

## Research Article

# Forced Air Precooling Enhanced Storage Quality by Activating the Antioxidant System of Mango Fruits

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Received 23 November 2018; Accepted 11 February 2019; Published 4 March 2019

Academic Editor: Antoni Szumny

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Effects of forced air precooling on storage quality and physiological metabolism of mangoes were evaluated in this study. Mango fruits were forced air precooled for 30 min at 0°C and then stored at 13°C. Control fruits were stored at 13°C directly. Results showed that forced air precooling treatment maintained fruit firmness, inhibited fruit peel coloration, retarded hydrolysis of polysaccharide to soluble sugar, and decreased fruit decay during storage. Biochemical studies revealed that precooling treatment could eliminate reactive oxygen species (ROS) effects by enhancing related antioxidant enzyme activities, such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), glutathione reductase (GR), and polyphenoloxidase (PPO). They all contributed to the delay of mango fruit ripening and senescence in storage. These results indicate that forced air precooling treatment could maintain mango fruit quality by enhancing antioxidant activity and delaying fruit ripening.

## 1. Introduction

Mango is a typical climacteric fruit that is characterized by increased ethylene production and a rise in respiration during fruit ripening [1]. To reduce postharvest losses, mango fruits are always harvested at the physiologically mature green stage and allowed to ripen after harvest. Then, mango fruits not only have the best quality for consumption but also are susceptible to pathogen invasion and begin to senesce. Postharvest respiration and ripening can constantly decompose fruit's nutritional compounds, resulting in high biochemical metabolism, fruit softening, and quality deterioration, and it is closely related to storage temperature [1–3]. Meanwhile, the temperature of mango fruits increased rapidly after harvest, due to both field heat and respiration heat. Therefore, it is important to cool down mango fruits immediately after harvest to delay fruit ripening after harvest.

Postharvest precooling is a common process to decrease the fruit temperature rapidly to the expected core

temperature. It not only slows down fruit respiration, water loss, and metabolism but also inhibits nutrition loss and pathogen development during subsequent storage [4]. Precooling has made a great significance, such as removing field heat, maintaining postharvest quality, and prolonging shelf-life, in cold-chain transportation for many fruits and vegetables [5, 6]. Now, precooling is regarded as an indispensable first step by many developed countries like Europe and Japan. Among various industrial postharvest precooling techniques, forced air precooling is widely accepted as an effective method to maintain postharvest quality and prolong shelf-life for many fruits like apple and plum [7–9]. Forced air cooling is much faster than other conventional cooling methods because the cool air comes in direct contact with the surfaces of the horticultural product [10].

During the late ripening stages, fruit senescence was accompanied by membrane deterioration, programmed cell death, and some other associated biochemical changes [11].

Excessive production of reactive oxygen species (ROS), such as  $O_2^{\cdot-}$  and  $H_2O_2$ , can cause progressive oxidative damage and ultimately cell death, accelerating fruit senescence [12]. Fruits also produce a natural defense system scavenging ROS, enhancing membrane stability and protecting fruits from senescence deterioration. The antioxidant enzyme system includes superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR). Mango fruits also include flavonoids which could eliminate  $O_2^{\cdot-}$  and inhibit senescence of plants [13].

So far, there have been fewer reports on the changes in quality and physiological metabolism of mango fruits treated with forced air precooling. In this study, the effect of forced air precooling on postharvest qualities of mango fruits during the storage period was investigated. The changes of reactive oxygen species and eliminate system were also discussed.

## 2. Materials and Methods

Tainong mango fruits (*Mangifera indica* L. cv. Tainong) were harvested from a nearby orchard and transported to the laboratory immediately. The maturity of the fruit was indicted by mature green. Fruits that were uniform in size, color, shape, and free of mechanical damage and pathogen infection were selected for experiments. Seventy-two fruits were placed in a 45 cm × 30 cm × 30 cm plastic box with 20% whole area. Each box was placed in a tube which contained an exhaust fan. Fruits were forced air precooled with 1 m/s 0°C air for 30 min until the central temperature reached 13°C and then stored at 13°C and 85%~95% relative humidity (RH). The control fruits were directly stored at 13°C and 85%~95% RH. The experiment contained two hundred and seventy fruits. Fruit quality was evaluated, and fruit flesh and peel were sampled on the 0<sup>th</sup>, 5<sup>th</sup>, 10<sup>th</sup>, 15<sup>th</sup>, and 20<sup>th</sup> day of storage.

Polyvinylpyrrolidone (PVPP), trichloroacetic acid (TCA), ethylene diamine tetraacetic acid (EDTA), nitroblue tetrazolium (NBT), polyethylene glycol (PEG), phosphate, acetate, perchloric acid, methyl alcohol, guaiacol, NaOH, HCl,  $KNO_3$ ,  $Na_2CO_3$ , Triton X-100, and  $H_2O_2$  were purchased from Sigma-Aldrich.

### 2.1. External Quality Evaluation

**2.1.1. Weight Loss.** Weight loss was determined by weighing fruit at harvest (initial weight) and every evaluation time and expressed as percentage loss of the initial weight.

**2.1.2. Firmness.** Firmness was measured by using a Bosch penetrometer (Model FT 327) at two opposite points on the equatorial region of mango fruits.

**2.1.3. Color.** The yellow index of fruit peel was visually evaluated and scored on a 0 to 4 scale from green to yellow. The yellow index was calculated using the following formula:

$$\text{yellow index (\%)} = \frac{1 \times n_1 + 2 \times n_2 + 3 \times n_3 + 4 \times n_4}{4 \times n} \times 100\%, \quad (1)$$

where  $n_1$  to  $n_4$  represent the number of fruits in different scores 1 to 4, respectively, and  $n$  represents the total number of fruit.

Fruit peel color was measured by using a chromameter (ADCI-60C, China) at two opposite points on the equatorial region of mango fruits and recorded “ $L^*$ ,” “ $a^*$ ,” and “ $b^*$ ” value.

**2.1.4. Pigment Contents.** For determination of chlorophyll a and chlorophyll b contents, 4 ml of 90% acetone was added into 1.0 g of mango flesh (0.5 g peel) and then centrifuged at 10,000 × g for 20 min at 4°C. Absorbance of the supernatant was measured at 663 nm and 645 nm.

For all external quality evaluation, there were three replicates in each treatment with one replicate containing ten fruits.

### 2.2. Internal Quality Evaluation

**2.2.1. Total Soluble Solid Content.** Total soluble solids (TSSs) content was expressed as % using a digital refractometer (Atago PAL-1, Japan).

**2.2.2. Titratable Acidity Content.** Titratable acidity (TA) content was titrated by NaOH.

**2.2.3. Soluble Sugar Content.** For soluble sugar content determination, 3 g of fruit flesh was mixed with 6.0 ml of distilled water. The mixture was heated in a boiling water bath for 20 min and then centrifuged at 10,000 × g for 15 min. Finally, the abstained supernatant was used for soluble sugar analysis. Soluble solid content (SSC) was measured by using an Atago digital refractometer (PAL-1, Japan).

