

## Research Article

# Effects of Chitosan Combined with $\epsilon$ -Polylysine Treatment on the Postharvest Quality and Antioxidant Status of Winter Jujube

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During storage, winter jujube often experiences physiological disorders that can result in postharvest quality deterioration. To address this concern, a combination of 1% chitosan (CTS) and varying concentrations (0.02, 0.2, and 2 mg mL<sup>-1</sup>) of  $\epsilon$ -polylysine ( $\epsilon$ -PL) was applied. The findings indicated that the treatment involving the combination of 1% CTS with 0.2 mg mL<sup>-1</sup>  $\epsilon$ -PL exhibited superior efficacy in preserving the hardness and titratable acid content of winter jujube. Additionally, the jujube fruits of this treatment exhibited a deceleration in the rise of decay incidence, red index, and weight loss rate. Simultaneously, the application of CTS combined with  $\epsilon$ -PL treatment improved the levels of antioxidant enzyme activities (SOD, CAT, POD, and APX) and enhanced contents of antioxidant compounds (AsA and GSH). Moreover, the generation of reactive oxygen species (H<sub>2</sub>O<sub>2</sub> and O<sub>2</sub><sup>•-</sup>) was suppressed. Consequently, CTS combined with  $\epsilon$ -PL treatment effectively enhanced postharvest quality and regulated antioxidant status of winter jujube.

## 1. Introduction

Winter jujube is highly regarded by individuals owing to its substantial nutritional value and favorable edibility. As bioactive compounds, ascorbic acid, flavonoids, and mineral constituents play significant roles in antioxidant and antimicrobial activities [1]. Research has indicated that the economic value of winter jujube is associated with its crisp texture, optimal moisture content, and elevated levels of protein and vitamins [2]. Notably, winter jujube at the white maturity stage is considered most suitable for commercialization and preservation [3]. However, challenges posed by environmental factors, maturation, microbial infection, and other physiological and biochemical factors within the postharvest supply chain render winter jujube fruit susceptible to deterioration [4], including dehydration, softening, browning, and decay. Therefore, the control of postharvest decay of winter jujube is of great importance for its storage and preservation. Currently, various physical [5], chemical [6], and biological [7] approaches have been recognized as effective

means to enhance the postharvest quality and prolong the shelf life of jujube fruits. Among these techniques, coating preservation technology is widely employed because of its exceptional effectiveness and safety [8].

Chitosan (CTS), a commonly found polysaccharide, has been widely utilized in postharvest preservation because of its film-forming, nontoxicity, and antimicrobial characteristics [9]. CTS, a naturally occurring alkaline polysaccharide with high molecular weight, is widely found in nature and can be absorbed by the body. Its application in preservation has proven effective not only for satsuma oranges [10] but also for pear [11], blueberry [12], and peach [13]. It could effectively enhance the antioxidant level and inhibit the deterioration of fruit.  $\epsilon$ -Polylysine ( $\epsilon$ -PL), derived from the fermentation of *Streptomyces albulus* in a glucose matrix [14], is a nontoxic, safe, and biodegradable compound that has attracted wide attention due to its great commercial potential [15]. According to the study of Tuersuntuoheti et al.,  $\epsilon$ -PL has been employed for preserving postharvest fruits and vegetables, such as apple, bamboo shoot, kiwi,

carrot, and citrus [14], and effectively inhibited the aging and extended the shelf life of fruits and vegetables. The application of  $\epsilon$ -PL solution and its composite solution has demonstrated a significant enhancement in the storage quality of fresh food [16], leading to a reduction in water loss. Additionally, numerous researchers have observed a synergistic effect between  $\epsilon$ -PL and various natural bacteriostatic agents [12].

Importantly, the combined treatment of CTS and  $\epsilon$ -PL has shown remarkable efficacy in preserving different types of food. Furthermore, certain studies have indicated that the covalent complex of polylysine and CTS exhibits antibacterial activity [17]. At present, Zhao et al. [18] found that a combined treatment of 2.0% CTS, 0.2% potassium sorbate, and  $0.05 \text{ g} \cdot \text{kg}^{-1}$   $\epsilon$ -PL effectively reduced the decay rate of blueberries. Additionally, this treatment slowed down the alterations in the levels of vitamin C, soluble solid content (SSC), and anthocyanins, effectively prolonged the period of freshness for blueberries. Previous studies applied CTS/ $\epsilon$ -PL coating for the preservation of white red carrots [19] and golden crown apple fruit [20], achieving positive results. Shen et al.'s research revealed that the film made from the combination of CTS and  $\epsilon$ -PL holds significant potential for preserving the taste and excellence of potatoes [21]. In a study conducted in 2022, cherries treated with a composite coating of  $\epsilon$ -polylysine hydrochloride and CTS exhibited reduced mass loss, depression, and rot rate [22].

However, empirical research exploring the impact of combining CTS with  $\epsilon$ -PL treatment on the preservation of postharvest winter jujubes is lacking. Consequently, this study is aimed at comprehensively investigating the effects of CTS combined with  $\epsilon$ -PL treatment on the postharvest quality and antioxidant-related status of winter jujube and contributing novel insights to the preservation technology of winter jujube fruits.

## 2. Materials and Methods

**2.1. Fruits and Treatments.** Following the selection process, we transported the winter jujubes (obtained from Yaoxiang Orchard, located in Linfen City, Shanxi Province, China) to the laboratory for storage at ambient temperature. Subsequently, the fruits were subjected to sterilization using a solution containing NaClO and then air-dried. The chitosan (CTS) and  $\epsilon$ -PL were obtained from Shanghai Alighting Reagent Company. A day later, the materials were soaked in different solutions for 10 min. The soaking solutions used were as follows: (a) 1% CTS+ $0.02 \text{ mg mL}^{-1}$   $\epsilon$ -PL, (b) 1% CTS+ $0.2 \text{ mg mL}^{-1}$   $\epsilon$ -PL, and (c) 1% CTS+ $2 \text{ mg mL}^{-1}$   $\epsilon$ -PL (1% chitosan was selected for the experiment according to previous experimental results of our laboratory). The control treatment involved soaking the fruits in distilled water for the equivalent duration. Each treatment involved jujubes for 7 kg. The fruits were arranged within a plastic crate and securely enclosed using cling wrap. These jujubes were stored at ambient temperature ( $23 \pm 2^\circ\text{C}$ ).

