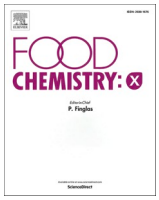
Food Chemistry: X 22 (2024) 101417

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x



Effect of polyethoxylated flavonoids (PMFs)-loaded citral and chitosan composite coatings on citrus preservation: From the perspective of fruit resistance

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ARTICLE INFO

Keywords:
PMFs-loaded citral nanoemulsion
Chitosan
Citrus
Preservation
Fruit resistance

ABSTRACT

Previous studies have shown that polymethoxylated flavonoids-loaded citral emulsion (PCT) can inhibit the growth and reproduction of Penicillium in citrus; however, PCT is difficult to apply to fruit preservation due to its high fluidity and volatility. Therefore, in this study, we combined PCT with chitosan (CS) to investigate the effect of a composite coating on citrus preservation. The results showed that compared to the control group, the CS-PCT group could effectively reduce the decay rate and maintain moisture availability, color difference, and hardness. Moreover, the contents of nonenzymatic antioxidants and volatile substances with antimicrobial activity were better preserved. In addition, the activities of related antioxidant enzymes were greater in the treatment group, and the expression of the corresponding enzyme-encoding genes was upregulated. Consequently, CS-PCT treatment could effectively maintain fruit quality and improve the resistance of citrus fruits during storage; moreover, it can be considered a nontoxic and efficient citrus preservative.

1. Introduction

Citrus fruits are popular worldwide due to their abundant yield, delightful flavor, and exceptional nutritional value (Liu, Heying, & Tanumihardjo, 2012). Nevertheless, the storage of these fruits can be challenging, and issues, such as weight loss, softening, and flavor degradation, can manifest due to postharvest processes, such as respiration, metabolism, and transpiration (López-Gómez, Ros-Chumillas, Buendía-Moreno, & Martínez-Hernández, 2020). As the storage duration increases, the antioxidant capacity of the fruit decreases, increasing its susceptibility to microbial infections, ultimately leading to fruit rot and substantial economic losses (Wang, Sui, Li, Tian, & Wang, 2022). Currently, the postharvest storage and preservation of citrus fruits primarily rely on chemical preservatives, and prochloraz, carbendazim, and 2,4-dichlorophenol are the most commonly used preservatives (Li et al., 2023; Zhang, Xu, Zhang, & Zhu, 2018). However, the prolonged use of chemical preservatives can result in the development of drug-resistant pathogenic microorganisms, environmental pollution, and adverse effects on human health (Li et al., 2023; Yang et al., 2022). Consequently, a growing research emphasis is to develop safe and efficient preservation technologies within this field.

Edible film preservation technology involves the application of a safe and nontoxic edible polymer as a coating agent on the surface of fruits and vegetables. This film is created by allowing the solvent to evaporate, forming a protective barrier that regulates the gas exchange between the product and the external environment (Sun, Cao, Kim, & Marelli, 2022). The film effectively inhibits respiration and transpiration, reduces nutrient depletion, minimizes water loss during storage, and acts as a shield against external microorganisms. This preservation method has demonstrated significant efficacy (Díaz-Montes & Castro-Muñoz, 2021). Chitosan (CS) is a natural polysaccharide known for its nontoxic, biodegradable, film-forming, and antimicrobial properties. CS has been extensively studied and employed in film coatings to preserve freshness (Arnon, Granit, Porat, & Poverenov, 2015). However, CS films suffer

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https://doi.org/10.1016/j.fochx.2024.101417
Received 17 January 2024; Received in revised form 20 April 2024; Accepted 25 April 2024
Available online 29 April 2024
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from poor water vapor resistance and are prone to brittleness; thus, their ability to effectively preserve freshness is limited, and they are susceptible to structural damage (Yang et al., 2019). Presently, a growing body of research is dedicated to enhancing the performance of the CS films. Researchers are exploring the use of polymer blending and the incorporation of nanomaterials such as titanium dioxide nanoparticles (Xing et al., 2021), zinc oxide nanoparticles (Dulta et al., 2022), and silver nanoparticles (He et al., 2022). While these materials have shown promise in improving the preservation properties of the CS film coatings, the potential unknown toxicity of these inorganic nanoparticles to the human body needs to be acknowledged. Consequently, in response to these safety concerns, current research trends are shifting toward the development of composite edible coatings that combine CS with other functional ingredients; these ingredients include antimicrobial agents, antioxidant enzymes, minerals, vitamins, and antioxidant composites (Pinedo-Guerrero et al., 2017; Saleem et al., 2021; Yu, Xu, Zhou, Zou, & Liu, 2021).

Numerous studies have demonstrated the exceptional antifungal properties of polyethoxylated flavonoids (PMFs) and citral, indicating that they are promising candidates for antifungal agents in the food industry (Wu et al., 2014). PMFs constitute a group of flavonoid compounds primarily derived from citrus peels that possess diverse potent biological activities, such as anti-inflammatory, antioxidant, antimicrobial, and anticancer properties (Ge et al., 2019). Citral is a mixture of geranial and neral and has exhibited robust antifungal activity against *Fusarium*, *Botrytis*, *Penicillium*, and *Aspergillus* (Magalhães et al., 2020). Our previous in vitro and in vivo research demonstrated that the incorporation of PMFs enhanced the antifungal activity of citral emulsions. Compared with citral emulsions, PMFs-loaded citral (PCT) nanoemulsions significantly inhibited the growth and reproduction of *Penicillium*, and their potential antifungal mechanism was related to the disruption of cell membranes; thus, these materials could be considered postharvest preservatives for citrus fruits (Guo et al., 2022; Guo, Mao, Li, & Zhou, 2023). However, due to their volatility, low stability and high fluidity, PCT emulsions have limited practical applications. To overcome these limitations, we integrated this emulsion with CS to broaden its utility. Nevertheless, research on whether the addition of these emulsions to the CS coating films can synergistically enhance film performance and improve citrus preservation through antimicrobial and antioxidant effects is lacking. Therefore, in this study, we incorporated a PCT emulsion into a CS coating film to investigate its impact on preserving citrus fruits from the perspective of fruit resistance.

Citrus fruits can resist external microorganisms and maintain fruit quality, primarily due to their high content of natural resistance compounds; these compounds include peel essential oils, flavonoids, and phenolic acids. Reports have shown a positive correlation between the fruit resistance and the content of polyphenols (Chen, Deng, Ruan, Yi, & Zeng, 2021; Yan, Zhang, Wang, Liu, & Zhang, 2022). Furthermore, the postharvest deterioration in fruit quality can be attributed to fruit aging processes induced by pathogenic or abiotic stresses (Zhang et al., 2016). When fruits are subjected to multiple stressors, they tend to accumulate significant amounts of reactive oxygen species (ROS) as part of their defense mechanism (Mittler, 2017). However, excessive ROS can be detrimental to fruits since lipid peroxidation in the cell membranes is promoted and malondialdehyde (MDA) is accumulated, damages lipid membranes and accelerates fruit aging (Gill & Tuteja, 2010). Fruits possess various antioxidants and antioxidant enzymes that help neutralize excess ROS, establishing an antioxidant network in postharvest stored fruits that plays a critical role in safeguarding against oxidative damage (Xiong et al., 2017). CS coatings have been shown to reduce ROS levels within postharvest fruit cells by inhibiting the production of ROS, increasing the concentration of free flavonoids, and enhancing the activities of antioxidant enzymes such as catalase (CAT) and ascorbate peroxidase (APX). Similar positive outcomes have been reported for other fruits, including nectarines (Zhang et al., 2020), cherries (Szarka, Tomasskovics, & Bánhegyi, 2012), and grapes (Shen & Yang, 2017).

The aim of this study was to develop a coating solution using a PCT emulsion and CS to extend the shelf life of citrus fruits while preserving their quality and nutritional value during storage. The objectives of this research were threefold: (1) to assess the impact of the composite coating on the essential fruit freshness parameters, including decay rate, weight loss, color, texture, total soluble solids (TSS), and titratable acidity (TA); (2) to investigate the influence of the composite coating on the levels of resistance-related components in the fruits, such as ascorbic acid (AsA), total phenolics (TPC), total flavonoids (TFC), organic acids, soluble sugars, and volatile compounds; and (3) to explore the effects of the composite coating on the activities of antioxidant enzymes in the peel and the expression of related genes. Our study provides valuable insights into the use of citrus coatings for freshness preservation.

2. Materials and methods

2.1. Plant material and chemicals

At commercial maturity, Yongquan tangerines (*Citrus reticulata* Blanco) were harvested from an orchard in Linhai city (Zhejiang, China) in early November 2021. Orange fruits free of mechanical injury and without any disease were selected based on their weight (50–60 g) and visible similarity in color and were subsequently sent to the laboratory at Southwest University (Chongqing, China) immediately after harvest. The fruits were then dipped in 1.0% (*v*/v) sodium hypochlorite solution for 2 min, washed with running tap water, and air-dried at 25 °C before the challenge test.

CS was purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). PMFs were extracted from 90% purity citrus fruit peels and purchased from Shanxi Huike Plant Development Co., Ltd. (Shanxi, China). Citral was purchased from Aladdin Reagent Database Co. (Shanghai, China). Caprylic/capric triglyceride (GTCC) was obtained from Shanghai Yuanye Biotechnology Co., Ltd. (Shanghai, China). Tween 80 (TW80) was obtained from Kelon Chemical Reagent Factory (Chengdu, China). The commercial preservative prochloraz (PCZ, 450 g/L) was purchased from Suzhou FMC Plant Protection Agent Co., Ltd. (Suzhou, China).