**2.2.4. Starch Content.** According to Elloumi et al. [14], the phenol-sulfuric acid method was used to determine the starch content. 1.0 g fruit flesh was added into 5.0 ml of distilled water and placed in a boiling water bath for 20 min. The residue was shifted to the graduate test tube, added with distilled water to 10 ml, and boiled for another 15 min. Then, 2.0 ml of 9.6 M perchloric acid was added to the graduated test tube to extract for 6 h. After centrifugation at 10,000 × g for 15 min, the supernatant was collected for starch content analysis. Starch content was measured by using a spectrophotometer (PerkinElmer, Lambda 35, UK).

**2.2.5. Flavonoid Content.** 1.0 g fruit flesh was added in 4 ml of 1% HCl-methyl alcohol, extracted for 2 h, and centrifuged at 10,000 × g at 4°C for 20 min. The absorbance of the

supernatant was measured at 325 nm. The content of the flavonoids was expressed as OD<sub>325/g</sub> at 325 nm by using a spectrophotometer (PerkinElmer, Lambda 35, UK).

For all the internal quality evaluations, three replicates were performed for each treatment, and ten fruits were used for each replicate.

**2.2.6. Decay Evaluation.** The decay rate of fruit was visually evaluated. Fruits with visible disease development were considered decayed, and the percentage of decay fruits was used for expressing decay rate:

$$\text{decay rate} = \frac{\text{total number of decayed fruits}}{\text{total number of fruits}} \times 100\%. \quad (2)$$

The decay index of fruit was visually evaluated and scored on from 0 to 4. There were three replicates in each treatment, and each replicate contained thirty fruits.

### 2.3. Reactive Oxygen Species and Related Enzymes

**2.3.1. O<sup>2-</sup> Production Rate.** According to Liu et al. [15], fruit flesh (2.0 g) was added with 4 ml of 100 mM phosphate buffer (pH 7.8, containing 2% PVPP, and precooled at 4°C) and grinded on ice. After centrifugation at 10,000 × g at 4°C for 20 min, the supernatant was collected for the measurement of O<sup>2-</sup> production rate. The KNO<sub>2</sub> solution was used to make the standard curve. The O<sup>2-</sup> production rate was expressed as nmol·g<sup>-1</sup>·FW·min<sup>-1</sup>.

**2.3.2. Malondialdehyde Content.** According to Hodges et al. [16], 1.0 g fruit flesh was added with 10% (w/v) TCA solution that was precooled at 4°C. After grinding and centrifugation at 10,000 × g at 4°C for 20 min, the supernatant was used for MDA measurement by using a spectrophotometer (PerkinElmer, Lambda 35, UK). The MDA content was calculated using the following formula:

$$C_{\text{MDA}} \left( \frac{\mu\text{mol}}{\text{L}} \right) = 6.45 \times (\text{OD}_{532} - \text{OD}_{600}) - 0.56 \times \text{OD}_{450}. \quad (3)$$

**2.3.3. Glutathione Content.** According to Brehe and Burch [17], fruit flesh (1.0 g) was homogenized on ice with 3 ml of 5% (w/v) TCA solution that contained 5 mM EDTA-Na<sub>2</sub> and was precooled at 4°C. After centrifugation at 10,000 × g at 4°C for 20 min, the supernatant was collected for GSH measurement. GSH content was expressed as nmol·g<sup>-1</sup>·FW.

**2.3.4. Superoxide, Catalase, Ascorbic Peroxidase, and Glutathione Reductase Activities.** Superoxide, catalase, ascorbic peroxidase, and glutathione reductase activities were expressed as 0.01 changes in absorbance at corresponding nm per minute per g flesh tissue by using a spectrophotometer (PerkinElmer, Lambda 35, UK).

Superoxide (SOD) activity was determined according to Prochazkova et al. [18] and Larrigaudière et al. [19]. The

amount of enzyme that inhibited 50% of NBT photochemical reduction was defined as a SOD activity unit.

Catalase (CAT) activity was determined according to Naima et al. [20] and expressed as 0.01 change in absorbance at 240 nm per minute per g flesh tissue.

Ascorbic peroxidase (APX) activity was determined according to Nakano and Asada [21] and expressed as 0.01 changes in absorbance at 290 nm per minute per g flesh tissue.

Glutathione reductase (GR) activity was determined according to Foyer and Halliwell [22] and expressed as 0.01 changes in absorbance at 340 nm per minute per g of flesh tissue.

### 2.3.5. Peroxidase and Polyphenoloxidase Activity.

Peroxidase (POD) activity was determined according to Zhu et al. [23]. 500 μL of 0.5% (v/v) guaiacol and 1.5 mL of 50 mM acetate buffer (pH 5.5) were added into 500 μL enzyme extraction. The reaction started when 500 μL of 0.059 M H<sub>2</sub>O<sub>2</sub> was added. The enzyme activity unit was determined by monitoring the increase of absorbance at 470 nm per min. The enzyme activity was expressed as U·g<sup>-1</sup>·FW<sup>-1</sup>. Polyphenoloxidase (PPO) activity was determined according to Zhu and Ma [24]. One PPO activity unit was defined as 1 change in absorbance at 420 nm per minute per g of flesh tissue by using a spectrophotometer (PerkinElmer, Lambda 35, UK).

**2.4. Statistical Analysis.** SPSS 11.0 for Windows (SPSS Inc., Chicago, IL) was used for data collection and analysis with a one-way analysis of variance (ANOVA). Duncan's multiple comparison was used to separate means at the 5% level.

## 3. Results

### 3.1. Effects of Forced Air Precooling on External Quality of Mango Fruits during Storage

**3.1.1. Firmness.** Mango fruits firmness decreased dramatically after harvest, while precooling treatment retarded the decrease. The precooled fruit had higher firmness than the control group during the whole period. On the 10<sup>th</sup> day and 15<sup>th</sup> day, precooled fruits had 33.3% and 62.4% higher firmness than control fruits, respectively (Figure 1(a)).

**3.1.2. Weight Loss.** As shown in Figure 1(b), weight loss of mango fruits increased steadily and precooling treatment increased fruit weight loss slightly. On the 20<sup>th</sup> day of storage, precooled fruits had 2.2% higher weight loss than control fruits.