**2.2. Determination of the Quality of Winter Jujube.** Measurements of decay occurrence, red index, and weight reduction

were recorded at intervals of 2 days. The decay incidence of the winter jujube fruits was determined by measuring the lesion diameter. The result was calculated by

$$\text{Decay incidence (\%)} = \frac{\sum (\text{the level of decay} \times \text{the number of this level})}{4 \times \text{total number of fruits}} \times 100. \quad (1)$$

The fruits were categorized into four levels based on the extent of red surface area: level 0: no red surface; level 1: 0-25% of the surface turned red; level 2: 25%-50% of the surface turned red; level 3: 50%-75% of the surface turned red; and level 4: over 75% of the surface turned red. Formula (2) was used to calculate red index.

$$\text{Red index (\%)} = \frac{\sum (\text{the level of turning red} \times \text{the number of this level})}{4 \times \text{total number of fruits}} \times 100. \quad (2)$$

Quantitative jujube fruits are weighed regularly, and the loss in weight was determined using formula (3). After peeling, the hardness was measured with a digital durometer, and the SSC in the pulp juice of winter jujube was determined using a refractive instrument.

$$\text{Weight loss (\%)} = \frac{\text{lost weight}}{\text{original weight}} \times 100. \quad (3)$$

Determination of titratable acid (TA) content [23]: three fruits were randomly selected and centrifuged for 15 min after grinding. Take 20 mL of supernatant, add 2 drops of 1% indicator (phenolphthalein), and then titrate with calibrated NaOH solution ( $0.01 \text{ mol} \cdot \text{L}^{-1}$ ) until the initial pink color and not fade within half a minute. According to the formula (4), the TA content could be calculated. The soluble solid content was measured with a handheld refractometer.

$$\text{Titratable acid content (\%)} = \frac{V \times c \times (V_1 - V_0) \times f}{V_s \times m} \times 100, \quad (4)$$

where  $V$  is the total volume of sample extract,  $c$  is the concentration of the NaOH titration solution,  $V_1$  is the volume of NaOH solution consumed by the supernatant,  $V_0$  is the volume of NaOH solution consumed by distilled water,  $f$  is the conversion coefficient,  $V_s$  is the volume of liquid taken in the titration, and  $m$  is the quality of sample.

The TCA solution ( $5 \text{ mL}$ ,  $100 \text{ g} \cdot \text{L}^{-1}$ ) was evenly shaken with jujube tissue ( $1 \text{ g}$ ) and centrifuged for 20 min. An extract was mixed with TBA for boiling (20 min) and centrifuged again after cooling. The MDA content was determined using

$$\text{MDA content } (\mu\text{mol} \cdot \text{g}^{-1}) = \frac{c \times V}{V_s \times m} \times 100, \quad (5)$$

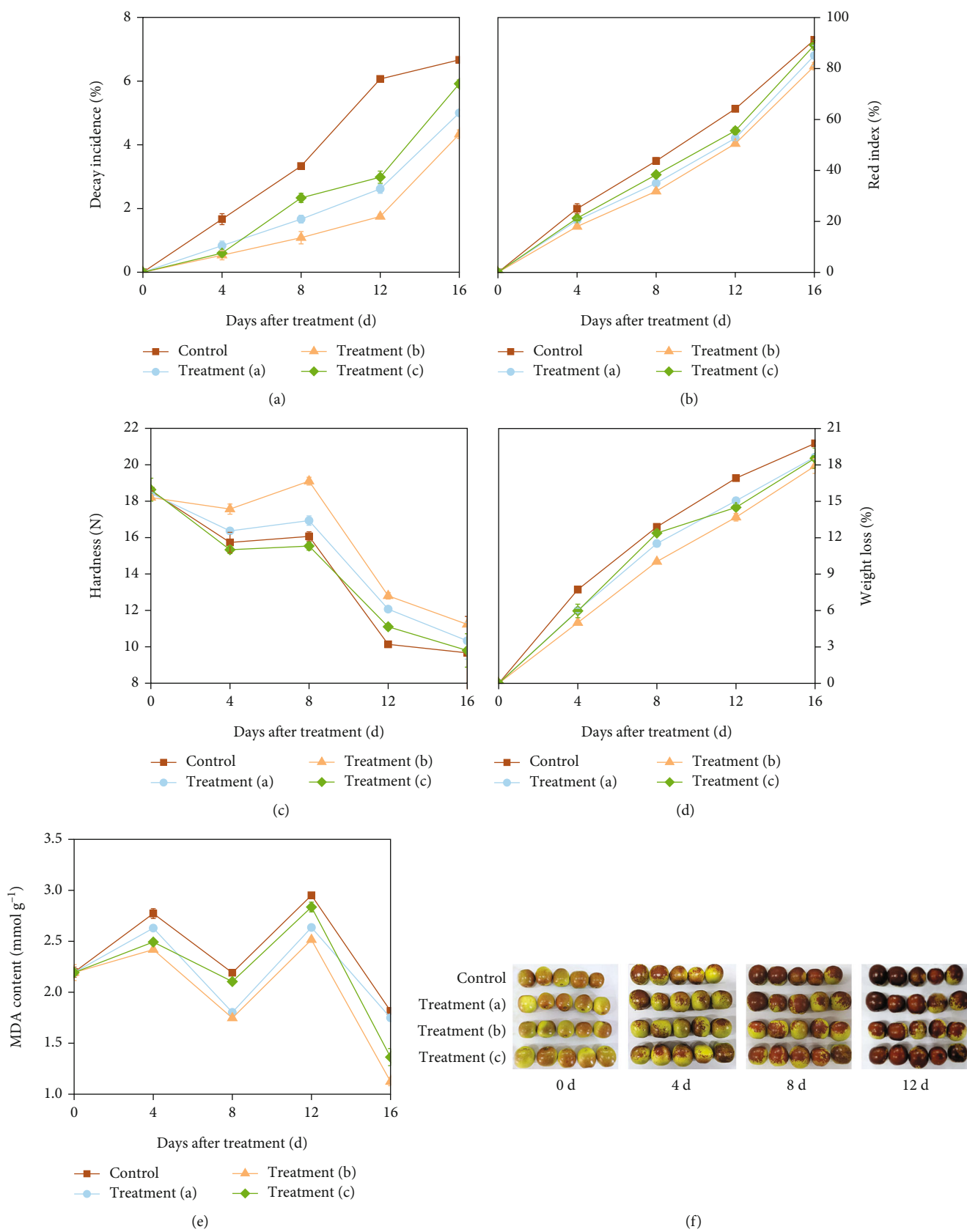


FIGURE 1: Effects of CTS combined with  $\epsilon$ -PL treatment on (a) decay incidence, (b) red index, (c) hardness, (d) weight loss, (e) MDA content, and (f) appearance status of winter jujube. Treatments (a), (b), and (c) express 1% CTS+0.02 mg mL<sup>-1</sup> $\epsilon$ -PL, 1% CTS+0.2 mg mL<sup>-1</sup> $\epsilon$ -PL, and 1% CTS+2 mg mL<sup>-1</sup> $\epsilon$ -PL, respectively. Error bars represent the standard deviations of the three measured values.

