



Exogenous melatonin treatment in the postharvest storage of pitaya fruits delays senescence and regulates reactive oxygen species metabolism

Liangjie BA¹, Sen CAO¹, Ning JI¹, Chao MA¹, Rui WANG¹, Donglan LUO^{1*} 

Abstract

Pitaya fruits have high senescence rates throughout their postharvest storage period. Additionally, studies have confirmed that melatonin plays a regulatory role in plant senescence. However, the involvement of melatonin in the postharvest senescence of fruits remains unclear. In this study, two cultivars of pitaya fruit, 'Zihonglong' and 'Jinghonglong', were treated with melatonin and then evaluated for characteristics of senescence while in storage for 10 days. The results showed that melatonin treatment delayed fruit senescence in both pitaya cultivars, as indicated by the inhibition of weight loss, decay incidence, relative membrane permeability, and malondialdehyde (MDA) content, as well as the maintenance of the total soluble solids and ascorbic acid contents and the reduced respiration intensity. In addition, melatonin treatment reduced the $O_2^{\cdot-}$ production rates, H_2O_2 contents, and lipoxygenase activities but enhanced the activities of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX) in both pitaya cultivars. These results indicate that melatonin may contribute to delaying senescence in pitaya fruits. This study shows the potential of the use of melatonin in the postharvest storage of pitaya fruits, as well as other horticultural fruits.

Keywords: pitaya; melatonin; senescence; antioxidant enzyme; storage.

Practical Application: The application of melatonin delays senescence and enhances antioxidant enzyme activity in postharvest pitaya fruits.

1 Introduction

Pitaya, also known as a dragon fruit, belongs to the genus *Hylocereus* of the order Caryophyllales, has been attracted much attention of growers worldwide. It is a colourful tropical and subtropical fruit rich in nutrients that is increasingly being utilised (Le Bellec et al., 2006). Among the different types of pitayas, the most popular are the red (*Hylocereus polyrhizus*) and white (*Hylocereus undatus*) cultivars, which are named according to their pulp colours (Suh et al., 2014). Fresh fruits and vegetables are very beneficial to human health (Grom et al., 2020; Wu et al., 2020). Pitaya fruits are sources of water-soluble dietary fiber, minerals and vitamins, which are rarely found in other plants (Wu et al., 2006; Ong et al., 2014). The total phenolic content and antioxidant activity of pitayas are valuable sources of antioxidants and anticancer properties, which can be utilized as a potential alternative for improving health of humans and animals (Dionísio et al., 2020a, b). Thus, it is significant to delay fruit senescence and maintain antioxidant activity of harvested pitaya fruit for improving fresh nutritive values.

Pitaya fruit can only be harvested during the hot and rainy seasons of summer and autumn, which may cause rapid fruit senescence, manifesting as rotting, shrivelling, dehydration, and reduced ascorbic acid content. Therefore, the senescence of pitaya fruits significantly reduces their commodity value. It has previously been reported that membrane lipid damage induces apoptosis and natural senescence in plants and that reactive

oxygen species (ROS), such as superoxide anion radical ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2), are associated with this damage (Wu et al., 2006). To date, several postharvest pitaya treatments, including storage at low temperatures (Liu et al., 2013), heat shock (Narvaez-Cuenca et al., 2011), modified atmosphere packaging (Van To et al., 2002), and administration of fungicides (Du et al., 2018) have been demonstrated to maintain redox equilibrium and inhibit lipid peroxidation by mediating ROS production. These methods also contribute to the maintained commodity value and longer storage of the pitaya fruits.