**3.1.3. Color.** As shown in Figure 2(a), the yellow index in mango fruits was increased steadily during storage, while precooling treatment inhibited the color change. On the 10<sup>th</sup> day and 15<sup>th</sup> day, the precooled fruits had 23.3% and 14.5% a lower yellow index than control fruits, respectively. The

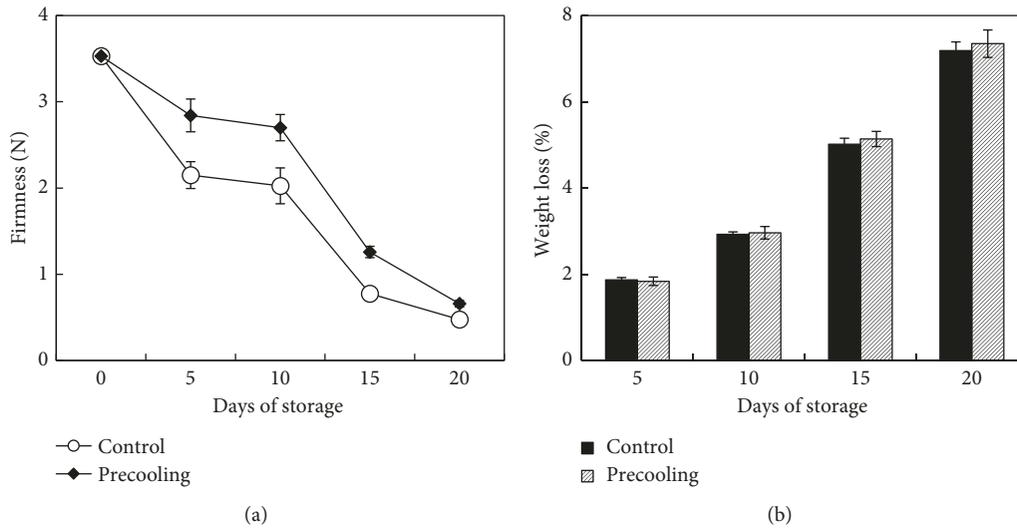


FIGURE 1: Effects of forced air precooling treatment on firmness (a) and weight loss (b).

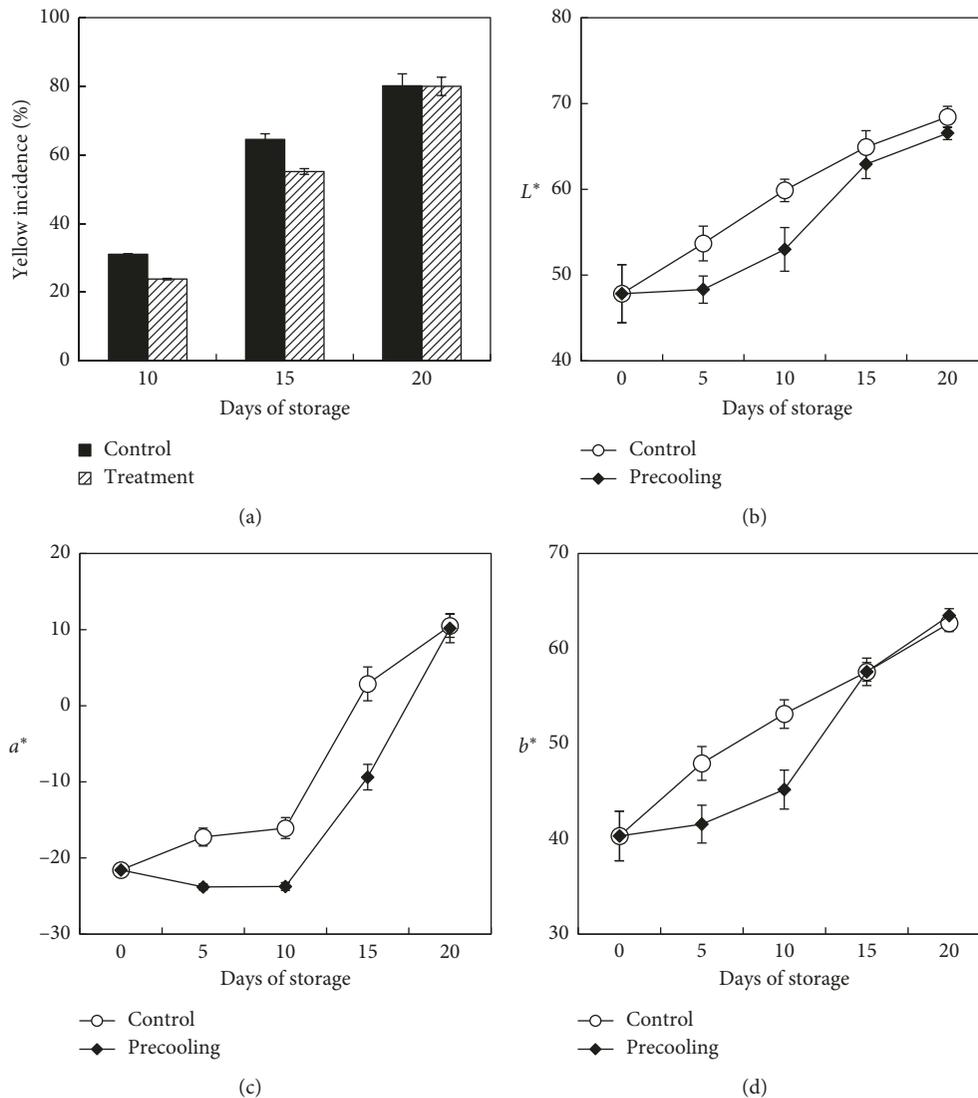


FIGURE 2: Effects of forced air precooling treatment on the (a) yellow index, (b)  $L^*$  value, (c)  $a^*$  value, and (d)  $b^*$  value.

difference between precooled and control fruit was not significant at the end of storage.

$L^*$  value from low to high indicates that the lightness increases.  $L^*$  value of mango fruits peel increased with the prolonging of the storage time.  $L^*$  value in precooled fruits was lower than control fruits during the entire storage period. On the 10<sup>th</sup> day, the  $L^*$  value of precooled fruits was 11.5% lower than that of control fruits (Figure 2(b)).  $a^*$  value from negative to positive represents that the color changes from green to red, and  $b^*$  was from blue to yellow. With the extension of storage,  $a^*$  and  $b^*$  values of mango fruits peel were all increased steadily, which showed that the peel color was changing from green to yellow. Precooling treatment retarded the increase of  $a^*$  and  $b^*$  during the beginning of the storage. On the 10<sup>th</sup> day, precooled fruits had 47.8% lower  $a^*$  and 14.9% lower  $b^*$  than that of control fruits, respectively ( $P < 0.05$ ). The differences between precooled and control fruits were not significant at the end of storage (Figures 2(c) and 2(d)).