2.2. Preparation of the PMFs-loaded citral/chitosan composite coatings (CS-PCT)

The preparation of PCT was based on the method reported in previous studies (Arnon et al., 2015; Guo et al., 2022), with some alterations. First, a certain amount of CS powder was weighed and dissolved in a glacial acetic acid solution (1%, *v*/v), stirred at room temperature for 24 h with a mechanical stirrer, and centrifuged at 5000 ×*g* for 10 min to remove impurities and bubbles to obtain a pure CS-acetic acid solution (2% *w/v*). Second, 1 g of citral containing 30 mg of PMFs was weighed and mixed with 0.5 g of caprylic glyceride and 3 g of TW80. After the mixture was stirred on a magnetic stirrer (DF-II, Jintan Youlian Instrument Research Institute, China) at 2600 r/min for 20 min, 45.5 g of distilled water and 50 g of pure CS-acetic acid solution (2% w/v) were successively added dropwise with constant stirring. Then, the harvested PCT was ultrasonicated with an ultrasonicator (KQ-500DE; Kunshan Ultrasound Instrument Co., Ltd., China) at an amplitude of 60 for 5 min to reduce the droplet size, and the sample was then preserved at 4 °C.

2.3. Fruit treatments

All selected citrus fruits were randomly divided into six groups, each comprising 72 fruits. Among these, three groups were allocated for sampling, physiological and biochemical analysis, RNA extraction, and relevant gene expression. Concurrently, an identical set of fruits was retained for determining weight loss rates and color changes.

The control group is designated as the CK group and did not undergo

any treatment. The commercial preservative prochloraz (PCZ) group was immersed in a PCZ solution diluted 1000 times for 2 min and subsequently air-dried at 25 °C. This group was labeled as the PCZ group. The coating groups were subjected to immersion in pure chitosan (CS) coating solution and a chitosan-polyethoxylated flavonoid-loaded citral (CS-PCT) composite coating solution for 2 min. Afterward, the samples were removed and dried at 25 °C and are referred to as CS and CS-PCT, respectively. Following air-drying at 25 °C, the fruits were placed in plastic boxes, and these boxes were subsequently placed in a constant temperature and humidity chamber. The chamber settings used a temperature of 25 °C and humidity of 85%. The experiment consisted of three replicates, and the total storage duration was 60 d. Fresh fruits were randomly selected from each group at different time points (0 d, 7 d, 15 d, 24 d, 34 d, 46 d, and 60 d) to assess their preservation indicators.

2.4. Determination of the fundamental preservation indices of the fruits

2.4.1. Decay rate and weight loss rate

The decay rate and weight loss rate of each group of citrus fruits were calculated according to the following formulas:

$$\text{Decay rate } (\%) = \frac{rotted\ fruits}{total\ fruit} \times 100$$

$$\text{Weight loss rate } (\%) = \frac{(W_0 - W_1)}{W_0} \times 100$$

where W_0 and W_1 are the initial and final weights of the same fruits, respectively.

2.4.2. Peel color

Using a HunterLab MiniScan XE Plus colorimeter (Hunter Associates Laboratory, Inc., Reston, VA), color measurements were recorded at four uniformly distributed positions along the equator of the fruit. The CIE 1976 Lab* color space was used.

The citrus color index (CCI) was calculated using the following formula:

$$\text{Citrus color index (CCI)} = \frac{1000 \times a^*}{L^* \times b^*}$$

where L^* denotes the brightness, and a greater value correlates to a brighter surface of the measured sample; a^* denotes the red–green difference; and b^* denotes the yellow–blue difference. The CCI values vary from small to large and denote the change in fruit color from green to yellow and then to orange–red.

2.4.3. Fruit firmness

The skin was removed from the area opposite the equator of each fruit. Fruit firmness was determined with a fruit firmness tester (GY-4, Zhejiang TOP Instrument Co. Ltd., Hang Zhou, China) fitted with an 11.1 mm diameter flat probe. The probe penetration depth was 10 mm. The firmness is expressed in N as the force.

2.4.4. TSS and TA of the pulp

The TSS and TA contents were determined by a PAL-BX/ACID F5 portable digital sugar meter (Guangzhou Aidang Scientific Instrument Co., Ltd., Guangzhou, China) and are expressed as percentages. The results are expressed as a percentage of the malic acid equivalent.

2.5. Resistance-related components in the citrus fruits

2.5.1. Organic acids and soluble sugars in the pulp

For the preparation of the fruit pulp extract, 8 mL of methanol was added to 2 g of fruit pulp, the mixture was homogenized, and ultrasonic-assisted extraction was performed at 50 °C for 30 min. Subsequently, the extract was centrifuged at 10000 ×*g* for 5 min at 25 °C. This extraction process was repeated twice. The supernatants from the three consecutive extractions were combined and stored in dark bottles at 4 °C.

Organic acids (citric, malic, and quinic acids) and soluble sugars (fructose, glucose, and sucrose) were analyzed using an HPLC system (Waters, MA, USA) following a previously described method with minor modifications (Tao, Chen, Li, Guo, & Zhou, 2022). The HPLC instrument parameters were set in accordance with previous methods. The contents of the soluble sugars and organic acids were calculated using standard curves, and the results are expressed in g/kg FW (fresh weight) with standard deviations.

2.5.2. AsA in the pulp

Ascorbic acid (AsA) was quantified following the method described by Nováková, Solich, and Solichová (2008) with slight modifications. A 50 mg sample (prepared in Section 2.4.1) was dissolved in 10 mL of 5% methanol containing citric acid (21 g/L) and EDTA (0.5 g/L). Subsequently, the mixture was centrifuged at 4000 r/min for 5 min, and the resulting supernatant was filtered through a 0.45 μm aqueous filtration membrane. The filtrate was purified using a C_{18} Sep-Pak cartridge (Waters, Milford, MA, US). HPLC analysis was carried out after derivatization of dehydroascorbic acid (DHAA) into the fluorophore 3-(1,2-dihydroxyethyl)fluorouracil [3,4-b]quinoxaline-1-one (DFQ) with 1,2-phenylenediamine dihydrochloride (OPDA). Qualitative analysis was based on the peak retention times, and quantitative analysis was performed using external standards according to the peak areas. The results are expressed as μg/g FW (fresh weight) with standard deviations.

2.5.3. TPC and TFC in the peel

For the preparation of peel extracts for TPC and TFC, 15 mL of 80% methanol were added to 2 g of ground citrus peel under liquid nitrogen, followed by ultrasonic extraction for 30 min at 25 °C. The mixture was then centrifuged at 4 °C and 4000 ×*g* for 5 min, and the supernatant was then collected. This extraction process was repeated three times, and the combined supernatants were stored in dark bottles at 4 °C.

The TPC was determined using the Folin–Ciocalteu method (Liu et al., 2012) with slight modifications. In brief, 0.3 mL of the test solution was mixed with 10 mL of ultrapure water, followed by the addition of 1 mL of Folin–Ciocalteu reagent. After shaking, the mixture was allowed to react in the dark for 5 min. Subsequently, 5 mL of 5% Na_2CO_3 was added, and the volume was adjusted to 25 mL with ultrapure water. After thorough shaking, the mixture was left in the dark for 60 min, and the absorbance was then measured at a wavelength of 750 nm. The results are expressed in terms of gallic acid equivalents (GAE).

The TFC was determined using the aluminum nitrate colorimetric method (Liu et al., 2012). In brief, 0.5 mL of the test solution was mixed with 0.7 mL of ultrapure water, 0.4 mL of 5% $NaNO_2$ was added, and the mixture was left in the dark for 6 min. Afterward, 0.4 mL of 10% $Al(NO_3)_3$ was added, and the mixture was left in the dark for an additional 6 min. Then, 0.7 mL of 40 g/L NaOH was added, and the volume was adjusted to 5 mL with ultrapure water. After thorough shaking, the mixture was left in the dark for 15 min, and the absorbance was then measured at a wavelength of 510 nm. The results are expressed in terms of the rutin equivalent (RE).

2.5.4. Volatile composition of the peel

The volatile compositions of the samples were determined using a GCMS-QP2010 gas chromatography–mass spectrometry system (Shimadzu Corporation, Kyoto, Japan), as described previously (Tao et al., 2022), with slight modifications. The extraction of volatile components from citrus peels by headspace solid-phase microextraction (HS-SPME). Two grams of the samples and 5 mL of saturated NaCl solution were added to 10 mL glass vials. The vials were exposed to fibers (DVB/CAR/PDMS, needle size 24 ga, length 1 cm; Sigma Aldrich) for extraction. The extraction conditions included equilibration at 42 °C for 6.21 min, desorption for 3.4 min and extraction for 45 min. The GC–MS instrument parameters were set according to previous methods. To screen out

substances with similarities of 80% and above, the mass spectra of the detected compounds were matched using a mass spectra computer library (NIST 2008 and Flavor 2.0), and the peak area normalization method was adopted to calculate the relative contents, with three replicates of each.

2.6. Malondialdehyde (MDA) content in the peel

The MDA content was determined via the thiobarbituric acid colorimetric method (Papastergiadis, Mubiru, Van Langenhove, & De Meulenaer, 2012). Three replicates were conducted for each treatment, and the results are expressed in μmol/g.

2.7. Defense-related enzyme activities in the peel

Catalase (CAT), superoxide dismutase (SOD), peroxidase (POD), and ascorbic peroxidase (APX) activity assay kits were purchased from Beijing Solarbio Science & Technology Co., Ltd. (Beijing, China). First, 0.1 g of citrus peel was extracted on ice with an extractant at a ratio of 1:5–10 (*w/v*) and then centrifuged at 8000 ×*g* for 10 min. Then, the supernatant was mixed with the reagent following the manufacturer's instructions, and the absorbances of CAT, SOD, POD, and APX were measured at 240 nm, 560 nm, 470 nm, and 290 nm, respectively. The results are expressed in U/g fresh weight.

2.8. Resistance-related gene expression in the peel

2.8.1. Total RNA extraction and cDNA synthesis

Total RNA was independently extracted from peel samples that were harvested from plants subjected to different coating treatments using a total RNA extraction kit. The total RNA extracted was quantified using a microsampler (Nanodrop 2000, Wilmington, DC, USA) and confirmed by plain agarose gel electrophoresis. Following the manufacturer's instructions, a PrimeScript RT reagent kit was used to synthesize the first-strand cDNA used as the template for real-time quantitative PCR analysis.

2.8.2. Primer design

Based on the target gene (CAT, SOD, POD, APX) sequences from the GENBANK database, the primers for the chosen genes were designed with Primer Premier 6.0 software (Premier Biosoft International, Palo Alto, CA, USA) and are listed in Table S1. Citrus actin was used as the reference gene for normalizing the expression of each sample.