Melatonin (N-acetyl-5-methoxytryptamine, MT) has primordially been identified as an important animal hormone related to various biological processes, such as antioxidant mechanisms, which is generally distributed among various plant tissues (Arnao & Hernández-Ruiz, 2007; Reiter et al., 2015). Arnao & Hernández-Ruiz (2009) found that exogenous application of MT on barley leaves delayed their dark-induced senescence and decreased their chlorophyll degradation. MT chlorophyll preservation has also been shown to control senescence in rosette and *Malus hupehensis* leaves (Wang et al., 2013; Shi et al., 2015). In addition, Wang et al. (2012) reported that exogenous MT treatment on apples reduced ROS levels and delayed the detachment of leaves. Furthermore, leaf senescence was delayed by MT treatment according to metabolomic and proteomic analyses (Wang et al., 2013). It has also been shown

Received 16 Mar., 2021

Accepted 10 Apr., 2021

¹Guiyang University, Guiyang, China

*Corresponding author: luodonglan1991@163.com

that MT treatment slowed senescence in peaches, which was associated with the maintenance of membrane integrity (Gao et al., 2016). These results provided reliable evidence that MT may be involved in plant senescence. However, the roles of MT in the storage quality and senescence of horticultural fruits are not well understood.

In this study, we investigated the effect of exogenous MT treatment on the senescence of pitaya fruit during storage using two cultivars (red and white pulp). Aging-related physiological indicators and ROS metabolism were also measured. This study may promote the application of MT to improve postharvest quality and delay senescence in pitaya fruits, as well as other horticultural fruits, in the future.

2 Material and methods

2.1 Plant material and treatment

'Zihonglong' (ZH, red peel with red pulp) and 'Jinghonglong' (JH, red peel with white pulp) pitaya fruits (*Hylocereus undatus*) were selected for this study. The plants were grown in a commercial orchard in Anshun, a city in the Guizhou province of China. The pitaya fruits were chosen based on uniformity in maturity and size, as well as the absence of visible defects. They were then transferred to the laboratory on the same day of collection.

A total of 300 pitayas from each cultivar were randomly divided into two groups and immersed into distilled water (control) and melatonin (MT) solution at 0.1 mmol/L for 15 min, respectively. To prepare the melatonin solution, 23.23 mg of melatonin (Sangon Biotech, A600605, Shanghai, China) was dissolved in 10 mL absolute ethanol, and the resulting mixture was diluted to 0.1 mmol/L using distilled water. The fruits were then air-dried and stored at 20 ± 0.5 °C and 80–90% humidity for up to 10 days. Every 2 days, the following physiological index were evaluated in the pitaya samples, including respiration intensity, weight loss, decay incidence, total soluble solids content, and relative membrane permeability.

Additionally, the samples of flesh tissues were derived from ten pitaya fruits, with each treatment on each cultivar being done in triplicate. The samples were collected and stored at -80 °C for measurement of ascorbic acid content, malondialdehyde (MDA) content, superoxide anion radical ($O_2^{\cdot-}$) production rate, hydrogen peroxide (H_2O_2) content, and activities of lipoxygenase (LOX), superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX).

2.2 Measurement of decay incidence and weight loss

Decay in the pitaya fruits was defined as having visible fungal growth, rot, and bacterial lesions. Decay incidence was defined as the number of fruits showing signs of decay relative to the total number of fruits in each treatment, expressed in percentage. Weight loss was evaluated by weighing the pitaya fruits before and after storage and presented as the percentage of weight loss compared to their initial weights (Gao et al., 2016).

2.3 Measurement of respiration intensity, relative membrane permeability, total soluble solids content, and ascorbic acid content

The respiration intensities were detected using a modified closed method. In each treatment, four pitaya fruits were randomly sampled and sealed in a glass container containing 20 mL of 0.4 M NaOH at 20 ± 0.5 °C for 2 h. Then, 5 mL of BaCl₂ saturated solution and 3 drops of phenolphthalein were added, and the resulting solution was titrated with 0.1 mol/L oxalic acid until the end point. The respiration intensities of the pitaya fruits were expressed as mg CO₂/(kg.h) (Jiang et al., 2012).