TABLE 1: Effects of CTS combined with  $\epsilon$ -PL treatment on TA content.

Treatment	Days after treatment (d)				
	0	4	8	12	16
Control	0.11 $\pm$ 0.00 <sup>a</sup>	0.13 $\pm$ 0.00 <sup>b</sup>	0.30 $\pm$ 0.00 <sup>a</sup>	0.25 $\pm$ 0.00 <sup>b</sup>	0.24 $\pm$ 0.00 <sup>b</sup>
1% CTS+0.02 mg mL <sup>-1</sup> $\epsilon$ -PL	0.13 $\pm$ 0.00 <sup>a</sup>	0.13 $\pm$ 0.00 <sup>b</sup>	0.29 $\pm$ 0.00 <sup>a</sup>	0.31 $\pm$ 0.00 <sup>a</sup>	0.28 $\pm$ 0.00 <sup>a</sup>
1% CTS+0.2 mg mL <sup>-1</sup> $\epsilon$ -PL	0.13 $\pm$ 0.00 <sup>a</sup>	0.19 $\pm$ 0.06 <sup>a</sup>	0.31 $\pm$ 0.00 <sup>a</sup>	0.35 $\pm$ 0.00 <sup>a</sup>	0.31 $\pm$ 0.00 <sup>a</sup>
1% CTS+2 mg mL <sup>-1</sup> $\epsilon$ -PL	0.11 $\pm$ 0.02 <sup>a</sup>	0.18 $\pm$ 0.00 <sup>a</sup>	0.30 $\pm$ 0.04 <sup>a</sup>	0.25 $\pm$ 0.00 <sup>b</sup>	0.21 $\pm$ 0.04 <sup>c</sup>

\*Values are the mean  $\pm$  standard deviation (SD) of three replicates. The letters in the same column indicate the level of significant differences ( $P < 0.05$ ).

TABLE 2: Effects of CTS combined with  $\epsilon$ -PL treatment on SSC (soluble solid content).

Treatment	Days after treatment (d)				
	0	4	8	12	16
Control	13.67 $\pm$ 0.51 <sup>a</sup>	15.67 $\pm$ 1.02 <sup>a</sup>	16.67 $\pm$ 1.08 <sup>a</sup>	15.67 $\pm$ 0.89 <sup>a</sup>	15.33 $\pm$ 1.16 <sup>a</sup>
1% CTS+0.02 mg mL <sup>-1</sup> $\epsilon$ -PL	15.33 $\pm$ 0.58 <sup>a</sup>	9.67 $\pm$ 1.15 <sup>a</sup>	15.33 $\pm$ 0.58 <sup>a</sup>	14.67 $\pm$ 1.21 <sup>a</sup>	14.33 $\pm$ 2.08 <sup>a</sup>
1% CTS+0.2 mg mL <sup>-1</sup> $\epsilon$ -PL	14.33 $\pm$ 0.79 <sup>a</sup>	10.33 $\pm$ 0.58 <sup>a</sup>	15.33 $\pm$ 0.89 <sup>a</sup>	13.00 $\pm$ 0.65 <sup>a</sup>	13.67 $\pm$ 1.52 <sup>a</sup>
1% CTS+2 mg mL <sup>-1</sup> $\epsilon$ -PL	15.33 $\pm$ 0.79 <sup>a</sup>	11.00 $\pm$ 1.73 <sup>a</sup>	13.33 $\pm$ 1.06 <sup>a</sup>	15.33 $\pm$ 1.15 <sup>a</sup>	13.67 $\pm$ 1.15 <sup>a</sup>

\*Values are the mean  $\pm$  standard deviation (SD) of three replicates. The letters in the same column indicate the level of significant differences ( $P > 0.05$ ).

where  $V$  is the total volume of sample extract,  $c$  is the concentration of malondialdehyde,  $V_S$  is the volume of liquid taken in the titration, and  $m$  is the quality of sample.

**2.3. Determination of Antioxidant Status.** After freezing, the fruits were stored at the ultracold storage freezer ( $-80^\circ\text{C}$ ).

An extraction buffer was used to blend the frozen tissue (4 g).  $\text{O}_2^-$  production rate was measured through supernatant. Data were expressed as  $\text{nmol min}^{-1} \text{g}^{-1}$ . Precooled acetone was used to extract  $\text{H}_2\text{O}_2$ . 1 mL sample was combined with a solution of peptide sulfate and hydrochloric acid (0.1 mL) before centrifuging. The precipitate was dissolved using sulfuric acid (3 mL). The measurements were reported in  $\mu\text{mol g}^{-1}$ .

Frozen tissue (2.0 g) was homogenized with sodium phosphate buffer (2 mL) for SOD and CAT and 2 mL of potassium phosphate buffer for APX. The resulting mixture was centrifuged at  $12,000 \times g$  for 30 min. Chen et al. [24] described the method used to determine the activities of SOD, CAT, and APX. The reaction system for CAT included a solution of hydrogen peroxide and crude enzyme. APX crude enzyme (0.1 mL) was incubated with potassium phosphate buffer. The response was triggered by the introduction of  $\text{H}_2\text{O}_2$  solution. The expression of SOD, CAT, and APX activities was measured as  $\text{U min}^{-1} \text{g}^{-1}$ . POD was extracted using an acetate-sodium acetate buffer. The reaction system included guaiacol solution and enzyme extraction. Data were represented as  $\text{U min}^{-1} \text{g}^{-1}$ .

In order to determine the AsA content, TCA (4 mL) was used to homogenize the frozen tissue. The reaction system involved 1 mL enzyme solution, TCA, and anhydrous ethanol, respectively, and phospho-ethanol (0.5 mL), BP-ethanol (1 mL), and ferric chloride-ethanol (0.5 mL). Data were represented as  $\mu\text{g g}^{-1}$ .

For GSH determination, frozen tissue (5 g) was evenly homogenized with precooled extraction. The supernatant (1 mL), phosphoric acid buffer (1 mL), and DTNB (0.5 mL)

were used to participate the reaction system. Data were expressed as  $\mu\text{mol g}^{-1}$ .

**2.4. Determination of the Activity of PAL, Flavonoid, and Phenolic Contents.** The determination of PAL activity was following a previously method described by Zhang et al. [25]. The means of centrifugation refer to SOD. The reaction included a mixture of 3 mL boric acid buffer and 0.5 mL phenylalanine solution. And then preserve the mixture at  $37^\circ\text{C}$  for 10 min. Ultimately, the response was concluded by adding HCl (0.1 mL). Data were represented as  $\text{U h}^{-1} \text{g}^{-1}$ .

