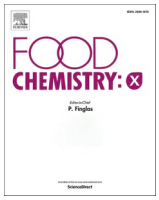
Food Chemistry: X 25 (2025) 102138

Contents lists available at ScienceDirect

Food Chemistry: X

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



Thymol maintains peppers quality by regulating antioxidant capacity, cell wall metabolism and membrane lipid metabolism

Xue Li [a,1], Xiaogang Wang [a,1], Jianye Chen [b], Donglan Luo [a], Sen Cao [a], Liangjie Ba [a,*]

[a] Guiyang University, Guiyang 550005, China
[b] State Key Laboratory for Conservation and Utilization of Subtropical Agro-bioresources/Guangdong Provincial Key Laboratory of Postharvest Science of Fruits and Vegetables/Engineering Research Center of Southern Horticultural Products Preservation, Ministry of Education, College of Horticulture, South China Agricultural University, Guangzhou, China

ARTICLE INFO

Keywords:
Antioxidant system
Cell membrane
Cell wall components
Postharvest
Storage

ABSTRACT

This study investigates the effect of 100 mg L^{-1} thymol treatment on the quality of post-harvest peppers stored at 10 °C. The results showed that thymol treatment significantly reduced decay rate, reactive oxygen species (ROS) accumulation, and saturated fatty acid levels in peppers. Moreover, unsaturated fatty acids, non-enzymatic antioxidants, and antioxidant enzyme levels increased after treatment. For example, catalase (CAT) activity rose by 1.29-fold at 18 d, while ascorbic acid (AsA) content increased by 3.38-fold at 36 d. Additionally, thymol application inhibited the hydrolysis of pepper cell wall components and decreased the activities of enzymes related to cell wall and membrane lipid metabolism. Specifically, cellulase and lipase treatments showed reductions of 26 % and 1.73-fold compared to control (CK) at 36 d; protopectin (PP) also exhibited a 1.2-fold increase relative to CK. In conclusion, thymol can maintain antioxidant activity under post-harvest conditions while slowing cell wall and lipid metabolism processes.

1. Introduction

Pepper is a herbaceous plant classified as either annual or perennial, belonging to the genus *Capsicum* within the family Solanaceae. It ranks among the most widely consumed vegetables in China. Characterized by its vibrant green colour, thick and crisp flesh, and high concentrations of vitamins, proteins, sugars, and minerals, pepper is highly nutritious and favored by consumers (Huang et al., 2023). The softening of pepper during storage is an inevitable phenomenon that significantly impacts postharvest transport. This process is closely associated with cell wall and membrane degradation. The softening directly influences firmness, which serves as a crucial indicator of commercial quality. An intact cell wall is essential for maintaining optimal firmness in peppers (Guo et al., 2022).

Cell membrane integrity is closely linked to cellular functions (Lu et al., 2023). In this context, Das (2021) proposed that there may be a significant correlation between cell membrane integrity and its compositional alterations during the process of senescence. Lipids serve as essential signaling molecules within cell membranes and play a crucial role in maintaining both the function and integrity of these membranes. Additionally, they are vital in coordinating responses to biotic and abiotic stressors (Oubohssaine et al., 2024). Moreover, lipids contribute to the damage sustained by cellular membranes throughout cellular senescence. This process is characterized by an increase in saturated fatty acids coupled with a decrease in unsaturation levels, such changes lead to reduced fluidity of the cell membrane and increased permeability, which collectively accelerate cellular senescence. Consequently, alterations in lipid composition are critical for fruit quality.

Furthermore, the senescence of peppers is closely associated with the accumulation of reactive oxygen species (ROS). It has been observed that storing plants under unfavorable conditions induces significant quantities of ROS production within cells, disrupting redox homeostasis and impairing cellular capacity to scavenge free radicals. This disruption leads to severe damage to cell membranes and disintegration of cell wall structures (Nukuntornprakit et al., 2015), which adversely affects the postharvest storage quality of peppers. Various methods have been demonstrated to maintain the postharvest quality of peppers, including Melatonin (MT) (Li et al., 2024), cold-excitation treatment (Mi et al., 2023), sodium alginate application (Castañeda et al., 2024), and hot air treatment (Wang et al., 2023). However, these approaches are not

* Corresponding author.
E-mail address: baliangjie@163.com (L. Ba).
[1] These authors contributed equally to this work

https://doi.org/10.1016/j.fochx.2024.102138
Received 9 November 2024; Received in revised form 16 December 2024; Accepted 26 December 2024
Available online 29 December 2024

without their limitations. For instance, the cost associated with air-conditioned storage can be considerable. Additionally, maintaining temperature consistency in cold storage presents challenges and may lead to cold damage. Furthermore, chemical reagents such as ClO_2 could potentially pose adverse effects on human health. Consequently, there is a pressing need for identifying more suitable preservation methods for peppers.

The utilization of plant essential oils as a method for food preservation has been extensively documented in the literature. Taban et al. (2024) demonstrated that treatments involving essential oils combined with edible coatings resulted in significantly enhanced firmness values, thereby confirming the preservative efficacy of these essential oils. Tzortzakis (2024) investigated the impact of oregano essential oil on controlling grey mould in peppers and found that it effectively inhibited mould propagation while maintaining post-harvest quality. Thymol is an essential oil derived from plants that is recognized for its safety and environmental friendliness. It exhibits a variety of beneficial properties, including antibacterial, antiviral, and antioxidant effects. Thymol has been widely employed in fruit and vegetable preservation efforts. Several studies have indicated that applying thymol to citrus fruits can reduce the incidence of green mould while improving preservation quality (Zhang, Shan, et al., 2023). Ding, Liu, et al. (2023) utilized thymol in coating blueberries, resulting in extended shelf life and increased activity of antioxidant enzymes. Furthermore, Ye et al. (2024) applied a thymol solution to blueberries and observed that thymol hindered cell wall deterioration while enhancing the energy status of blueberries and effectively preserving their post-harvest quality.

Additionally, Wang et al. (2024) employed thymol-bearing microcapsules for the treatment of okra and found that this approach could mitigate cell membrane damage and reduce the levels of ROS. The studies have demonstrated that thymol treatment provides a protective effect on both cell membranes and cell walls while preserving antioxidant capacity. Consequently, it can be postulated that thymol treatment may be advantageous for maintaining the quality of peppers during postharvest storage. Previous research has established that thymol possesses excellent preservative properties and offers protection to cell membranes and cell walls. Furthermore, it has been shown to sustain antioxidant capacity. However, the effects of thymol on the metabolism of cell walls and membranes in peppers remain undocumented. Therefore, a comprehensive investigation into the preservation effects of thymol on peppers is highly warranted.

The objective of this study was to investigate the effects of thymol on the postharvest physiological quality, antioxidant activity, membrane lipid, and cell wall metabolism in peppers. Furthermore, this research aims to establish a theoretical foundation for the practical application of thymol.

2. Methods and materials

2.1. Chemical reagents

Thymol (AR, China), capsaicin (standard products, China), NaCl (AR, China), DTT (dithiothreitol) (AR, China), phosphatidylcholine (AR, China), GSH (AR, China), docosahexaenoic acid (AR, China), 4-hydroxyethylpiperazine ethanesulfonic acid (AR, China), dimethyl sulfoxide (AR, China), o-nitrobenzene-β-D-galactoside (AR, China), salicin (BR, China), horseradish POD (BR, China), choline oxidase (BR, China), fast-blue B ($\geq$ 95 %, China), anhydrous sodium sulfate (AR, China), phosphoric acid (AR, China), 2,2-bipyridine (AR, China), NADPH (AR, China), carbazole (AR, China), $CaCl_2$ (AR, China), $ZnSO_4.7H_2O$ (AR, China), α-acetic acid naphthyl ester ($\geq$ 99 %, China), DTNB (5,5'-Dithiobis) (AR, China), N-ethylmaleimide (AR, China), sodium linoleate (AR, China), pectin (AR, China), Triton X-100 (AR, China), cysteine hydrochloride (BR, China), Thiobarbituric acid (TBA) (AR, China), 4-aminoantipyrine ($\geq$ 98 %, China), hydroxylamine HCl (AR, China), α-naphthylamine (AR, China), Na_2HPO_4 (AR, China), NaH_2PO_4 (AR, China), PVPP (polyvinylpolypyrrolidone)(AR, China), EDTA(AR, China), AsA(AR, China), EDTA-2Na(AR, China), TCA (trichloroacetic acid TCA) (AR, China), glu (AR, China), bromomuscovanillin blue (AR, China), n-hexane (AR, China), quartz sand (AR, China), $FeCl_3$ (AR, China), 3,5-Dinitrosalicylic acid (AR, China), potassium sodium tartrate (AR, China), p-aminobenzenesulphonic acid (AR, China), phenol (AR, China), titanium tetrachloride (CP, China), ammonia (AR, China), H_2O_2 (AR, China), HCl (AR, China), sulfuric acid (AR, China), acetone (AR, China), chloroform (AR, China), K_2HPO_4 (AR, China), KH_2PO_4 (AR, China), potassium hydride (AR, China), sodium carbonate (AR, China), acetic acid (AR, China), petroleum ether (AR, China), ethyl anthrone acetate (AR, China), ethanol (AR, China), methanol (AR, China), benzene (AR, China), CMC (sodium carboxymethylcellulose) (AR, China), polygalacturonic acid (GR, China), CH_3COOK (AR, China), H^+-ATPase, Ca^{2+}-ATPase kits are from Nanjing Jianjian Bioengineering Institute.

2.2. Plants and treatments

Fresh 'jumbo' peppers were sourced from a cultivation area in Guizhou Province and transported to the laboratory immediately after harvesting. A total of 30 kg of ripe, uniformly sized peppers exhibiting no visible mechanical damage were selected for this study. The peppers were randomly divided into four groups and treated with different concentrations of thymol (50 mg L^{-1}, 100 mg L^{-1}, 150 mg L^{-1}) as well as distilled water (CK). Following treatment, the peppers were naturally dried, placed in microporous film bags (PE), and stored in a chromatographic refrigerator at a temperature of 10 $\pm$ 0.5 °C and relative humidity of 80 % for a duration of 36 d. Physiological parameters were assessed at intervals of 0, 9, 18, 27, and 36 d. Samples weighing approximately 1.5 kg were collected from each group at each time point. The remaining portion of each group (1.5 kg) was preserved in an ultra-low temperature freezer at −80 °C for subsequent analysis related to antioxidant metabolism, membrane lipid metabolism, and cell wall metabolism indicators.

2.3. Measurement of firmness, ΔE, chlorophyll, total flavonoid and decay rate

To ascertain the firmness of the peppers. Eight fresh peppers were randomly selected, and the middle part was identified. Test conditions: probe type P 2, speed 1 mm s^{-1} for pre-measurement, 1 mm s^{-1} for detection speed, 2 mm s^{-1} for post-measurement speed, penetration distance 10 mm, and the results are expressed as Newton (N).

To evaluate the firmness of the peppers, eight fresh specimens were randomly selected, and their central portions were identified. The testing conditions included: probe type P 2; pre-measurement speed at 1 mm s^{-1}; detection speed maintained at 1 mm s^{-1}; post-measurement speed set to 2 mm s^{-1}; and a penetration distance of 10 mm. The results are expressed in Newtons (N).

The colour differences of eight fresh peppers were randomly selected and measured using a portable colorimeter to record the values of L*, a*, and b*. The ΔE was calculated utilizing Eq. 1.

$$\Delta E = \sqrt[2]{\Delta L^2 + \Delta a^2 + \Delta b^2} \quad (1)$$

Chlorophyll content was determined according to the method described by Zhang et al. (2024). 1 g of fresh pepper was ground with 80 % alcohol, followed by filtration. The precipitate was rinsed with 80 % alcohol until it became colorless. The two filtrates were combined and diluted to a final volume of 25 mL. Absorbance was measured at 625 nm, and the results are expressed in mg kg^{-1}.

The total flavonoid content was determined according to the method described by Shen et al. (2025). 1 g of fresh pepper was ground with 75 % ethanol and diluted to a final volume of 25 mL, followed by extraction in the dark for 1 h. The mixture was centrifuged, and the supernatant was collected. The supernatant (1 mL) and 0.6 % TBA (4 mL) should be

measured and incubated in a water bath for 15 min. Subsequently, take 1 mL of the resulting mixed solution, combine it with 2 mL 0.1 mol L^{-1} $AlCl_3$ and 3 mL of 1 mol L^{-1} CH_3COOK, then bring the total volume to scale. The absorbance is to be measured at 420 nm. The results were expressed in μmol kg^{-1}.