Relative membrane permeabilities were represented by the relative electrolytic leakages. Ten discs of pitaya peel tissue were removed from five pitaya fruits per replicate using a brass cork borer (8 mm in diameter). The pitaya peel tissues were rinsed 3 times with redistilled water and then transferred into a conical flask containing 15 mL of redistilled water. They were then shaken with a table concentrator for 20 min at room temperature, and their relative electrolytic leakages (P_1) were measured. Subsequently, the samples were boiled for 10 min and their relative electrolytic leakages (P_2) were measured again. The results were calculated as follows (Niu et al., 2018, Equation 1):

$$\text{Relative electrolytic leakage}(\%) = \frac{P_1}{P_2} \times 100\% \quad (1)$$

For measurement of the total soluble solids, 2 g of the pitaya flesh tissues were pulverised and centrifuged at $5,000 \times g$ for 20 min. The content of total soluble solids in the resulting supernatants was measured using a PAL-1 hand-held refractometer (ATAGO, Japan) and expressed as Brix.

To measure the ascorbic acid content, 5 g of the pitaya flesh tissue was homogenised in 25 mL of 2% oxalic acid solution and centrifuged at $8,000 \times g$ for 15 min at 4°C. After centrifugation, 10 mL of the supernatant was transferred to a 50 mL triangular bottle and titrated with a calibrated 2,6-dichlorophenolindophenol solution until the solution became permanently pink. The ascorbic acid contents of the pitaya fruits were expressed on a fresh weight basis as mg/100 g.

2.4 Measurement of MDA content, $O_2^{\cdot-}$ production rate, and H_2O_2 content

Pitaya flesh tissues (2 g) were homogenised with 10 mL of 10% trichloroacetic acid containing 0.5% (w/v) thiobarbituric acid. The mixtures were then heated at 100°C for 10 min. After rapid cooling, the mixtures were centrifuged at $5,000 \times g$ for 15 min. The absorbances of the supernatants were measured at 450, 532, and 600 nm. The MDA contents of the pitaya fruits were expressed on a fresh weight basis as $\mu\text{mol/g}$ (Dhindsa et al., 1981).

The $O_2^{\cdot-}$ production rates of 3 g of pitaya flesh tissues were determined using a modified method (Wang et al., 2013). The $O_2^{\cdot-}$ production rates were calculated using NaNO₂ as a standard and were expressed on a fresh weight basis as nmol/g/min.

Pitaya flesh tissues (5 g) were homogenised in 5 mL of cold acetone and centrifuged at $5,000 \times g$ and 4°C for 15 min.

Volumes of 1 mL of the supernatants were mixed with 0.1 mL of 22 mmol/L titanium sulphate and 0.2 mL of ammonia. The resulting solutions were then centrifuged at $5,000 \times g$ and 4°C for 10 min. Then, the pellets were dissolved in 3 mL of 1 mol/L sulfuric acid and centrifuged for 10 min at $5,000 \times g$. The H_2O_2 contents of the pitaya fruits were calculated using H_2O_2 as a standard and were expressed on a fresh weight basis as mmol/g (Patterson et al., 1984).

2.5 Enzyme assays

Pitaya flesh tissues (2 g) were homogenised in various precooled buffers (4°C) to prepare the extracts for assays to measure activities of various enzymes. 6 mL of 50 mmol/L sodium phosphate buffer (pH=7.8) containing polyvinylpyrrolidone for SOD; 8 mL of 50 mmol/L sodium phosphate buffer (pH=6.8) containing polyvinylpyrrolidone for POD, CAT, and LOX; 8 mL of 100 mmol/L potassium phosphate buffer (pH=7.5) containing 0.1 mmol/L ethylene diamine tetraacetic acid, 1 mmol/L ascorbic acid, and polyvinylpyrrolidone for APX. The tissue homogenates were then centrifuged at $12,000 \times g$ and 4°C for 15 min. The resulting supernatants were used for the enzyme assays.