The flavonoids and phenolics were extracted by 80% ethanol (12 mL). The methods described by Guo et al. [26] and Li et al. [27] were employed to determine the flavonoid and phenolic contents. Data were expressed as  $\text{mg g}^{-1}$ .

**2.5. Statistical Analysis.** The experiments were repeated three times. SPSS was used for data analysis and Duncan's test. Diagrams were drawn by Origin 2022.

### 3. Results

**3.1. The Effect of Combined Treatments on the Quality of Winter Jujube.** To comprehensively evaluate the quality of winter jujube in different treatments ((a) 1% CTS + 0.02 mg mL<sup>-1</sup> $\epsilon$ -PL, (b) 1% CTS + 0.2 mg mL<sup>-1</sup> $\epsilon$ -PL, and (c) 1% CTS + 2 mg mL<sup>-1</sup> $\epsilon$ -PL), the experiment determined decay incidence, red index, hardness, weight loss, and other indicators that can characterize the quality of winter jujube. Throughout the storage period, there was a general upward trend observed in decay incidence (Figure 1(a)), red index (Figure 1(b)), and weight loss (Figure 1(d)). However, the hardness of the winter jujubes gradually decreased (Figure 1(c)). At the end of storage, the red index of fruit in the control group was significantly higher than that in the other treatment groups (Figure 1(f)).

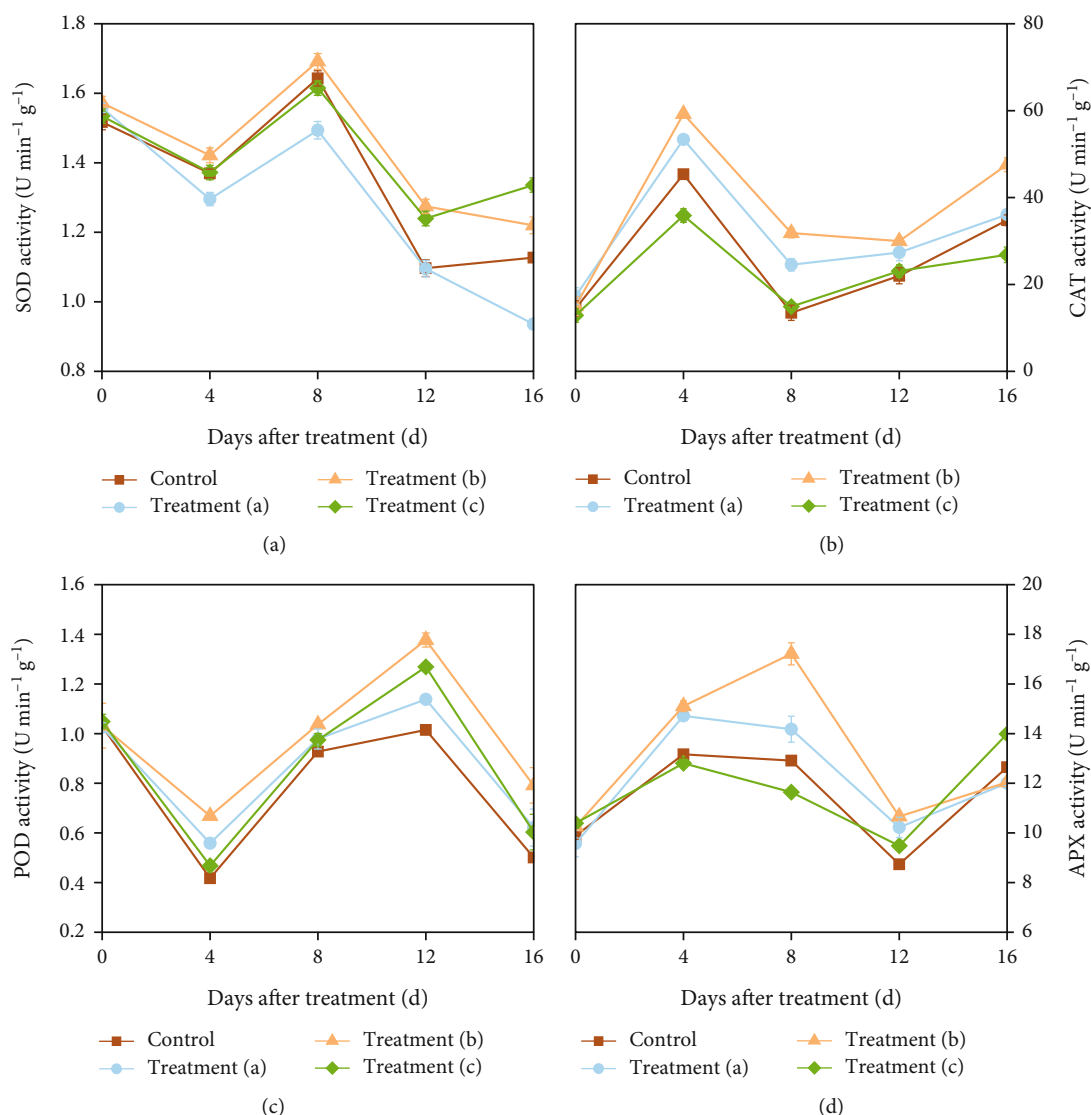


FIGURE 2: Effects of CTS combined with  $\epsilon$ -PL treatment on antioxidant status: (a) SOD, (b) CAT, (c) POD, and (d) APX. Treatments (a), (b), and (c) express 1% CTS+0.02  $\text{mg mL}^{-1}$   $\epsilon$ -PL, 1% CTS+0.2  $\text{mg mL}^{-1}$   $\epsilon$ -PL, and 1% CTS+2  $\text{mg mL}^{-1}$   $\epsilon$ -PL, respectively. Error bars represent the standard deviations of the three measured values.

During the whole stage of storage, the control exhibited a higher decay incidence than the treatment groups. On the 8th day, the decay incidence of the control group was 2.06, 3.05, and 1.43 times higher than that of the treatment groups, respectively. On day 12, the decay incidence of the treatment groups was 56.9%, 71.1%, and 50.8% lower in comparison to the control group. At the last day, the decay incidence of the control group was 1.53 times higher than that of treatment (b) (Figure 1(a)). The red index (Figure 1(b)) of both control group and treatment groups exceeded 80% on the 16th day of storage, with values of 91.2%, 85.1%, 80.9%, and 89.2%, respectively. In addition, the red index of treatment (b) was the lowest throughout the experimental period. As shown in Figure 1(c), the peak of hardness during storage occurred on day 8, with values of 2 N, 3 N, 5 N, and 5 N for the control group and treatment groups, respectively. Moreover, on day 16, the control group

and treatment groups exhibited decreases in hardness of 48.1%, 44%, 38.5%, and 47.3%, respectively, compared to the initial day. On the 12th day, the control group and treatment groups achieved weight loss percentages of 16.9%, 15.1%, 13.7%, and 14.5%, respectively. On the 16th day, minimal differences were observed among the different groups, with treatment (b) showing the lowest weight loss at 17.9% (Figure 1(d)).