The decay rate of peppers was assessed by measuring the number of decayed peppers during storage, and the decay rate was calculated using eq. 2.

$$\text{Decay rate}\ (\%) = \text{Number of rotten fruits/total number of fruit} \times 100 \quad (2)$$

2.4. Determination of hydrogen peroxide (H_2O_2) contents, superoxide anion ($O_2^{\cdot-}$) production rate, catalase (CAT), peroxidase (POD), superoxide dismutase (SOD) and ascorbate peroxidase (APX) activities

H_2O_2 content and $O_2^{\cdot-}$ production rate were determined according to Shen et al. (2025). For the determination of H_2O_2. Grind 1 g of fresh pepper with 5 mL of acetone and centrifuge to obtain the supernatant. A volume of 1 mL of the supernatant is pipetted and mixed with an equal volume of 0.1 mmol L^{-1} H_2O_2-acetone, 0.1 mL 10 % titanium tetrachloride-hydrochloric acid, 0.2 mL ammonia. The precipitate was washed with acetone until the pigment was removed. The precipitate was dissolved in 2 mol L^{-1} sulfuric acid (3 mL) and the absorbance measured at 412 nm. The results are expressed as μmol kg^{-1}.

For the determination of $O_2^{\cdot-}$ production rate. 1 g of fresh pepper should be ground with 5 mL of extraction bufferand centrifuge to obtain the supernatant. Take 1 mL of the supernatant, 1 mL of 0.05 mol L^{-1} pH 7.8 PBS (phosphate buffer saline), and 1 mL of 0.001 mol L^{-1} hydroxylamine HCl, and incubate for 1 h at 25 °C. A supplementary 1 mL of 0.017 mol L^{-1} p-aminobenzenesulphonic acid (1 mL) and 1 mL of 7 mmol L^{-1} α-naphthylamine should be added, and the mixture should be incubated at 25 °C for 0.5 h. The absorbance should be determined at 530 nm. The results are expressed as μmol kg^{-1}.

The CAT assay was conducted by Wu, Li, et al. (2024). 1 g of fresh pepper was ground with 5 mL of buffer solution (comprising 5 mmol L^{-1} DTT (dithiothreitol) and 5 % PVPP (polyvinylpolypyrrolidone)), then centrifuged. The resulting supernatant was retained. The solution subjected to analysis comprised 2.9 mL of 0.02 mol L^{-1} H_2O_2 and 0.1 mL of enzyme extract. The absorbance was measured at 240 nm, and the results are expressed in U kg^{-1}.

POD was determined according to the method described by Wu, Li, et al. (2024). 1 g of fresh pepper was ground in 5 mL of buffer solution. The resulting supernatant was collected following centrifugation. The assay solution consisted of 200 μL of 0.5 mol L^{-1} H_2O_2, 500 μL of enzyme solution, and 3 mL of a 0.025 mol L^{-1} guaiacol solution. Absorbance was measured at a wavelength of 470 nm. The results are expressed in U kg^{-1}.

SOD was measured according to the method described by Marklund and Marklund (1974). 1 g of fresh pepper was ground in 15 mL of water, and the supernatant was retained after centrifugation. The assay solution consisted of 2.35 mL of distilled water, 1.45 mL of liquid A (100 mmol L^{-1} Tris-HCl buffer at pH 8.2 containing 2.0 mmol L^{-1} EDTA), and 700 μL of 0.01 mol L^{-1} HCl as a blank. For the determination, the solution included 2.35 mL of liquid A, 1.45 mL of water, and 700 μL of liquid B (4.5 mol L^{-1} o-benzenetriol). Absorbance readings were taken at intervals of 30 s at 325 nm and expressed as autoxidation ΔA325 (min^{-1}). Additionally, to another test tube should be added: further amounts consisting of 2.35 mL of liquid A, 850 μL of distilled water, along with the same volume (700 μL) from liquid B, and this mixture is processed similarly while noting Δ′A325 (min^{-1}). The results are expressed in U kg^{-1}.

The measurement of APX was performed following the methodology established by Wu, Li, et al. (2024). A 5 mL PBS buffer at pH 7.5, containing 2 % PVPP, 0.1 mmol L^{-1} EDTA, and 1 mmol L^{-1} AsA, was utilized to homogenize 1 g of fresh pepper. The mixture was centrifuged, and the supernatant was collected for further analysis. The assay solution consisted of 2.6 mL of reaction buffer, 0.1 mL of enzyme solution, and 0.3 mL of 2 mmol L^{-1} H_2O_2 solution. Absorbance readings were taken at a wavelength of 290 nm. The results are expressed in U kg^{-1}.

2.5. Determination of glutathione (GSH), oxidized glutathione (GSSG), ascorbic acid (AsA), dehydroascorbate (DHA) contents, AsA/DHA rate, monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR) and glutathione reductase (GR) activities

GSH and GSSG contents were determined following the method described by Zhao et al. (2011). Initially, 1 g of fresh pepper was ground with 5 mL of 0.05 g mL^{-1} trichloroacetic acid (TCA) containing 5 mmol L^{-1} EDTA-2Na. The resulting supernatant was collected after centrifugation. Two test tubes were prepared: to one tube, 1 mL of the supernatant was added along with an equal volume of 0.1 mol L^{-1} PBS at pH 7.7 to the other tube (control), 0.5 mL of a 4 mmol L^{-1} solution of DTNB (5,5'-Dithiobis) was added alongside an equal volume of PBS at pH 6.8 for the sample tube. The samples were incubated at a temperature of 25 °C for a duration of 30 min. Absorbance values at a wavelength of 412 nm were recorded as optical densities (ODs) for both control and sample tubes (ODc). The results are expressed in mg kg^{-1}.

The contents of AsA and DHA were determined according to the method described by Shen et al. (2025). 2 g of fresh pepper was ground with 5 mL of 60 g L^{-1} TCA. The resulting supernatant was collected after centrifugation. The AsA assayed solution comprised 200 μL of supernatant, 1 mL of 100 g L^{-1} TCA, 0.5 mL of PBS (pH 7.4), 800 μL of 20 g L^{-1} 2,2-bipyridine, 800 μL of 42 % phosphoric acid, and 400 μL of 30 g L^{-1} $FeCl_3$, placed at 42 °C for 40 min. The total AsA assayed solution comprised 200 μL of supernatant, 0.2 mL of DTT and 0.5 mL of PBS (pH 7.4), placed at 42 °C for 15 min, adding 200 μL of N-ethylmaleimide, and then proceeding as described above for AsA. The result is expressed in mg kg^{-1}.

DHAR, MDHAR, and GR activities were determined following Huang et al. (2022). 1 g of fresh pepper was ground with 5 mL of 0.05 mol L^{-1} pH 7.8 phosphate buffer (containing 2 mmol L^{-1} AsA, 0.2 mmol L^{-1} EDTA and 2 g L^{-1} PVPP). The resulting homogenate should be centrifuged and the supernatant retained. The reactive solution for GR comprised 2 mL of 0.1 mol L^{-1} pH 7.5 PBS, 200 μL of supernatant, 100 μL of 5 mmol L^{-1} GSH, and 50 μL of 3 mmol L^{-1} NADPH. The MDHAR reaction solution comprised 200 μL of supernatant, 1.7 mL of 0.05 mol L^{-1} PBS (pH 7.5), 0.2 mL of 2 mmol L^{-1} AsA, and 0.1 mL of 4 mmol L^{-1} NADPH. The DHAR determined solution comprised 200 μL of supernatant, 0.1 mL of 8 mmol L^{-1} docosahexaenoic acid, 0.1 mL of 20 mmol L^{-1} glu and 1.6 mL of 0.1 mmol L^{-1} 4-hydroxyethylpiperazine ethanesulfonic acid-potassium hydride buffer. The activity of DHAR was measured at 265 nm, GR and MDHAR activities were measured at 340 nm. The results are expressed in U kg^{-1}.

2.6. Measurement of cellulose, protopectin (PP), soluble pectin (SP), ionic soluble pectin (ISP), covalent soluble pectin (CSP) and water-soluble pectin (WSP) contents

The cellulose content was determined according to the method described by Sun, Liu, et al. (2024). Initially, 1 g of fresh pepper was ground with 60 mL of 50 % sulfuric acid and then diluted to a final volume of 100 mL, allowing it to stand for 0.5 h. Subsequently, 5 mL of the supernatant was taken and further diluted to a total volume of 100 mL, followed by thorough shaking to prepare the cellulose determination solution. A volume of 1 mL from this test solution was combined with 500 μL of a 2 % ethyl anthrone acetate solution and 5 mL of sulfuric acid, then boiled for 10 min. The absorbance was measured at a wavelength of 630 nm. The results are expressed in mg kg^{-1}.

The content of PP and SP was measured following the methodology described by Li, Qiu, et al. (2023). Initially, 1 g of fresh pepper was ground with 50 mL of 95 % methanol, followed by a water bath at 100 °C

for 10 min, and then centrifuged for 5 min. This procedure was repeated three times. Subsequently, 20 mL of distilled water was added to the precipitate, which was incubated at 50 °C for 30 min. After centrifugation, the supernatant was collected and adjusted to a fixed volume. This solution represents soluble pectin. Next, to the precipitate, 50 mL of 0.5 mol L^{-1} sulfuric acid was added and boiled for 1 h. The resulting mixture was then diluted to a final volume of 100 mL. This constitutes the protopectin determination solution. A sample volume of 1 mL from this solution should be taken and mixed with 6 mL of sulfuric acid before boiling for another 20 min. Following this step, add 200 μL of a carbazole-alcohol solution at a concentration of 1.5 g L^{-1}. After allowing the reaction to proceed in darkness for 2 h, absorbance measurements were conducted at a wavelength of 530 nm. The results are expressed in mg kg^{-1}.

The determination of ISP, CSP, and WSP was conducted by Sun, Tian, et al. (2024). 1 g of fresh pepper was ground with 3 mL of 80 % ethanol and boiled for 20 min. The resulting precipitate was collected after centrifugation. This precipitate was washed three times with both 80 % ethanol and acetone, then suspended in 3 mL of 90 % dimethyl sulfoxide and left overnight. Subsequently, the precipitate was dried at 45 °C for 10 h and weighed to obtain the cell wall material (CWM). The extraction of different components followed a sequential approach as outlined below: WSP was extracted using 5 mL of acetate buffer (pH 6.5, 0.05 mol L^{-1}); ISP was extracted with volume of the same acetate buffer containing 0.05 mol L^{-1} CDTA; CSP was extracted with 5 mL of 0.05 mol L^{-1} pH 6.5 sodium carbonate buffer (0.05 mol L^{-1} CDTA). For analysis, to each milliliter of supernatant, we added 0.2 mL of carbazole-ethanol solution (1.5 g L^{-1}) along with 6 mL of sulfuric acid, followed by incubation at 85 °C for 10 min. The absorbance was measured at a wavelength of 530 nm, and results were expressed in mg kg^{-1}.

2.7. Determination of cellulase (Cx), pectin methylesterase (PME), β-Galacturonidase (β-gal), polygalacturonase (PG), pectate lyase (PL) and β-glucosidase (β-Glu) activities

The Cx activity was quantified according to the method described by Wang et al. (2020). 3 g of fresh pepper was homogenized with 15 mL of acetate buffer. Following centrifugation, the supernatant was collected for further analysis. The reaction mixture consisted of 2 mL of CMC, 1 mL of acetate buffer, and 500 μL of the supernatant. This mixture was incubated at 50 °C for a duration of 30 min. Subsequently, after adding 2 mL of DNS, the solution was heated in a boiling water bath for 5 min. The final volume was then adjusted to 100 mL before measuring the absorbance at a wavelength of 540 nm. The results are expressed in mg kg^{-1}.

The measurement of PME activity was performed by Alagie et al. (2023). 2 g of fresh pepper was homogenized with 5 mL of 5 % NaCl solution. The resulting supernatant was collected following centrifugation. The assay mixture consisted of 2 mL of 0.5 % pectin, 150 μL of 0.01 % bromomuscovanillin blue solution, and 750 μL of distilled water, which were incubated at 37 °C for 15 min. Subsequently, an aliquot of 400 μL from the supernatant was added to the mixture. The absorbance was measured rapidly at a wavelength of 620 nm over a period of 2 min. The results are expressed in U kg^{-1}.

