rates exceeding 30% in some cases (Zhu *et al*., 2023). Microbial interactions become more pronounced, with spoilage microorganisms readily colonizing fruit surfaces and wounds. This not only accelerates quality deterioration but also poses potential safety risks, such as mycotoxin production (Shankar *et al*., 2024). The aforementioned issues directly result in the challenge of maintaining the quality of tree-ripened fruits throughout the supply chain. Consequently, investigating postharvest quality preservation techniques for tree-ripened fruits has emerged as a critical research focus within the domain of agricultural product storage and preservation.

Currently, mango preservation research predominantly centers on low-maturity fruits, whereas investigations into preservation techniques for high-maturity mangoes remain limited. The postharvest preservation of highly mature mangoes encounters significant challenges, with a strong correlation observed between their physiological traits and disease incidence. As typical climacteric fruits, mangoes undergo characteristic changes during ripening, marked by a pronounced rise in respiration intensity and a substantial increase in metabolic activity (Hewitt & Dhingra, 2020). This physiological process involves the conversion of sugars, degradation of organic acids, and synthesis and accumulation of characteristic aromatic compounds. Simultaneously, it creates favorable conditions for the proliferation of pathogenic microorganisms, thereby significantly increasing the incidence of postharvest diseases such as stem-end rot and anthracnose (Liu *et al*., 2022). Among these, anthracnose (*Colletotrichum gloeosporioides*) is the primary pathogen responsible for postharvest losses in mangoes (Li *et al*., 2024). Relevant studies indicate that the yield loss attributed to this pathogen can range from 30% to 60% (Worku *et al*., 2025). During postharvest storage and transportation, fruit rot caused by anthracnose is an important subject, severely impacting the commercial value and marketability of mangoes. The 14th Five-Year Plan for Promoting the Modernization of Agriculture and Rural Areas is identified as a key research focus within the Green Storage and Transportation Technology Research and Development (Wang *et al*., 2024). Consequently, the development of a specialized green antagonistic bacterial preservation technology tailored for the highly mature mangoes holds significant importance in reducing postharvest losses and enhancing the commercial value of the fruit.

Molinos *et al*. (2008) utilized Entercin AS-48 to preserve small berries, including mature strawberries, blackberries, and raspberries, which had been precontaminated with *Listeria monocytogenes*. The results demonstrated that treatment with Entercin AS-48 effectively suppressed *L. monocytogenes* in all tested berries. Liu *et al*. (2019) discovered that three insect-derived peptides—thanatin, ponericin W1, and mastoparan-S—could reduce the spoilage rate of mature citrus fruits by 20% to 30% while inducing shriveling and deformation of the mycelia of Xanthomonas citri. Huang *et al*. (2021) found that the recombinant *Pichia pastoris* antimicrobial peptides could reduce the disease incidence of mature pears by 42% (GS115/Ac-AMP2 [recombinant strain of *P. pastoris*]). However, research on the use of antimicrobial peptides for preserving mature mangoes remains limited, particularly in the optimization of techniques for controlling anthracnose, where in-depth investigation is warranted. In this study, *Lactobacillus plantarum* NMGL2, which exhibits broad-spectrum antibacterial and antifungal activities, was isolated from Inner Mongolia cheese. The combination of this strain with chitosan coating treatment effectively suppressed the development of anthracnose in mangoes. At the end of storage, mangoes treated with *L. plantarum* NMGL2 in conjunction with chitosan coating exhibited lower levels of soluble solids and ethylene release compared to the control group (Wang *et al*., 2024). Nevertheless, the specific substances within *L. plantarum* NMGL2 that contribute to the inhibition of *C. gloeosporioides* on mango surfaces remain unclear. Furthermore, the antibacterial peptide PNMGL2 was isolated and purified from this strain. Its molecular weight is 761.95 Da (Dalton) that has a specific LNFLKK sequence, which is highly hydrophilic, stable in solution, and has a random coil structure (Ren *et al*., 2023). This study utilized the antimicrobial peptide PNMGL2, which has well-defined structural

characteristics, as the antagonistic agent and mature mangoes as the research subject to examine the preservative and disease-prevention effects of PNMGL2. This investigation aims to further identify the specific antagonistic components that effectively inhibit anthracnose development in mangoes and to establish a theoretical basis for the future application of this peptide in the preservation of mature mangoes.

Material and Methods

Experimental materials and reagents

The Tainong No. 1 mango (Mangifera indica L.), which had reached commercial maturity (80% ripe), was sourced from Baise City, Guangxi. Immediately after harvest, the fruits were transported via air freight by SF Express and arrived at the laboratory in Beijing within 24 hours for experimental analysis. Upon arrival, all fruit samples were inspected, and those exhibiting diseases, physical damage, decay, or significant variation in maturity were excluded. The remaining samples were then allowed to rest for 12 hours under controlled conditions prior to the commencement of the experiment.

Hydrogen peroxide (H_2O_2) assay kit, superoxide anion detection kit, total antioxidant capacity (T-AOC) assay kit, 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity kit, superoxide dismutase (SOD) activity assay kit, catalase (CAT) activity assay kit, ascorbate peroxidase (APX) activity assay kit, peroxidase (POD) activity assay kit, glutathione reductase (GR) activity assay kit, A005-1 glutathione peroxidase (GSH-Px) activity assay kit, plant total phenol content assay kit, vitamin C/ascorbic acid (ASA) content assay kit, reduced glutathione (GSH) content assay kit, and malondialdehyde (MDA) content assay kit (Nanjing Jiancheng Bioengineering Research Institute Co., Ltd., Nanjing, Jiangsu, China).

Experimental instruments and equipment

LQ = C5002 Electronic Balance (Shenzhen Feiya Weighing Instrument Co., Ltd., Shenzhen, Guangdong, China), UV-1800P UV-Visible Spectrophotometer (Mapada Instruments Co., Ltd., Shanghai, China), HH-2A Electric Heating Constant Temperature Water Bath (Beijing Kewei Yongxing Instrument Co., Ltd., Taihu Town, Tongzhou Qu, China), TGL-16 gR High-Speed Refrigerated Centrifuge (Shanghai Yitian Scientific Instrument Co., Ltd., Shanghai, China), 101 Electric Heating Blower Drying Oven (Beijing Zhongxing Weiyang Century Instrument Co., Ltd., Chaoyang District, Beijing, China), LAC-350HPY-2 Artificial Climate Chamber (Shanghai Longyue Instrument Equipment Co., Ltd., Songjiang District, Shanghai, China), THZ-C Constant Temperature Shaker (Taicang Haocheng Experimental Instrument Manufacturing Co., Ltd., Shenzhen, Guangdong Province, China), DNW-9602 Enzyme Labeling Instrument (Beijing Plang New Technology Co., Ltd., Wangjingxilu, Beijing, China), A11 Basic Liquid Nitrogen Grinder (IKA Instrument Equipment Co., Ltd., Staufen im Breisgau, Germany), FX950 Ethylene Detector (Beijing Sunshine Yishida Technology Co., Ltd., Beijing, China), and Rapid-TA (Texture Analyzer) (Shanghai Tengba Instrument Technology Co., Ltd., Shanghai, China).

Experimental methods

Sample processing methods and group allocation

Preparation of PNMGL2 Antimicrobial Peptide Solution: Accurately weigh 0.25 mg, 0.50 mg, and 0.70 mg of the antimicrobial peptide, dissolve each in water, and adjust the final volume to 1000 mL to prepare solutions with concentrations of 0.25 mg/L, 0.50 mg/L, and 0.70 mg/L, respectively.

Preparation of Chitosan Compound Solution: According to the method described by Tokatl & Demirdöven, 2020, accurately weigh 1 g of chitosan and dissolve it in 100 mL of a sterile glacial acetic acid aqueous solution (1% v/v [volume per volume]) containing 1 mL of sterile glycerol. Place the mixture in a water bath at 40°C and stir continuously until the chitosan is completely dissolved. Subsequently, apply ultrasonic treatment to remove any bubbles from the solution, thereby preparing a 1% (w/v [weight per volume]) chitosan solution.

Preparation of Chitosan-Antimicrobial Peptide PNMGL2 Compound Solution: Accurately weigh 2 g of chitosan and dissolve it in an appropriate volume of 1% (v/v) sterile glacial acetic acid aqueous solution. Add 1 mL of sterile glycerol as a plasticizer, then adjust the final volume to 100 mL with 1% (v/v) glacial acetic acid aqueous solution. Place the mixture in a 40°C water bath and stir continuously until the chitosan is completely dissolved. Subsequently, remove any residual gas from the solution using the ultrasonic treatment to prepare a 2% (w/v) chitosan solution. On this basis, mix different concentrations of antimicrobial peptide solutions with the 2% chitosan solution at a 1:1 volume ratio to obtain the antimicrobial peptide PNMGL2-chitosan compound solution.