Table 1 reveals distinct variations in TA content among the different groups on the 8th day, with treatment (b) displaying significantly higher TA content compared to other groups by the end of storage. According to Table 2, the SSC content remained relatively stable throughout the storage period, and there were no significant differences observed within each group. These results indicated that different concentrations of CTS and  $\epsilon$ -PL had little effect on SSC content in jujube fruits.



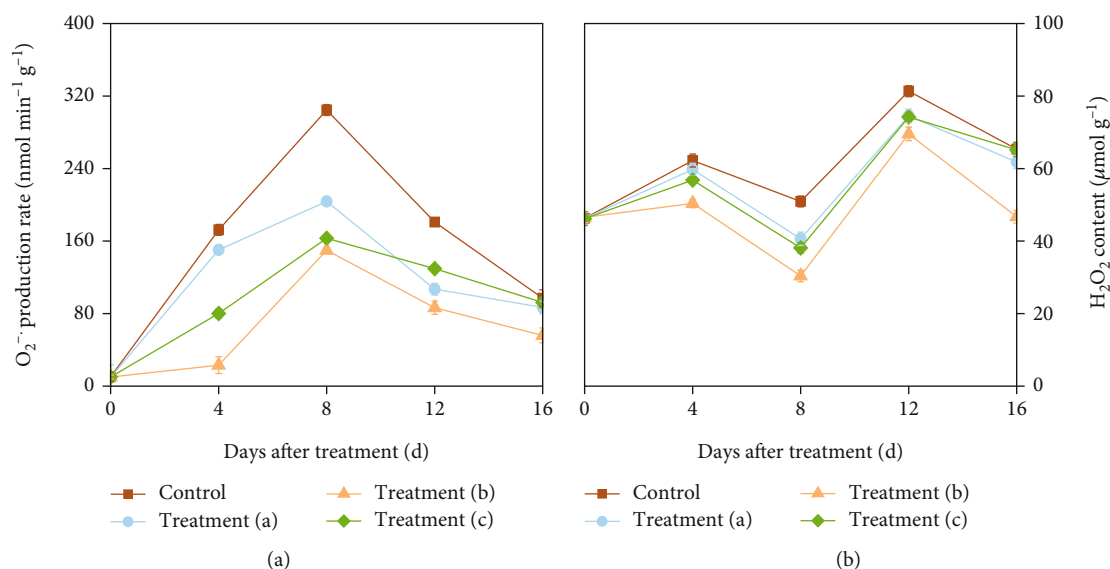


FIGURE 3: Effects of CTS combined with  $\epsilon$ -PL treatment on production rate of (a)  $O_2^-$  and (b)  $H_2O_2$  content. Treatments (a), (b), and (c) express 1% CTS+0.02 mg mL $^{-1}$  $\epsilon$ -PL, 1% CTS+0.2 mg mL $^{-1}$  $\epsilon$ -PL, and 1% CTS+2 mg mL $^{-1}$  $\epsilon$ -PL, respectively. Error bars represent the standard deviations of the three measured values.

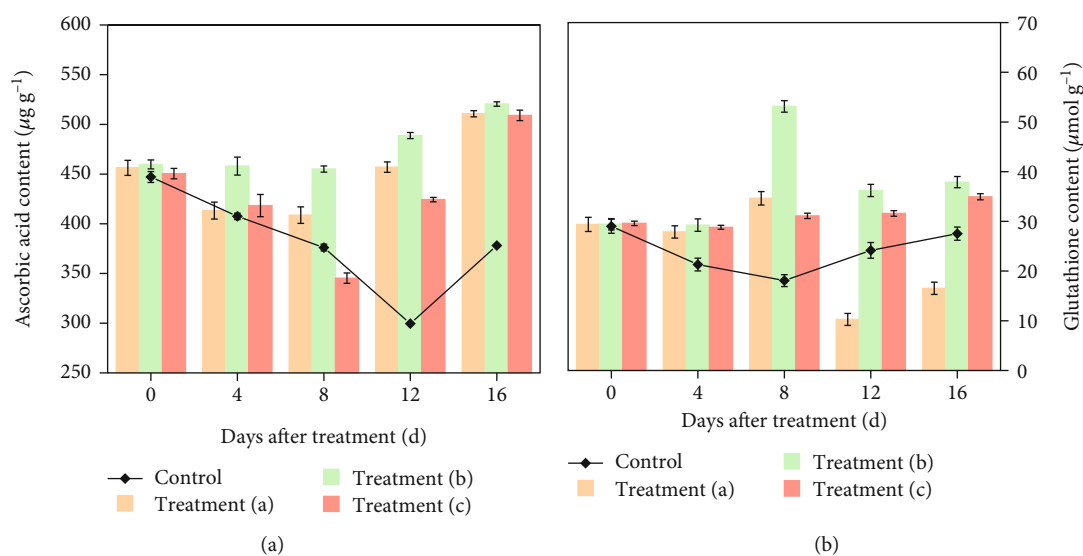


FIGURE 4: Effects of CTS combined with  $\epsilon$ -PL treatment on contents of (a) ascorbic acid and (b) glutathione. Treatments (a), (b), and (c) express 1% CTS+0.02 mg mL $^{-1}$  $\epsilon$ -PL, 1% CTS+0.2 mg mL $^{-1}$  $\epsilon$ -PL, and 1% CTS+2 mg mL $^{-1}$  $\epsilon$ -PL, respectively. Error bars represent the standard deviations of the three measured values.

According to the results, MDA content presented a cyclical trend of initial increase followed by decrease, and two peaks of MDA content occurred during storage (Figure 1(e)). On the 4th day, MDA contents in the control group and treatment groups were 2.77, 2.62, 2.41, and 2.49 mmol g $^{-1}$ , respectively. The control group displayed a MDA content that was 1.15 times higher than the content in treatment (b). On the 12th day, MDA contents in the control group and treatment groups were 2.95, 2.63, 2.51, and 2.83 mmol g $^{-1}$ , respectively. At the last day, MDA content rapidly decreased, which was lower than that on the first day.