For the determination of β-Gal activity, please refer to Wang et al. (2020). 1 g of fresh pepper was ground with 4 mL of pH 5.6 acetate buffer (0.05 mol L^{-1}). After centrifugation, the supernatant was retained. To this supernatant (0.1 mL), 1 mL of 1 mol L^{-1} o-nitrobenzene-β-D-galactoside was added along with a control consisting of o-nitrobenzene-β-D-galactoside (1.1 mL). Subsequently, 2 mL of 1 mol L^{-1} sodium carbonate was incorporated into the mixture and incubated at 37 °C for 30 min. The absorbance should be measured at a wavelength of 400 nm. The results are expressed in μmol kg^{-1}.

PG activity was quantified following the methodology described by Alagie et al. (2023). 1 g of fresh pepper was ground in 10 mL of 0.1 mol L^{-1} acetate buffer at pH 4.0, and the supernatant was retained after centrifugation. Subsequently, 0.1 mL of the supernatant was combined with 3.8 mL of a 0.5 % pectin solution and incubated at 37 °C for 30 min. After this incubation period, 2 mL of DNS reagent was added, followed by boiling for an additional 5 min before cooling to room temperature. The absorbance was measured at a wavelength of 540 nm, and the results are expressed as mg kg^{-1}.

PL activity was measured following the methodology outlined by Gwanpua et al. (2014). 1 g of fresh pepper was ground with 50 mmol L^{-1} pH 7.0 PBS, which contained 1 % (*w/v*) Triton X-100, 20 mmol L^{-1} cysteine hydrochloride, and 1 % (w/v) PVPP. The mixture was incubated for 2 h at 4 °C. The resulting supernatant was centrifuged and retained. Subsequently, we added 1.5 mL of supernatant, mixed it with 2.5 mL of a 1 % (w/v) polygalacturonic acid solution and followed by the addition of 1 mL of 0.01 mol L^{-1} $CaCl_2$ solution at a temperature of 37 °C for a duration of 1.5 h. Following this incubation period, we introduced an aliquot consisting of 300 μLof a saturated solution of $ZnSO_4.7H_2O$ along with 0.05 mol L^{-1} NaOH. Next, we took Take 2.5 mL of the supernatant, along with 0.75 mL of 0.01 mol L-1 HCl, 1.5 mL of 0.6 % TBA and 0.25 mL of water and boil for 30 min. The absorbance values were recorded at 550 nm. The results are expressed in U kg^{-1}.

The activity of β-Glu was determined by Alagie et al. (2023). Fresh pepper (2 g) was ground with 20 mL of 95 % methanol, followed by extraction with 20 mL of 80 % ethanol for 10 min. The mixture was centrifuged, and the supernatant was retained. Then, it was extracted with 5 mL of 50 mmol L^{-1} pH 5.5 acetic acid buffer (1.8 mol L^{-1} NaCl) for 20 min. Subsequently, the supernatant was retained again and the volume was adjusted to 25 mL after centrifugation. 0.5 mL of enzyme solution and 1.5 mL of 10 g L^{-1} salicin were placed at 37 °C for 1 h. Subsequently, 1.5 mL of DNS was boiled for 5 min. The absorbance was assayed at 540 nm. The result is expressed in μg kg^{-1}.

2.8. Determination of phospholipase D (PLD), lipase (LPS), lipoxygenase (LOX) activities, fatty acids, MDA

The activity of PLD was assayed by Yavar et al. (2021). Grind 1 g of fresh pepper with distilled water (5 mL), the supernatant was retained after centrifugation. A volume of 30 μL of the enzyme solution was combined with an equal volume 500 mmol L^{-1} pH 5.5 acetate buffer (0.5 mmol L^{-1} $CaCl_2$), and 10 μL of PC (phosphatidylcholine), placed at 37 °C for 10 min. Subsequently, 20 μL of 1 mol L^{-1} pH 8.0 Tris-HCl (0.1 mol L^{-1} EDTA), 30 μL of 0.1 mol L^{-1} Tris-HCl (containing 0.2 U horseradish POD, 1.5 mmol L^{-1} 4-aminoantipyrine, 2.1 mmol L^{-1} phenol, and 3 U choline oxidase) were mixed and incubated at 37 °C for 45 min. The change in absorbance over 3 min at 492 nm was determined. The result is expressed in U kg^{-1}.

LPS activity was assessed according to the method described by Kuang et al. (2023). 1 g of fresh pepper was ground with 5 mL of 0.2 mol L^{-1} PBS at pH 7.8. The mixture was then subjected to centrifugation, and the supernatant was retained for further analysis. Subsequently, 1 mL of the supernatant, along with 2.3 mL of 0.2 mol L^{-1} PBS (pH 7.8) and 500 μL of 0.2 mol L^{-1} α-acetic acid naphthyl ester solution, were combined and incubated at 25 °C for a duration of 30 min. Following incubation, 200 μL of a fast-blue B solution (0.15 %) was added. The change in absorbance at 520 nm over a period of three minutes was measured, with results expressed in U kg^{-1}.

LOX activity was evaluated by Kuang et al. (2023). 1 g of fresh pepper was ground with 0.1 mol L^{-1} PBS at pH 6.8, supplemented with 1 % Triton X-100 and 4 % PVPP. Following centrifugation, the supernatant was collected for analysis. The assay solution comprised 2.75 mL of a 0.1 mol L^{-1} sodium phosphate buffer at pH 5.5, along with 50 μL of a 0.1 mol L^{-1} sodium linoleate solution and 0.2 mL of the enzyme extract. The change in absorbance at 234 nm over a period of 3 min was recorded, and the results were expressed in U kg^{-1}.

The fatty acid content, including linolenic acid, linoleic acid, palmitic acid, and stearic acid, was analyzed according to the method described by Lin et al. (2017). 3 g of fresh pepper was combined with a

chloroform-methanol (2:1) and subjected to sonication for 1 h before being filtered. Saturated sodium chloride was added to the filtrate; the upper layer contained the fat extract, which was subsequently dried over anhydrous sodium sulfate. Benzene-petroleum ether (1:1) and 0.4 mol L^{-1} KOH-methanol were introduced to the fat extract and incubated at 45 °C for 30 min. Following this incubation, n-hexane and saturated sodium chloride were added. The supernatant was then carefully pipetted into injection vials for further analysis.

The determination of malondialdehyde (MDA) was carried out according to the method described by Sun, Liu, et al. (2024). Briefly, 1 g of pepper was ground with 0.1 g mL^{-1} TCA (5 mL), centrifuged and the supernatant retained. A 2 mL sample of the supernatant was taken (the control tube was replaced with 2 mL of 0.1 g mL^{-1} TBA) and combined with an equal volume of TBA. Boil in a water bath for 1/3 h and determine the absorbance at 600 nm, 450 nm and 532 nm. The unit is expressed as μmol kg^{-1}.

2.9. Statistical analysis

The data were expressed as the standard deviation of means obtained from three independent experiments and subsequently analyzed for statistical significance using GraphPad Prism 10.1.0 and Origin 2021 software, respectively. Statistical differences between means at $P < 0.05$ were analyzed using ANOVA.

3. Results and discussion

3.1. Thymol on contents of firmness, ΔE, chlorophyll, total flavonoid and decay rate of peppers

During the pre-storage period, no differences were observed in the phenotypic changes of the peppers. However, as the storage period progressed, the peppers exhibited progressive wrinkling and yellowing. The treatment with 100 mg L^{-1} demonstrated the least significant changes. At 36 d, the peppers in the CK group exhibited multiple instances of rot, while the peppers in the 50 mg L^{-1} and 150 mg L^{-1} treatment groups displayed indications of yellowing of the skin, wrinkling, and slight rotting. In contrast, the peppers in the 100 mg L^{-1} treatment group did not demonstrate any instances of rotting and retained a organoleptic quality. The firmness of peppers exhibited a gradual decline throughout the storage period, with the 100 mg L^{-1} treatment group consistently exhibiting higher firmness levels compared to the other experimental groups. At 36 d, firmness of the 100 mg L^{-1} treatment group was 5.127 N, while the firmness of the CK group was 3.344 N. This represents a 1.53-fold increase in firmness for the 100 mg L^{-1} treatment group relative to the CK group (Fig. 1B). Furthermore, the colour difference exhibited a gradual increase during storage period. The 100 mg L^{-1} treatment group demonstrated the smallest shift in colour difference value, suggesting that the 100 mg L^{-1} treatment group was more effective in maintaining the colour of the pepper and preserving its sensory quality (Fig. 1C). The chlorophyll content exhibited a declining trend throughout the storage period. The highest chlorophyll content was observed in the 100 mg L^{-1} treatment group, which was 1.24 times greater than that of the CK group. This finding indicates that the treatment group effectively inhibited the reduction in chlorophyll levels and maintained the quality of the peppers. (Fig. 1D) The flavonoid content demonstrated a tendency to increase and subsequently decline throughout the entire storage period. Conversely, the flavonoid content of the CK group consistently exhibited a lower level than that of the treatment group. At the 36 d, the flavonoid content of the 100 mg L^{-1} treatment group reached its peak, while the flavonoid content of the CK group reached its lowest point (Fig. 1E). Notably, the observed increase in flavonoid content may be attributed to the inhibitory effect of thymol-treated on the conversion of flavonoids and the stimulatory effect on the conversion of phenols to flavonoids (Mahfujul et al., 2023). The decay rate exhibited a linear increase, with the onset of decay observed in the CK group after 18 d and in the treated group until 27 d. At 36 d, the decay rate reached 43.45 % in the CK group and only 10.1 % in the 100 mg L^{-1} treatment group. This represented a 4.3-fold difference between the two groups (Fig. 1F). These findings align with those of Grzegorzewska et al. (2020), Kong et al. (2020), and Ma et al. (2023), which demonstrated that MT, $CaCl_2$, and 1-MCP treatments mitigated pepper softening and preserved storage quality. Based on these observations, it can be postulated that thymol may have a comparable effect.

The results showed that the 100 mg L^{-1} treatment group could maintain the firmness, flavonoids and chlorophyll content of the peppers, reduce the decay rate of the peppers and reduce the change of colour difference to ensure the storage quality of the peppers. Therefore, we chose the 100 mg L^{-1} treatment group to study the effects of thymol on the antioxidant system, membrane lipid metabolism and cell wall metabolism in peppers.

3.2. Effect of thymol-treated on the rate of H_2O_2 content and O_2^- production rate, CAT, POD, SOD and APX activities in peppers

ROS is intimately associated with lipid peroxidation and cell softening, and excessive reactive oxygen species can lead to a disruption of the dynamic equilibrium, thus accelerating fruit senescence and softening (Mittler, Zandalinas, Fichman, & Breusegem, 2022). POD, CAT, and SOD are very common and essential anti-oxidant enzymes with the function of scavenging ROS. In this article, the H_2O_2 content and the O_2^- production rate demonstrated an upward trajectory during the storage period. At 36 d, the H_2O_2 content of CK was 1.46 times greater than that of the thymol-treated (Fig. 2A). At 18 d, the O_2^- production rate in the thymol-treated was only 79 % of that in CK (Fig. 2B). Similarly, Wu, Li, et al. (2024) observed that the treatment resulted in a reduction in H_2O_2 and O_2^- content. At 6 d, the fumigation of H_2O_2 content exhibited a 23.33 % decrease in comparison to the control, indicating that the treatment was effective in maintaining ROS levels. The findings of this study indicate that CAT activity exhibits a fluctuating pattern, with an initial increase followed by a subsequent decrease during the storage period. At 18 d, the CAT activity of the thymol-treated was 88.5 U g^{-1}, while that of the CK was 68.5 U g^{-1}. This represented a 1.29-fold increase in CAT activity relative to the CK (Fig. 2C). The activity of POD exhibited a gradual increase throughout the storage period, with differences observed at the later stages (Fig. 2D). The same findings were found in Li et al. (2024), who revealed that exogenous MT could efficiently scavenge ROS by regulating the levels of *CaCAT* and *CaPOD* and maintaining high antioxidant enzyme activity, thereby protecting pepper cells from ROS-induced damage. The activity of SOD exhibited an initial increase followed by a subsequent decrease during the storage period. Throughout this period, the SOD activity in the thymol-treated consistently exceeded that of the CK. The highest SOD activity was observed at 18 d, with the enzyme activity of the thymol-treated being 1.36 times that of the CK (Fig. 2E). APX activity demonstrated a tendency to increase and subsequently decline. The highest APX activity was observed in the thymol-treated at 9 d, with an activity of 325.5 U g^{-1}, and an APX activity of 262 U g^{-1} in the CK. The thymol-treated exhibited a 1.24-fold increase in APX activity compared to the CK (Fig. 2F). This is comparable to the recent study conducted by Wu et al. (2023), who discovered that APX activity in the treated group was markedly enhanced at 14 d, exhibiting a 1.27-fold increase relative to the control group. Furthermore, SOD activity after 28 d of storage was 21.25 ± 0.11 in the treated group and 14.43 ± 0.07 in the CK. In summary, thymol-treated dramatically increased SOD, POD and CAT activities and suppressed the rise of ROS levels.