**3.1.4. Pigment Content.** Figure 3 showed pigment changes in mango fruit flesh and peel. With the ripening of mango fruits, chlorophyll a in mango fruits peel decreased dramatically and the decrease of control fruits was higher than precooled fruits. On the 20<sup>th</sup> day of storage, precooled fruits peel had 84% higher chlorophyll a than control fruits (Figure 3(a)). Chlorophyll a in mango fruits flesh was also decreased dramatically. On the 10<sup>th</sup> day, precooled fruits fresh had a significantly higher level of chlorophyll a than that of control fruits. At other times, there was no significant difference (Figure 3(b)).

The change tendency of chlorophyll b in mango fruits peel was similar to chlorophyll a, and precooling treatment also retarded chlorophyll b reduction. On the 15<sup>th</sup> and 20<sup>th</sup> day of storage, precooled fruits peel had 188% and 200% higher chlorophyll b than that of control fruits, respectively (Figure 3(c)). However, there was no significant difference in chlorophyll b between precooled fruits flesh and control fruits flesh during the storage period (Figure 3(d)).

### 3.2. Effects of Forced Air Precooling on Internal Quality of Mango Fruits during Storage

**3.2.1. Total Soluble Solids.** As shown in Figure 4(a), TSS of control fruits increased during the first 15 days and then decreased. Precooling treatment retarded the increase of TSS, and there was no decrease until the end of storage. Therefore, on the 20<sup>th</sup> day of storage, the TSS in precooled fruits was 11.6% higher than the control.

**3.2.2. Titratable Acidity.** Figure 4(b) showed the changes of TA content in mango fruits during storage. TA in mango fruits changed slightly during first 10 days, after which TA decreased rapidly. Precooled fruits exhibited less decrease compared to the control. On the 15<sup>th</sup> day, precooled fruits had 39.5% higher TA content than control fruits.

**3.2.3. Soluble Sugar Content.** Soluble sugar content of mango fruits in both precooled fruits and control fruits increased steadily with the increase of storage time. At the beginning of storage, soluble sugar content in precooled fruits was slightly lower than that of control fruits. At the end of the storage, however, soluble sugar content in precooled fruits was slightly higher than that of control fruit. But there was no significant difference between the two groups (Figure 4(c)).

**3.2.4. Starch Content.** Figure 4(d) showed that starch content decreased continuously during the whole storage period. On the 20<sup>th</sup> day, starch content was only 25% of the initial value. Precooling treatment delayed the decrease of starch content during the beginning of the storage period. On the 5<sup>th</sup>, 10<sup>th</sup>, and 15<sup>th</sup>, precooled fruits had 27.3%, 22.3%, and 37.7% higher starch content than the control group, respectively.

**3.2.5. Flavonoid Content.** As shown in Figure 5, flavonoids content was decreased in both precooled and control fruits and then increased slightly at the first 5 days. Flavonoid in precooled fruits increased more rapidly than that in the control. On the 10<sup>th</sup>, 15<sup>th</sup>, and 20<sup>th</sup> day, precooled fruits had 10.3%, 11.2%, and 6.1% higher flavonoids content than control, respectively.

**3.3. Effects of Forced Air Precooling Treatment on Decay Incidence and Decay Severity of Mango Fruits during Storage.** Decay incidence and decay severity of mango fruits that were stored at 13°C and 85%~95% RH was shown in Figures 6(a) and 6(b). Precooling treatment decreased fruit decay, in both decay incidence and decay index. On the 20<sup>th</sup> day, precooled fruits had 39.7% lower decay rate and 41.3% lower decay index than control fruits, respectively.

### 3.4. Effects of Forced Air Precooling Treatment on Reactive Oxygen Species and Related Enzymes of Mango Fruits during Storage

**3.4.1.  $O_2^-$  Production.** There was no significant change of  $O_2^-$  production rate in mango fruits during the beginning of storage. After 10 days, rate of  $O_2^-$  production in control fruits increased rapidly and suddenly, while that in precooled fruits increased slightly. On the 15<sup>th</sup> and 20<sup>th</sup> day, rate of  $O_2^-$  production in precooled fruits was only 67.8% and 64.5% of control fruits, respectively (Figure 7(a)).

**3.4.2. Malondialdehyde Content.** Figure 7(b) showed that MDA content in control fruits increased rapidly at the first 5 days of storage and then kept steady, while MDA content in precooled fruits increased steadily during the whole storage. On the 5<sup>th</sup> and 10<sup>th</sup> day, MDA content in precooled fruits was only 66.1% and 80.2% of that in control fruits. In the storage period, MDA content of precooled fruits was gradually close to the control. At the end of the storage