The mango samples were divided into five groups and treated as follows:

- Chitosan Treatment Group (CH): The samples were soaked in the chitosan compound solution for 2 minutes and subsequently air-dried.

- The CH-0.25P Group (Chitosan-0.25 mg/L PNMGL2 Compound Treatment): The samples were soaked in the chitosan-0.25 mg/L PNMGL2 antimicrobial peptide compound solution for 2 minutes, removed, and subsequently air-dried.
- The CH-0.50P Group (Chitosan-0.50 mg/L PNMGL2 Compound Treatment): The samples were soaked in the chitosan-0.50 mg/L PNMGL2 antimicrobial peptide compound solution for 2 minutes, removed, and subsequently air-dried.
- The CH-0.75P Group (Chitosan-0.75 mg/L PNMGL2 Compound Treatment): The samples were soaked in the chitosan-0.75 mg/L PNMGL2 antimicrobial peptide compound solution for 2 minutes, removed, and subsequently air-dried.
- Blank Control Group (CK): No treatment was applied.

The processed mangoes were stored at 25 ± 1°C for 12 days; the samples were collected every 2 days. Each time, eight mangoes were randomly selected from each group to measure the physiological parameters of the fruits. Subsequently, the mangoes were cut into pieces, ground in liquid nitrogen, homogenized, and stored at –80.0°C for the analysis of relevant indicators.

Determination of rot rate and lesion diameter

The fruit rot rate was assessed using the counting method. Specifically, eight mangoes were randomly sampled from each group and the number of rotten fruits as well as the diameter of lesions on both the peel and flesh were recorded every two days. The calculation formula for the fruit rot rate is presented in Equation 1.

$$\text{Corruption rate} = \frac{\text{Number of corrupt fruits}}{\text{Total number of fruits}} \times 100\% \quad (1)$$

Determination of disease index

The inhibitory effect of the antimicrobial peptide PNMGL2 on anthrax was assessed by slightly modifying the method described by Fan *et al*. (2022). Specifically, the disease severity was categorized into five levels according to the proportion of lesion area: level 0 (no lesions), level 1 (< 10%), level 2 (10–20%), level 3 (20–50%), and level 4 (≥ 50%). The disease index was then calculated using Formula 2.

$$DI = \frac{\sum(m \cdot n)}{z \cdot s} \quad (2)$$

Where n is the numerical value representing that grade, z represents the total number of fruits, and s corresponds to the numerical value of the highest disease grade.

Determination of ethylene release rate and respiratory intensity

Based on the method described by X. Zhou *et al*. (2023) with minor modifications, the F-950 portable ethylene analyzer was employed to simultaneously measure the postharvest physiological parameters of mangoes. Specifically, three mango samples were placed in a sealed gas chamber and equilibrated for 30 minutes. Once the concentrations of ethylene and carbon dioxide (CO_2) reached the detection threshold, the ethylene release rate (mg/kg/hour) and respiratory intensity (mg/kg/hour) were measured over a unit time.

Measurement of titratable acidity and soluble solids content

The titratable acidity and soluble solids content were determined according to the method described by Wang *et al*. (2024). Specifically, 1 g of tissue sample was accurately weighed, homogenized, and mixed with distilled water. After standing for 30 minutes, the mixture was filtered. Subsequently, 200 µL of the filtrate was measured using a PAL-1 digital refractometer. The titratable acidity was expressed as malic acid equivalent (percentage), while the soluble solids content was expressed as soluble sugar equivalent (percentage).

Hardness testing

The Rapid-TA equipped with a P/10 cylindrical probe was used to perform the test in the texture profile analysis (TPA) mode. The equatorial region of the mango was sectioned into blocks and used as the testing site. For each group, eight fruit samples were analyzed, and the hardness values were recorded in Newtons (N). The final measurement result was determined by calculating the mean value.

Color determination

The L*, a*, and b* values of the mango peel were measured using a precision color difference meter and the total color difference ΔE was calculated according to Equation 3:

$$\Delta E = \sqrt{(a_0^* - a_1^*)^2 + (b_0^* - b_1^*)^2 + (L_0^* - L_1^*)^2} \quad (3)$$

Where L_0^*, a_0^*, b_0^*, represent the L_0^*, a_0^*, b_0^* values at day 0, respectively, while L_1^*, a_1^*, and b_1^* represent the corresponding values at the time of sampling.

Determination of MDA, cell membrane permeability, H_2O_2, and superoxide anion radical content

Based on the research of Zhou *et al*. (2023), the determination of fruit electrical conductivity was modified. The specific steps were as follows: accurately weigh 3 g of fruit flesh slices with a thickness of 1 mm, add 25 mL of deionized water, and oscillate at 110 r/minute (revolutions) for 30 minutes to extract. The initial electrical conductivity (P1) was measured. After boiling the sample for 10 minutes, the volume was adjusted to the original volume and the final electrical conductivity (P2) was measured. Simultaneously, the blank value of deionized

water (P0) was measured. The relative electrical conductivity was calculated according to Formula 4.

$$P = \frac{P_1 - P_0}{P_2 - P_0} \quad (4)$$

The specific methods for determining the contents of H_2O_2, $O_2^{\bullet-}$, and MDA are as follows: Accurately weigh 1 g of the sample, add 9 mL of physiological saline, and homogenize. After centrifugation at 2500× g for 10 minutes, collect the supernatant. Perform colorimetric reactions according to the instructions provided in the reagent kits and measure the absorbance values at the characteristic wavelengths using a spectrophotometer (H_2O_2: 405 nm; $O_2^{\bullet-}$: 550 nm; MDA: 532 nm). The final contents of H_2O_2 and MDA are expressed as mmol/g fresh weight, while the content of $O_2^{\bullet-}$ is expressed as U/g fresh weight.

Enzyme activity assays related to reactive oxygen species scavenging

The specific methods for determining the activities of SOD, CAT, APX, and POD are as follows: Accurately weigh 1 g of the sample, add 9 mL of physiological saline (0.9%), homogenize, and then centrifuge at 4°C for 10 minutes at 3500× g. Collect the supernatant for subsequent determination. Following the instructions provided in the reagent kits, measure the enzyme activities of SOD, CAT, APX, and POD. Use a spectrophotometer to determine the absorbance values at their characteristic wavelengths (SOD: 412 nm; CAT: 405 nm; APX: 290 nm; POD: 420 nm). The units of enzyme activity are expressed as follows: SOD, POD, and APX are U/g fresh weight and CAT is U/g protein (based on protein concentration).

Determination of DPPH radical scavenging ability and total antioxidant capacity

The specific method for determining the DPPH free radical scavenging capacity is as follows: Accurately weigh 1.0 g of the sample, add 10 mL of 80% methanol, homogenize in an ice bath, and centrifuge at 4°C for 10 minutes at 12000× g. Collect the supernatant and measure the absorbance at 517 nm according to the instructions provided. The specific method for determining the T-AOC is as follows: Weigh 1 g of the sample, add 9 mL of physiological saline, homogenize in an ice water bath, and centrifuge at 12000× g for 10 minutes. Collect the supernatant and measure the absorbance at 405 nm according to the instructions provided. The unit for DPPH free radical scavenging capacity is μg/g and the unit for T-AOC is mmol/g.

Determination of total phenolic content and ascorbic acid in plants

The specific methods for determining the total phenols and ASA content in fruits are as follows: Accurately weigh 0.1 g of the sample, add 2 mL of the extraction solution, homogenize in an ice bath, and then centrifuge at 4°C (4000 r/minute, 10 minutes). Collect the supernatant for testing. Follow the instructions to conduct the determination and measure the absorbance value at the specific wavelength (total phenols: 760 nm; Vc: 536 nm). The unit of total phenols content is μmol/g and the unit of ASA content is ug/g.

Determination of GSH content, GR activity, and GPX activity

The specific methods for determining the content of GSH, the activity of GR, and the activity of GPX are as follows: Accurately weigh 1 g of the sample, add 9 mL of 0.9% physiological saline, homogenize in an ice bath, and centrifuge at 4°C for 10 minutes at 3500× g. Collect the supernatant for subsequent determination. Follow the instructions provided in the assay kit to measure the absorbance values at specific wavelengths (GSH: 420 nm; GR: 340 nm; GPX: 550 nm). The GSH content is expressed as mg/g fresh weight (FW), the GR activity as U/g FW, and the GPX activity as U/mg protein (prot).

Statistical analysis

All experiments were performed with three biological replicates. Data processing was conducted using Microsoft 365 Excel to calculate the mean ± standard deviation. Graphs were generated using Origin 2022 software and statistical analyses, including one-way analysis of variance (ANOVA) and correlation tests, were carried out using SPSS 27. The significance level was set at $p < 0.05$.