3.3. Effect of thymol on GSH, GSSG, AsA, DHA contents, AsA/DHA rate, MDHAR, DHAR and GR activities of peppers

The AsA-GSH cycle is a very essential ROS removal system (Ding, Yao, et al., 2023). In the present study, the GSH content exhibited a

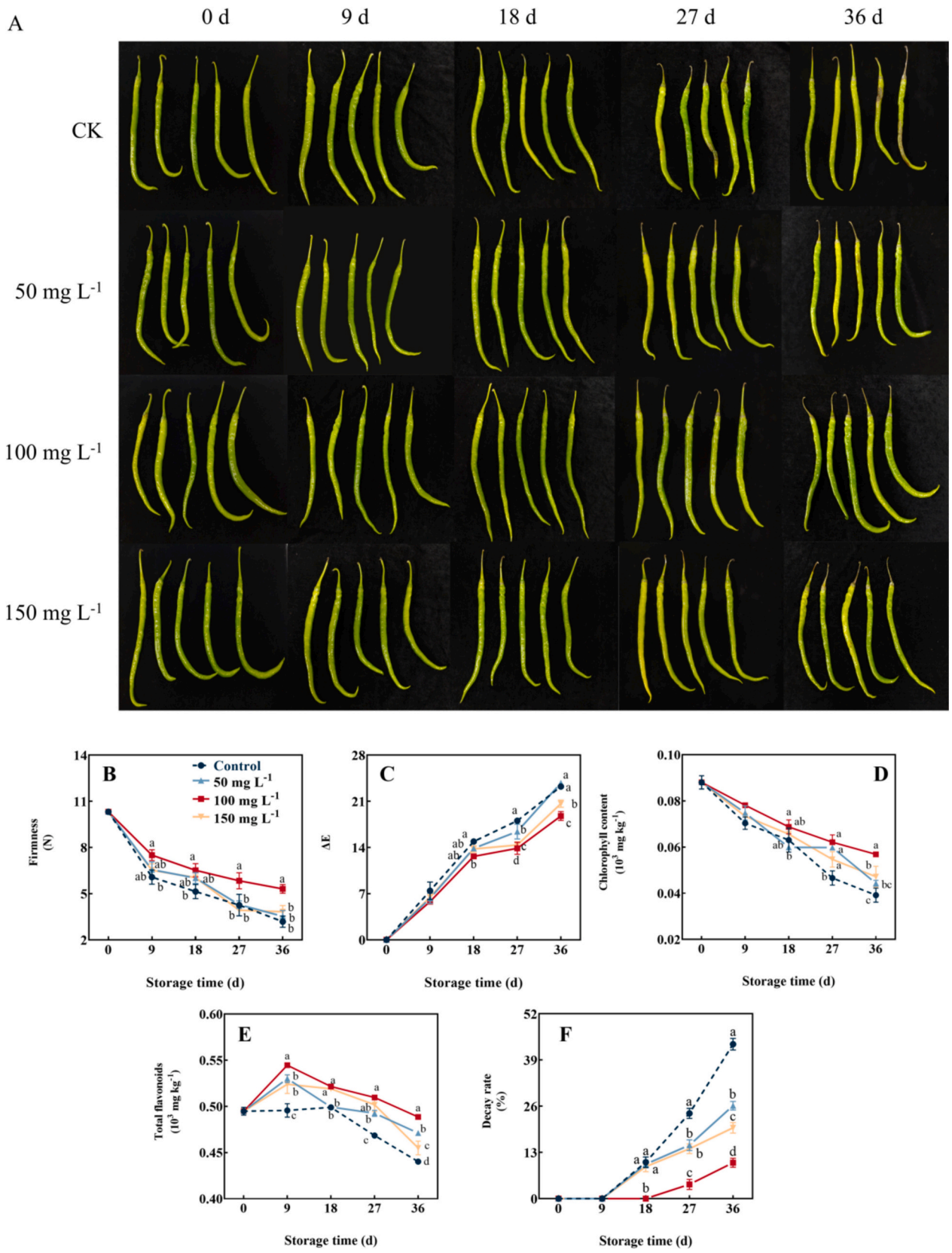


Fig. 1. Effect of thymol-treated on the appearance (A), firmness (B), ΔE (C), chlorophyll (D), total flavonoids (E) and decay rate (F) of peppers at 10 ± 0.5 °C, the statistics are the averages values ± SD ($n = 3$). * $P < 0.05$; ** $P < 0.01$. The following labels are identical.

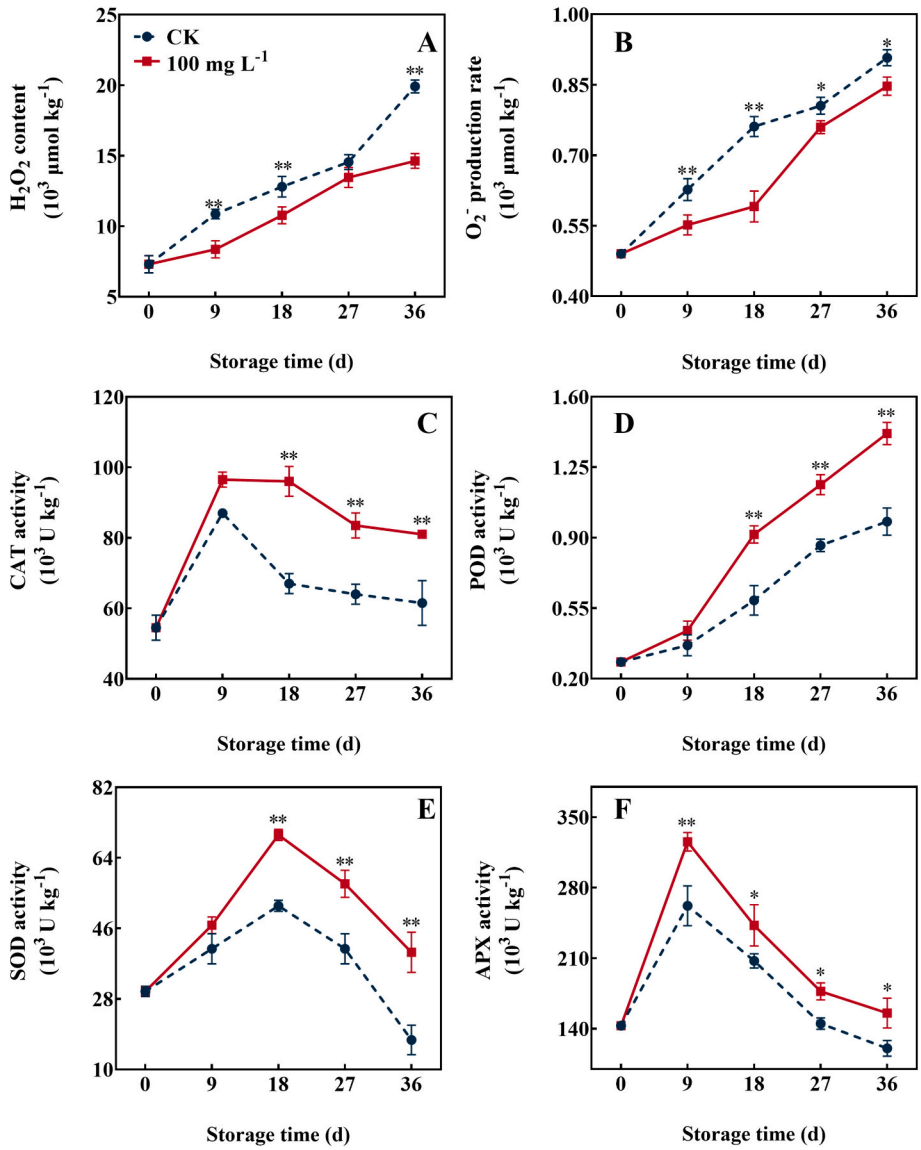


Fig. 2. Effect of thymol-treated on H_2O_2 (A), O_2^- production rate (B), CAT (C), POD (D), SOD (E), and APX (F) of peppers at 10 ± 0.5 °C. The data are the average of three values.

precipitous decline at 9 d, which is likely attributable to the alteration in pepper environment at the beginning of storage. Subsequently, the GSH content demonstrated a tendency towards stabilization throughout the duration of storage with a discrepancy (Fig. 3A). The GSSG content exhibits initially rising and subsequently declining, the highest value is reached at 9 d (Fig. 3B). AsA content demonstrated a downward trajectory during the storage period. The AsA content of the thymol-treated consistently exceeded that of CK. At the 36 d, the AsA content of the thymol-treated reached a value that was 3.38 times greater than that of the CK (Fig. 3C). During the storage period, the DHA content of the CK consistently exceeded that of the thymol-treated. At 27 d, the DHA content of CK was 1.21 times greater than that of the thymol-treated. The AsA/DHA ratio exhibited a gradual decline throughout the storage period. In the treatment group, the AsA/DHA ratio, which reflects the antioxidant capacity of the peppers, remained consistently higher than that of CK. At the 36 d, the ratio of the thymol-treated was 2.15 times greater than that of CK (Fig. 3D and E). Similarly, Guo et al. (2025) observed that the irradiation treatment of pakchoi stimulated the AsA-GSH cycle. The RL (Red light-emitting diode irradiation) group exhibited 65–142 % higher AsA levels compared to the CK throughout the storage period. Additionally, the AsA/DHA ratio was significantly increased during storage, ranging from 15 % to 161 % higher than the CK, the findings indicate that the homeostasis of ROS can be maintained by regulating the AsA-GSH cycle, which may slow down the ageing process of fruits and vegetables. Similar variations have been identified in previous studies, including those on water chestnuts (Wu, Zhang, et al., 2024) and pepper (Narin, Nam, Jessada, & Karthikeyan, 2024). In this study, the MDHAR activity initially increased and subsequently decreased. At 9 d, the MDHAR activity of the thymol-treated was observed to be 1.59 times higher than that of the CK (Fig. 3F). DHAR activity initially increased and subsequently declined, reaching its peak at the 18 d. The thymol-treated exhibited a 1.45-fold higher DHAR activity compared to the CK (Fig. 3G). The trend of the GR enzyme activity was like that of MDHAR, with the enzyme activity reaching its highest value at the 18 d (Fig. 3H). Similarly, we find similar results in Yao et al. (2021), who demonstrated that exogenous GSH improved the antioxidant capacity of pepper by up-regulating *CaGR2*, inducing the expression of *CaAPX1*, *CaMDHAR1*, and *CaDHAR1*, thereby mitigating cold damage and protecting the cellular membrane system from damage. As demonstrated by Zhang et al. (2022), sulfur dioxide has the capacity to regulate the AsA-GSH cycle in grapes, inhibit the accumulation of H_2O_2, and reduce O_2^- production rate. Shen et al. (2025) demonstrated that H_2S