LOX activity was estimated by measuring the increase in absorbance at 234 nm and was expressed on a fresh weight basis as U/g using the following equation (Surrey, 1964, Equation 2):

$$U = 0.01\Delta A_{234\text{ nm}} / \text{min} \quad (2)$$

The reaction mixture for determination of SOD activity contained 50 mmol/L sodium phosphate buffer (pH=7.8), 14.5 mmol/L methionine, 2.25 mM nitro blue tetrazolium, 30 $\mu\text{mol/L}$ EDTA, 60 $\mu\text{mol/L}$ riboflavin, and 30 μL of supernatant. SOD activity was determined by measuring the absorbance at 560 nm to estimate the inhibition of nitro blue tetrazolium formazone production and was expressed on a fresh weight basis as U/g, where U is the amount of SOD that inhibits 50% of nitro blue tetrazolium production in 1 min (Surrey, 1964; Gao et al., 2016).

The reaction mixture for the determination of POD activity contained 0.05 mol/L phosphate buffer (pH=6.8), 0.16 mol/L guaiacol, 0.88 mol/L H_2O_2 , and 0.4 mL of supernatant (Kochba et al., 1997). POD activity was determined by measuring the increase in absorbance at 470 nm and was expressed on a fresh weight basis as U/g FW using the following equation (Kochba et al., 1997, Equation 3):

$$U = 0.01\Delta A_{470\text{ nm}} / \text{min} \quad (3)$$

CAT activity was determined by measuring the decrease in absorbance at 240 nm and was expressed on a fresh weight basis as U/g using the following equation (Dhindsa et al., 1981, Equation 4):

$$U = 0.01\Delta A_{240\text{ nm}} / \text{min} \quad (4)$$

The reaction mixture for determination of APX activity contained 2.4 mL of 50 mmol/L sodium phosphate buffer (pH=7.5), 0.2 mL of 2 mmol/L H_2O_2 , and 0.4 mL of supernatant. APX activity was estimated by measuring the decrease in absorbance at 290 nm and was expressed on a fresh weight basis as U/g using the following equation; (Gao et al., 2016, Equation 5):

$$U = 0.01\Delta A_{290\text{ nm}} / \text{min} \quad (5)$$

2.6 Statistical analysis

All assays in this study were performed on at least three independent biological replicates. Values are represented as mean \pm SE (standard error) of three or six biological replicates. Student's t-test was used for statistical analysis ($*P < 0.05$). All figures were produced using Origin 7.5.

3 Results

In the preliminary experiments, the effects of MT on pitaya fruits were investigated at concentrations of 0.01, 0.05, 0.1, 0.15, 0.2, 0.25, 0.3, 0.4, and 0.5 mmol/L. The results showed that 0.1 mmol/L was the concentration that displayed the most optimal effect in delaying senescence in the pitaya fruits. In contrast, concentrations of MT greater than or equal to 0.15 mmol/L significantly promoted ripening of the fruits, as observed by the increased respiration intensity, weight loss, and decay incidence. Thus, the optimal concentration 0.1 mmol/L of MT was used for all subsequent experiments.

3.1 Effect of MT treatment on weight loss and decay incidence in the pitaya fruits

Throughout the storage period, it was observed that the weight loss and decay incidence gradually increased with an increase in storage time (Figure 1). After 10 days of storage, weight loss in the 'ZH' fruit was around 8.5% and in the 'JM' fruit was around 8%, which was 31.5% and 17.2% lower than the corresponding control groups, respectively (Figure 1a, b).

Decay in the pitaya fruits was first observed in both the 'ZH' and 'JM' fruits after 2 days of storage. Furthermore, decay incidence increased as storage time was extended (Figure 1c, 1d). In the MT treatment groups of the 'ZH' and 'JM' pitaya fruits, decay incidence was effectively inhibited. Compared with the control fruits, the decay incidence in the MT treatment fruits at the end of the storage period was reduced by 39.9% and 45.3% in the 'ZH' and 'JM' pitaya fruits, respectively.