Results and Analysis

Effects of different concentrations of antimicrobial peptides on ripening status and disease indices of mangoes during storage

The morphological changes in the peel and flesh of mangoes can serve as an intuitive indicator of the fruit's freshness and ripening status. As illustrated in Figure 1a, the mango peel progressively turns yellow with extended storage time. Compared with the CK group, the mango peel in the group treated with chitosan film combined with antimicrobial peptide PNMGL2 exhibited less noticeable yellowing. Furthermore, the CH-0.50P treatment group demonstrated a superior inhibitory effect on mango anthracnose. On the 12th day posttreatment, no obvious disease symptoms were observed in the peel or flesh of the fruit in this group. In contrast, the CK group displayed numerous disease spots as early as the 6th day, accompanied by progressive disease aggravation and increased rot severity, rendering the fruit inedible by the 8th day. These results indicate that the combination of

chitosan film and antimicrobial peptide PNMGL2 effectively inhibits disease development in mangoes.

Anthracnose is the primary disease responsible for mango rot. During the postharvest ripening process, the pathogen induces black lesions on the fruit peel and stem, which subsequently progresses to decay, thereby diminishing or completely eliminating the commercial value of the mangoes (Anusha *et al*., 2023). The impact of different concentrations of antimicrobial peptides on the rot rate during storage is presented in Figure 1b. The final rot rates of the CH-0.50P and CH-0.75P treatment groups increased to 75%, whereas the CK group exhibited the most pronounced increase, reaching a 100% rot rate by day 10. These results suggest that the combination of antimicrobial peptide PNMGL2 with chitosan coating effectively suppresses the rise in mango rot rate and preserves the appearance quality of the fruit.

The disease index serves as a direct indicator of the degree of fruit rot—specifically, higher the disease index, more the severe rot (Wang *et al*., 2024). The effects of different concentrations of antimicrobial peptides on the disease index of mangoes during storage are presented in Figure 1c and 1d. As storage time increased, the disease index of all treatment groups exhibited an upward trend. During the storage period, fruits treated with the combination of antimicrobial peptide PNMGL2 and chitosan coating showed a suppressed disease index in both peel and flesh compared to other groups. On day 12, the disease index of the peel and flesh in the CK group was three times and seven times higher than that of the CH-0.5P group, respectively. These results indicate that the CH-PNMGL2 coating treatment effectively inhibits disease progression in mangoes during shelf life.

The effects of different concentrations of antimicrobial peptides on the diameter of disease spots in mangoes during storage are presented in Figure 1e and 1f. Between days 8 and 12, the diameters of both peel and flesh disease spots in the CH-PNMGL2 treatment group were significantly smaller than those in the CK group ($P < 0.05$). On day 12, the diameters of the peel and flesh disease spots in the CH-0.5P treatment group were reduced by 56.98% and 74.95%, respectively, compared to the CK group. These results indicate that the CH-0.5P treatment effectively delays fruit senescence, inhibits bacterial growth, and enhances fruit self-protection mechanisms.

Effects of different concentrations of antimicrobial peptides on ethylene release and respiratory intensity during storage of mangoes

Respiratory climacteric fruits exhibit a respiratory peak during the postharvest ripening process, which signifies the onset of the fruit's ripening stage. During this phase, the edible quality and economic value of the fruit gradually increase. Therefore, investigating changes in respiratory intensity is one of the key indicators for assessing whether respiratory climacteric fruits have entered the ripening stage (Li *et al*., 2024). The effects of different concentrations of antimicrobial peptides on ethylene release and respiratory intensity in mangoes during storage are presented in Figure 2a and 2b. In the early stages of storage, all four treatment groups showed an upward trend in both respiratory intensity and ethylene release. The CK, CH, and CH-0.25P treatment groups reached their respective peaks in ethylene release and respiratory intensity on the 4th day. However, compared with the CK group, the CH-0.50P and CH-0.75P treatment groups exhibited a delay of 2 days in reaching their peaks, with reductions of 19.39% and 2.01%, respectively, in respiratory intensity peaks and decreases of 14.31% and 3.06%, respectively, in ethylene release peaks. These results suggest that the CH-0.50P treatment effectively suppresses ethylene release and respiratory intensity in mangoes. As the maturation process progresses, the weakened respiratory climacteric pattern in fruits treated with the antimicrobial peptide PNMGL2 may be attributed to the corresponding deceleration of ethylene release and the associated decline in metabolic activities that drive the maturation process (Chomba *et al*., 2025).

Effects of different concentrations of antimicrobial peptides on titratable acid, soluble solids, firmness, and total color difference Δe in mango peel during storage

Soluble solids and titratable acid are key indicators for evaluating the maturity of fruits and vegetables as well as fruit quality (He *et al*., 2025). The titratable acid content of mangoes decreased progressively with extended storage time (Figure 3a). The soluble solid content of the CH-0.50P group was consistently and significantly higher than that of the CK group ($P < 0.05$). On day 12 of storage, the titratable acid content of the CH-0.50P treatment was 1.67%, which was 43.96% higher than that of the CK group. During the storage period, the soluble solid content of mangoes exhibited an overall upward trend (Figure 3b). At the end of storage, the soluble solid mass fractions of the CH, CH-0.25P, CH-0.50P, CH-0.75P, and CK groups were 18.67%, 18.20%, 19.20%, 19.53%, and 20.80%, respectively. The increase in TSS content may be attributed to the metabolic conversion of pectin into soluble pectin and the transformation of starch and other polysaccharides into soluble oligosaccharides, thereby enhancing the TSS content (Wang *et al*., 2021).

Fruit firmness is a critical indicator for assessing fruit maturity and edible quality as well as the most direct measure of fruit senescence and deterioration. Fruit firmness

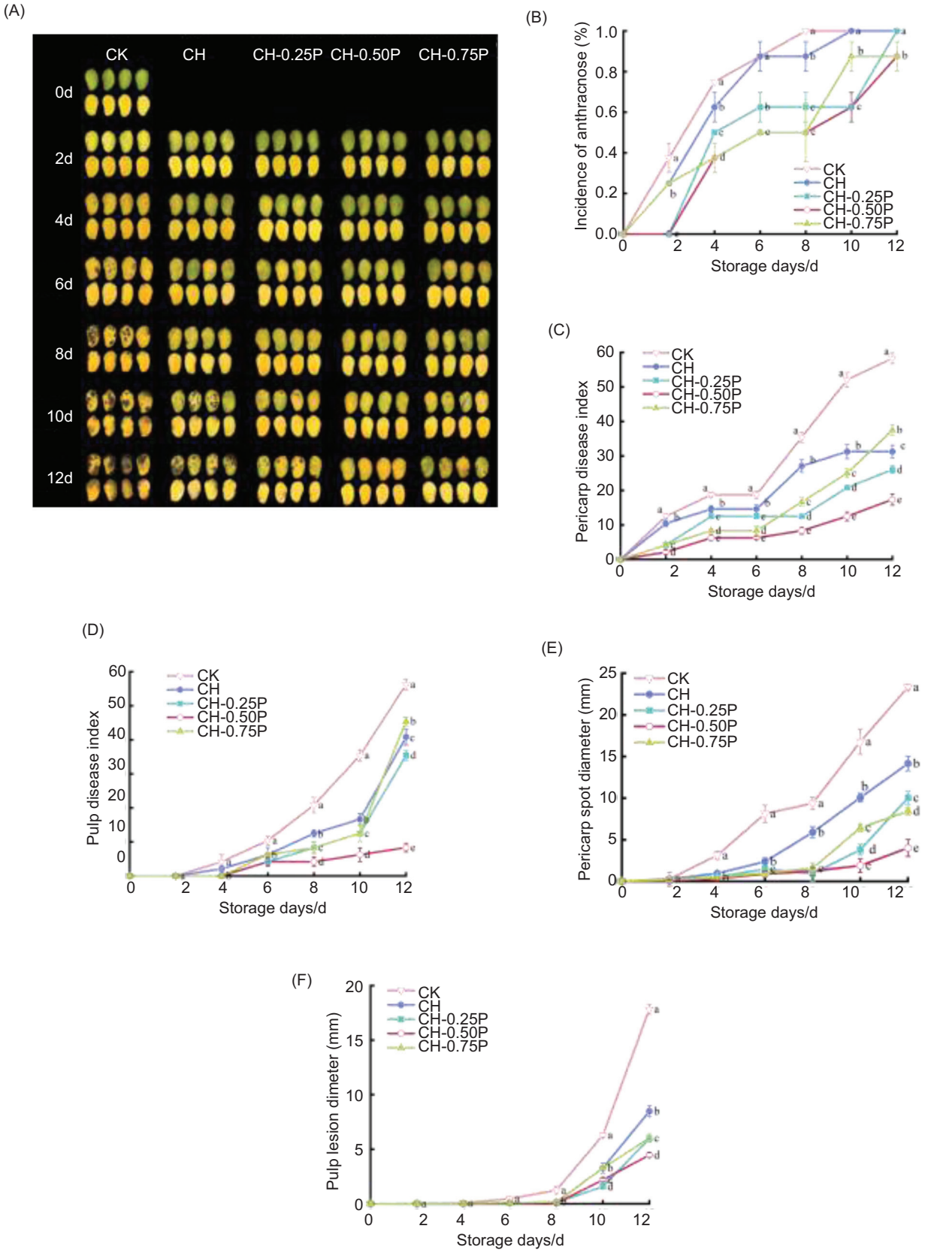

Figure 1. Effects of different concentrations of antimicrobial peptides on the incidence of anthracnose, skin disease index, pulp disease index, skin spot diameter, and pulp spot diameter of mango during storage. In the figure, different lower case letters indicate significant differences (P < 0.05). (A) Apparent phenomenon of storage for 12 days; (B) Incidence of anthracnose; (C) Pericarp disease index; (D) Pulp disease index; (E) Pericarp spot diameter; (F) Pulp lesion diameter/mm.