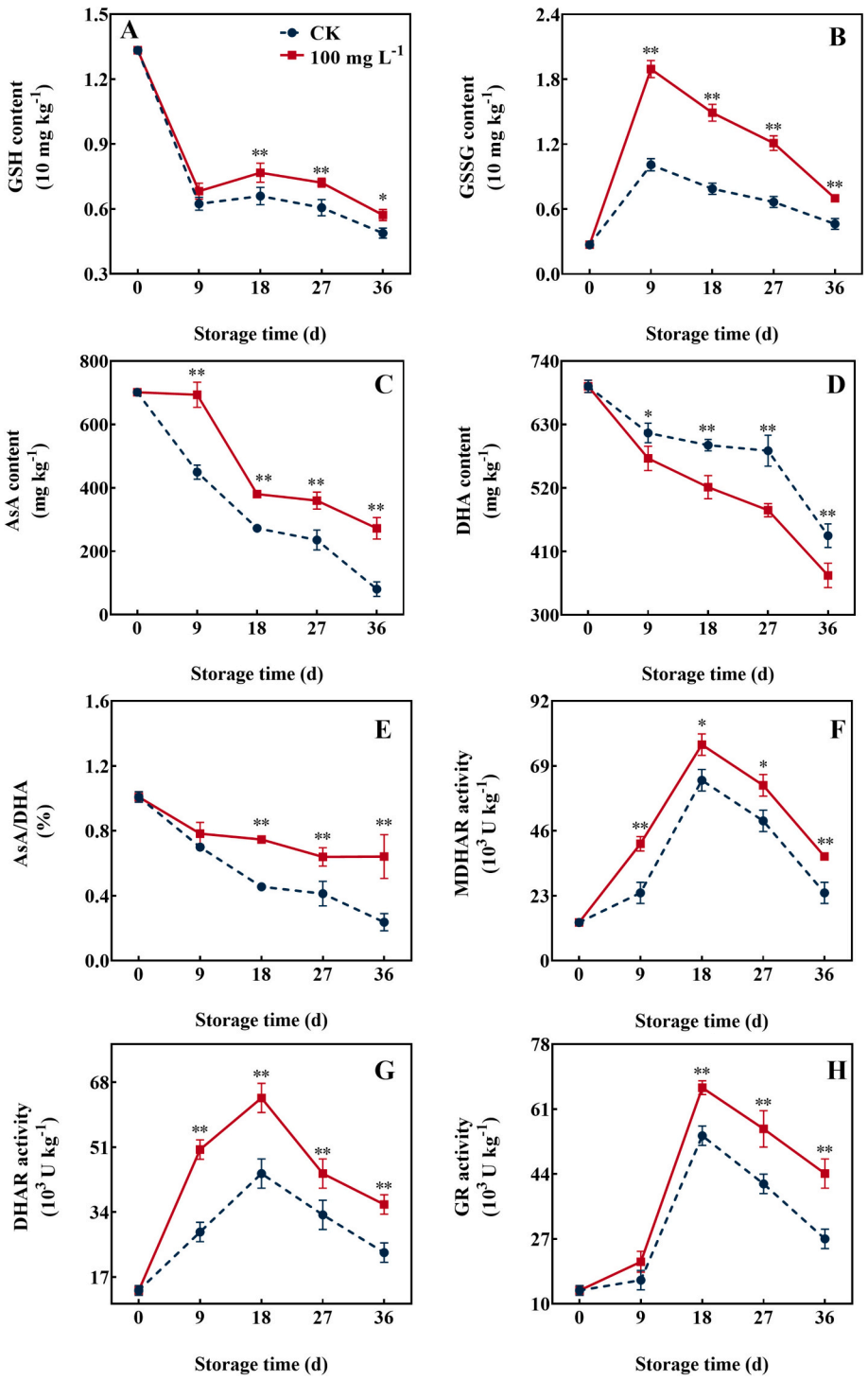


Fig. 3. Effect of thymol-treated on GSH (A), GSSG (B), AsA (C), DHA (D), AsA/DHA (E), MDHAR (F), DHAR (G), and GR (H) of peppers at 10 ± 0.5 °C, and the data are the average of three values.

treatment induces gene expression in the AsA-GSH cycle and markedly suppresse ROS production, thereby maintaining a high antioxidant capacity. This, in turn, enhances the vitality of key enzymes involved in the AsA-GSH cycle, thereby maintaining a state of equilibrium with respect to ROS. It was determined that the application of thymol enhanced the antioxidant capacity of peppers, augmented the scavenging capacity of reactive oxygen species by elevating the expression of the AsA-GSH cycle, and mitigated the deleterious effects of ROS, thereby improving the quality of preservation.

3.4. Effect of thymol contents of cellulose, PP, SP, WSP, ISP and CSP of peppers

The cell wall is primarily comprised of pectin, cellulose, and hemicellulose (Rapin et al., 2023). The cellulose content demonstrated a declining trend over the course of the storage period. The thymol-treated exhibited consistently higher cellulose content than the CK. At 9 d, the cellulose content of the CK was 81 % of that of the thymol-treated (Fig. 4A). The PP content exhibited a similar trend to that of cellulose. At 36 d, the thymol-treated was 1.2 times greater than that of the CK

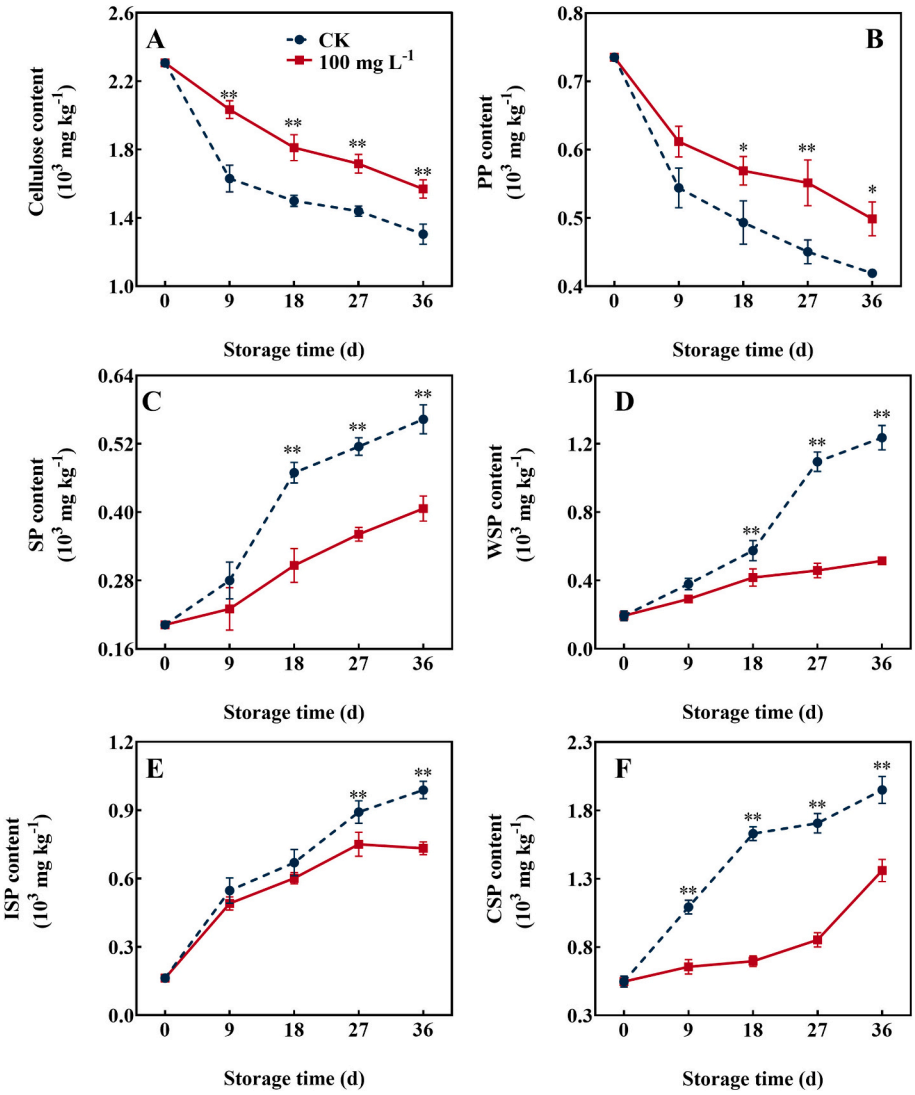


Fig. 4. Effect of thymol-treated on cellulose (A), PP (B), SP (C), WSP (D), ISP (E), and CSP (F) of peppers at 10 ± 0.5 °C. The data are the average of three values.

(Fig. 4B). Consistent with our result, Li, Qiu, et al. (2023) demonstrated elevated levels of PP and cellulose in okra treated with GA, accompanied by a reduced expression of cell wall-degrading enzymes. The contents of SP, WSP, ISP and CSP exhibited an upward trajectory during the storage period. The thymol-treated consistently exhibited lower values than the CK. Furthermore, each index demonstrated disparities during the late storage phase, suggesting that the thymol-treated may have impeded the solubilisation of capsicum pectin (Fig. 4C-F). The same results were found in different treatments of peppers, Alagie et al. (2023) demonstrated that the reduction of cell wall degradation by MT could maintain pepper firmness.

3.5. *Effect of thymol-treated on cx, PME, β-gal, PG, PL, β-Glu activities of peppers*

Polysaccharides in the cell wall are destroyed by cell wall degrading enzymes (PME, PG, *β*-Glu, *β*-Gal, Cx, etc.), which ultimately leads to the collapse of the cell wall structure (Ji et al., 2021). Zeng et al. (2024) found that inhibition of *β*-Gal, PME activity could delay the degradation of pectin and cellulose and maintain the integrity of cell wall structure, and found that inhibition of key genes, such as *Miβ-Gal* and *MiPME*, could delay the degradation of the cell wall. The results of our study are in accordance with the aforementioned findings, the activity of the Cx in question exhibited a tendency to increase over the course of the storage period, with the rate of this increase becoming more pronounced after 27 d. At 36 d, the thymol-treated activity was observed to be 74 % of the CK (Fig. 5A). The data indicated a fluctuating trend in PME activity, exhibiting an increase followed by a decline. At 27 d, the activity of the PME in the thymol-treated was observed to be 1.81 times lower than that of the CK. Following the 27 d, a decline in enzyme activity was observed, with a decrease evident in the thymol-treated compared to the CK (Fig. 5B). During the storage period, *β*-Gal, PL and PG activity exhibited a linear increase, and at 36 d, PG and PL activities in CK were 1.2 and 1.1 times higher than those in the thymol treatment, respectively (Fig. 5C, E and D). *β*-Glu demonstrated a proclivity for an initial increase, followed by a decline, with the enzyme activity beginning to diminish after 27 d. At 18 d, CK exhibited a 2.24-fold higher enzyme activity compared to that of the thymol-treated (Fig. 5F). Our findings in Li, Tu, et al. (2023) that the generation of free radicals could be inhibited by elevated oxygen atmosphere modulation, thereby delaying date fruit softening and reducing the activities of PG, PME and Cx. Previous studies have demonstrated that thymol can control cell wall degradation and maintain the normal structure of the cell wall by inhibiting ROS generation, as evidenced by Ye et al. (2024) and others.

These findings suggest that the application of thymol can effectively maintain the polysaccharide content within pepper cells during storage, while also demonstrating the ability to suppress the observed increase in *β*-Glu, PME, PG and other enzyme activities.