3.2 Effect of MT treatment on respiration intensity, relative membrane permeability, total soluble solids content, and ascorbic acid content of the pitaya fruits

Throughout the storage process, the respiration intensities of both variants of the pitaya fruits exhibited a downward trend throughout the storage period, but no respiratory peaks were observed until significant rotting was seen in the fruits (Figure 2a, 2b). The respiratory intensities of the MT treatment groups were significantly lower than those of the control groups

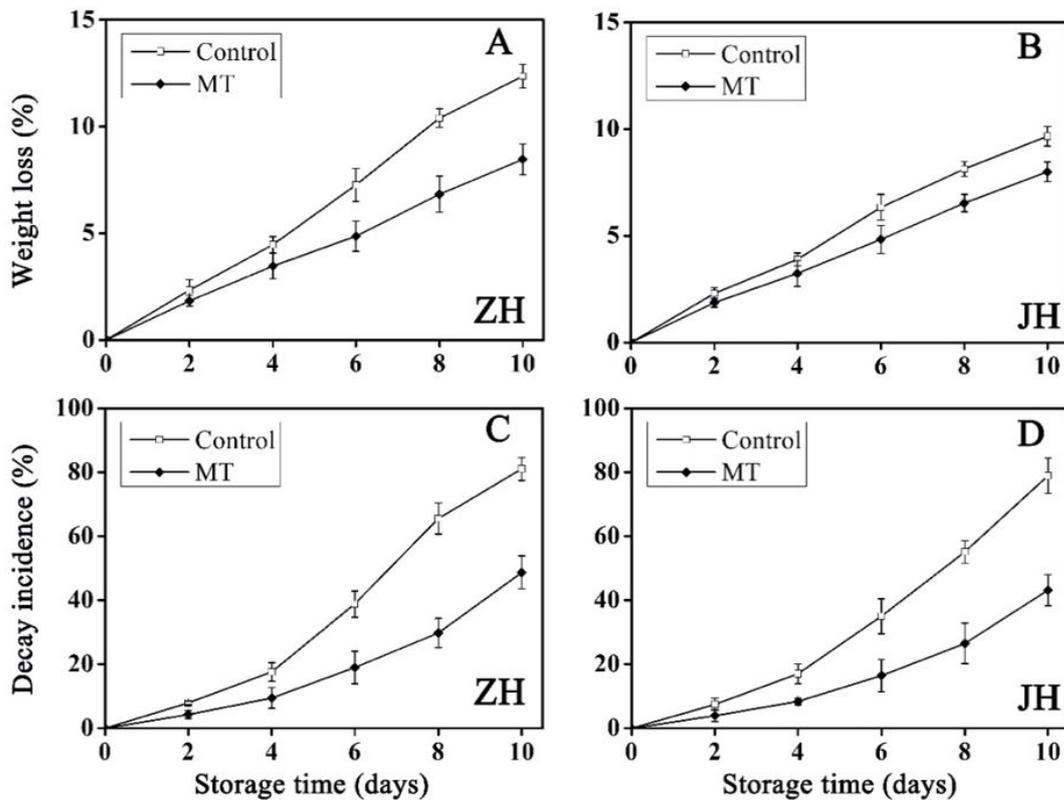


Figure 1. Weight loss (A, 'ZH'; B, 'JH') and decay incidence (C, 'ZH'; D, 'JH') of pitaya fruits after treatment with 0.1 mmolL^{-1} MT during storage at $(20 \pm 0.5)^\circ\text{C}$ for 10 days. 'ZH' and 'JH' represent pitaya cultivars of 'Zihonglong' and 'Jinghonglong', respectively. Vertical bars represent the standard errors of the means of triplicate assays.

in the late storage period (from day 4 to 10), for both the 'ZH' and 'JM' fruits.