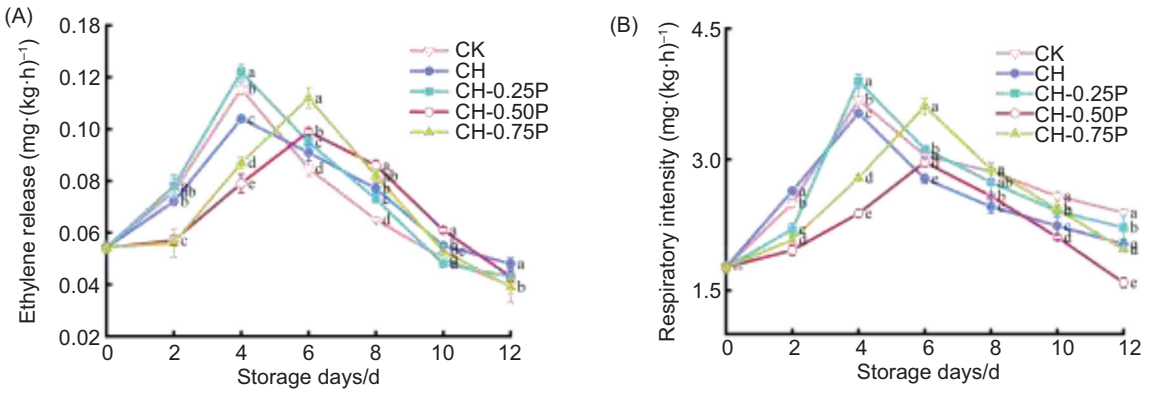

Figure 2. Effects of different concentrations of antimicrobial peptides on ethylene release and respiratory intensity of mango during storage. (A) Ethylene release; (B) Respiratory intensity.

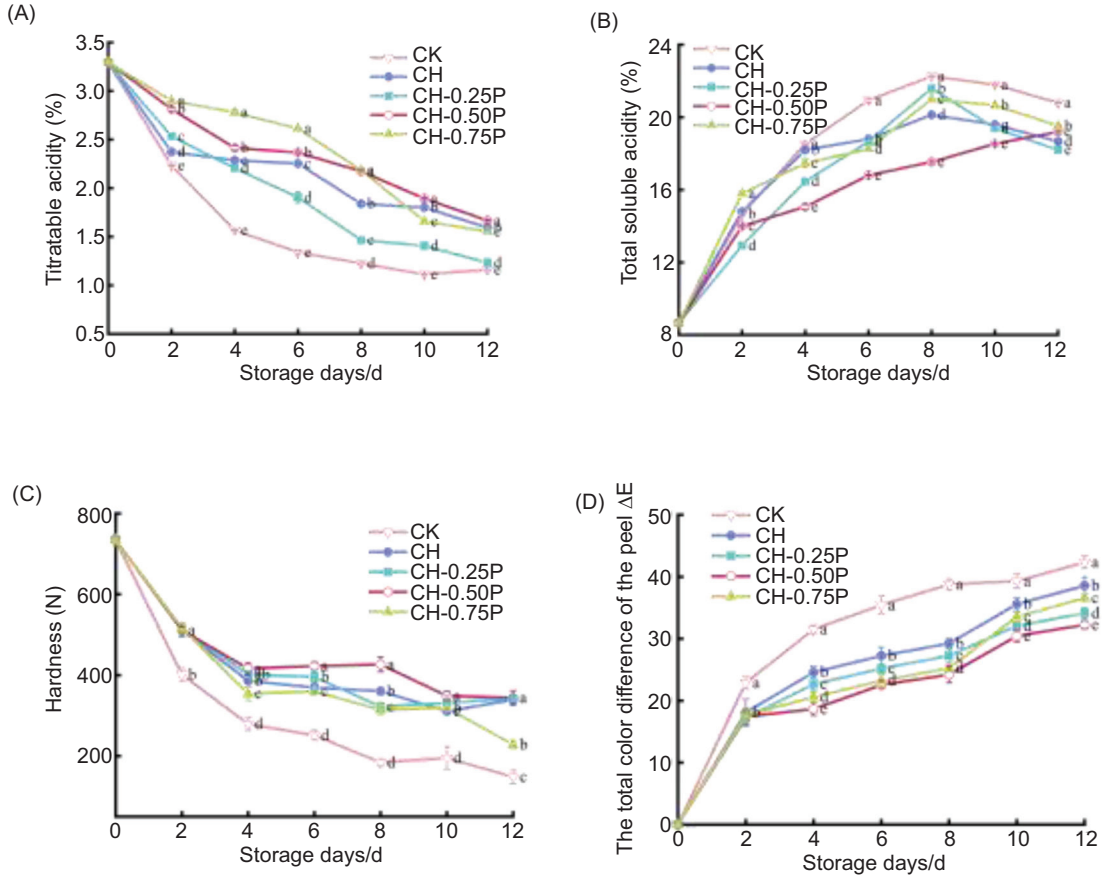

Figure 3. Effects of different concentrations of antimicrobial peptides on titrable acid, soluble solid, hardness, and total skin color difference ΔE of mango during storage. (A) Titrable acidity; (B) Total soluble slid; (C) Hardness; (D) The total color difference of the peel ΔE.

decreased continuously throughout the entire storage period (Figure 3c). On day 12 of storage, the firmness values of CH, CH-0.25P, CH-0.50P, and CH-0.75P mangoes were 338.97 N, 342.45 N, 344.25 N, and 228.25 N, respectively, which were significantly higher than that of the CK group (148.63 N). These results suggest that the combination of chitosan coating with the antimicrobial peptide PNMGL2 effectively delays the reduction in mango firmness and slows down mango softening. It is hypothesized that this treatment facilitates the preservation of

cell wall structural components and water-soluble pectin in mangoes, thereby contributing to the maintenance of fruit firmness (Pang *et al*., 2024).

The total color difference change in fruits is a critical physiological indicator that directly reflects the extent of fruit ripening.The variations in the total color difference of mango peels during storage under different concentrations of antimicrobial peptide treatments are presented in Figure 3d. A larger ΔE value indicates a more pronounced color difference between the sample and its color at the previous sampling time. During the 12-day storage period, the other treatment groups exhibited significantly lower ΔE values than the CK group ($P < 0.05$) (Figure 3d). From day 4 to day 12, the ΔE value of the CH-0.50P treatment group was the lowest, suggesting that the degreening and yellowing process of mangoes in the CH-0.50P group was the slowest. This treatment could effectively retard mango ripening.

Effects of different concentrations of antimicrobial peptides on MDA, electrical conductivity, H_2O_2, and $O_2^{\cdot-}$ during storage of mangoes

When plants are injured, lipid peroxidation of cell membranes significantly increases. MDA, one of the key products of membrane lipid peroxidation, is an important indicator for evaluating the extent of cell membrane damage and plant stress tolerance based on its content changes (Balcı *et al*., 2021). Compared with the CK group, the MDA content in all treatment groups decreased significantly, and the MDA content in the CK group remained consistently higher than that in the other groups (Figure 4a). At the end of storage, the MDA contents in the CH-0.25P, CH-0.50P, and CH-0.75P groups were 52.70 μmol/g, 45.48 μmol/g, and 50.92 μmol/g, respectively. These results suggest that the CH-0.50P treatment was more effective in reducing MDA production and delaying the senescence process of mangoes. The decrease in MDA content indicates an enhanced activity of antioxidant enzyme systems (such as SOD, CAT, APX, and POD), thereby alleviating the lipid peroxidation damage of cell membranes.

The cell membrane exhibits selective permeability, which is essential for maintaining normal physiological functions of cells; however, external stress or post ripening processes may compromise the integrity of the cell membrane, thereby increasing its permeability (Su *et al*., 2025). The combination of antimicrobial peptide PNMGL2 and chitosan coating significantly suppressed the increase in relative conductivity (Figure 4b). Specifically, the change in relative conductivity of the CH-0.50P group was significantly lower than that of the other groups ($P < 0.05$). It is speculated that the combination of the antimicrobial peptide PNMGL2 and chitosan coating can reduce the damage to mangoes during transportation by inhibiting the activity of cell wall degrading enzymes and maintain the integrity of their cell membrane functions (Wei *et al*., 2021).