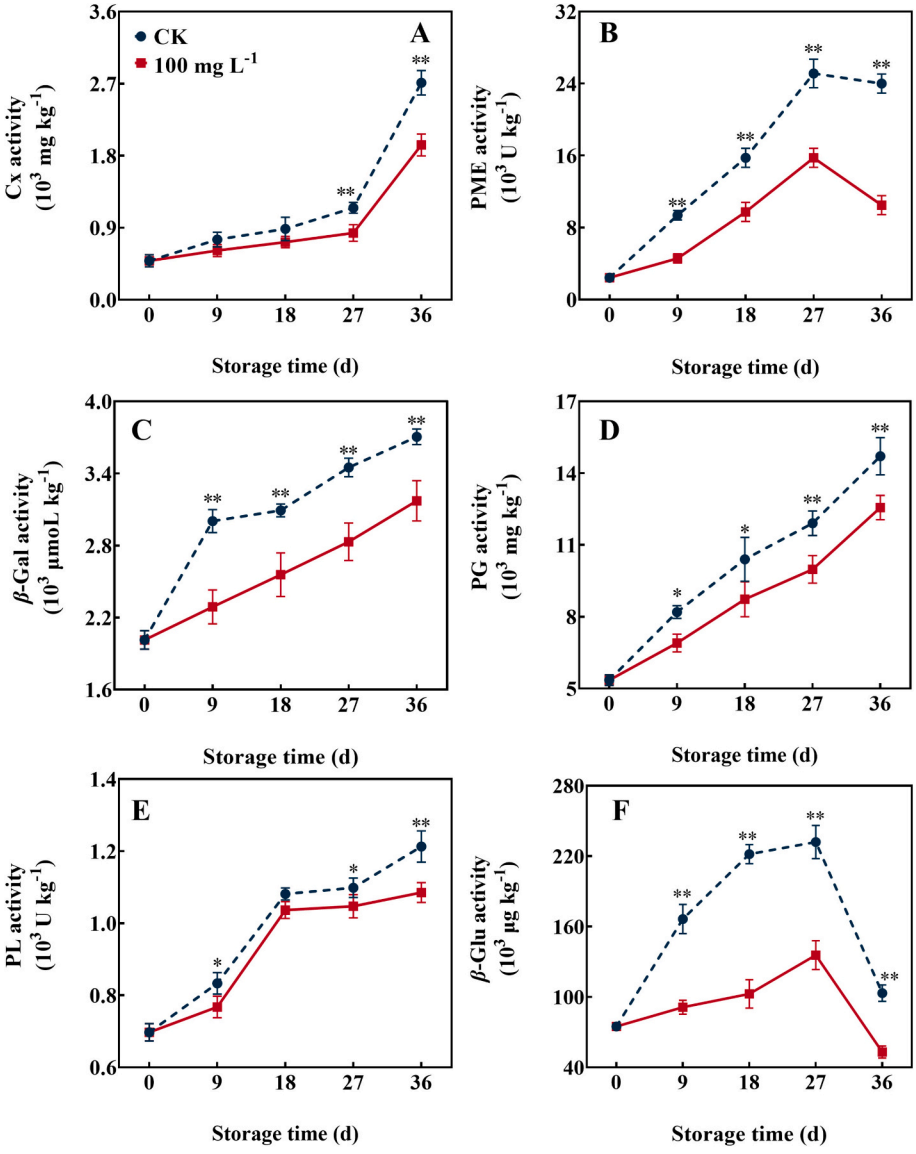


Fig. 5. Effect of thymol-treated on Cx (A), PME (B), *β*-Gal (C), PG (D), PL (E), and *β*-Glu (F) of peppers at 10 ± 0.5 °C. The data are the average of three values.

3.6. Effect of thymol-treated on PLD, LPS, LOX activities, fatty acids contents and MDA of peppers

The cell membrane and cell wall collectively form the cell's barrier, and an intact cell membrane system is essential for maintaining normal physiological processes in plants (Liu et al., 2022). Cell membranes are primarily composed of lipids, and the hydrolysis and oxidation of lipids can result in the disruption of membrane structure and function, this process is regulated by phospholipid metabolism enzymes, including PLD, LPS, and LOX (Lin, Lin, et al., 2024). The activities of PLD and LPS exhibited a linear trend, with values being lower in the treated than in the CK. At 36 d, the thymol-treated exhibited a 1.73-fold reduction in LPS activity relative to the CK (Fig. 6A and B). Consistent with our result, Kong et al. (2020) observed that MT mitigated membrane lipid peroxidation in green peppers. Additionally, they noted a marked increase in *CaPLD* expression in the CK during the initial five days, which was nearly twice as high as that observed in the treated fruits. Furthermore, MT was found to significantly reduce the activity of phospholipid oxidases and preserve the unsaturated fatty acid content. The activity of LOX exhibited a trend of increase, followed by a decline, reaching a peak at 18 d. The thymol-treated demonstrated 65 % of the activity observed in the CK (Fig. 6C). The linolenic acid content initially increased and subsequently declined, reaching a maximum at 9 d, after which it began to decrease and exhibited differences. At 18 d, the thymol-treated exhibited a linolenic acid content that was 1.6 times higher than that of the CK (Fig. 6D). (Fig. 6E) linoleic acid content initially declined and subsequently increased, reaching a nadir at 9 d, before exhibiting a gradual rise. At 9 d, the CK exhibited a 1.6-fold lower concentration than the thymol-treated. The palmitic acid content demonstrated an initial increase, followed by a decline, reaching its highest value at 27 d. Throughout the storage period, the CK content consistently exceeded that of the thymol-treated, exhibiting notable differences (Fig. 6F). The content of stearic acid increased and then decreased, reaching a maximum at 18 d. The thymol-treated exhibited a value that was 1.3 times lower than that of the CK (Fig. 6G). Similarly, Kuang et al. (2023) found that storage at 8 °C was more favourable for the accumulation of unsaturated fatty acids, and the PC content was 1.9 times higher at 8 °C than at 2 °C on the last day of storage. The U/S ratio exhibited a tendency to increase and was elevated in the thymol-treated relative to the CK, with the thymol-treated exhibiting a ratio that was 1.42 times higher than that of the CK (Fig. 6H). The MDA, which serves as an important indicator of cell membrane lipid peroxidation, continued to increase

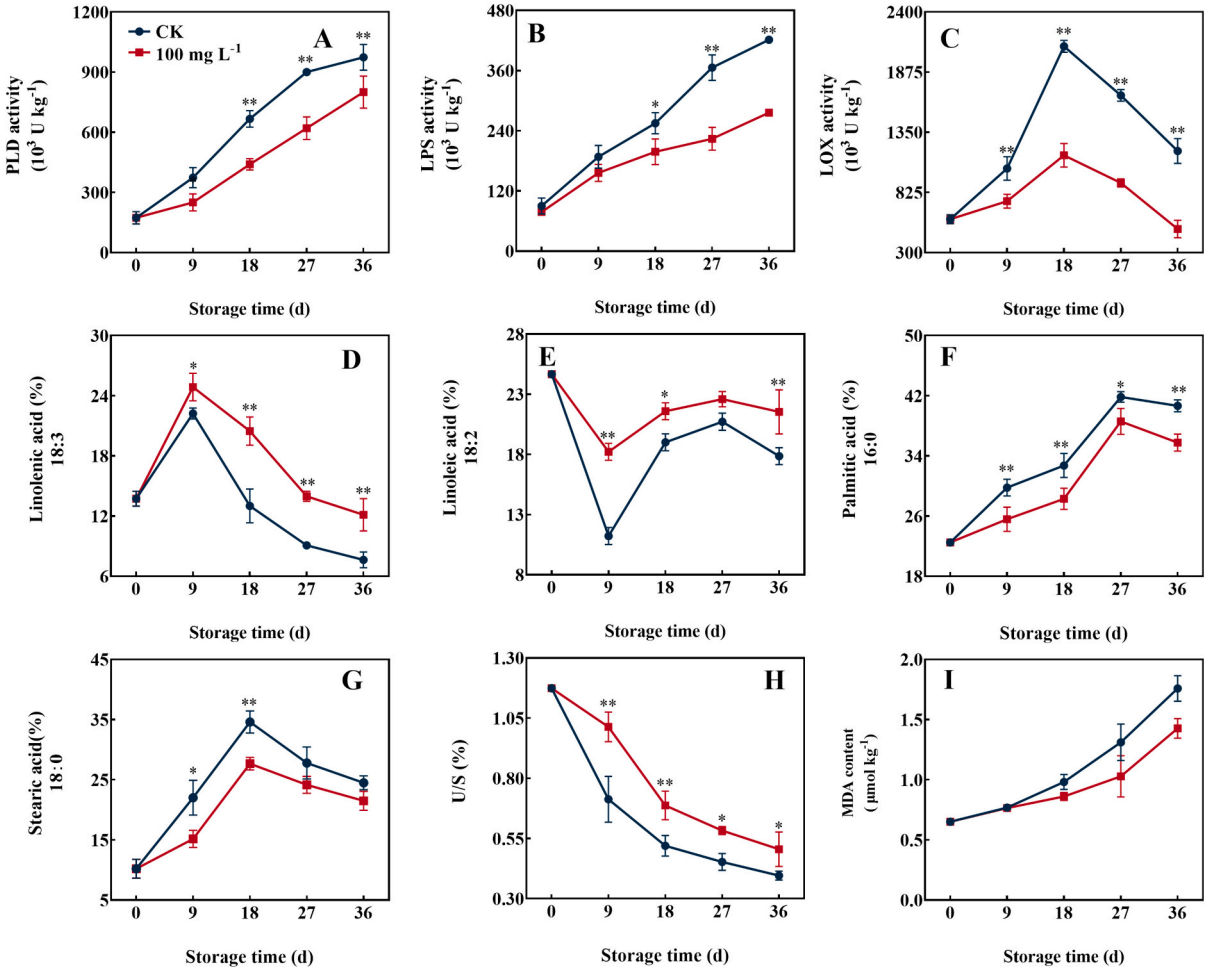


Fig. 6. Effect of thymol-treated on PLD (A), LPS (B), LOX (C), linolenic acid (D), linoleic acid (E), palmitic acid (F), stearic acid (G), ratio of unsaturated to saturated fatty acid (U/S) (H), and MDA (I) of peppers at 10 ± 0.5 °C. Data is the average of three values.

during the storage period. After 36 d, the CK was observed to be 1.2 times higher than that of the treatment group, which suggests that the treatment group effectively inhibited cell membrane peroxidation (Fig. 6I). In a study conducted by Lv et al. (2023), figs were treated with UV-C + 1-MCP. At 35 d, the MDA content of 1-MCP was found to be 1.64 nmol kg^{-1}, with no difference observed between the UV-C + 1-MCP and 1-MCP. In contrast, the CK content reached 1.84 nmol kg^{-1}, demonstrating a statistically significant difference. Additionally, the antioxidant and free radical scavenging capacities were markedly enhanced by the UV-C + 1-MCP treatment, indicating the potential for a correlation between antioxidant membrane lipids and the notion that the inhibition of ROS generation may serve to mitigate membrane lipid peroxidation. It can therefore be hypothesized that the treatment with thymol inhibited the peroxidation of cell membrane lipids and retard ed. damage to the cell membrane, thus effectively delaying the onset of pepper senescence, as confirmed by latest findings (Ye et al., 2024; Zhang, Tan, et al., 2023).

3.7. Correlation analysis

In this study, we analyzed the correlations among physiological indicators, the AsA-GSH cycle, cell wall metabolism, and membrane lipid metabolism using Pearson correlation coefficients. Notably, the content of AsA/DHA exhibited a negative correlation with stearic acid and fat-degrading enzymes. This finding suggests that thymol treatment may help maintain cell membrane integrity by modulating ROS levels. Furthermore, as indicated by our correlation analysis (Fig. 7), there was a significant negative correlation ($P < 0.01$) between LPS and PLD activity and both proline production (PP) and cellulose content. Lin, Chen, et al. (2024) demonstrated that the antioxidant system plays a crucial role in maintaining membrane lipids and cell walls; ATP treatment was shown to extend the shelf life of longan fruit by reducing ROS accumulation while mitigating softening and decomposition through alleviating PLD, LPS, LOX, PME, PG, Cx, and β-galactosidase activities. Similarly, Cao et al. (2024) found that melatonin could alleviate blueberry senescence by regulating the AsA-GSH cycle, controlling ROS generation, reducing membrane lipid peroxidation, down-regulating VcLOX and VcPLD expression levels, thereby maintaining cell membrane fluidity which subsequently delayed membrane lipid metabolism.

These findings provide compelling evidence linking the antioxidant system—particularly the AsA-GSH cycle—to both membrane lipid metabolism and cell wall metabolism in pepper fruits. It can be surmised that there exists a relationship between the integrity of cellular membranes and cell wall structures. Consequently, it can be inferred that the preservative effect observed in thymol-treated peppers may stem from thymol's ability to preserve quality by lowering ROS levels within peppers to sustain both cell wall integrity and cellular membrane stability, the possible mechanism of thymol treatment on pepper is shown in Fig. 8.