2.8.3. Real-time quantitative polymerase chain reaction analysis

RT–qPCR was performed in 96-well PCR plates using a Bio-Rad CFX96 Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA) according to the instructions of the kit under the following procedures: pre-denaturation at 95 °C for 30 s, followed by 40 cycles at 95 °C for 5 s, 60 °C for 10 s and 72 °C for 15 s. Each gene was subjected to three biological replicates. The relative RNA expression was calculated using the $2^{-\Delta\Delta Ct}$ method.

2.9. Statistical analysis

All data were analyzed and processed using SPSS 25.0 statistical software. Analysis of the variance was performed to analyze the significance of differences among the different groups using Duncan's multiple range test ($p < 0.05$) following one-way ANOVA.

3. Results

3.1. Effect of CS-PCT on the key fruit freshness indicators

After 60 d of citrus storage, fruits in the CK and PCZ groups had reduced quantities of fruits, accompanied by varying degrees of blackening and shriveling on their surfaces, while fruits in the CS and CS-PCT groups were more abundant and displayed a vibrant appearance (Fig. 1 A). This observation indicated that the coating treatments effectively preserved the visual appeal of the fruit.

The decay rate of the fruits continued to increase during storage (Fig. 1 B). The number of deteriorated fruits after storage was significantly greater in the CK and CS groups than in the PCZ and CS-PCT groups, with the decay rate in the CS-PCT group being lower than that in the PCZ group. After 60 d of storage, the decay rates for CK, PCZ, CS, and CS-PCT were 51.39%, 37.50%, 41.67%, and 30.56%, respectively.

The rate of the fruit weight loss progressively increased with increasing storage time (Fig. 1 C). This rate was notably lower in the CS and CS-PCT groups than in the CK and PCZ groups, highlighting the ability of the coating treatments to mitigate fruit weight loss. After 60 d of storage, the weight loss rates in the CS and CS-PCT treatment groups were 8.63% and 8.11%, respectively, which were significantly lower than those in the CK group (10.14%) and the CS group (9.88%).

As depicted in Fig. 1 D, as the storge time increased, the color indices of the fruits progressively increased. The CS and CS-PCT treatments were effective at preserving the color of the fruits in contrast to the CK and PCZ treatments; these results were consistent with those shown in Fig. 1A. The results for the CS and PCZ groups mirrored those for the CK and PCZ groups.

Regarding the fruit firmness (Fig. 1 E), both the CK and CS treatments had more pronounced effects, while the CS and CS-PCT treatments significantly decelerated the change in fruit hardness. After 60 d of storage, the hardnesses of the CK, PCZ, CS, and CS-PCT groups were 8.63 N, 8.47 N, 9.03 N, and 9.4 N, respectively. These values were considerably lower than the initial value of 12.27 N.

3.2. Effect of CS-PCT on the TSS and TA contents of the pulp

The TSS accumulation in fruits exhibited a pattern of an initial increase followed by a decrease during the early stages of storage (Fig. 1 F). The coating treatment contributed to delaying the peak TSS levels, and the TSS content in the fruits of the CK and CS groups remaining significantly greater than that in the coated group until 24 d of storage. In the control group, the maximum TSS content reached 12.97%. The maximum TSS content in the CS and CS-PCT groups reached 11.93% and 12.22%, respectively, at 35 d. Subsequently, the TSS content in the fruits gradually decreased. At the end of storage, the TSS contents in the CK, PCZ, CS, and CS-PCT groups were 10.83%, 10.36%, 11.07%, and 11.47%, respectively.

The content of TA in the mandarin fruits tended to decrease with increasing storage time; moreover, the CS and CS-PCT treatments effectively reduced the TA content during storage (Fig. 1 G). After 60 d of storage, the TA content significantly decreased. The TA contents of CS and CS-PCT were 0.57% and 0.62%, respectively ($p < 0.05$); these values were significantly greater than those in the CK (0.54%) and PCZ (0.49%) groups.

3.3. Effect of CS-PCT on the organic acid content in the pulp

The content of organic acids, including citric, malic, and quinic acids, in fruits was monitored throughout the storage period. Among these acids, citric acid was the primary organic acid present in the pulp, constituting 74.6% of the total acidity. In both the CK and CS groups, the organic acid content generally decreased with increasing storage time (Fig. 2 A, 2 B, and 2C). Specifically, the CS group exhibited a continuous decrease in citric acid and quinic acid, while malic acid showed an initial increase followed by a subsequent decrease, reaching its peak value of 1.28 g/kg FW on the seventh day. The CS-PCT group demonstrated an initial increase in the content of all three organic acids, followed by a decrease in the content of all three organic acids. Citric acid and malic acid reached peak values of 4.09 g/kg FW and 1.36 g/kg FW, respectively, on the fifteenth day, while quinic acid reached its maximum

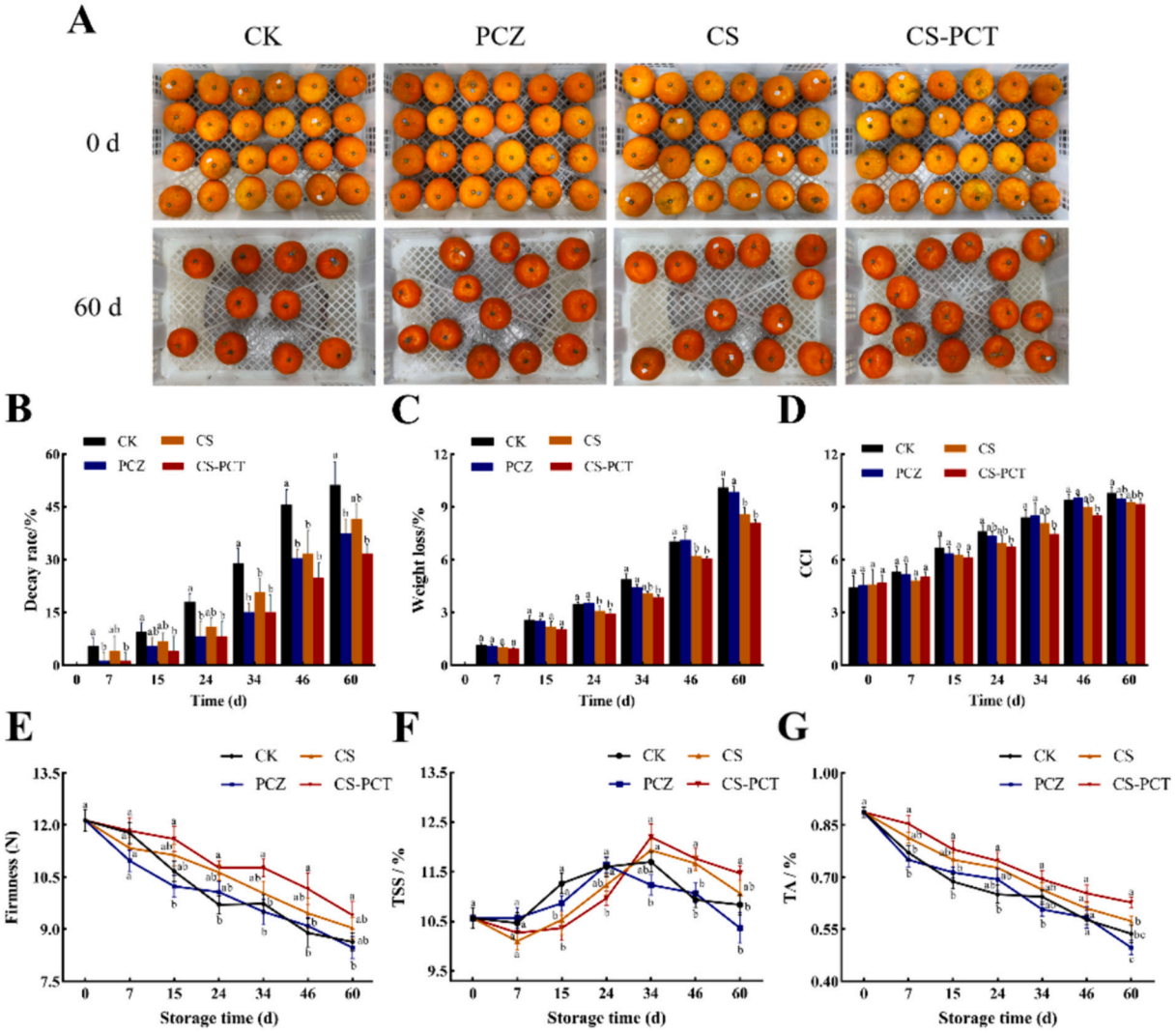

Fig. 1. Effect of CS-PCT treatment on the appearance (A), decay rate (B), weight loss (C), color index (D), firmness (E), TSS content (F), and TA content (G) of citrus fruits during storage at 25 °C for 60 d. Values followed by different superscripts (a–b) are significantly different ($p < 0.05$) at the same sampling time. The vertical bars represent the standard errors of the means.

value of 0.33 g/kg FW on the seventh day. Consequently, the composite coating was more effective at preserving the organic acids in the fruits and maintaining their flavor.

3.4. Effect of CS-PCT on the soluble sugar content in the pulp

The changes in the levels of the three soluble sugars (fructose, glucose, and sucrose) in the pulp during storage are illustrated in Fig. 2 D, 2 E and 2 F. The glucose and sucrose contents exhibited an initial increasing trend followed by a decreasing trend, while the fructose content demonstrated a relatively stable pattern. As shown in Fig. 2 D, both the glucose and sucrose contents in the CK group reached their lowest levels at 34 d of storage, and after 46 d, they showed an increasing trend. Notably, the sugar content in the CS-PCT group were consistently greater than those in the other three treatment groups. At the end of the 60 d storage period, the glucose contents in the CK, PCZ, CS, and CS-PCT groups were 20.90 g/kg FW, 13.43 g/kg FW, 18.96 g/kg FW, and 22.77 g/kg FW, respectively. As shown in Fig. 2 E, a significant reduction in the sucrose content was observed after 7 d, followed by relative stabilization and an increasing trend after 46 d. At 60 d, the sucrose contents in the CK, PCZ, CS, and CS-PCT groups were 64.46 g/kg FW, 65.40 g/kg FW g, 71.65 g/kg FW, and 75.05 g/kg FW, respectively. As shown in Fig. 2 F, the fructose contents in the CK, PCZ, and CS groups exhibited an overall fluctuating downward trend with increasing storage duration, and their contents were significantly lower than their initial values. In contrast, the fructose content in the CS-PCT group showed an increase during the initial storage period, followed by a decrease after 24 d; during the storage time, the fructose content in the CS-PCT group was consistently greater than those of the other treatment groups and the initial value throughout the entire storage period. Thus, compared to the other treatments, the composite coating effectively preserved the levels of glucose and sucrose and reduced the loss of fructose in the fruits.