ROS are generated during aerobic respiration and play a critical role in inducing oxidative damage to macromolecules, such as $O_2^{\cdot-}$ and H_2O_2 (Yan *et al*., 2024). During the storage period, the H_2O_2 content in the CH-0.50P group exhibited a continuous upward trend, whereas in the other groups, it initially increased and subsequently decreased (Figure 4c). The H_2O_2 content in the CK group peaked at 0.85 mmol/g on the 4th day of storage, while that in the CH-0.50P group was significantly lower at 0.46 mmol/g ($p < 0.05$). The $O_2^{\cdot-}$ content in all treatment groups followed a similar trend of first increasing and then decreasing, with the superoxide anion content in fruits reaching its peak on the 2nd day of storage (Figure 4d). Compared to the CK group, the peak values for CH-0.25P, CH-0.50P, and CH-0.75P were reduced by 5.59%, 8.40%, and 12.91%, respectively, and were significantly lower than those of the CK group ($P < 0.05$). These results suggest that the combination of chitosan coating with the antimicrobial peptide PNMGL2 effectively inhibited the generation of ROS in the fruits. The reduction of H_2O_2 content can decrease peroxidation damage to cells and maintain the balance of ROS in fruits.

Effects of different concentrations of antimicrobial peptides on enzyme activities related to ROS metabolism during storage of mangoes

SOD is recognized as the primary ROS scavenging enzyme, which converts superoxide radicals into H_2O_2 and molecular oxygen. This enzyme plays a critical role in eliminating ROS (Feng *et al*., 2024). Compared with the CK group, the APX and SOD contents in all treatment groups exhibited a downward trend (Figure 5a and 5b). Notably, the SOD content in the CH-0.50P group remained consistently higher than to that in the other groups throughout the storage period. At the end of storage, the APX contents in the CH-0.25P, CH-0.50P, and CH-0.75P groups were 0.21 U/g, 0.42 U/g, and 0.35 U/g, respectively. In contrast, the increase in SOD content in the CH-0.50P group was significantly greater than that in the other treatment groups ($P < 0.05$). These results suggest that the CH-0.50P treatment effectively delayed the decline of APX and SOD, thereby slowing down the aging process of mangoes.

In the ROS scavenging enzyme system, CAT and POD play critical roles in directly eliminating excessive ROS in fruits and vegetables, thereby maintaining cellular redox balance (Cao *et al*., 2021). During storage, both CAT and

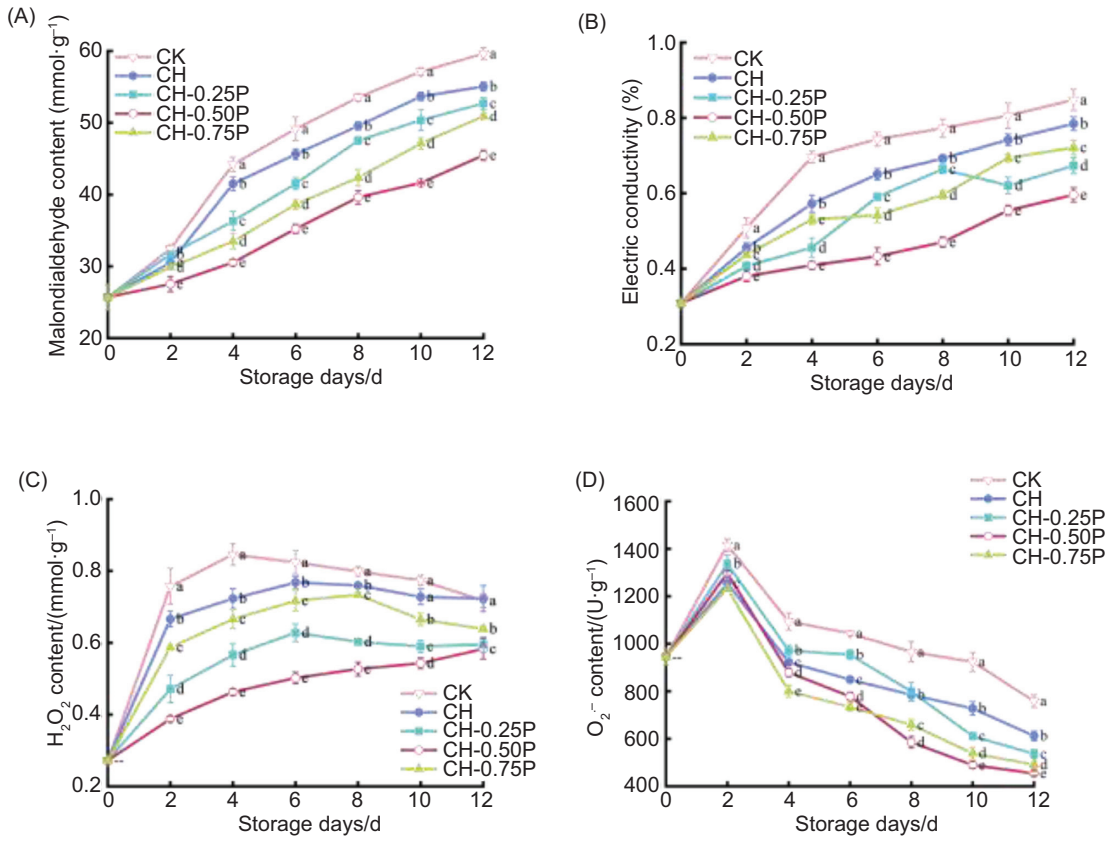

Figure 4. The effects of different concentrations of antimicrobial peptides on MDA, conductivity, H_2O_2, and $O_2^{\cdot-}$ during storage of mango. (A) Malondialdehyde content; (B) Electric conductivity; (C) H_2O_2 content; (D) $O_2^{\cdot-}$.

POD activities initially increased and then decreased, reaching their peaks on the 2nd day (Figure 5c and 5d), and the activities of CAT and POD in the CH-0.50P treatment group were significantly higher than those in the CK group ($P < 0.05$), being 1.19 and 1.55 times higher than the CK group on the 12th day, respectively. Similarly, the activities of CAT and POD in the PNMGL2 antimicrobial peptide chitosan coating treatment group were also significantly higher than those in the CK group ($P < 0.05$). These results suggest that the combination of PNMGL2 antimicrobial peptide with chitosan coating enhances the activities of ROS metabolism-related enzymes in mangoes during storage. These antioxidants can undergo redox reactions with ROS, thereby preventing ROS from causing damage to plants (Wei *et al.*, 2025).

GR is one of the key enzymes in the plant antioxidant system, primarily responsible for catalyzing the reduction of oxidized glutathione (GSSG) to reduced GSH, thereby maintaining the dynamic balance of GSH/GSSG within cells (Hasanuzzaman *et al.*, 2017). GR activity in all groups initially increased and then decreased, reaching its peak on the 2nd day (Figure 5e and 5f). Significant differences were observed between the treatment groups and the CK group ($P < 0.05$). Meanwhile, GPX activity in all groups rapidly increased on the second day and subsequently rose slowly. At the end of storage, the GR and GPX activities in the groups treated with the combination of antimicrobial peptide PNMGL2 and chitosan coating were significantly higher than those in the CK group, increasing by 28.91%, 33.22%, and 17.64%, respectively, compared to the CK group. These results suggest that the treatment combining antimicrobial peptide PNMGL2 with chitosan coating effectively enhances GR and GPX activities during mango storage.