3.8. Mechanism of thymol-treated on antioxidant, cell wall metabolism and membrane lipid metabolism in peppers

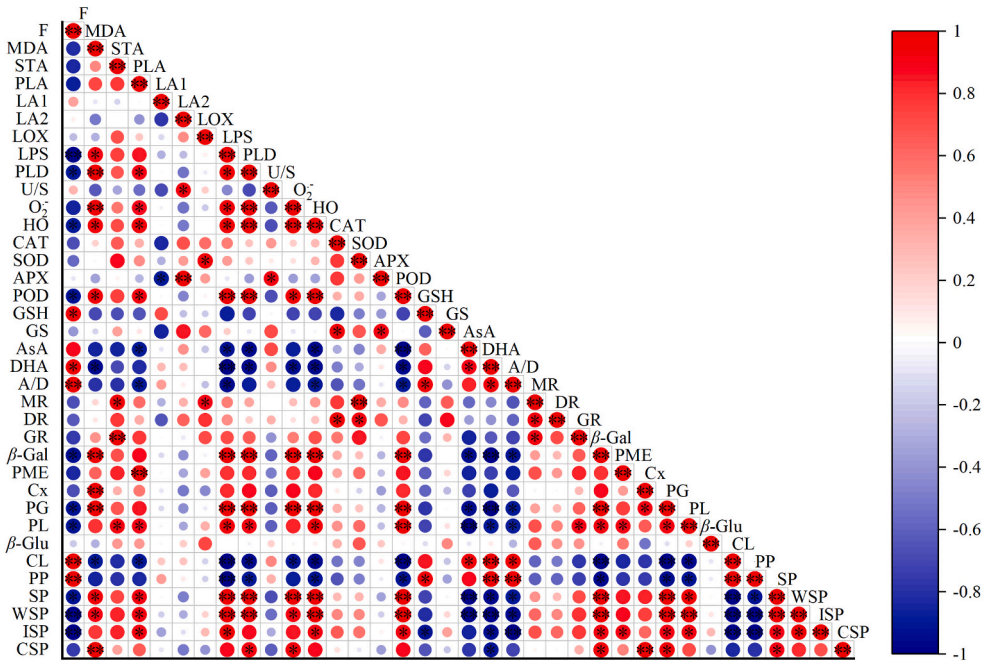


Fig. 7. Heat map of correlation analysis of various parameters of peppers after storage at 10 ± 0.5 °C for 36 d. Heat map of correlation of physiological, antioxidant, cell wall, and membrane lipid metabolism of thymol-treated during storage. F is firmness; SLA is stearic acid; PLA is palmitic acid; LA 1 is linoleic acid; LA 2 is linolenic acid; U/S is unsaturated acid/saturated acid ratio; HO is H_2O_2; GSG is GSSG; MR is MDHAR; CL is cellulose.

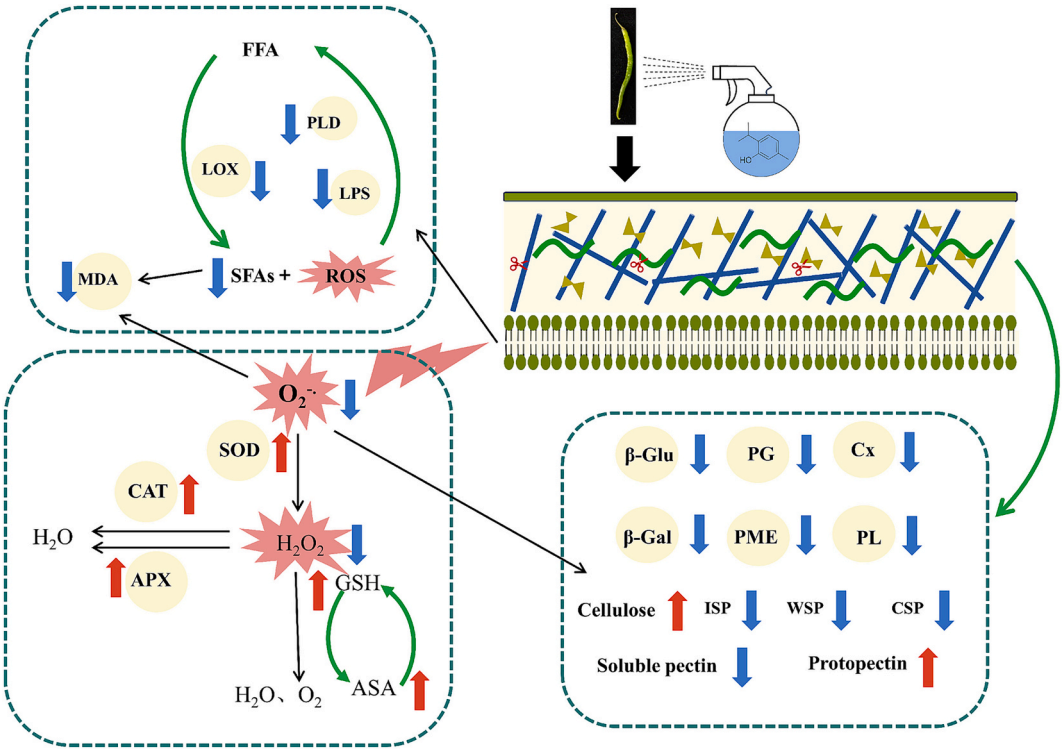


Fig. 8. Mechanism of thymol-treated on antioxidant, cell wall metabolism and membrane lipid metabolism in peppers at 10 ± 0.5 °C.

4. Conclusions

The objective of this study was to investigate the impact of thymol treatment on the senescence and quality deterioration of peppers. The findings indicated that the application of thymol (100 mg L^{-1}) inhibited enzymatic activities such as PG, PL, and *β*-Gal. This phenomenon contributed to the maintenance of pectin and cellulose content, thereby preserving the integrity of the cell wall. Furthermore, thymol treatment resulted in a reduction in hydrogen peroxide concentration, an increase in antioxidant enzyme activities including APX, SOD, and MDHAR, while also regulating the AsA-GSH cycle to mitigate ROS effects on peppers. Additionally, there was an inhibition in the production of stearic and palmitic acids alongside an increase in linoleic and linolenic acid content, which led to a decrease in membrane lipid peroxidation.

Nevertheless, the precise molecular mechanisms through which thymol influences pepper quality remain unclear. Future investigations could utilize advanced techniques such as proteomics, transcriptomics, and genomics to gain deeper insights into the regulatory mechanisms governing the effects of thymol application on pepper shelf-life.

Funding

This work was supported by the Guizhou Province 2024 Graduate Research Fund Project "Study on the Effect of Thymol on the Storage Quality of Peppers" (No. 2024-396) and the sixth batch of “Thousand” level innovative talent projects in Guizhou Province (Zhu Ke He Tong GCC [2022]008). This work was also supported by the Guizhou Provincial Key Technology R&D Program (Qian Ke He support [2024] General 089).

CRediT authorship contribution statement

Xue Li: Writing – original draft, Data curation. **Xiaogang Wang:** Writing – review & editing, Methodology. **Jianye Chen:** Supervision, Data curation. **Donglan Luo:** Investigation, Conceptualization. **Sen Cao:** Formal analysis. **Liangjie Ba:** Project administration, Methodology.

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.

References

Alagie, N., Dong, X. Q., Liu, Q. G., Lu, C. Y., Pan, X. J., & Zhang, W. E. (2023). Melatonin treatment inhibits mango fruit (cv. ‘Guiqi’) softening by maintaining cell wall and reactive oxygen metabolisms during cold storage. *Postharvest Biology and Technology, 205*, Article 112500. https://doi.org/10.1016/j.postharvbio.2023.112500

Cao, S., Qiao, L. X., Huang, T. Y., Zhang, Y., Qu, G. F., & Kou, X. H. (2024). Melatonin reduces postharvest decay of blueberries by regulating ascorbate–glutathione cycle and membrane lipid metabolism[J]. *Postharvest Biology and Technology, 218*, Article 113185. https://doi.org/10.1016/j.postharvbio.2024.113185

Castañeda, L. A., Hernández, F. O. M., Gómez, Y. G. M. L. D. Y., & Rios, C. J. L. (2024). Physical properties of zein-alginate-glycerol edible films and their application in the preservation of chili peppers (*Capsicum annuum* L.). *Food Science and Biotechnology, 33*(4), 889–902. https://doi.org/10.1007/S10068-023-01393-Z

Das, U. N. (2021). “Cell membrane theory of senescence” and the role of bioactive lipids in aging, and aging associated diseases and their therapeutic implications. *Biomolecules, 11*(2). https://doi.org/10.3390/biom11020241. Article 241.

Ding, J., Liu, C. Y., Huang, P., Li, H. Y., Liu, Y. E., S. D, et al. (2023). Effects of konjac glucan-nan/low-acyl gellan edible coatings loaded thymol-*β*-cyclodextrin microcapsules on postharvest blueberry. *Food Chemistry, 430*, Article 137080. https://doi.org/10.1016/j.foodchem.2023.137080

Ding, X. C., Yao, Q. P., Zhu, D. Q., & Dong, B. Y. (2023). Hydrogen-rich water maintains the quality of rosa roxburghii by regulating AsA biosynthesis and regeneration. *Postharvest Biology and Technology, 195*, Article 112136. https://doi.org/10.1016/j.postharvbio.2022.112136

Grzegorzewska, M., Badełek, E., Wrzodak, A., Fabiszewski, K., & Ciecierska, A. (2020). Quality and storage ability of fresh-cut pepper treated by 1-methylcyclopropene. *Journal of Horticultural Research, 28*(1), 101–110. https://doi.org/10.2478/johr-2020-0007

Guo, F., Sun, Y., Hu, H., Zhou, H. S., Zhang, Y. T., Liu, X. T., et al. (2025). Red light-emitting diode irradiation delays the senescence of postharvest pakchoi through promoting antioxidant capacity and regulating ascorbate-glutathione cycle. *Postharvest Biology and Technology, 222*, Article 113351. https://doi.org/10.1016/j.postharvbio.2024.113351

Guo, Y. T., Wu, B. G., Guo, X. Y., Liu, D. D., Qiu, C. C., & Haile, M. (2022). Effect of thermosonication on texture degradation of carrot tissue in relation to alterations in cell membrane and cell wall structure. *Food Chemistry, 393*, Article 133335. https://doi.org/10.1016/j.foodchem.2022.133335

Gwanpua, G. S., Buggenhout, V. S., Verlinden, E. B., Christiaens, S., Shpigelman, A., Vicent, V., et al. (2014). Pectin modifications and the role of pectin-degrading enzymes during postharvest softening of jonagold apples. *Food Chemistry, 158*, 283–291. https://doi.org/10.1016/j.foodchem.2014.02.138

Huang, J. L., Sun, R. L., Cao, X., Hu, N., Xia, B., Yi, Y. J., et al. (2023). Preservation effect of lactobacillus plantarum O_2 fermentation supernatant on postharvest pepper and its induced resistance to phytophthora capsici. *Plant Physiology and Biochemistry, 204*, Article 108098. https://doi.org/10.1016/j.plaphy.2023.108098

Huang, R., Cheng, Y., Li, C. Y., Guo, M., Zhu, J., Ge, Y. H., et al. (2022). Postharvest application of acibenzolar-s-methyl delays the senescence of pears by mediating the ascorbate-glutathione cycle. *Scientia Horticulturae, 293*, Article 110741. https://doi.org/10.1016/j.scienta.2021.110741

Ji, Y. R., Hu, W. Z., Liao, J., Xiu, Z. L., Jiang, A. L., Guan, Y. G., et al. (2021). Ethanol vapor delays softening of postharvest blueberry by retarding cell wall degradation during cold storage and shelf life. *Postharvest Biology and Technology, 177*, Article 111538. https://doi.org/10.1016/j.postharvbio.2021.111538

Kong, X. M., Ge, W. Y., Wei, B. D., Zhou, Q., Zhao, Y. B., & Ji, S. J. (2020). Melatonin ameliorates chilling injury in green bell peppers during storage by regulating membrane lipid metabolism and antioxidant capacity. *Postharvest Biology and Technology, 170*, Article 111315. https://doi.org/10.1016/j.postharvbio.2020.111315

Kuang, X. Y., Chen, Y. Z., Lin, H. T., Lin, H., Chen, G., Lin, Y. F., et al. (2023). Comprehensive analyses of membrane lipids and phenolics metabolisms reveal the developments of chilling injury and browning in Chinese olives during cold storage. *Food Chemistry, 416*, Article 135754. https://doi.org/10.1016/j.foodchem.2023.135754

Li, L., Tu, M. Y., Li, W. X., Bao, N. N., & Luo, Z. S. (2023). Elevated oxygen atmosphere maintains antioxidant capacity and inhibits cell wall metabolism in postharvest jujube. *Postharvest Biology and Technology, 199*, Article 112301. https://doi.org/10.1016/j.postharvbio.2023.112301

Li, P. P., Zhang, R. H., Zhou, H. D., Mo, Y. R., Wu, S., Zhang, X., et al. (2024). Melatonin delays softening of postharvest pepper fruits (Capsicum annuum L.) by regulating cell wall degradation, membrane stability and antioxidant systems. *Postharvest Biology and Technology, 212*, Article 112852. https://doi.org/10.1016/j.postharvbio.2024.112852