3.2. *The Effect of Combined Treatments on the Antioxidant Status of Winter Jujube.* The activities of APX and CAT demonstrated a pattern of initial increase, subsequent decrease, and finally increase, whereas the activities of SOD and POD exhibited an initial decrease, followed by an increase and then a decrease (Figure 2). Noteworthy differences in CAT and APX activities were observed on day 4 and day 8, respectively. On the 4th day, the CAT activities in the treatment groups were measured 53.38, 59.23, and 35.86 U min $^{-1}$  g $^{-1}$ , which were 1.18, 1.31, and 0.79 times than that in the control group, respectively. Moreover, on the

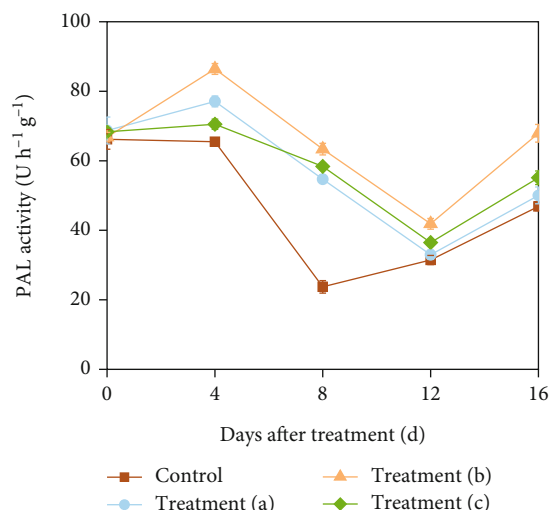


FIGURE 5: Effects of CTS combined with  $\epsilon$ -PL treatment on PAL activity. Treatments (a), (b), and (c) express 1% CTS+0.02 mg mL<sup>-1</sup>  $\epsilon$ -PL, 1% CTS+0.2 mg mL<sup>-1</sup>  $\epsilon$ -PL, and 1% CTS+2 mg mL<sup>-1</sup>  $\epsilon$ -PL, respectively. Error bars represent the standard deviations of the three measured values.

16th day, the CAT activity of the (b) treatment group exhibited a noteworthy rise compared to the control and other treatment groups, with increments of 26.9%, 24.15%, and 43.6%, respectively. On day 8, the activities of APX in the control and treatment groups were 12.91, 14.17, 17.21, and 11.64 U min<sup>-1</sup> g<sup>-1</sup>, respectively. Treatments (a) and (b) exhibited higher APX activities than the control group, whereas only treatment (c) displayed lower APX activity than the control group.

The O<sub>2</sub><sup>-</sup> production rate indicated a pattern of initial growth followed by decline, and the peaks of the control and treatment groups appeared on the 8th day of storage, which were 304.58, 203.77, 149.87, and 163.05 nmol·min<sup>-1</sup>·g<sup>-1</sup>, respectively (Figure 3(a)). Notably, the (b) treatment group was always maintained the lowest level. H<sub>2</sub>O<sub>2</sub> content peaked on day 4 and day 12, and significant differences in H<sub>2</sub>O<sub>2</sub> content were apparent among all groups on the 8th day. Specifically, H<sub>2</sub>O<sub>2</sub> contents in the control group and treatment groups measured 50.92, 40.63, 30.38, and 38.11  $\mu$ mol·g<sup>-1</sup>, respectively. The findings indicated that the control group exhibited H<sub>2</sub>O<sub>2</sub> content that were 20.2%, 40.3%, and 25.2% higher than those observed in the treatment groups (Figure 3(b)).

As shown in Figure 4, the treatment groups exhibited higher levels of AsA and GSH compared to the control group. At the last day, the treatment groups shown 35.0%, 37.6%, and 34.6% increase in AsA contents in comparison to the control group (Figure 4(a)). In addition, the control group displayed a decline followed by an increase in GSH content, whereas the treatment groups demonstrated the opposite pattern (Figure 4(b)). The GSH content in the treatment group (b) reached a peak (53.12  $\mu$ mol·g<sup>-1</sup>) on day 8.

**3.3. The Effect of Different Treatments on PAL, Flavonoids, and Phenolics of Winter Jujube.** The treatment (b) exhibited

the highest PAL activity during storage. On 8th day, PAL activities were recorded as 23.72, 54.71, 63.36, and 58.38 U·h<sup>-1</sup>·g<sup>-1</sup> for the control group and treatment groups, respectively (Figure 5). On day 16, PAL activity in the (b) treatment group was 1.45, 1.35, and 1.23 times higher than that in the control group and other treatment groups.

During the initial 12 days of storage, the flavonoid content of the (b) treatment group was the highest. On days 4, 6, and 8 of storage, the flavonoid contents in the (b) treatment group were 1.37, 1.23, and 1.06 times higher than that in the control group, respectively (Figure 6(a)). Phenolic content gradually increased from day 0 to day 16. At the end of storage, the phenolic contents in the control group and treatment groups were 5.94, 5.99, 6.35, and 6.18 mg·g<sup>-1</sup>, respectively, which were 1.26, 1.20, 1.31, and 1.27 times higher than that in the early storage period (Figure 6(b)).

**3.4. Correlation Analysis.** Positive correlation showed in red, and negative correlation showed in blue. As shown in Figure 7(a), several significant relationships were displayed. The incidence of postharvest winter jujube fruit was positively correlated with the levels of H<sub>2</sub>O<sub>2</sub>, and the correlation coefficient is 0.53. Conversely, it exhibited a negatively relationship with the activities of SOD, POD, and PAL, and the correlation coefficients are -0.64, -0.24, and -0.58, respectively. Distinct correlations were observed among the various antioxidant enzymes. Specifically, CAT and APX displayed a positive correlation (0.56), while POD exhibited a negative correlation with both APX (-0.50) and CAT (-0.60). Furthermore, a noteworthy association was observed between the levels of flavonoids and phenolics in fruits. Additionally, we also found a negative correlation between the levels of antioxidant substances (AsA and GSH) and the contents of reactive oxygen species. In the meantime, a certain negative correlation surfaced between the activities of disease-resistant enzymes and the antioxidant status. For example, a higher PAL activity corresponded to a lower level of reactive oxygen species.