Effects of different concentrations of antimicrobial peptides on T-AOC and DPPH free radical scavenging capacity in mangoes during storage

T-AOC reflects the combined effects of various antioxidants and antioxidant enzymes, such as vitamin C, vitamin E, β-carotene, and enzymatic antioxidants. Therefore, it can serve as an effective indicator for evaluating the overall antioxidant capacity of bioactive substances (Pérez-Lamela *et al.*, 2021). On the 2nd day, the total antioxidant capacities of CH-0.25P, CH-0.50P, and

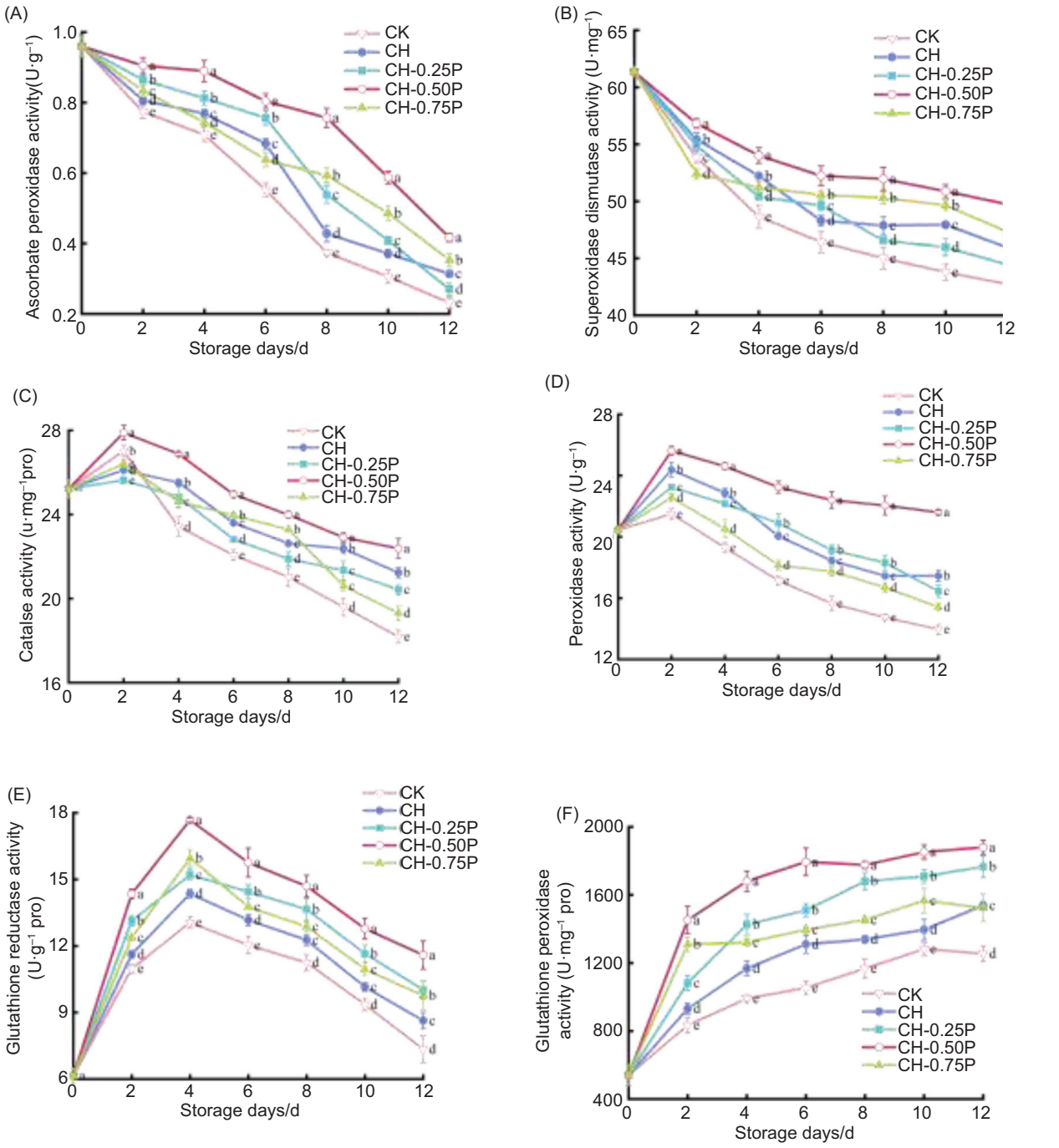

Figure 5. Effects of different concentrations of antimicrobial peptides on APX activity, SOD activity, CAT activity, POD activity, GR activity, and GPX activity of mango during storage. (A) Ascorbate peroxidase activity; (B) Superoxide dismutase activity; (C) Catalase activity; (D) Peroxidase activity; (E) Glutathione reductase activity; (F) Glutathione peroxidase activity.

CH-0.75P reached their respective peaks at 1.00 mmol/g, 1.01 mmol/g, and 0.98 mmol/g (Figure 6a). At the end of storage, the T-AOC of the CH-0.50P treatment group was significantly higher than that of the CK group ($P < 0.05$). These results suggest that the CH-0.50P treatment effectively protects cells and tissues from oxidative stress induced by ROS, thereby maintaining fruit quality during storage.

The DPPH radical scavenging capacity is an important indicator reflecting the antioxidant capacity of fruits and vegetables (Ge *et al.*, 2015). During the storage period, the DPPH content initially increased and then decreased (Figure 6b). Notably, the CK group exhibited a significant decline in scavenging rate during the middle and later stages of storage, whereas the CH-0.50P treatment group showed a relatively smaller decrease. On the 2nd day,

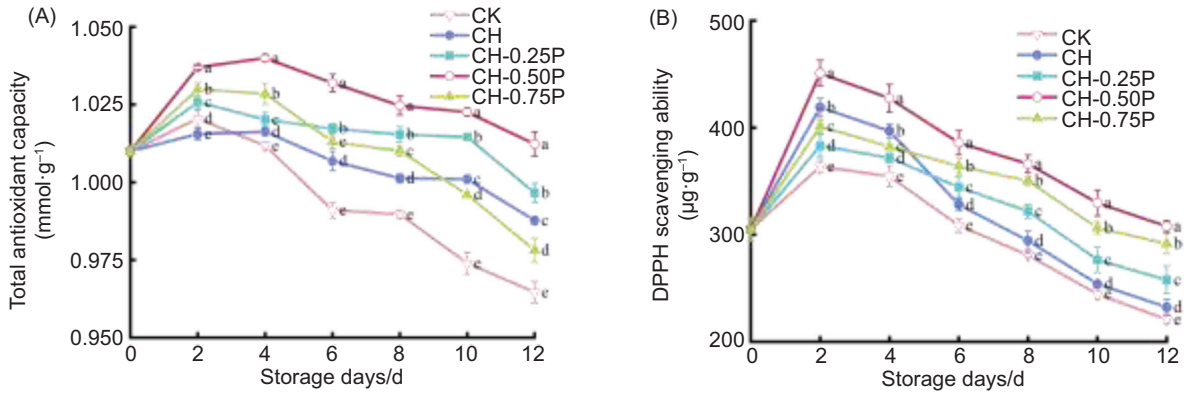

Figure 6. Effects of different concentrations of antimicrobial peptides on T-AOC and DPPH free radical scavenging ability of mango during storage. (A) Total antioxidant capacity; (B) DPPH scavenging ability.

the DPPH content of the CH-0.50P treatment group reached its maximum value of 307.91 ug/g, which was significantly higher than that of the CK group ($P < 0.05$). At the end of storage, the DPPH content of the CH-0.50P treatment group remained higher than that of the other treatment groups, suggesting that the CH-0.50P treatment effectively enhanced the free radical scavenging ability, thereby facilitating the rapid clearance of free radicals accumulated in plant tissues.

Effects of different concentrations of antimicrobial peptides on total phenols, ASA, and GSH contents in mangoes during storage

ASA and GSH serve as critical antioxidants in the nonenzymatic ROS scavenging system and play a pivotal role in degrading H_2O_2 through the ASA-GSH and GPX cycles (Zhang *et al*., 2022). The total phenolic content in mango fruits during storage initially increased and then decreased (Figure 7A). Each treatment group reached its peak on the 2nd day, with values of 54.53 μmol/g, 60.96 μmol/g, 70.73 μmol/g, and 57.02 μmol/g, respectively. Notably, the total phenolic content in the CH-0.50P treatment group remained significantly higher than that in the CK group throughout the 12-day storage period, being 29.92% higher than the CK group on the 12th day. The ASA content in mango fruits exhibited a continuous downward trend during storage (Figure 7B). There was a significant difference between the CH-0.50P group and the chitosan treatment group ($P < 0.05$), and the content of the CH-0.50P treatment group on the 12th day was 37.25% higher than that of the CK group. The GSH content in mango fruits during storage demonstrated an upward trend (Figure 7C). Throughout the storage period, the GSH content in the treatment groups was significantly higher than that in the CK group ($P < 0.05$). In summary, the CH-0.50P treatment effectively enhances GSH accumulation in mangoes and mitigates the decline of total phenols and ASA.

Discussion

This study revealed that the combined application of the antimicrobial peptide PNMGL2 and chitosan coating treatment could efficiently inhibit the occurrence of postharvest anthracnose in mangoes. These findings imply that PNMGL2 exerts antibacterial and preservative functions throughout the mango preservation process. Currently, the primary antibacterial mechanisms of antimicrobial peptides secreted by antagonistic bacteria include the following:

1. Interacting with the cell membrane of pathogenic bacteria to disrupt its integrity, leading to leakage of intracellular substances, or inhibiting the growth and altering the morphology of pathogenic bacterial hyphae, thereby suppressing pathogenic bacterial growth.

2. Modulating the microbial community structure on the surface of mangoes to inhibit pathogenic bacterial growth, promote the proliferation of beneficial microorganisms, and enhance disease resistance in mangoes.