Li, S. S., Qiu, C. Y., Yang, M. J., Shi, L. Y., Cao, S. F., Yang, Z. F., et al. (2023). Effect of gibberellic acid on cell wall degradation and softening in postharvest okras. *LWT, 186, Article 115223*. https://doi.org/10.1016/j.lwt.2023.115223

Lin, L. J., Lin, H. T., Zheng, S. J., Chen, Y. Z., Zhang, H. L., Lin, M. S., et al. (2024). Metabolisms of ROS and membrane lipid participate in pestalotiopsis microspora-induced disease occurrence of harvested Chinese olives. *Postharvest Biology and Technology, 210*, Article 112720. https://doi.org/10.1016/j.postharvbio.2023.112720

Lin, Y. F., Chen, J., Lin, Y. X., Lin, M. S., Wang, H., Fan, Z. Q., et al. (2024). DNP and ATP modulate the pulp softening and breakdown in fresh longan by acting on the antioxidant system and the metabolisms of membrane lipids and cell wall polysaccharides. *Food Chemistry, 460*(P1), Article 140531. https://doi.org/10.1016/j.foodchem.2024.140531

Lin, Y. F., Lin, Y. X., Lin, H. T., Ritenour, A. M., Shi, J., Zhang, S., et al. (2017). Hydrogen peroxide-induced pericarp browning of harvested longan fruit in association with energy metabolism. *Food Chemistry, 225*, 31–36. https://doi.org/10.1016/j.foodchem.2016.12.088

Liu, Q. Q., Xie, H. L., Chen, Y. H., Lin, M. S., Hung, Y. C., & Lin, H. T. (2022). Alleviation of pulp breakdown in harvested longan fruit by acidic electrolyzed water in relation to membrane lipid metabolism. *Scientia Horticulturae, 304*, Article 111288. https://doi.org/10.1016/j.scienta.2022.111288

Lu, Y. Q., Zhang, Y., Lian, N., & Li, X. J. (2023). Membrane dynamics regulated by cytoskeleton in plant immunity. *International Journal of Molecular Sciences, 24*(7), Article 6509. https://doi.org/10.3390/ijms24076059

Lv, Y. H., Fu, A. Z., Song, X. X., Wang, Y. F., Chen, G. G., & Jiang, Y. (2023). 1-Methylcyclopropene and UV-C treatment effect on storage quality and antioxidant activity of ‘Xiaobai’apricot fruit. *Foods, 12*(6). https://doi.org/10.3390/foods12061296. article 1296.

Ma, L. L., Zheng, Y. Y., Sang, Z. Z., Ge, Y. H., Bai, C. M., Fu, A. Z., et al. (2023). Multi-omics analysis reveals the mechanism of calcium-reduced quality deterioration in mechanically injured green pepper fruit. *Postharvest Biology and Technology, 204*, Article 112437. https://doi.org/10.1016/j.postharvbio.2023.112437

Mahfujul, A., Mrityunjoy, B., Kanti, M. D., Meahadi, M. H., Rakibul, M. I., & Avishek, J. (2023). Evaluation of green chili stored at the chilling condition: Effects of thermal and ultrasound pretreatments. *Food and Humanity, 1*, 1274–1281. https://doi.org/10.1016/j.foohum.2023.09.023

Marklund, S., & Marklund, G. (1974). Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *European Journal of Biochemistry, 47*, 469–474. https://doi.org/10.1111/j.1432-1033.1974.tb03714.x

Mi, S., Li, T., Sang, Y. X., Wang, X. H., & Duan, Y. Q. (2023). Effect of cold shock precooling on the physicochemical, physiological properties and volatile profiles of chili peppers during postharvest storage. *LWT, 183*, Article 115300. https://doi.org/10.1016/j.lwt.2023.115300

Mittler, R., Zandalinas, S. I., Fichman, Y., & Breusegem, F. V. (2022). Reactive oxygen species signalling in plant stress responses. *Nature reviews. Molecular cell biology, 23*(10), 663–679. https://doi.org/10.1038/s41580-022-00499-2

Narin, C., Nam, H. P., Jessada, R., & Karthikeyan, V. (2024). Exogenous application of melatonin on the preservation of physicochemical and enzymatic qualities of pepper fruit from chilling injury. *Horticulturae, 10*(6), 550. https://doi.org/10.3390/horticulturae10060550

Nukuntornprakit, O., Chanjirakul, K., Doorn, V. G. W., & Siriphanich, J. (2015). Chilling injury in pineapple fruit: Fatty acid composition and antioxidant metabolism.

Postharvest Biology and Technology, 99, 20–26. https://doi.org/10.1016/j.postharvbio.2014.07.010

Oubohssaine, M., Hnini, M., & Rabeh, K. (2024). Exploring lipid signaling in plant physiology: From cellular membranes to environmental adaptation. *Journal of Plant Physiology, 300*, Article 154295. https://doi.org/10.3390/plants11182367

Rapin, N. M., Murray, L., Sadler, H. I., Bothwell, H. J., & Fry, C. S. (2023). Same but different — pseudo-pectin in the charophytic alga chlorokybus atmophyticus. *Physiologia Plantarum, 175*(6), Article e14079. https://doi.org/10.1111/ppl.14079

Shen, X. Y., Liu, Y., Zeng, Y., Zhao, Y. Q., Bao, Y. Q., Shao, X. F., et al. (2025). Hydrogen sulfide attenuates chilling injury in loquat fruit by alleviating oxidative stress and maintaining cell membrane integrity. *Food Chemistry, 463*(P2), Article 141094. https://doi.org/10.1016/j.foodchem.2024.141094

Sun, P. P., Liu, C., Yu, Y. C., Zhou, J. J., & Ren, Y. Y. (2024). Regulation effect of magnetic field combined with low temperature storage on postharvest quality and cell wall pectic-polysaccharide degradation of *Clausena lansium* (Lour.) Skeels. *Food Chemistry: X, 22*, Article 101253. https://doi.org/10.1016/j.fochx.2024.101253

Sun, X. Y., Tian, S. H., Li, B., Zhang, Y. H., Li, D. N., Yang, N., et al. (2024). Space electric field impacts on softening and cell wall modification of strawberry during cold storage. *Postharvest Biology and Technology, 216*, Article 113068. https://doi.org/10.1016/j.postharvbio.2024.113068

Taban, A., Haghighi, M. T., Mousavi, S. S., & Sadeghi, H. (2024). Are edible coatings (with or without essential oil/extract) game changers for maintaining the postharvest quality of strawberries? A meta-analysis. *Postharvest Biology and Technology, 216*, Article 113082. https://doi.org/10.1016/j.postharvbio.2024.113082

Tzortzakis, N. (2024). Origanum dictamnus essential oil in vapour or aqueous solution application for pepper fruit preservation against botrytis cinerea. *Agronomy, 14*(2). https://doi.org/10.3390/agronomy14020257. Article 257.

Wang, J., Pei, Y. P., Chen, C., Yang, X. H., An, K. J., & Xiao, H. W. (2023). High-humidity hot air impingement blanching (HHAIB) enhances drying behavior of red pepper via altering cellular structure, pectin profile and water state. *Innovative Food Science & Emerging Technologies, 83*, Article 103246. https://doi.org/10.1016/j.ifset.2022.103246

Wang, K., Li, T. T., Chen, S. Q., Li, Y. L., & Rashid, A. (2020). The biochemical and molecular mechanisms of softening inhibition by chitosan coating in strawberry fruit (*Fragaria x ananassa*) during cold storage. *Scientia Horticulturae, 271*, Article 109483. https://doi.org/10.1016/j.scienta.2020.109483

Wang, Z. W., Zhang, Q. Q., Bukvicki, D., Xu, Y., Peng, Y., Li, F., et al. (2024). Konjac glucomannan/microcapsule of thymol edible coating reduces okra pericarp browning by regulating antioxidant activity and ROS synthesis. *International Journal of Biological Macromolecules, 276*, Article 133641. https://doi.org/10.1016/j.ijbiomac.2024.133641

Wu, C. C., Hao, W. Z., Yan, L., Zhang, H. Z., Zhang, J., Liu, C. H., et al. (2023). Postharvest melatonin treatment enhanced antioxidant activity and promoted GABA biosynthesis in yellow-flesh peach. *Food Chemistry, 419*, Article 136088. https://doi.org/10.1016/j.foodchem.2023.136088

Wu, X. P., Li, Q. Y., Yi, Y., Wang, L. M., Hou, W. F., Ai, Y. W., et al. (2024). Cinnamaldehyde affects the storage quality of freshly cut water chestnuts through the regulation of reactive oxygen species and the AsA-GSH cycle. *Scientia Horticulturae, 332*, Article 113199. https://doi.org/10.1016/j.scienta.2024.113199

Wu, X. T., Zhang, H. S., Liu, X. J., Liu, Z. A., Wang, C. L., & Liao, W. B. (2024). Molecular hydrogen prolongs Lanzhou lily (Lilium davidii var. unicolor) postharvest shelf-life via improving antioxidant capacity. *Scientia Horticulturae, 336*, Article 113431. https://doi.org/10.1016/j.scienta.2024.113431

Yao, M. M., Ge, W. Y., Zhou, Q., Zhou, X., Luo, M. L., Zhao, Y. B., et al. (2021). Exogenous glutathione alleviates chilling injury in postharvest bell pepper by modulating the ascorbate-glutathione (AsA-GSH) cycle. *Food Chemistry, 352*, Article 129458. https://doi.org/10.1016/j.foodchem.2021.129458

Yavar, S., Abbasali, J., Rezapour, J. F., & Soleimani, M. A. (2021). Melatonin treatment delays senescence and improves antioxidant potential of sweet cherry fruits during cold storage. *Scientia Horticulturae, 288*, Article 110304. https://doi.org/10.1016/j.scienta.2021.110304

Ye, S. J., Chen, J. Y., Cao, S., Luo, D. L., & Ba, L. J. (2024). Thymol application delays the decline of fruit quality in blueberries via regulation of cell wall, energy and membrane lipid metabolism. *Food Chemistry, 458*, Article 140193. https://doi.org/10.1016/j.foodchem.2024.140193

Zeng, Y., Bao, Y. Q., Shen, X. Y., Liu, Y., Yi, B. H., Ren, H. Y., et al. (2024). Phytosulfokine-α treatment maintains fruit quality and alleviates chilling injury of peach fruit by regulating relative oxygen species and cell wall metabolisms. *Postharvest Biology and Technology, 216*, Article 113073. https://doi.org/10.1016/j.postharvbio.2024.113073

Zhang, H. L., Shan, T. T., Chen, Y., Lin, M. S., Chen, Y. Z., Lin, L. J., et al. (2023). Salicylic acid treatment delayed the browning development in the pericarp of fresh longan by regulating the metabolisms of ROS and membrane lipid. *Scientia Horticulturae, 318*, Article 112073. https://doi.org/10.1016/j.scienta.2023.112073

Zhang, S. R., Li, C. Y., Wang, M., Xu, H. P., Guo, Y., & Ge, Y. H. (2024). Phenyllactic acid maintains the storage quality of 'Zaosu' pears by regulating respiration and energy metabolism. *Postharvest Biology and Technology, 207*, Article 112607. https://doi.org/10.1016/j.postharvbio.2023.112607

Zhang, Y. H., Tan, Y. Z., OuYang, Q. L., Duan, B., Wang, Z. T., Meng, K. X., et al. (2023). γ-Cyclodextrin encapsulated thymol for citrus preservation and its possible mechanism against penicillium digitatum. *Pesticide Biochemistry and Physiology, 194*, Article 105501. https://doi.org/10.1016/j.pestbp.2023.105501

Zhang, Z., Wu, Z. H., Yuan, Y. T., Zhang, J. X., Jia, W., & Wu, B. (2022). Sulfur dioxide mitigates oxidative damage by modulating hydrogen peroxide homeostasis in postharvest table grapes. *Postharvest Biology and Technology, 188*, Article 111877. https://doi.org/10.1016/j.postharvbio.2022.111877

Zhao, R. P., Hao, J. X., Xue, J., Liu, H. J., & Li, L. T. (2011). Effect of high-voltage electrostatic field pretreatment on the antioxidant system in stored green mature tomatoes. *Journal of the Science of Food and Agriculture, 91*(9), 1680–1686. https://doi.org/10.1002/jsfa.4369