3.5. Effect of CS-PCT on the AsA content in the pulp and the TPC and TFC contents in the peel

Regarding the AsA content (Fig. 3 A), all groups of fruits exhibited a fluctuating downward trend, which was likely attributed to the disruption of pericarp cell membranes in the middle and late stages of fruit storage. At the end of storage, in the CK, PCZ, and CS groups, the AsA content was 37.59%, 41.79%, and 41.89% of the initial value, respectively. In contrast, the AsA content in the CS-PCT treatment group was maintained at 47.05% of the initial value at the end of storage; this value was significantly greater than those in the other treatment groups. Furthermore, throughout the storage period, the AsA content of the

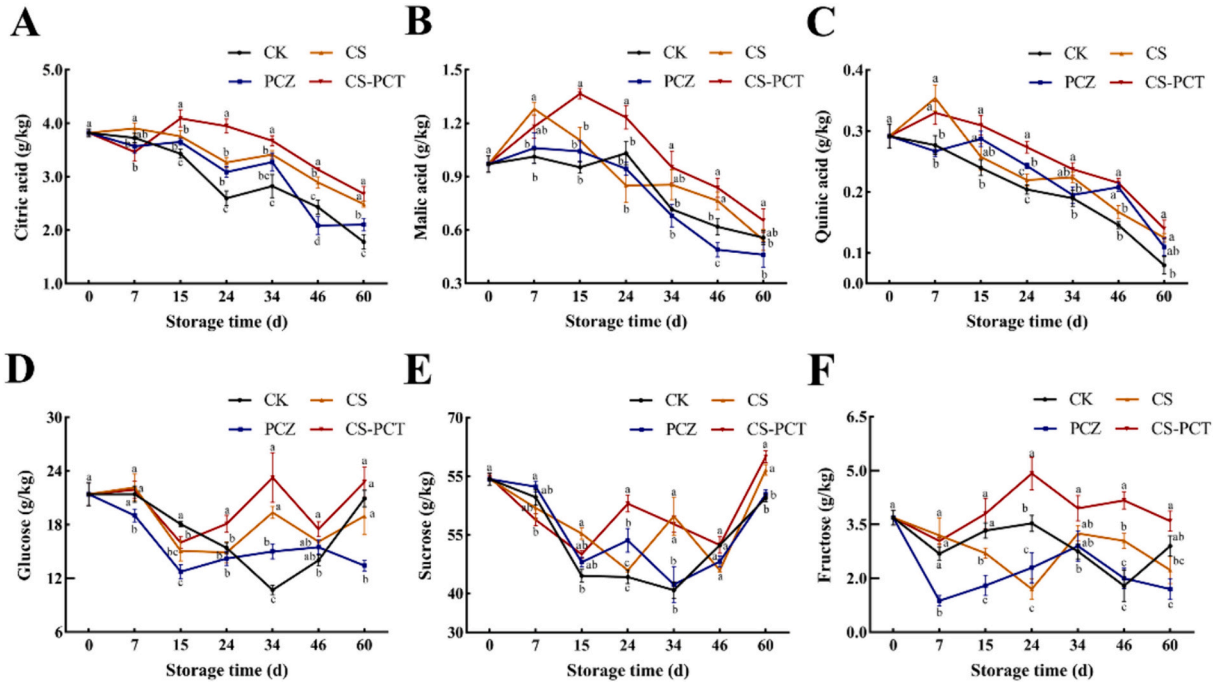

Fig. 2. Effect of the CS-PCT treatment on the levels of citric acid (A), malic acid (B), quinic acid (C), glucose (D), sucrose (E), and fructose (F) in the pulp during storage at 25 °C for 60 d. Values followed by different superscripts (a–d) are significantly different ($p < 0.05$) at the same sampling time. The vertical bars represent the standard errors of the means.

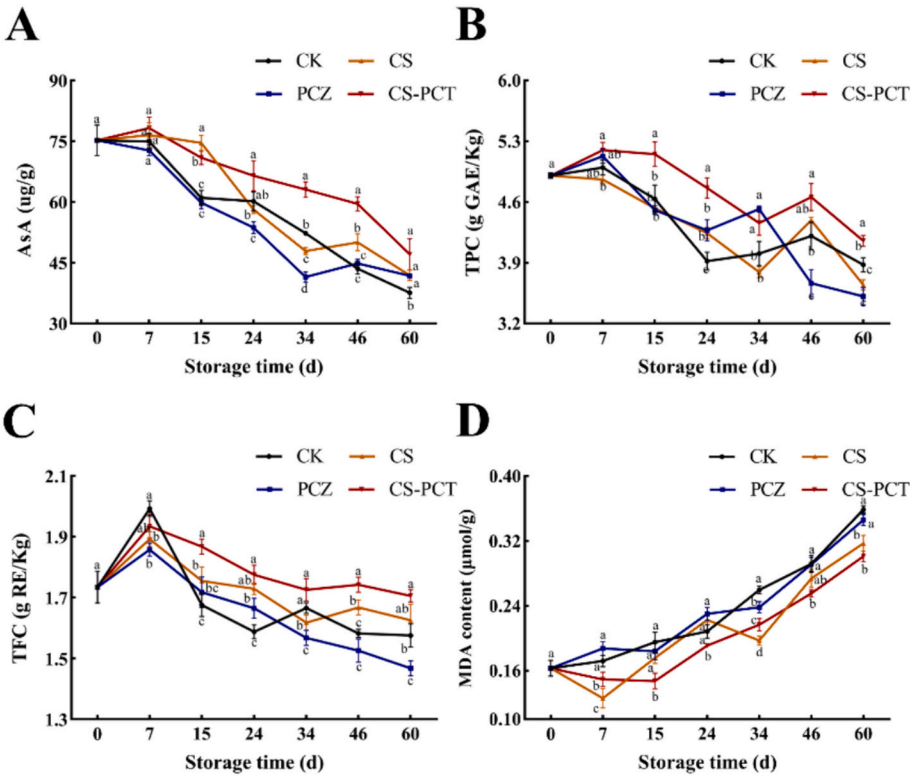

Fig. 3. Effect of the CS-PCT treatment on the contents of AsA (A), TPC (B), and TFC (C) in the pulp and MDA (D) in the peel during storage at 25 °C for 60 d. Values followed by different superscripts (a–d) are significantly different ($p < 0.05$) at the same sampling time. The vertical bars represent the standard errors of the means.

fruits in each coating group rapidly decreased. The impact of various coating treatments on the TPC and TFC of citrus peels is illustrated in Fig. 3 B and 3C. The trend for the TPC gradually decreased throughout the storage period. In contrast, the TFC increased after 7 d of storage, followed by a gradual decrease. Notably, the total phenolic and total flavonoid contents were greater in the CS-PCT treatment group than in the other treatment groups. These results indicated that the application of a CS film containing PCT before storage could delay the degradation

of total phenolics and TFC in the rinds of Yongquan tangerines after harvest, thereby preserving their nutritional value for a longer duration.

3.6. Effect of CS-PCT on the volatile components in the peel

A total of 34 volatile compounds, including terpenes, alcohols, aldehydes, lipids, and acids, were detected in the Yongquan tangerines, and the primary compounds are listed in Table S2. Over the storage period, the overall content of volatile compounds in Yongquan tangerines gradually decreased. Specifically, on the seventh day, the total volatile compound contents in the CK and PCT groups were 89.75% and 86.89% of the initial values, which subsequently decreased to 22.72% and 23.54%, respectively, by the end of the 60-d period. The CS-treated group experienced a more substantial decrease in the total volatile compound content, and the content in the CS group reached 33.66% of the initial value at the end of the storage period. In contrast, the CS-PCT treatment resulted in a slower loss of volatiles, and the total content was 42.38% of the initial value occurring by the end of the 60-d period; this value was significantly greater than those of the other three groups. Furthermore, a detailed analysis of the key volatile compounds, including D-limonene, γ-pinene, β-myrcene, and α-pinene, was conducted. D-limonene was the predominant volatile compound in Yongquan tangerines and constituted >50% of the total volatiles; its content exhibited a significant decreasing trend during the storage period, especially after 46 d. The reduction was rapid, and the CS-PCT-treated group experienced a notably slower decrease than the more-treated group. Compounds of α-pinitol, nerolidol, hexenal, and anisaldehyde had relatively low initial concentrations, gradually decreased throughout the storage process, and ultimately reached minimal concentrations. In summary, the total volatile component content in the fruits decreased during the storage period. Several major volatile compounds, including D-limonene, β-pinene, and α-phellandrene, exhibited decreasing trends.

3.7. Effect of CS-PCT on the MDA content in the peel

MDA serves as the end product of membrane lipid peroxidation and is recognized as a pivotal indicator of the extent of membrane oxidative damage in plant tissues (Chen, Tait, & Kitts, 2017). Throughout the 60-d storage period, the MDA content in the peels of the various coating treatment groups exhibited a consistent increase (Fig. 3 D). Nonetheless, the MDA content in Yongquan tangerines treated with the CS film containing the PCT emulsion was consistently lower than those in the CK, PCZ, and CS groups. This observation indicated that treatment of the CS membrane with the PCT emulsion effectively mitigated membrane lipid peroxidation damage and preserved the antioxidant capacity of the citrus peels.