3. Activating the immune system of mangoes to suppress the metabolism of pathogenic bacteria and reduce their pathogenicity.

4. The specific mechanism of the action of PNMGL2 in mango preservation in this study may be associated with the induction and enhancement of resistance-related responses (K R *et al*., 2024).

The disease resistance of postharvest fruits during storage is closely associated with the production and regulation of ROS within the plant. Upon infection by pathogenic bacteria, plants generate ROS, primarily in the form of H_2O_2, which inhibits the germination of pathogen spores

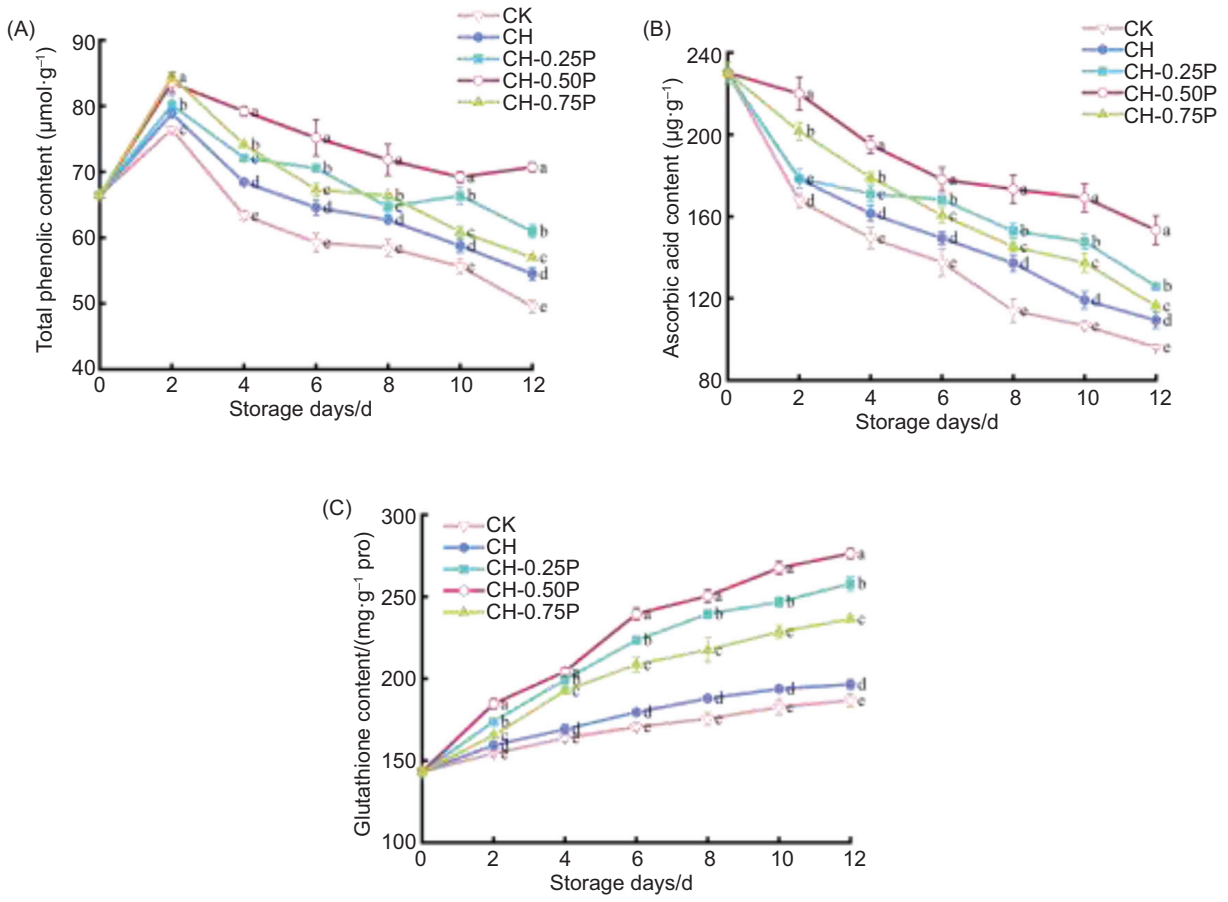

Figure 7. Effects of different concentrations of antimicrobial peptide treatment on total phenol content, ASA content, and GSH content of mango during storage period. (A) Total phenolic content; (B) Ascorbic acid content (C) Glutathione content.

and may even lead to pathogen death (Chang *et al*., 2024). Moreover, H_2O_2 serves as a critical signaling molecule in plant responses, acting as a secondary messenger in signal transduction pathways. It activates and regulates stress-related genes or proteins within the plant, thereby amplifying the defense signal and enabling the production of various disease-resistant substances (Kong *et al*., 2019); however, the overaccumulation of ROS can also induce damage to the plant's cellular membranes. Consequently, plants activate both enzymatic and nonenzymatic ROS scavenging systems to mitigate the adverse effects of excessive ROS accumulation . This study found that the treatment with the antimicrobial peptide PNMGL2 effectively inhibited anthracnose development in highly mature mangoes. The inhibitory effect was strongly and positively correlated with MDA, electrical conductivity, GSH, H_2O_2, $O_2^{\bullet-}$, APX, SOD, and ASA levels (Figure 8). The antimicrobial peptide PNMGL2 enhances the anthracnose resistance of mangoes primarily by leveraging the ASA-GSH cycle, which regulates ROS generation and scavenging to maintain the balance of the ROS production and scavenging system. Nevertheless, not all preservative and antibacterial treatments depend on the ASA-GSH cycle to enhance resistance. Lin *et al*. (2020) demonstrated that H_2O_2 treatment of longan pulp downregulated the activities of SOD, CAT, and APX in the ROS cycle system, leading to lysis of the longan pulp. This indicates that H_2O_2 promotes ROS accumulation and lipid peroxidation of cell membranes. (Xu *et al*., 2021) treated pitaya with anisaldehyde (PAA), which significantly reduced the levels of H_2O_2 and the generation rate of $O_2^{\bullet-}$ while markedly enhancing the activities of antioxidant enzymes such as SOD, POD, and CAT. This treatment also maintained higher levels of ASA and reduced GSH. Furthermore, SOD exhibits a strong negative correlation with H_2O_2 elimination. This suggests that during the anthracnose resistance induced by the antimicrobial peptide PNMGL2, SOD serves as the primary mechanism for ROS scavenging.

In this study, a comparison was made among three different concentrations of antimicrobial peptides. The results indicated that only when the antimicrobial peptides are present at an appropriate concentration can they stimulate the signal transduction pathways within fruit cells. This stimulation subsequently activates the relevant

enzyme systems, ultimately leading to an enhanced generation of ROS. If the concentration of antimicrobial peptides is too low, it may fail to effectively activate the enzyme system associated with ROS generation, leading to reduced ROS production, consequently compromising the bactericidal and preservative effects. Conversely, an excessively high concentration may exert cytotoxicity on fruit cells, impair their normal physiological functions, and potentially induce cell death. This study revealed that during the process of ROS generation and clearance, 0.5 mg/L of the antimicrobial peptide PNMGL2, as an optimal treatment concentration, could more rapidly and effectively enhance the activity of all enzymes involved in ROS metabolism in mangoes compared to concentrations of 0.25 mg/L and 0.75 mg/L. On the 2nd day, mangoes treated with 0.5 mg/L of PNMGL2 exhibited a rapid increase in the accumulation of GPX, CAT, GR, and ASA. Hassam *et al*. (2024) and Tahir *et al*. (2024) also found that the content of antioxidant enzymes significantly increased during the ripening of mangoes, indicating that they play an important role in eliminating high concentrations of H_2O_2 and lipid peroxides, thereby reducing oxidative damage during the fruit's ripening process. By the 8th day, the accumulation rates of SOD and APX had significantly accelerated (Vašková *et al*., 2023). The activity of GPX is modulated by the concentration of