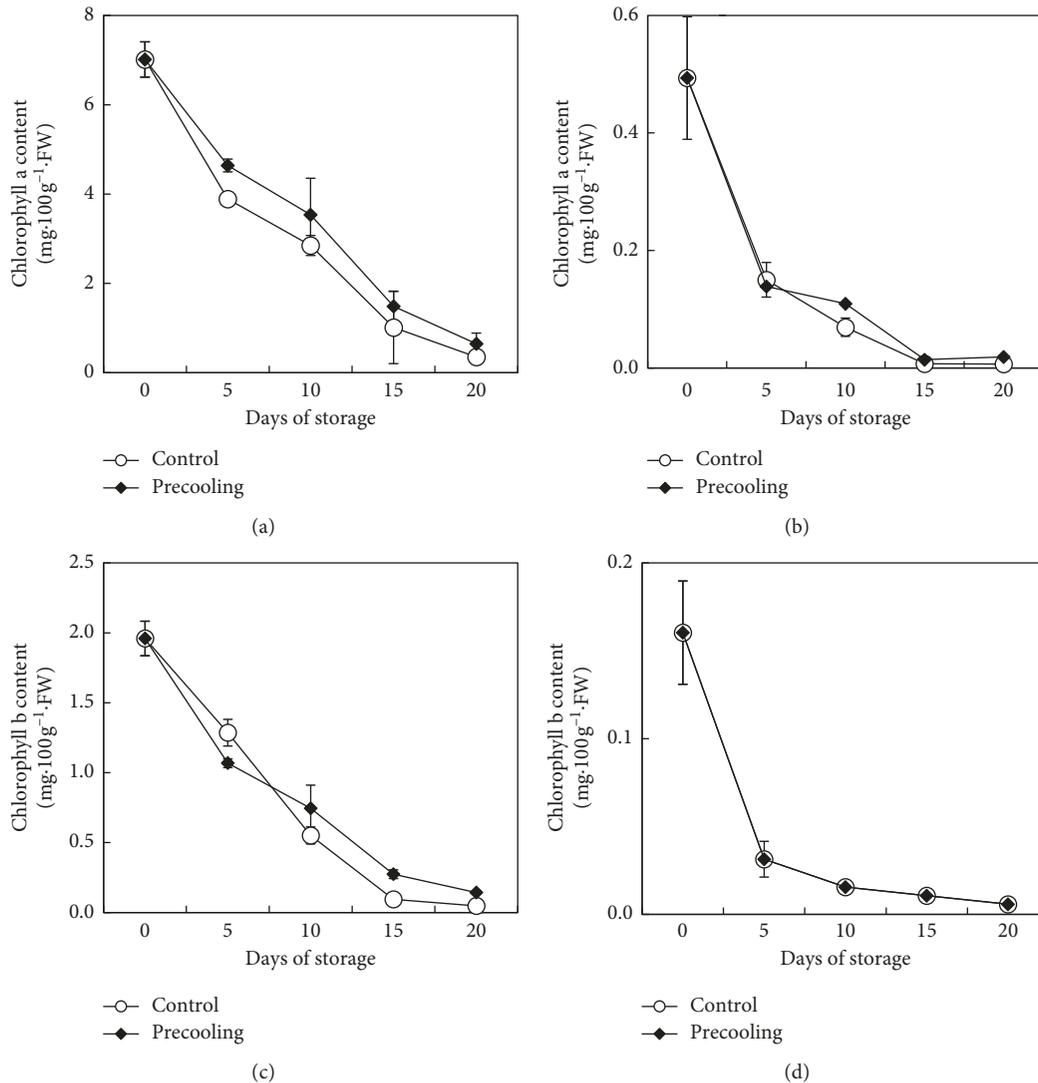


FIGURE 3: Effects of forced air precooling treatment on chlorophyll a of peel (a) and flesh (b) and chlorophyll b of mango fruit peel (c) and flesh (d).

period, there was no significant difference between the MDA content of two groups.

**3.4.3. Superoxide, Catalase, Peroxidase, and Ascorbic Peroxidase Activities.** As shown in Figure 8, during the whole storage period, the activities of SOD, CAT, POD, and APX in mango fruits showed a constant increase tendency. Precooled fruits exhibited higher SOD and CAT activities in the entire storage period. On the 20<sup>th</sup> day, treated fruits had 23.2% higher CAT activity and 2.7% higher SOD activity than control fruits, respectively (Figures 8(a) and 8(b)).

Precooling treatment enhanced POD activity in mango fruits, except on the 5<sup>th</sup> day of storage. On the 15<sup>th</sup> and 20<sup>th</sup> day of storage, precooled fruits had 23.6% and 10.5% higher POD activity than control fruits, respectively (Figure 8(c)). At the beginning of storage, precooled fruits showed higher APX activity, which was 126.6% higher than the control. While at the end of storage, precooling treatment had no

significant influence on APX activity in mango fruits (Figure 8(d)).

**3.4.4. Glutathione Content and Glutathione Reductase Activity.** As shown in Figure 9(a), GSH content in mango fruits showed a gradually increase during first 10 days and then decreased slowly. Precooled fruits had higher GSH content than control fruits during the whole storage. On the 10<sup>th</sup> and 15<sup>th</sup> day of storage, treated fruits had 22.1% and 12.7% higher GSH content than control fruits, respectively.

GR activity in mango fruits increased with the prolonging of the storage time. Precooled fruits had lower GR activity than control fruits at earlier storage. At the end of the storage, the GR activity in precooled fruits was slightly higher, but the difference was not significant ( $P > 0.05$ , Figure 9(b)).

**3.4.5. Polyphenoloxidase Activity.** As shown in Figure 10, PPO activity in control fruits increased dramatically at

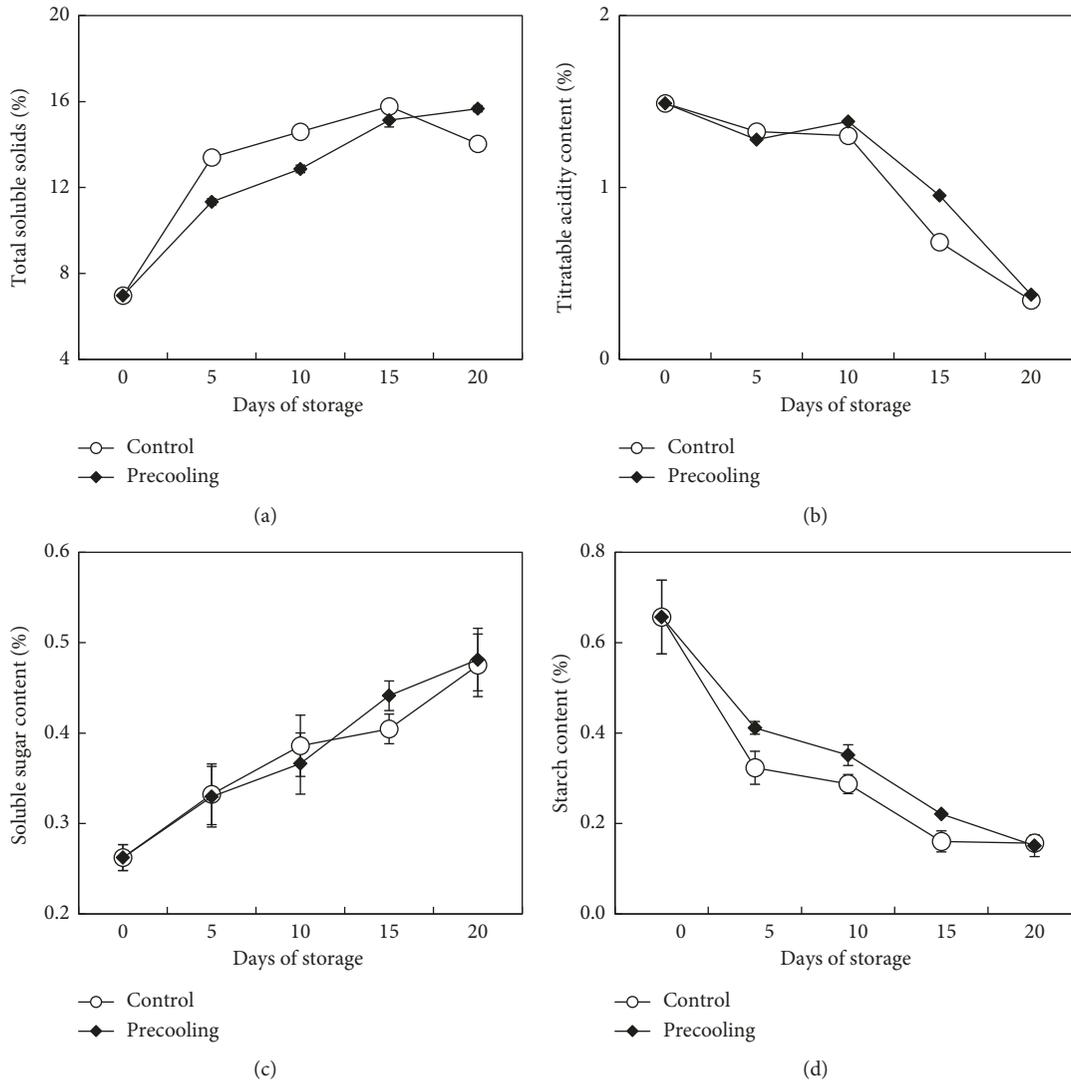


FIGURE 4: Effects of forced air precooling treatment on TSS (a), TA (b), soluble sugar (c), and starch (d) content.