3.8. Effect of CS-PCT on antioxidant enzyme activity in the peel

As shown in Fig. 4 A, the CAT activity in the fruits of all four treatment groups exhibited a rapid increase, reaching a maximum at 34 d, followed by a sharp decrease. Notably, the activity of CAT in the pericarp of the composite-coated fruit at 34 d significantly surpassed those of the other treatment groups. As illustrated in Fig. 4 B, the SOD activity in all four groups displayed an increasing trend in the pre-storage phase, reached a maximum at 24 d, and then gradually decreased. During this period, the composite-coated group exhibited notably greater activity than the other treatment groups. As shown in Fig. 4 C and 4 D, the trends in the POD and APX activities during the storage period followed similar patterns, with activities in the fruits of the four different treatment groups showing a consistent increase. The activities of both enzymes reached their maximum at 34 d and then gradually decreased.

3.9. Effect of CS-PCT on the defense-related genes in the peel

In this study, we assessed the transcript levels of four defense-related genes (CAT, SOD, PPO, and APX) via RT–qPCR (Fig. 5). Fig. 5 shows the

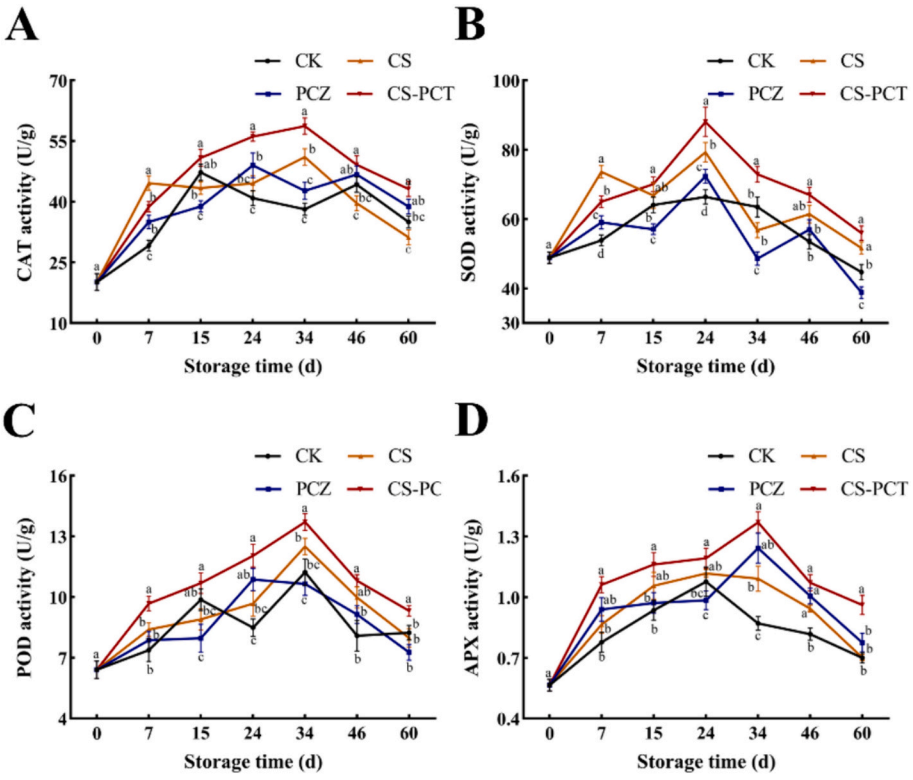

Fig. 4. Effect of the CS-PCT treatment on the activities of CAT (A), SOD (B), POD (C), and APX (D) in the peel during storage at 25 °C for 60 d. Values followed by different superscripts (a–c) are significantly different ($p < 0.05$) at the same sampling time. The vertical bars represent the standard errors of the means.

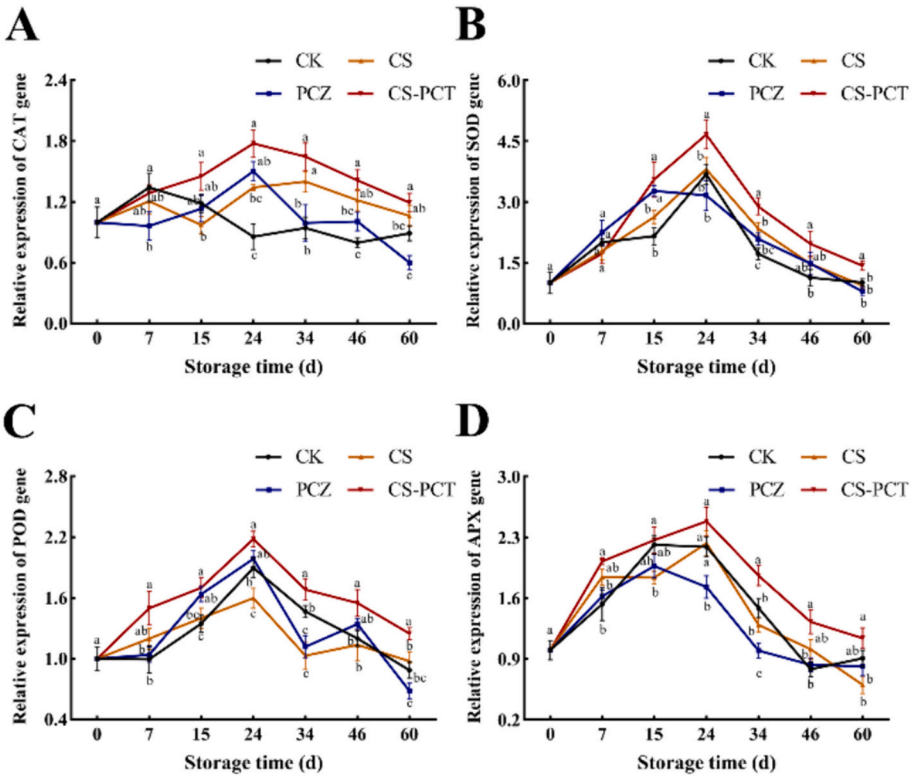

Fig. 5. Effect of the CS-PCT treatment on the expression of CAT (A), SOD (B), POD (C), and APX (D) in the peel during storage at 25 °C for 60 d. Values followed by different superscripts (a–c) are significantly different ($p < 0.05$) at the same sampling time. The vertical bars represent the standard errors of the means.

relative expression of these four genes in the citrus peels, and their expression profiles displayed similar patterns. Over the course of the 60-d storage, the gene expression in the orange peels exhibited an initial rapid increase, followed by a more gradual increase toward the end of the storage period. In the CK and PCZ treatment groups, the expression of these genes exhibited an initial fluctuating increase in the early stages of storage and then decreased. Conversely, in the CS and CS-PCT treatment groups, the genes were significantly upregulated before 24 d of storage, and their expression decreased afterward. Importantly, compared to those of the CK, PCZ, and CS groups, the composite CS-PCT coating treatment group showed significantly greater gene expression levels. These results indicated that the composite emulsion treatment effectively stimulated the expression of pericarp genes (CAT, SOD, PPO, and APX), which subsequently led to increased production of the corresponding antioxidant enzymes; this then enhanced the resistance of the fruit to stress factors.

4. Discussion

In this study, to address the volatility and long-lasting effects of emulsions in citrus postharvest preservation applications, a composite coating solution (CS-PCT) that combines a PMFs-loaded citral (PCT) nanoemulsion with chitosan (CS) was prepared. We explored the feasibility of this preservative by assessing its efficacy in improving postharvest citrus fruit resistance. Our research not only provides the foundation for expanding the utilization of active components derived from citrus plants and their emulsions but also facilitates the creation of innovative composite preservatives. Furthermore, these findings enhance the understanding of the preservation mechanisms employed by these preservatives.

The assessment of fruit storage quality relies on key indicators such as decay rate, weight loss, hardness, TSS, TA, and color. The dynamics of postharvest storage are intricately linked to the following factors: fruit variety, storage conditions, ripening, and senescence stages (López-Gómez et al., 2020; Nie, Huang, Chen, Wan, & Chen, 2020). CS possesses notable water absorption properties and excellent film-forming capabilities, effectively acting as a barrier to gases and moisture. This property reduces fruit metabolism and minimizes nutrient consumption (Arnon et al., 2015). Chen, Nie, Wan, and Chen (2019) showed the effects of adding *Ficus hirta* Vahl. The coating of Xinyu tangerines with a flavonoid extract-chitosan coating significantly reduced the risk of fungal infection and extended the storage lifespan of the plants. The CS-PCT prophylactic coating serves dual purposes by acting as a deterrent or eradicator of surface pathogens on citrus plants while also mitigating the attachment of airborne pathogens through physical isolation. This multipronged approach significantly lowers the risk of fruit rot. Anjum, Akram, Zaidi, and Ali (2020) showed that the use of film coatings, either alone or supplemented with plant extracts, decelerated the degradation rate of TA in guinea plants. Incorporating CS-PCT composite coatings contributes to reduced water evaporation, limited gas exchange, delayed nutrient degradation, minimized weight loss, and superior color preservation in fruits.

Citric, malic, and quinic acids are essential organic acids found in Yongquan tangerines and play vital roles in respiration and sugar anabolism processes. Typically, these acids are preferentially utilized as respiratory substrates (Osorio, Scossa, & Fernie, 2013). The changes in the concentrations of these compounds have significant impacts on the fruit flavor, the degree of senescence, and the ability of fruits to adapt to postharvest storage conditions (Sun et al., 2013). In a study by Taş et al. (2021) involving strawberries, the application of 1-methylcyclopropane (1-MCP) resulted in reduced biochemical changes, mitigating the decomposition of citric, malic, and oxalic acids and ultimately preserving the postharvest quality of the fruits. Energy metabolism in fruits occurs predominantly through glycolysis and the tricarboxylic acid (TCA) cycle within mitochondria and involves coupled electron transfer and oxidative phosphorylation. These processes produce intermediates, including citric acid and malic acid (Wang et al., 2022). Consistent with our findings, under the CS-PCT treatment, the concentrations of citric

acid, malic acid, and quinic acid were notably greater than those in the control group during the late storage stage. These findings indicated that the coating treatment could inhibit fruit respiration or energy metabolism, ultimately enhancing the ability of fruits to adapt to postharvest conditions.