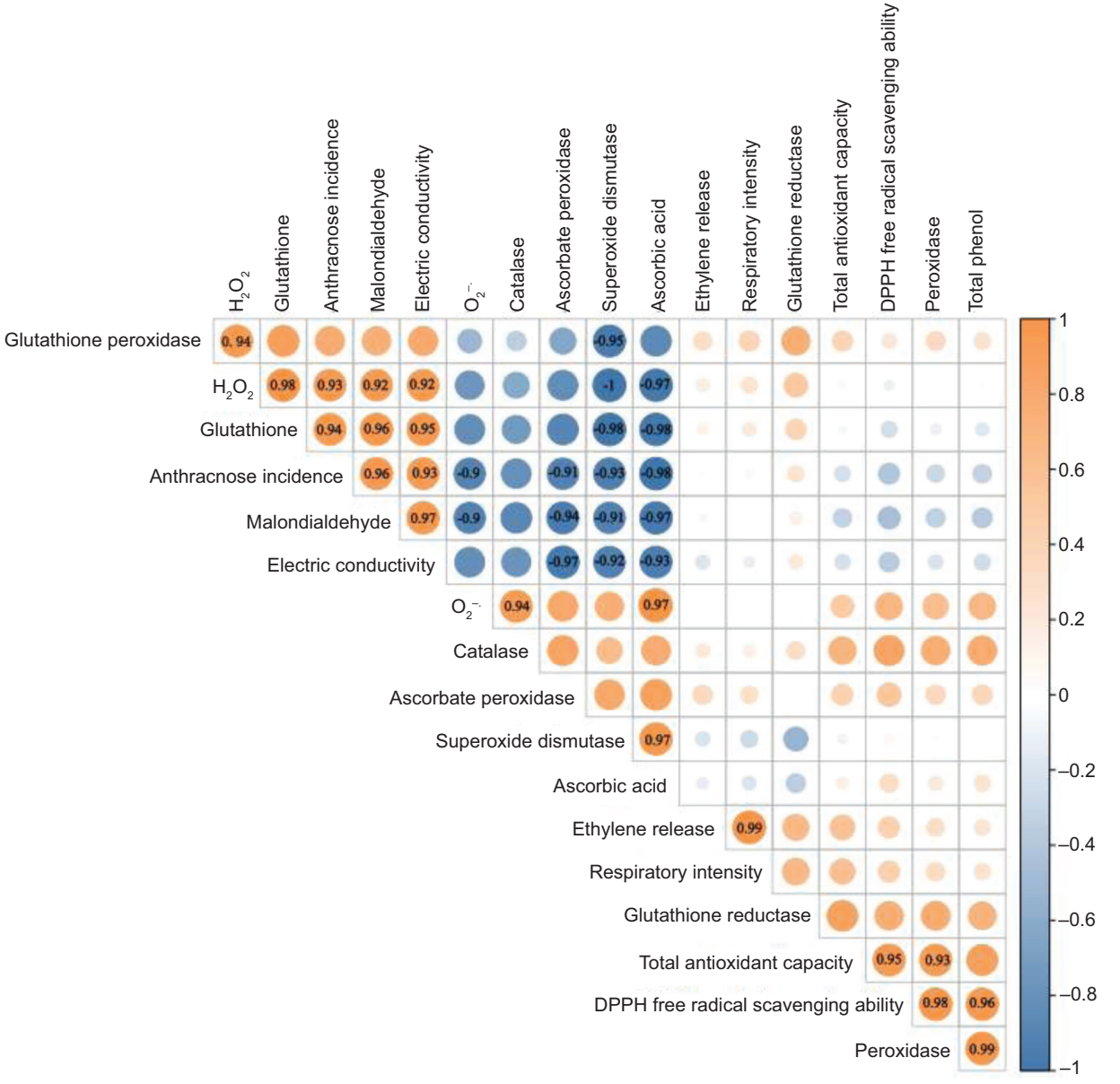

Figure 8. Correlation analysis of mango treated with antimicrobial peptide PNMGL2 during storage. The depth of the color gradation indicates the strength of correlation where 0~1 is positive correlation and –1~0 is negative correlation. The marked value indicates the correlation coefficient, and the absolute correlation coefficient is greater than 0.9.

GSH and the activity of GR. As nonenzymatic antioxidants, ASA and GSH, in conjunction with GPX and GR, eliminate peroxides via the synthesis and reduction of GSH mediated by glutathione synthetase (GS) and GR, respectively (Averill-Bates, 2023; Mishra *et al*., 2023). These enzymes and antioxidants collectively function to scavenge ROS within the plant cells, thereby maintaining redox homeostasis (Panday *et al*., 2020). This suggests that 0.5 mg/L of the antimicrobial peptide PNMGL2 can effectively enhance the activity of enzymes involved in the ROS metabolic pathway, facilitating both the generation and clearance of ROS and thus sustaining cellular redox equilibrium. In contrast, the CH-0.25P treatment group exhibited a slower accumulation rate of superoxide anions and POD levels remained largely unchanged during storage. In the CH-0.75P treatment group, the accumulation rate of antioxidant enzymes was also delayed, with rapid increases observed only around the 8th day of storage. This imbalance in ROS generation and clearance likely compromised cellular integrity, induced lipid peroxidation, and accelerated fruit decay . These findings suggest that the combination of 0.5 mg/L antimicrobial peptide PNMGL2 and chitosan coating significantly enhances H_2O_2 content in postharvest mangoes. Furthermore, this treatment markedly increased the activities of disease-related enzymes (SOD, APX, GPX) and the concentration of ASA, a key disease-related compound. It is hypothesized that following combined treatment, H_2O_2 may function as a signaling molecule to mediate disease resistance signal transduction within mango fruits.

Conclusion

This study systematically investigated the effects of varying concentrations of the antimicrobial peptide PNMGL2 on the postharvest preservation of mangoes. The results demonstrated that the treatment combining 0.50 mg/L PNMGL2 with chitosan coating (CH-0.50P) exhibited a significant antibacterial effect, effectively suppressing the incidence of postharvest anthracnose in mangoes. This treatment also significantly preserved higher levels of soluble solids, titratable acid, and vitamin C, while markedly inhibiting the decline in fruit firmness and the browning of the flesh—key indicators of senescence. During storage, the CH-0.50P treatment mitigated the reduction in activities of SOD, APX, POD, CAT, and GR, effectively inducing the active oxygen/GSH cycle in mangoes. It rapidly eliminated excess H_2O_2 and superoxide anions within cells, maintaining a balanced production and scavenging capacity of ROS, thereby enhancing the antioxidant capacity of mangoes and inhibiting anthracnose development. In conclusion, PNMGL2, as a safe and effective antibacterial agent, has broad application prospects in the field of fruit and vegetable preservation,

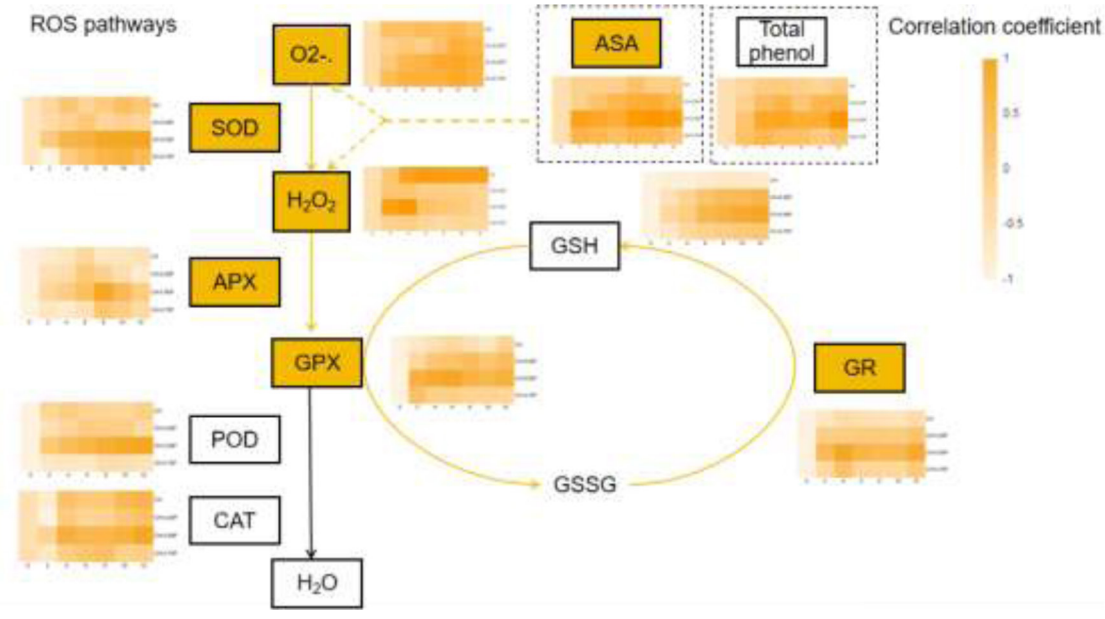

Figure 9. Mechanistic diagram of the effects of different treatment methods on mango ROS metabolism. The change of color level indicates the level of metabolite accumulation. The small heat map shows the accumulation of ROS metabolis-related enzymes in mango from four treatment groups during 0, 2, 4, 6, 8, 10, and 12 days of storage. The arrows are the relationships between the materials. SOD: superoxide dismutase; APX: ascorbate peroxidase; POD: peroxidase; GPX; glutathione peroxidase; GSH: glutathione; GR: glutathione reductase; GSSG: oxidized glutathione; ASA: ascorbic acid.

providing new ideas and methods for the preservation technology of mangoes and other perishable fruits.

Author Contributions

All authors contributed equally to this article.

Competing Interests

The authors had no relevant financial or nonfinancial interests to disclose.

Conflicts of Interest

The authors declare no conflict of Interest.

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