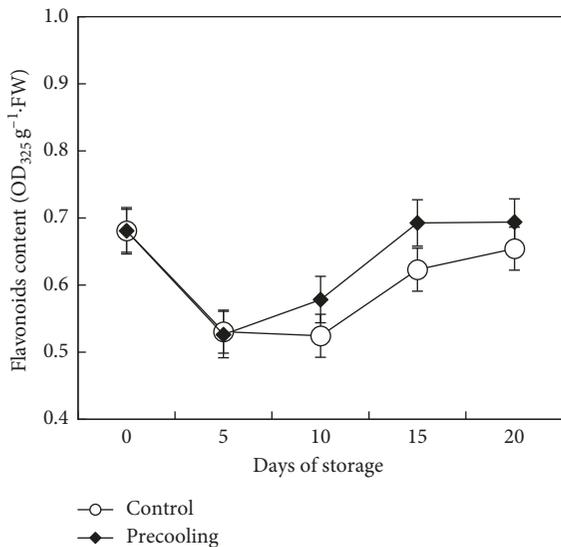


FIGURE 5: Effects of forced air precooling treatment on flavonoid content.

earlier storage and had a peak activity on the 5<sup>th</sup> day of storage, after which PPO activity decreased and was kept stable. PPO activity in precooled fruits was always at a lower level. On the 5<sup>th</sup> day and 20<sup>th</sup> day, precooled fruits had 81.9% and 67.4% lower PPO activity than control fruits, respectively.

#### 4. Discussion

Mango fruits are a typical climacteric fruit and commonly harvested at a green mature stage to prevent the postharvest loss [25]. After harvest, fruits begin to ripen with the characteristic changes on peel coloration, textural softening, sugar maintenance suffer from over softening, and ripening during storage, which result in postharvest quality deterioration and short shelf-life [26]. In this study, the results showed that precooling treatment could retard fruit softening, peel coloration, accumulation of sugar and organic acid, and disease development on mango fruits, and all of which were closely related to the maintenance of fruit ripening [27].

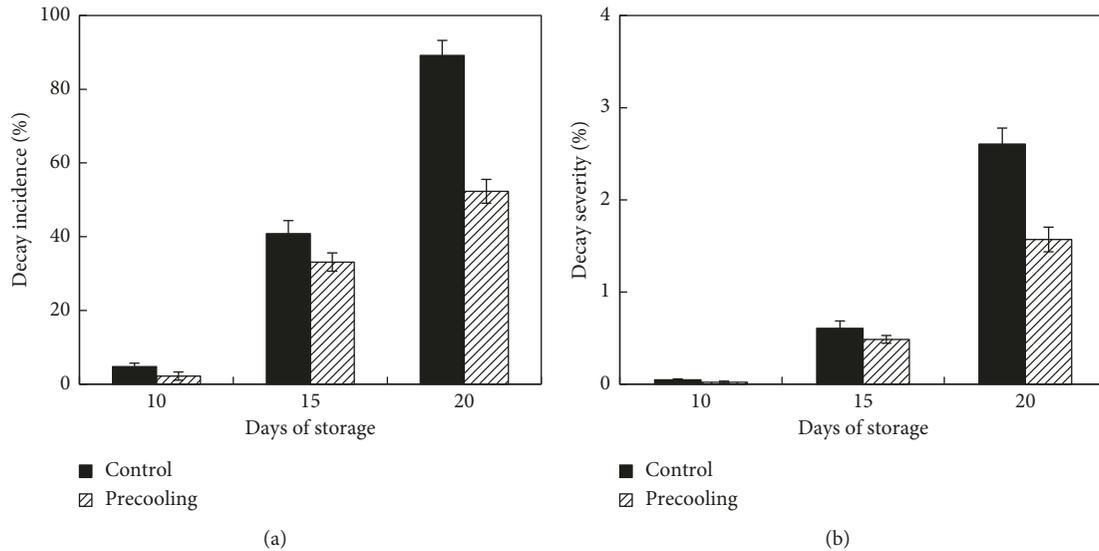


FIGURE 6: Effects of forced air precooling treatment on decay incidence (a) and decay severity (b).

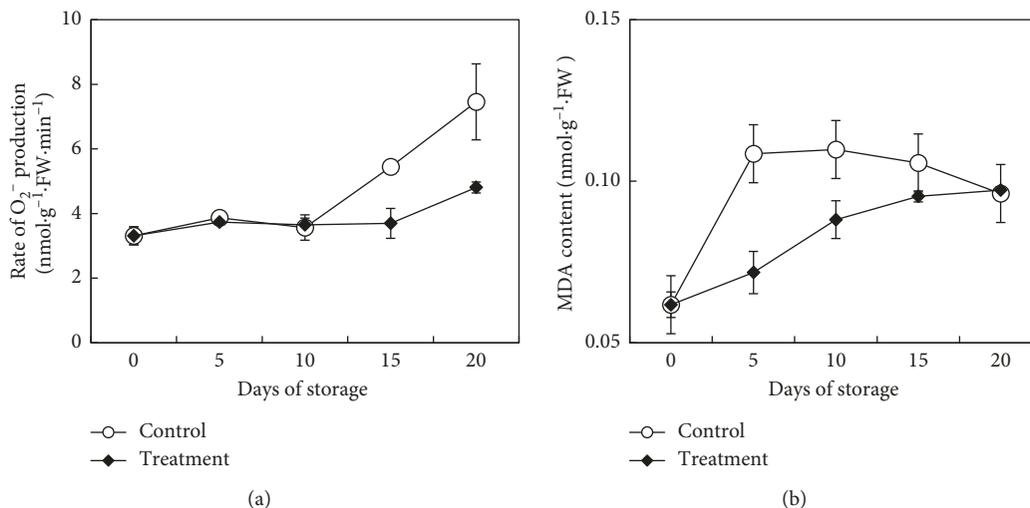


FIGURE 7: Effects of forced air precooling treatment on  $O_2^-$  production (a) and MDA content (b).