Plants possess a nonenzymatic defense system that strengthens their antioxidant capacity. Nishizawa, Yabuta, and Shigeoka (2008) introduced the concept of "sugars as antioxidants;" the role of soluble sugars, particularly sucrose, fructose, and glucose, were highlighted, and these sugars are considered crucial compounds that shield plants from oxidative stress and show ROS scavenging properties (Cao, Yang, & Zheng, 2013). Glucose serves as a precursor for AsA biosynthesis. Studies on passion fruit have shown a strong positive correlation between the fructose and glucose levels and the ASA content (Devi Ramaiya, Bujang, Zakaria, King, & Shaffiq Sahrir, 2013); thus, these two substances likely contribute to the overall antioxidant properties of the fruit. Sucrose and sucrose-based oligosaccharides (SOSs) are linked to oxidative defense and scavenging and can potentially stabilize membrane-associated peroxidases. SOS-derived free radicals potentially play an intermediary role in redox reactions near cell membranes (Van Den Ende & Valluru, 2008). In our study, the CS-PCT treatment effectively maintained the glucose and sucrose levels and reduced the loss of fructose in the fruit. Our comprehensive analysis of the oxidative stress within the fruit indicated that these soluble sugars could also act as antioxidants during the storage process. This dual role preserves the fruit's flavor and extends its storage period.

AsA plays a significant role in the antioxidant activity of citrus plants and protects plant tissues against various biotic and abiotic stresses; it also contributes to maintaining fruit disease resistance during storage (Nie et al., 2020). Typically, substrates utilized for ASA synthesis are gradually consumed by respiration and other metabolic processes over time, and the loss of ASA is accelerated by stimuli from pathogenic bacteria (Kalt, Forney, Martin, & Prior, 1999). For instance, Khaliq, Ullah, Memon, Ali, and Rashid (2021) reported a substantial reduction in the ASA content in custard apple (*Annona squamosa L.*) during storage, and the pathogenic bacteria accelerated this loss. In our study, we noted a significant decrease in the ASA concentration in the control group of Yongquan tangerines. However, this decrease was less pronounced in the film-coated treatment group than in the control group; additionally, the AsA content in the CS-PCT treatment group did not significantly decrease until 46 d. The variations in the AsA content were influenced by the fruit condition, metabolism-related enzyme activities, and the extent of fruit spoilage after coating treatment; these results were consistent with those from Chien and Chou (2006).

TPC and TFC are essential secondary metabolites in fruit and exert a significant positive influence on enhancing the fruit resistance (Ge et al., 2019). TPC directly inhibits the growth of the pathogenic fungi and plays a role in lignin synthesis (Yan et al., 2022). Phenols can also chelate metal ions, inhibit the activity of certain enzymes in microorganisms, and reduce the destruction of the plant bodies by the pathogenic bacteria (Chen et al., 2021). A reduction in flavonoid content directly compromises the disease resistance of postharvest fruit. Wei et al. (2023) and Deng et al. (2015) associated the decreased resistance to *Penicillium* in ripening citrus plants with a decrease in the flavonoid synthesis and content as the fruits ripened. Various phytoantimicrobial constituents include p-coumaric acid, coumarins, polymethoxy flavonoids in citrus peel, and flavanones found in the white peel layer (e.g., naringenin and hesperidin); these constituents have been proposed to enhance the fruit resistance to pathogens. Deng et al. (2015) reported that the accumulation of the total phenolics and flavonoids improved melon resistance to the pathogenic fungi. In our study, we observed significantly greater TPC and TFC in the CS-PCT-treated Yongquan tangerine group than in the other treatment groups. We postulated that the reduced fruit decay rate was potentially attributed to the potential activation of the phenylpropanoid pathway by CS-PCT in Yongquan tangerine rinds. Subsequently, this maintenance of high levels of total phenolics and TFC in the rind enhances the resistance of the fruit to pathogenic fungi.

Citrus peels contain a variety of volatile organic compounds (VOCs) with antimicrobial properties; these compounds include olefins, terpenes, aldehydes, and alcohols. Previous studies have demonstrated that these constituents can act as natural antimicrobial and antioxidant agents and prevent pathogenic bacterial infections in citrus plants (Frassinetti, Caltavuturo, Cini, Della Croce, & Maserti, 2011; Magalhães et al., 2020). For instance, bergamot essential oil exhibits antifungal activity against fungi such as *Aspergillus niger* and *Penicillium expansum* and has antioxidant properties (Cebi & Erarslan, 2023). Several volatiles, including D-limonene, γ-pinene, β-myrcene, α-pinene, and β-pinene, identified in our study have been previously reported to inhibit fungal growth and enhance fruit resistance. Notably, the changes in the volatile constituents during citrus storage have been documented. Factors such as exposure to cold damage, blue light irradiation, and fade-to-green treatment (Liao, Alferez, & Burns, 2013; Sdiri, Rambla, Besada, Granell, & Salvador, 2017) can affect the content of the volatile constituents. In our experiment, we observed a gradual decrease in the total volatile content, but at the end of the 60-d storage period, the CS-PCT group exhibited a significantly greater volatile content than the other two groups. These findings indicated that CS-PCT effectively inhibited the reduction in volatile compound concentrations. However, the levels of the major volatile components, including D-limonene, γ-pinene, and β-myrcene, were greater in the treated group than in the control group, but the differences were not statistically significant. Due to the large number of volatile components and their inconsistent changes, the CS-PCT coating treatment considering the total volatile compounds more effectively preserved the fruit resistance.

When a plant is stressed, its defense system is triggered, leading to increased activity and content of resistance enzymes. This response helps reduce the accumulation of harmful substances and restore the redox homeostasis of tissues during storage; this ultimately enhances the disease resistance (Panda, Rangani, & Kumar Parida, 2019). Enzymes such as SOD, CAT, APX, and POD play crucial roles in eliminating excess ROS from fruits, while the accumulation of MDA exacerbates membrane damage and accelerates the fruit senescence and decay (Nie et al., 2020). Certain postharvest treatments, such as biocontrol and antimicrobial agent applications, have been shown to significantly increase the activities of these enzymes (Yan et al., 2022). For instance, CS treatment was found to enhance the activities of polyphenol oxidase (PPO) and POD in tomato fruits (Liu et al., 2017). As fruit storage time increased, the activities of antioxidant enzymes tended to decrease; the ROS accumulation surpassed the scavenging capacity, and the fruits were more susceptibility to damage. Conversely, CS-PCT treatment enhanced antioxidant enzyme activities. Enzyme activities significantly increased from 0 to 34 d, effectively protecting citrus fruits. APX is a key enzyme involved in cellular hydrogen peroxide scavenging by catalyzing AsA, helps regulate signal transduction in response to plant stress and controls the ROS levels (Imahori, 2014). POD converts carbohydrates into lignin and strengthens the cell defenses against pathogenic bacterial infestations through increased cell lignification (Christensen, Bauw, Gjesing Welinder, Van Montagu, & Boerjan, 1998). In our study, the CS-PCT composite coating promoted increased antioxidant enzyme activity, reduced MDA accumulation, and minimized membrane lipid peroxidation in Yongquan tangerine peels. These findings indicated that coating helped maintain the membrane integrity and enhanced the resistance of postharvest mandarin oranges to adverse conditions.

Increasing the expression levels of resistance-related genes can increase disease resistance in fruits (Segal & Wilson, 2018). To elucidate the potential defense mechanisms involved, in this study, we investigated the relative expression of antioxidant enzyme-encoding genes (CAT, SOD, POD, and APX) in citrus fruit peels. The results indicated that the expression of defense-related enzymes was significantly greater in the CS-PCT composite-coated fruits than in the control fruits. These findings aligned with previous research. For instance, Jiwanit,

Pitakpornpreecha, Pisuchpen, and Leelasuphakul (2018) explored the impact of *an aloe vera* gel coating supplemented with *Pichia guilliermondii* BCC5389 as a postharvest citrus coating; this led to a substantial increase in the expression of resistance-related genes such as LOX, PAL, and POD in treated citrus fruits. Similarly, Ma et al. (2021) utilized melatonin in the treatment of "Newhall" navel oranges. Their study revealed that melatonin enhanced the activity of related enzymes by upregulating the expressions of the APX, CAT, GR, and SOD genes. This treatment alleviated lipid peroxidation and effectively activated antioxidative stress. Furthermore, CS-PCT was found to increase the cell wall temperature, improving resistance to oxidative stress by increasing the expression of POD. Yang, Zhang, Lu, Wang, and Wang (2015) reported a consistent relationship between the POD expression and the changes in lignin content during pear fruit development. POD catalyzed the formation of lignin and ligand-like substances when the tissues or cells were damaged; this, in turn, strengthened the cell wall and served as an effective barrier against pathogen penetration and spread. The CS-PCT-coated membranes helped maintain the balance of the ROS metabolism in citrus fruits, activate the defense system, enhance resistance to pathogenic fungi, reduce postharvest decay, preserve postharvest quality, and extend shelf life.

5. Conclusion

In this study, we investigated the effects of treating Yongquan tangerines with a PMFs-loaded citral nanoemulsion and a CS composite coating. The findings showed that CS-PCT treatment significantly reduced the decay rate of the citrus fruits, delayed fruit aging, and maintained excellent fruit quality. Furthermore, the coating protected the essential antioxidant components, such as vitamin C, phenolic compounds, and total flavonoids, and enhanced the ability of the fruit to withstand environmental stressors. The physical barrier provided by the coating also minimized the loss of volatile components from the fruit peel and strengthened the peel resistance to pathogens. Moreover, research has shown the impact of the CS-PCT treatment on the expression of the resistance enzymes and defense-related genes in Yongquan tangerines. These effects on resistance enzyme activity and the expression of defense-related genes in citrus fruits could be pivotal factors in enhancing fruit storage resilience. In conclusion, CS-PCT treatment has demonstrated potential for preserving the quality and prolonging the shelf life of citrus fruits.

Funding

This work is supported by the Natural Science Foundation of Chongqing (Ref: CSTB2022NSCQ-MSX0293) and the Science and Technology Research Program of Chongqing Municipal Education Commission (Grant No. KJQN202303240).