Figure 2c and 2d show that the relative membrane permeabilities of the pitaya fruits exhibited an upward trend throughout the storage period. Furthermore, these permeabilities were found to be lower in the MT treatment groups than those in the control groups. After storage for 10 days, the relative membrane permeabilities of the MT treatment groups in the 'ZH' and 'JM' pitaya fruits were 33.6% and 34.5%, respectively. These values were lower than those of their respective control groups at 38.9% and 41.5%, respectively. Thus, the MT treatment on the pitaya cultivars was found to inhibit their relative membrane permeabilities, and the differences between the MT and control groups were significant throughout the storage period.

In the 'ZH' fruits, the total soluble solids contents in the control group initially decreased, exhibited a subsequent increase, and then decreased again as the storage period was prolonged for 'ZH' fruit. On the other hand, the control group of the 'JH' fruits exhibited a gradual decrease throughout the same storage period. In the MT treatment groups, there was no change in the content of the total soluble solids of the 'ZH' and 'JH' pitaya fruits, throughout the storage period (Figure 2e, 2f).

Based on the results shown in Figure 2g and 2h, it can be concluded that the ascorbic acid content decreased in both the 'ZH' and 'JH' pitaya fruits throughout the storage period

of 10 days. On the other hand, this decrease in ascorbic acid content was significantly delayed in the MT treatment groups. By the end of the storage period, the ascorbic acid content in the control and MT treatment groups of the 'ZH' fruits was 13.5 and 14.6 mg/100 g, respectively, and that of the 'JH' fruits was 13.0 and 14.3 mg/100 g, respectively.

3.3 Effect of MT treatment on the MDA content, $\text{O}_2^{\cdot-}$ production rate, H_2O_2 content, and LOX activity of the pitaya fruits

MDA content is an indicator of the degree of lipid peroxidation in cell membranes. Therefore, the higher the MDA content, the more serious the cell damage. Throughout the storage period, MDA content increased gradually but was significantly lower in the MT treatment group ($P < 0.05$) than in the control group (Figure 3a, 3b). At the end of the storage period, the MDA content in the control and MT treatment groups of the 'ZH' fruits was 2.2 and 1.7 $\mu\text{mol/g}$, respectively, and that of the 'JH' fruits was 2.3 and 1.7 $\mu\text{mol/g}$, respectively. Hence, MT treatment inhibited the increase in MDA content of the pitaya fruits during storage.

The $\text{O}_2^{\cdot-}$ production rates in the corresponding control groups of the 'ZH' and 'JH' pitaya fruits gradually increased with the prolongation of storage period, while production rates were more delayed in the MT treatment groups (Figure 3c, 3d). After a storage period of 10 days, the $\text{O}_2^{\cdot-}$ production rates in the MT