## 4. Discussion

**4.1. The Combined Treatments Enhanced the Quality of Winter Jujube.** In China, winter jujube is highly valued due to its widespread consumption as a popular fruit. However, the winter jujube during storage and transportation may be susceptible to deterioration due to the characteristics of delicate skin and postharvest vulnerability. The quest for effective and secure preservation technique of winter jujube has consistently been a subject of great interest among scientists. During storage, a series of physiological and biochemical reactions transpired within winter jujube tissues, which may result in a decline in fruit quality and an escalation in decay incidence, thus significantly impacting the economic worth of the fruit. The decay incidence serves as a direct indicator of this transformation. After the combined treatment of CTS and  $\epsilon$ -PL, the decay incidence (Figure 1(a)) was reduced by nearly a half comparing to the control. In accordance with prior study, the utilization of chitosan/enoki mushroom foot polysaccharide in the preservation of

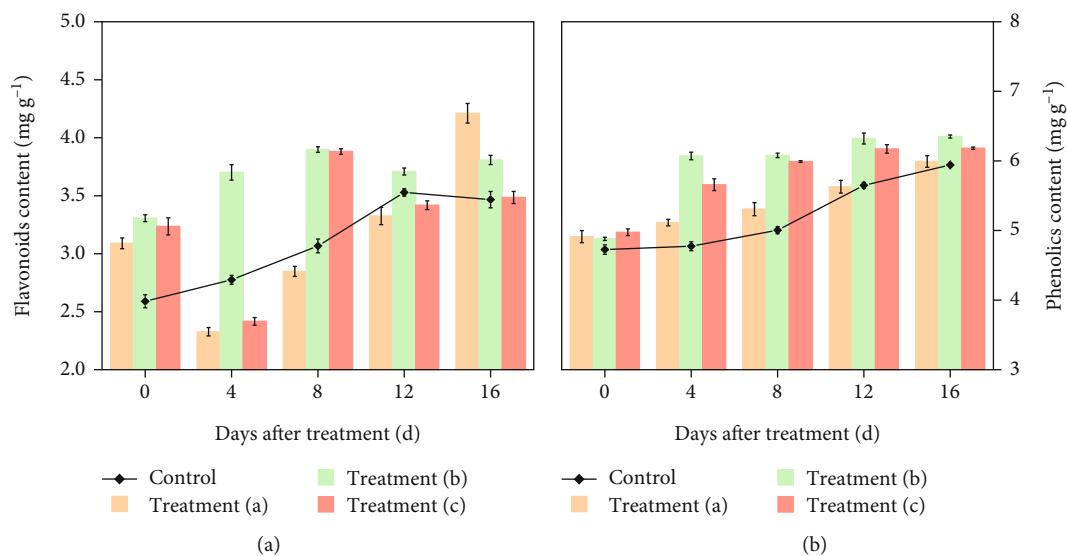


FIGURE 6: Effects of CTS combined with  $\epsilon$ -PL treatment on contents of (a) flavonoids and (b) phenolics. Treatments (a), (b), and (c) express 1% CTS+0.02 mg mL<sup>-1</sup> $\epsilon$ -PL, 1% CTS+0.2 mg mL<sup>-1</sup> $\epsilon$ -PL, and 1% CTS+2 mg mL<sup>-1</sup> $\epsilon$ -PL, respectively. Error bars represent the standard deviations of the three measured values.

blueberries resulted in a notable reduction in decay incidence [12], which is similar to our results. The determination results of the red index (Figure 1(b)), hardness (Figure 1(c)), and weight loss (Figure 1(d)) demonstrated that combined treatment exhibited a lesser degree of alteration compared to the control group. The observation verified that the respiration and fruit mass loss were significantly slowed down after treatment, aligning with the conclusions of Liu et al. [23]. SSC and TA contents are indispensable indexes in evaluating the flavor and taste of fruits. Our results showed that there was little difference in SSC contents (Table 2), while 1% CTS+0.2 mg mL<sup>-1</sup> $\epsilon$ -PL composite treatment exhibited the highest TA content (Table 1). In the study on winter jujube, the application of methionine treatment successfully preserved the TA content [8], which was consistent with our results. MDA content serves as a representative indicator for fruit senescence and lipid peroxidation in cell membrane. Our results showed the significant reduction in MDA content within the treatment groups when contrasted with the control group. The results of similar experiments demonstrated that the coated jujube fruit exhibited the lowest MDA content, thereby impeding peroxidation within cells [28].

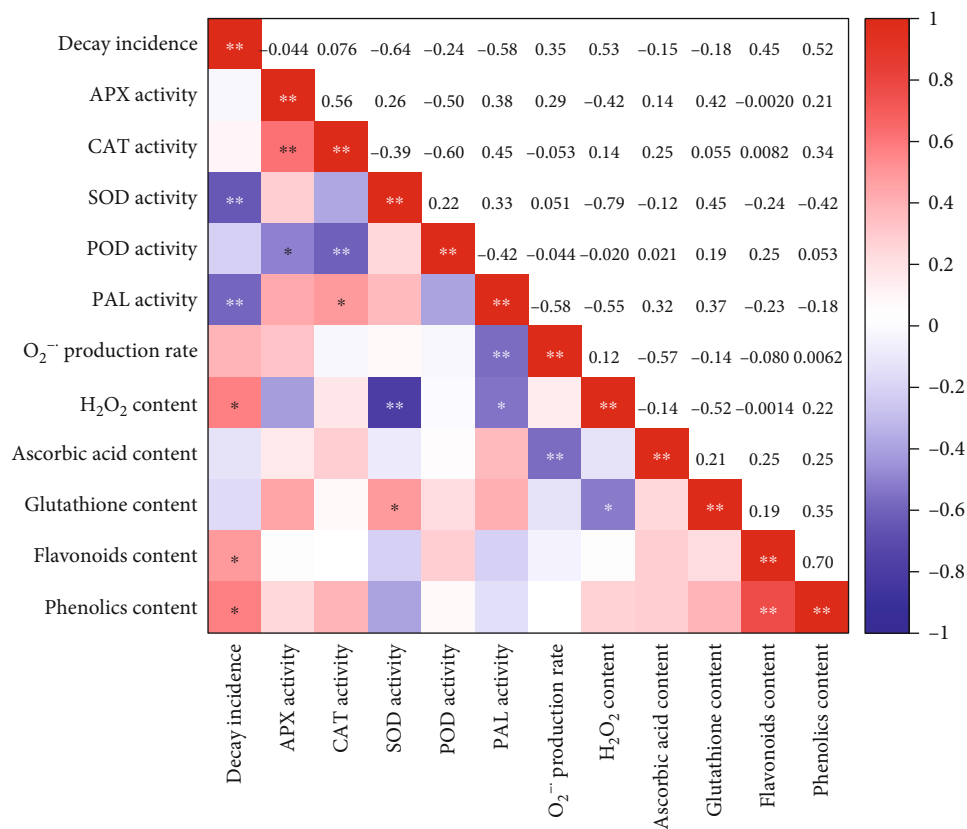
**4.2. The Combined Treatments Regulated the Antioxidant Status of Winter Jujube.** Maintaining the quality of postharvest fruit requires the preservation of the relative equilibrium of reactive oxygen species status, as an elevated amount of reactive oxygen species is usually linked to cellular senescence [24]. Notably, H<sub>2</sub>O<sub>2</sub> and O<sub>2</sub><sup>-</sup> are the predominant reactive oxygen species observed during the storage of postharvest fruits [29]. SOD, CAT, POD, and APX are effective in regulating the generation and elimination of reactive oxygen species within plant cells. SOD can promote the decomposition of O<sub>2</sub><sup>-</sup>, APX decomposes H<sub>2</sub>O<sub>2</sub> through AsA-GSH cycle, and CAT and POD also contribute to the decomposition of H<sub>2</sub>O<sub>2</sub> into H<sub>2</sub>O and O<sub>2</sub> [30]. The 1%