CRediT authorship contribution statement

Yurong Li: Writing – original draft, Software, Methodology, Data curation. **Long Guo:** Writing – review & editing, Resources, Methodology, Data curation. **Juanjuan Wei:** Resources, Methodology, Data curation. **Yijun Yao:** Writing – review & editing, Data curation. **Li Xu:** Writing – review & editing, Validation, Supervision. **Zhiqin Zhou:** Writing – review & editing, Validation, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101417.

References

Anjum, M. A., Akram, H., Zaidi, M., & Ali, S. (2020). Effect of gum arabic and Aloe vera gel based edible coatings in combination with plant extracts on postharvest quality and storability of 'Gola' guava fruits. *Scientia Horticulturae, 271*, Article 109506. https://doi.org/10.1016/j.scienta.2020.109506

Arnon, H., Granit, R., Porat, R., & Poverenov, E. (2015). Development of polysaccharides-based edible coatings for citrus fruits: A layer-by-layer approach. *Food Chemistry, 166*, 465–472. https://doi.org/10.1016/j.foodchem.2014.06.061

Cao, S., Yang, Z., & Zheng, Y. (2013). Sugar metabolism in relation to chilling tolerance of loquat fruit. *Food Chemistry, 136*, 139–143. https://doi.org/10.1016/j.foodchem.2012.07.113

Cebi, N., & Erarslan, A. (2023). Determination of the antifungal, antibacterial activity and volatile compound composition of Citrus bergamia Peel essential oil. *Foods, 12*, 203. https://doi.org/10.3390/foods12010203

Chen, C., Nie, Z., Wan, C., & Chen, J. (2019). Preservation of Xinyu tangerines with an edible coating using Ficus hirta Vahl. Fruits Extract-Incorporated Chitosan. *Biomolecules, 9*, 46. https://doi.org/10.3390/biom9020046

Chen, O., Deng, L., Ruan, C., Yi, L., & Zeng, K. (2021). *Pichia galeiformis* induces resistance in postharvest Citrus by activating the Phenylpropanoid biosynthesis pathway. *Journal of Agricultural and Food Chemistry, 69*, 2619–2631. https://doi.org/10.1021/acs.jafc.0c06283

Chen, X.-M., Tait, A. R., & Kitts, D. D. (2017). Flavonoid composition of orange peel and its association with antioxidant and anti-inflammatory activities. *Food Chemistry, 218*, 15–21. https://doi.org/10.1016/j.foodchem.2016.09.016

Chien, P., & Chou, C. (2006). Antifungal activity of chitosan and its application to control post-harvest quality and fungal rotting of tankan citrus fruit (*Citrus tankan Hayata*). *Journal of the Science of Food and Agriculture, 86*, 1964–1969. https://doi.org/10.1002/jsfa.2570

Christensen, J. H., Bauw, G., Gjesing Welinder, K., Van Montagu, M., & Boerjan, W. (1998). Purification and characterization of peroxidases correlated with lignification in poplar Xylem1. *Plant Physiology, 118*, 125–135. https://doi.org/10.1104/pp.118.1.125

Deng, J., Bi, Y., Zhang, Z., Xie, D., Ge, Y., Li, W., Wang, J., & Wang, Y. (2015). Postharvest oxalic acid treatment induces resistance against pink rot by priming in muskmelon (Cucumis melo L.) fruit. *Postharvest Biology and Technology, 106*, 53–61. https://doi.org/10.1016/j.postharvbio.2015.04.005

Devi Ramaiya, S., Bujang, J. S., Zakaria, M. H., King, W. S., & Shaffiq Sahrir, M. A. (2013). Sugars, ascorbic acid, total phenolic content and total antioxidant activity in passion fruit (*Passiflora*) cultivars. *Journal of the Science of Food and Agriculture, 93*, 1198–1205. https://doi.org/10.1002/jsfa.5876

Díaz-Montes, E., & Castro-Muñoz, R. (2021). Edible films and coatings as food-quality preservers: An overview. *Foods, 10*, 249. https://doi.org/10.3390/foods10020249

Dulta, K., Koşarsoy Ağçeli, G., Thakur, A., Singh, S., Chauhan, P., & Chauhan, P. K. (2022). Development of alginate-chitosan based coating enriched with ZnO nanoparticles for increasing the shelf life of Orange fruits (Citrus sinensis L.). *Journal of Polymers and the Environment, 30*, 3293–3306. https://doi.org/10.1007/s10924-022-02411-7

Frassinetti, S., Caltavuturo, L., Cini, M., Della Croce, C. M., & Maserti, B. E. (2011). Antibacterial and antioxidant activity of essential oils from *Citrus* spp. *Journal of Essential Oil Research, 23*, 27–31. https://doi.org/10.1080/10412905.2011.9700427

Ge, Y., Chen, Y., Li, C., Zhao, J., Wei, M., Li, X., Yang, S., & Mi, Y. (2019). Effect of sodium nitroprusside treatment on shikimate and phenylpropanoid pathways of apple fruit. *Food Chemistry, 290*, 263–269. https://doi.org/10.1016/j.foodchem.2019.04.010

Gill, S. S., & Tuteja, N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiology and Biochemistry, 48*, 909–930. https://doi.org/10.1016/j.plaphy.2010.08.016

Guo, L., Li, Y., Mao, X., Tao, R., Tao, B., & Zhou, Z. (2022). Antifungal activity of Polymethoxylated flavonoids (PMFs)-loaded Citral Nanoemulsion against Penicillium italicum by causing cell membrane damage. *Journal of Fungi, 8*, 388. https://doi.org/10.3390/jof8040388

Guo, L., Mao, X., Li, Y., & Zhou, Z. (2023). Polymethoxylated flavonoids (PMFs)-loaded citral nanoemulsion controls green mold in citrus by damaging the cell membrane of Penicillium digitatum. *Fungal Biology, 127*, 854–864. https://doi.org/10.1016/j.funbio.2022.12.003

He, W., Zhu, Y., Chen, Y., Shen, Q., Hua, Z., Wang, X., & Xue, P. (2022). Inhibitory effect and mechanism of chitosan–ag complex hydrogel on fungal disease in grape. *Molecules, 27*, 1688. https://doi.org/10.3390/molecules27051688

Imahori, Y. (2014). Role of ascorbate peroxidase in postharvest treatments of horticultural crops. In *Oxidative Damage to Plants* (pp. 425–451). Elsevier. https://doi.org/10.1016/B978-0-12-799963-0.00014-9.

Jiwanit, P., Pitakpornpreecha, T., Pisuchpen, S., & Leelasuphakul, W. (2018). The use of Aloe vera gel coating supplemented with Pichia guilliermondii BCC5389 for

enhancement of defense-related gene expression and secondary metabolism in mandarins to prevent postharvest losses from green mold rot. *Biological Control, 117*, 43–51. https://doi.org/10.1016/j.biocontrol.2017.08.023

Kalt, W., Forney, C. F., Martin, A., & Prior, R. L. (1999). Antioxidant capacity, vitamin C, Phenolics, and anthocyanins after fresh storage of small fruits. *Journal of Agricultural and Food Chemistry, 47*, 4638–4644. https://doi.org/10.1021/jf990266t

Khaliq, G., Ullah, M., Memon, S. A., Ali, A., & Rashid, M. (2021). Exogenous nitric oxide reduces postharvest anthracnose disease and maintains quality of custard apple (Annona squamosa L.) fruit during ripening. *Food Measure, 15*, 707–716. https://doi.org/10.1007/s11694-020-00658-z

Li, M., Zhao, S., Kong, Z., Li, L., Yang, L., Feng, B., Cui, Y., Lin, X., Fan, B., Simal-Gandara, J., & Wang, F. (2023). Preservation of citrus fruit, and dissipation by diffusion and degradation of postharvest pesticides during storage. *Journal of Food Composition and Analysis, 122*, Article 105456. https://doi.org/10.1016/j.jfca.2023.105456

Liao, H.-L., Alferez, F., & Burns, J. K. (2013). Assessment of blue light treatments on citrus postharvest diseases. *Postharvest Biology and Technology, 81*, 81–88. https://doi.org/10.1016/j.postharvbio.2013.02.019

Liu, Y., Heying, E., & Tanumihardjo, S. A. (2012). History, global distribution, and nutritional importance of Citrus fruits. *Comprehensive Reviews in Food Science and Food Safety, 11*, 530–545. https://doi.org/10.1111/j.1541-4337.2012.00201.x

Liu, Y., Wang, W., Zhou, Y., Yao, S., Deng, L., & Zeng, K. (2017). Isolation, identification and in vitro screening of Chongqing orangery yeasts for the biocontrol of Penicillium digitatum on citrus fruit. *Biological Control, 110*, 18–24. https://doi.org/10.1016/j.biocontrol.2017.04.002

López-Gómez, A., Ros-Chumillas, M., Buendía-Moreno, L., & Martínez-Hernández, G. B. (2020). Active cardboard packaging with encapsulated essential oils for enhancing the shelf life of fruit and vegetables. *Frontiers in Nutrition, 7*, Article 559978. https://doi.org/10.3389/fnut.2020.559978

Ma, Q., Lin, X., Wei, Q., Yang, X., Zhang, Y., & Chen, J. (2021). Melatonin treatment delays postharvest senescence and maintains the organoleptic quality of 'Newhall' navel orange (Citrus sinensis (L.) Osbeck) by inhibiting respiration and enhancing antioxidant capacity. *Scientia Horticulturae, 286*, Article 110236. https://doi.org/10.1016/j.scienta.2021.110236

Magalhães, M. L., Ionta, M., Ferreira, G.Á., Campidelli, M. L. L., Nelson, D. L., Ferreira, V. R. F., … Cardoso, M. D. G. (2020). Biological activities of the essential oil from the Moro orange peel (*Citrus sinensis* (L.) Osbeck). *Flavour and Fragrance Journal, 35*, 294–301. https://doi.org/10.1002/ffj.3561

Mittler, R. (2017). ROS are good. *Trends in Plant Science, 22*, 11–19. https://doi.org/10.1016/j.tplants.2016.08.002