Firmness is an important parameter in postharvest storage and marketing. Loss of firmness caused by degradation of cell wall and starch may result in physical damages in transportation and fruit susceptibility to pathogen attacks [2]. In this study, pre-cooled fruits showed higher firmness during the entire storage. It is in coincidence with previous study on waxberry and black mulberry fruits [1]. It indicated that precooling treatment could retard mango fruit's softening and ripening, and it might be related to the decreased respiration in the early of storage. During postharvest ripening, the chlorophyll content in mango peel decreased and the peel color changes from green to yellow [28]. Usually, the peel color was considered as an important indicator of fruit maturity and a critical parameter in fruit marketing [29]. In this study, pre-cooled fruits showed a lower yellow index and  $b^*$  value than control fruits, and it was closely related to

higher chlorophyll a and chlorophyll b content in mango fruits peel during the storage [13]. However, the pigment in mango flesh was not influenced by precooling treatment. Fruit weight loss is an important postharvest parameter influencing fruit storage quality, shelf-life, and economic value. In the current study, however, precooling treatment promoted fruit weight loss in storage. That might be because that precooling treatment speeded water transpiration on mango fruit's surface and enhanced fruit water loss. Forced air precooling was done without humidified air leading to higher weight loss.

After harvest, mango fruits showed high respiration rate and metabolism activity, which accelerated the consumption of nutritional compounds and fruit ripening. With fruit ripening, SSC and soluble sugar increase because of polysaccharide hydrolysis while starch, a vital polysaccharide in

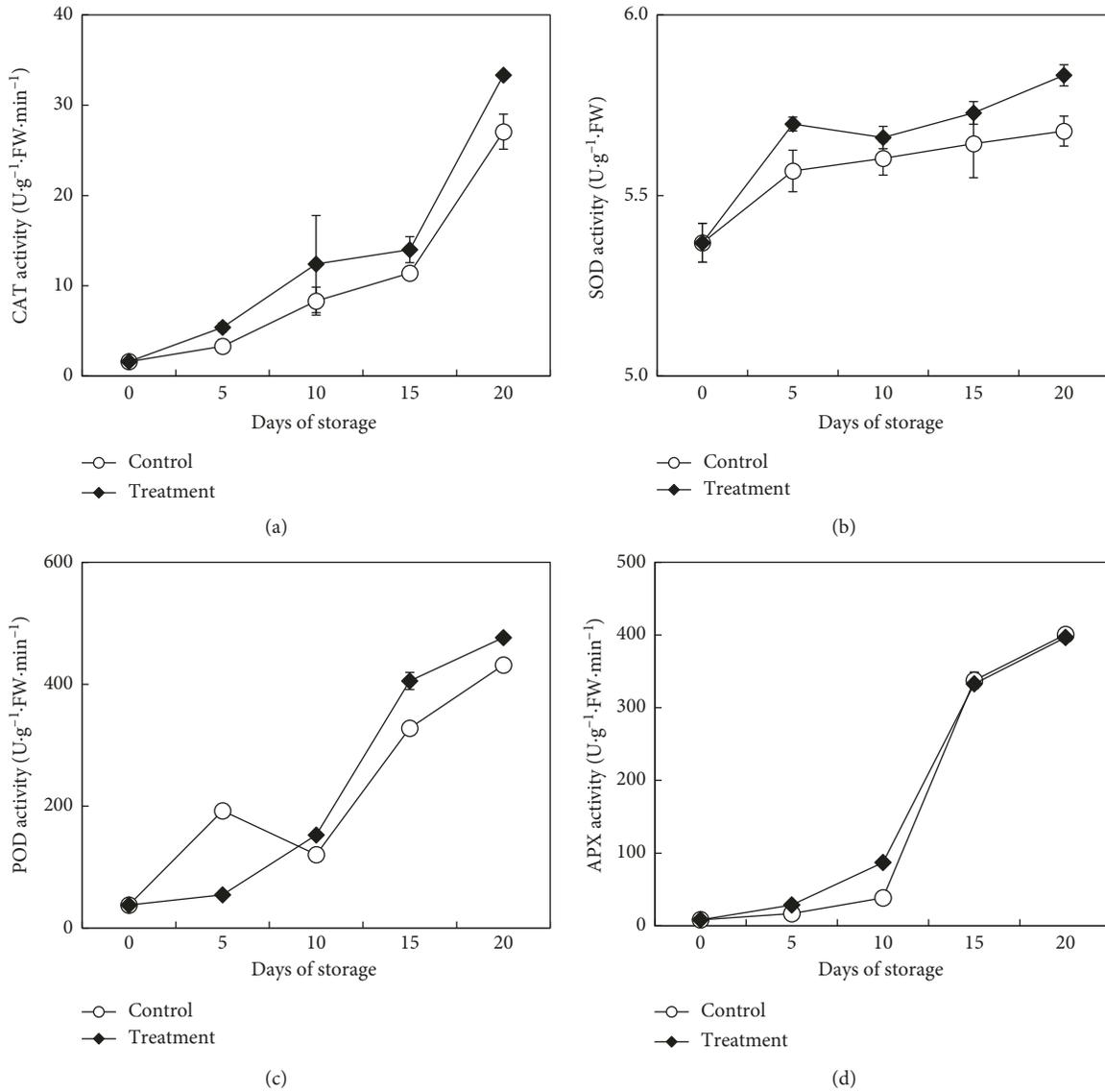


FIGURE 8: Effects of forced air precooling treatment on CAT (a), SOD (b), POD (c), and APX (d) activity.

fruit, decreases continuously [30]. In the current study, pre-cooled fruits showed lower SSC and higher starch content during the entire storage. It indicated precooling treatment retarded the hydrolysis of polysaccharides to SSC. Meanwhile, starch degradation is closely related with fruit softening. Inhibiting the degradation of starch to SSC also contributed to the fruit firmness maintenance [15]. The content of TA represents the concentration of organic acid in fruits. Fruit respiration can promote organic acid consumption and reduce TA content [31]. During storage, the content of TA in mango fruits decreased rapidly and pre-cooling treatment inhibited the tendency. The change of SSC, TA, and starch content might be related to the delay in the ripening process and reduction of respiration rate. In coincidence with the previous study on apricot [10], pre-cooling treatment enhanced flavonoids content in mango fruits in the late stage of storage. Flavonoids content is an important indicator of fruit's antioxidant capacity [32], and

precooling treatment might contribute to mango fruits antioxidant capacities. However, possible mechanisms need to be studied further.

With the prolonging of storage, fruit resistance to pathogen decreases because of ripening and softening, and decay happens. The current study found that precooling treatment reduced decay on mango fruits, including decay incidence and decay index (decay severity), which is similar to the study on green asparagus and broccoli [33]. It is possible because that precooling treatment retarded mango fruit ripening and maintained fruit resistance to postharvest pathogens.