CTS+0.2 mg mL<sup>-1</sup> $\epsilon$ -PL treatment exhibited the most significant effect and regulate the aging of fruit and contributed to restore the equilibrium of intracellular reactive oxygen status. Zhang et al. [31] found that controlling the antioxidant metabolism of winter jujube is beneficial to prevent postharvest softening. In addition, Zhang et al. [25] found that boosting the performance of antioxidant enzymes has a positive impact on improving the postharvest value of sweet cherries.

Ascorbic acid and glutathione are nonenzymatic antioxidants [30], which play a crucial effect in the removing of ROS under room temperature. The AsA-GSH cycle is responsible for maintaining cellular redox homeostasis and regulating oxidative stress-induced cell damage by eliminating ROS. Recent research [30] demonstrated that exogenous GSH treatment effectively alleviated the postharvest chilling damage of sweet pepper and improved the antioxidant capacity of strawberry. For this study, the AsA and GSH contents in the treatment groups were higher compared to the control group, and 1% CTS+0.2 mg mL<sup>-1</sup> $\epsilon$ -PL treatment maintained the highest level, which was consistent with the high activities of antioxidant enzymes, showing that this treatment inhibited the oxidation in the pulp cells of winter jujube and slowed down the quality reduction caused by physiological activities of fruits.

**4.3. The Combined Treatments Regulated the PAL Activity and the Contents of Flavonoids and Phenolics of Winter Jujube.** In addition, PAL is a key enzyme of phenylpropane metabolism, while flavonoids and phenolics are essential secondary compounds. Previous studies have reported that PAL and the plant secondary metabolites (such as flavonoids and phenolics) have been identified as important makers to reflect the antioxidant activity of plant tissue [32]. A scientific evidence indicated that the increase in metabolic enzyme activities (PAL, C4H, and 4CL) within the





\*  $p < 0.05$  \*\*  $p < 0.01$

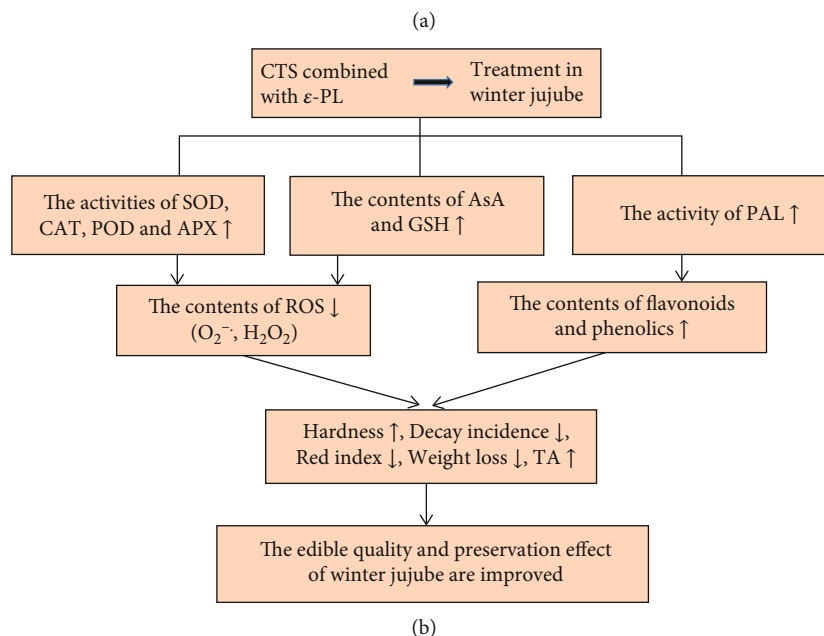


FIGURE 7: Correlation analysis of quality and antioxidant status of winter jujube (a). Preservation mechanism of CTS combined with ε-PL treatment on winter jujube (b).

phenylpropane pathway is helpful in increasing the phenolic compounds and reducing the decay incidence of fruits [25]. The findings of our study explained that the combination of CTS and ε-PL treatment improved PAL activity and accelerated the accumulation of flavonoids and phenolics; in addition,

the previous researchers have also verified the roles of PAL and secondary metabolites (flavonoids and phenolics) in irradiation treatment [32, 33]. Hence, there is sufficient evidence to explain that these changes effectively inhibited the deterioration of winter jujube fruit.

## 5. Conclusion

According to the results, the treatment of CTS combined with  $\epsilon$ -PL is a beneficial strategy for controlling the postharvest quality of winter jujube. Results showed that the composite coating of CTS and  $\epsilon$ -PL enhanced the activities of antioxidant enzymes (SOD, CAT, POD, and APX) and the levels of antioxidant compounds (AsA and GSH) inhibited the accumulation of reactive oxygen species ( $O_2^{\cdot-}$  and  $H_2O_2$ ) and then significantly enhanced the activity of PAL and resistant substance contents (flavonoids and phenolics). CTS combined with  $\epsilon$ -PL treatment observably preserved the hardness and TA content of winter jujube, concurrently slowed down the rise of decay incidence, red index, and weight loss of winter jujube (Figure 7(b)). As mentioned above, CTS (1%) combined with  $\epsilon$ -PL ( $0.2\text{ mg mL}^{-1}$ ) treatment not only mitigates the extent of fruit decay but also maintains the flavor and texture quality of winter jujube fruit. Therefore, the composite coating of CTS and  $\epsilon$ -PL has great potential in postharvest jujube preservation.

## Data Availability

The figure and table data used to support the results of this study are included in the data file.

## Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

## Authors' Contributions

Lulu Chang performed the experiments, analyzed the data, wrote the manuscript, and edited the manuscript. Shaoying Zhang revised the manuscript.

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## Supplementary Materials

The experimental data are recorded in the data file. (*Supplementary Materials*)

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