Nie, Z., Huang, Q., Chen, C., Wan, C., & Chen, J. (2020). Chitosan coating alleviates postharvest juice sac granulation by mitigating ROS accumulation in harvested pummelo (Citrus grandis L. Osbeck) during room temperature storage. *Postharvest Biology and Technology, 169*, Article 111309. https://doi.org/10.1016/j.postharvbio.2020.111309

Nishizawa, A., Yabuta, Y., & Shigeoka, S. (2008). Galactinol and Raffinose constitute a novel function to protect plants from oxidative damage. *Plant Physiology, 147*, 1251–1263. https://doi.org/10.1104/pp.108.122465

Nováková, L., Solich, P., & Solichová, D. (2008). HPLC methods for simultaneous determination of ascorbic and dehydroascorbic acids. *TrAC Trends in Analytical Chemistry, Advanced MS Analysis of Metabolites and Degradation Products - I, 27*, 942–958. https://doi.org/10.1016/j.trac.2008.08.006

Osorio, S., Scossa, F., & Fernie, A. R. (2013). Molecular regulation of fruit ripening. *Frontiers in Plant Science, 4*, 198. https://doi.org/10.3389/fpls.2013.00198

Panda, A., Rangani, J., & Kumar Parida, A. (2019). Cross talk between ROS homeostasis and antioxidative machinery contributes to salt tolerance of the xero-halophyte Haloxylon salicornicum. *Environmental and Experimental Botany, 166*, Article 103799. https://doi.org/10.1016/j.envexpbot.2019.103799

Papastergiadis, A., Mubiru, E., Van Langenhove, H., & De Meulenaer, B. (2012). Malondialdehyde measurement in oxidized foods: Evaluation of the spectrophotometric Thiobarbituric acid reactive substances (TBARS) test in various foods. *Journal of Agricultural and Food Chemistry, 60*, 9589–9594. https://doi.org/10.1021/jf302451c

Pinedo-Guerrero, Z. H., Hernández-Fuentes, A. D., Ortega-Ortiz, H., Benavides-Mendoza, A., Cadenas-Pliego, G., & Juárez-Maldonado, A. (2017). Cu nanoparticles in hydrogels of chitosan-PVA affects the characteristics of post-harvest and bioactive compounds of Jalapeño pepper. *Molecules, 22*, 926. https://doi.org/10.3390/molecules22060926

Saleem, M. S., Anjum, M. A., Naz, S., Ali, S., Hussain, S., Azam, M., Sardar, H., Khaliq, G., Canan, İ., & Ejaz, S. (2021). Incorporation of ascorbic acid in chitosan-based edible coating improves postharvest quality and storability of strawberry fruits. *International Journal of Biological Macromolecules, 189*, 160–169. https://doi.org/10.1016/j.ijbiomac.2021.08.051

Sdiri, S., Rambla, J. L., Besada, C., Granell, A., & Salvador, A. (2017). Changes in the volatile profile of citrus fruit submitted to postharvest degreening treatment. *Postharvest Biology and Technology, 133*, 48–56. https://doi.org/10.1016/j.postharvbio.2017.07.001

Segal, L. M., & Wilson, R. A. (2018). Reactive oxygen species metabolism and plant-fungal interactions. *Fungal Genetics and Biology, 110*, 1–9. https://doi.org/10.1016/j.fgb.2017.12.003

Shen, Y., & Yang, H. (2017). Effect of preharvest chitosan-g-salicylic acid treatment on postharvest table grape quality, shelf life, and resistance to Botrytis cinerea-induced spoilage. *Scientia Horticulturae, 224*, 367–373. https://doi.org/10.1016/j.scienta.2017.06.046

Sun, H., Cao, Y., Kim, D., & Marelli, B. (2022). Biomaterials technology for AgroFood resilience. *Advanced Functional Materials, 32*, 1–39. https://doi.org/10.1002/adfm.202201930

Sun, X., Zhu, A., Liu, S., Sheng, L., Ma, Q., Zhang, L., … Deng, X. (2013). Integration of metabolomics and subcellular organelle expression microarray to increase understanding the organic acid changes in post-harvest Citrus fruit. *JIPB, 55*, 1038–1053. https://doi.org/10.1111/jipb.12083

Szarka, A., Tomasskovics, B., & Bánhegyi, G. (2012). The ascorbate-glutathione-α-tocopherol triad in abiotic stress response. *International Journal of Molecular Sciences, 13*, 4458–4483. https://doi.org/10.3390/ijms13044458

Tao, R., Chen, Q., Li, Y., Guo, L., & Zhou, Z. (2022). Physicochemical, nutritional, and phytochemical profile changes of fermented citrus puree from enzymatically hydrolyzed whole fruit under cold storage. *LWT, 169*, Article 114009. https://doi.org/10.1016/j.lwt.2022.114009

Taş, A., Berk, S. K., Orman, E., Gundogdu, M., Ercişli, S., Karatas, N., … Mlcek, J. (2021). Influence of pre-harvest gibberellic acid and post-harvest 1-methyl cyclopropane treatments on phenolic compounds, vitamin C and organic acid contents during the shelf life of strawberry fruits. *Plants (Basel), 10*, 121. https://doi.org/10.3390/plants10010121

Van Den Ende, W., & Valluru, R. (2008). Sucrose, sucrosyl oligosaccharides, and oxidative stress: Scavenging and salvaging? *Journal of Experimental Botany, 60*, 9–18. https://doi.org/10.1093/jxb/ern297

Wang, Z., Sui, Y., Li, J., Tian, X., & Wang, Q. (2022). Biological control of postharvest fungal decays in citrus: A review. *Critical Reviews in Food Science and Nutrition, 62*, 861–870. https://doi.org/10.1080/10408398.2020.1829542

Wang, Z., Yang, H., Ma, Y., Jiang, G., Mei, X., Li, X., … Dong, C. (2022). WGCNA analysis revealing molecular mechanism that bio-organic fertilizer improves pear fruit quality by increasing sucrose accumulation and reducing citric acid metabolism. *Frontiers in Plant Science, 13*, 1039671. https://doi.org/10.3389/fpls.2022.1039671

Wei, L., Wang, W., Li, T., Chen, O., Yao, S., Deng, L., & Zeng, K. (2023). Genome-wide identification of the CsPAL gene family and functional analysis for strengthening green mold resistance in citrus fruit. *Postharvest Biology and Technology, 196*, Article 112178. https://doi.org/10.1016/j.postharvbio.2022.112178

Wu, T., Cheng, D., He, M., Pan, S., Yao, X., & Xu, X. (2014). Antifungal action and inhibitory mechanism of polymethoxylated flavones from Citrus reticulata Blanco peel against aspergillus Niger. *Food Control, 35*, 354–359. https://doi.org/10.1016/j.foodcont.2013.07.027

Xing, Y., Yue, T., Wu, Y., Xu, Q., Guo, X., Wang, X., … Yang, P. (2021). Effect of chitosan composite coatings with salicylic acid and titanium dioxide nanoparticles on the storage quality of blackcurrant berries. *Coatings, 11*, 738. https://doi.org/10.3390/coatings11060738

Xiong, B., Ye, S., Qiu, X., Liao, L., Sun, G., Luo, J., … Wang, Z. (2017). Transcriptome analyses of two Citrus cultivars (Shiranuhi and Huangguogan) in seedling etiolation. *Scientific Reports, 7*, 46245. https://doi.org/10.1038/srep46245

Yan, F., Zhang, D., Wang, X., Liu, C., & Zhang, F. (2022). Reduction of postharvest diseases of loquat fruit by serine protease and possible mechanisms involved. *Scientia Horticulturae, 304*, Article 111246. https://doi.org/10.1016/j.scienta.2022.111246

Yang, G., Weng, Y., Zhao, Y., Wang, D., Luo, T., & Jin, Y. (2022). Transcriptomic and targeted metabolomic analysis revealed the toxic effects of prochloraz on larval zebrafish. *Science of the Total Environment, 822*, Article 153625. https://doi.org/10.1016/j.scitotenv.2022.153625

Yang, K., Dang, H., Liu, L., Hu, X., Li, X., Ma, Z., Wang, X., & Ren, T. (2019). Effect of syringic acid incorporation on the physical, mechanical, structural and antibacterial properties of chitosan film for quail eggs preservation. *International Journal of Biological Macromolecules, 141*, 876–884. https://doi.org/10.1016/j.ijbiomac.2019.08.045

Yang, S.-L., Zhang, X.-N., Lu, G.-L., Wang, C.-R., & Wang, R. (2015). Regulation of gibberellin on gene expressions related with the lignin biosynthesis in 'Wangkumbae' pear (Pyrus pyrifolia Nakai) fruit. *Plant Growth Regulation, 76*, 127–134. https://doi.org/10.1007/s10725-014-9982-0

Yu, K., Xu, J., Zhou, L., Zou, L., & Liu, W. (2021). Effect of chitosan coatings with cinnamon essential oil on postharvest quality of mangoes. *Foods, 10*, 3003. https://doi.org/10.3390/foods10123003

Zhang, J., Wang, M., Cheng, F., Dai, C., Sun, Y., Lu, J., Huang, Y., Li, M., He, Y., Wang, F., & Fan, B. (2016). Identification of microRNAs correlated with citrus granulation based on bioinformatics and molecular biology analysis. *Postharvest Biology and Technology, 118*, 59–67. https://doi.org/10.1016/j.postharvbio.2016.03.010

Zhang, R., Xu, Q., Zhang, Y., & Zhu, F. (2018). Baseline sensitivity and toxic actions of Prochloraz to *Sclerotinia sclerotiorum*. *Plant Disease, 102*, 2149–2157. https://doi.org/10.1094/PDIS-01-18-0148-RE

Zhang, Z., Zhao, P., Zhang, P., Su, L., Jia, H., Wei, X., … Jia, H. (2020). Integrative transcriptomics and metabolomics data exploring the effect of chitosan on postharvest grape resistance to Botrytis cinerea. *Postharvest Biology and Technology, 167*, Article 111248. https://doi.org/10.1016/j.postharvbio.2020.111248