Although reactive oxygen species (ROS) was beneficial to the improvement of plant disease resistance, excessive ROS can affect DNA replication and protein synthesis, resulting in fruit senescence [34]. According to free radical theory, senescence was a process of active oxygen metabolism disorders and accumulation. When the fruits are forced by

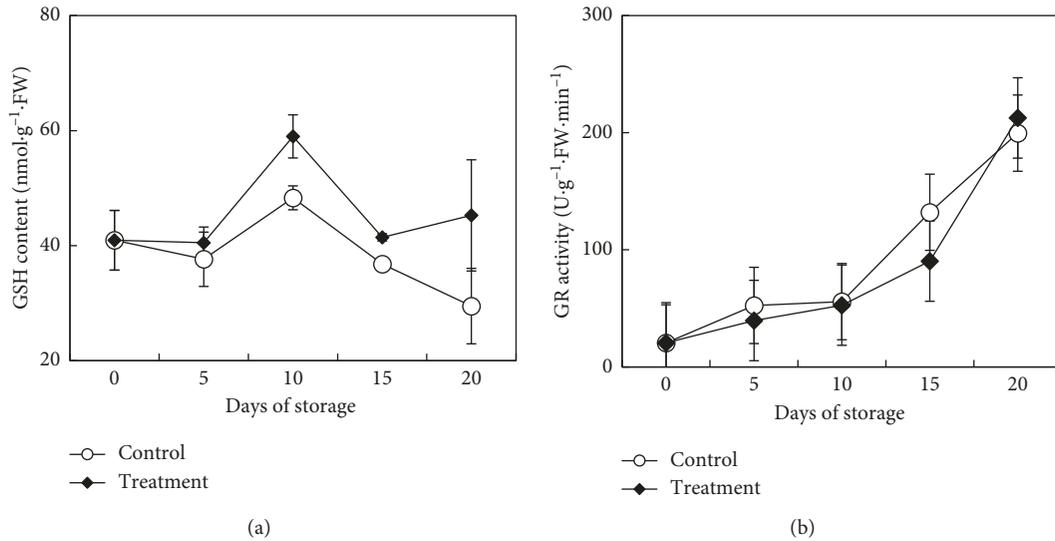


FIGURE 9: Effects of forced air precooling treatment on GSH content (a) and GR activity (b).

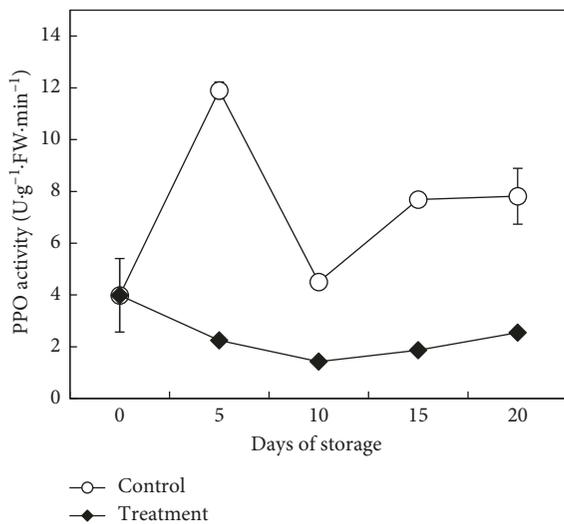


FIGURE 10: Effects of forced air precooling treatment on PPO activity.

outside stresses, reactive oxygen species metabolism balance is broken, free radicals are accumulated in abundance, and the peroxidation of membrane lipid is improved or aggravated, all of which induce the fruit senescence [13].  $O^{2-}$  is a kind of reactive oxygen species with relatively high virulence, playing an important role in horticultural plant ripening and senescence processes, particularly in membrane deterioration [35]. This study showed that the  $O^{2-}$  production rate in mango fruits increased rapidly at the end of storage, leading to senescence of mango. Precooling treatment inhibited the increase of  $O^{2-}$  production rate and subsequent fruit senescence. Moreover, being as the product of membrane lipid peroxidation, MDA was usually used to evaluate the degree of membrane damage [36] and lipid peroxidation [37]. In this study, MDA content was increased rapidly during the later storage period and was always at a higher level. This verified that the mango fruit's cellulose was

peroxidated and the integrity of the cell membrane was destroyed.

There are many active oxygen removal systems in plant tissue. The excessive active oxygen was eliminated by different antioxidant enzymes [13].  $O^{2-}$  can be transformed into  $H_2O_2$  by SOD in the plants. This study found that precooling treatment enhanced the SOD activity of mango fruits during storage. That might be why precooled fruit had lower  $O^{2-}$  production rate than control fruits. In addition, we also found that precooled fruits had higher CAT and POD activities than control fruits. We could include that precooled fruits had lower reactive oxygen species than control fruits. However, there was no significant difference of APX between the precooled fruits and control fruits in this study. This could be due to the higher activity of CAT and POD in mango fruits, and APX had not played a major part in elimination of  $H_2O_2$ . Therefore, the balance between SOD, POD, CAT, and APX activities in plants is important for determining the steady-state levels of  $H_2O_2$  and ROS [38].

Glutathione (GSH) is also a very important kind of the active oxygen removal material [35]. In plant tissue, GSH can reduce dehydroascorbic acid (DHA) to ascorbic acid (ASA), and ASA can eliminate  $H_2O_2$  directly. Glutathione reductase (GR) is the key enzyme involved in the GSH generation from oxidized glutathione (GSSC) [13, 35]. This study showed that precooled fruits had a higher GSH content and GR activity than control fruits during the storage, all of which contributed to the delay of fruit ripening and senescence.

Previous reports indicated that PPO was directly related to browning and decay. Decrease in its activity would lead to an induced resistance to browning and decay [25]. In normal circumstances, PPO usually exists in organelles membrane and cell membrane, so the PPO activity is low [13]. When the structure of cell membrane is destroyed, PPO is released, and the activity increases. In this study, the PPO activity in

precooled fruits was always kept in a low level, and this might be because the precooling treatment enhanced the antioxidant defense capabilities and helped to maintain cell integrity.

## 5. Conclusion

Mango fruit's quality was significantly improved by forced air precooling treatment. Precooling treatment delayed fruit ripening and senescence, with inhibition of ROS and enhancement of active oxygen removal enzymes such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), glutathione reductase (GR), and polyphenoloxidase (PPO). The firmness was maintained, and fruit decay was inhibited during storage. These results indicate that forced air precooling treatment could maintain mango fruit's quality by enhancing antioxidant activity and delaying fruit ripening. Therefore, it is suggested that mango fruits should be precooled immediately after harvest, for maintaining fruit quality and prolonging shelf-life.

## Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

## Conflicts of Interest

The authors declare that there are no conflicts of interest associated with the publication of this manuscript.

## Acknowledgments

This research was supported by Support Project of High-Level Teachers in Beijing Municipal Universities in the Period of the 13th Five-Year Plan (CIT&TCD201704037), Construction of Scientific Research Innovation Service Ability-Basic Scientific Research Operating Expense-Food Feature Project (PXM2018\_014213\_000033), and Support Project of High-Level Teachers in Beijing Municipal Universities (IDHT20180506).

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