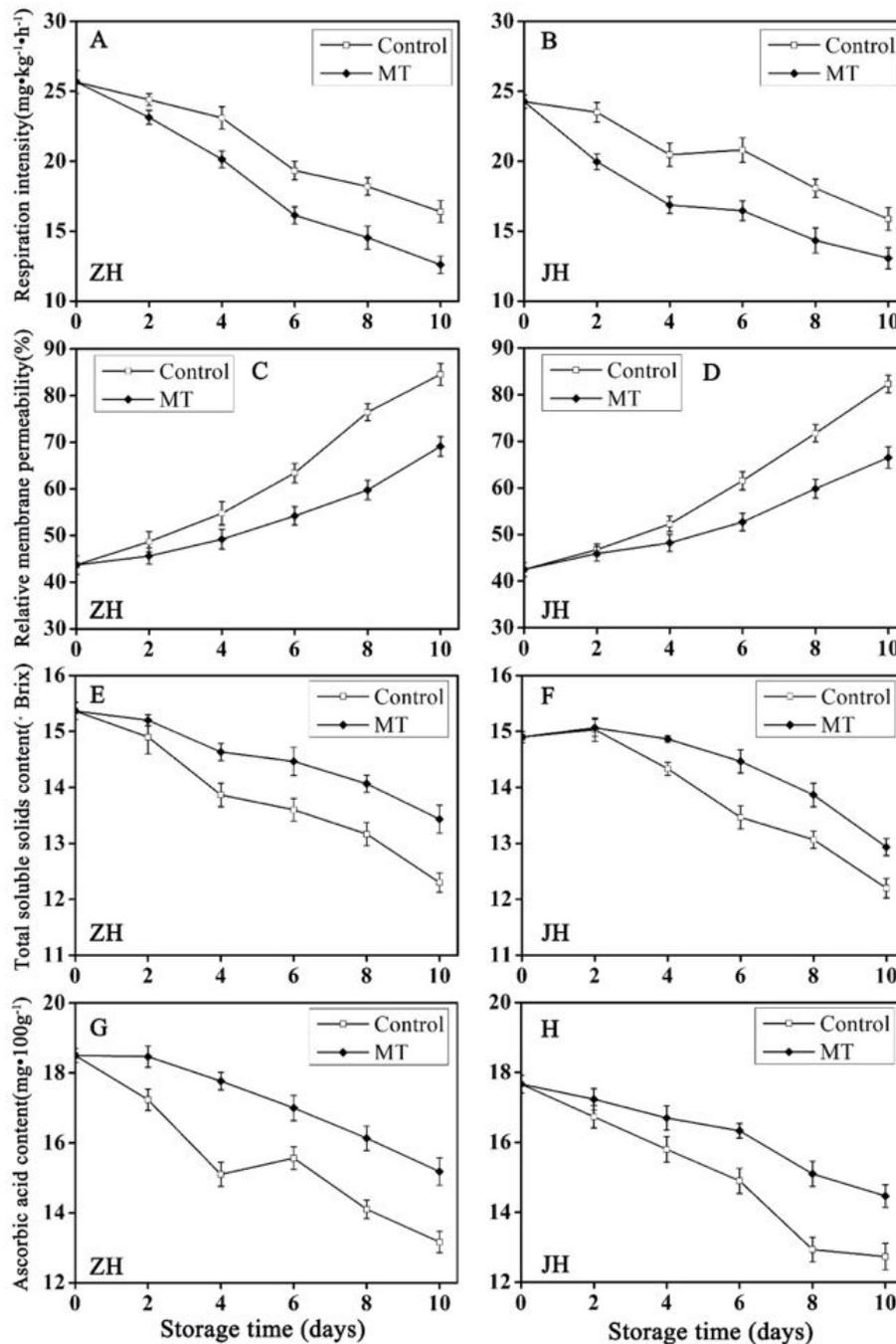


Figure 2. Respiration intensity (A, 'ZH'; B, 'JH'), relative membrane permeability (C, 'ZH'; D, 'JH'), total soluble solids content (E, 'ZH'; F, 'JH') and ascorbic acid content (G, 'ZH'; H, 'JH') of pitaya fruits after treatment with 0.1 mmol·L⁻¹ MT during storage at (20 ± 0.5)°C for 10 days. 'ZH' and 'JH' represent pitaya cultivars of 'Zihonglong' and 'Jinghonglong', respectively. Vertical bars represent the standard errors of the means of triplicate assays.

treatment groups were 0.78 and 0.81 nmol/g/min for the 'ZH' and 'JM' pitaya fruits, respectively. These were lower than the production rates of their corresponding control groups, which were 0.91 and 0.90 nmol/g/min, respectively.

Similarly, throughout the storage period, the H₂O₂ contents of the pitaya fruits in the MT treatment groups were significantly lower than those in the control groups. Compared with the control groups, the pitaya fruits of the MT treatment groups

exhibited a reduction in H₂O₂ content, by the end of the storage period, of 16.7% and 11.9% for the 'ZH' and 'JM' pitaya fruits, respectively (Figure 3e, 3f).

In the 'ZH' pitaya fruits, the LOX activities in the control and MT treatment groups gradually increased throughout the storage period, with the values in the MT treatment groups being significantly lower compared with the control groups. Although the MT treatment groups exhibited gradual

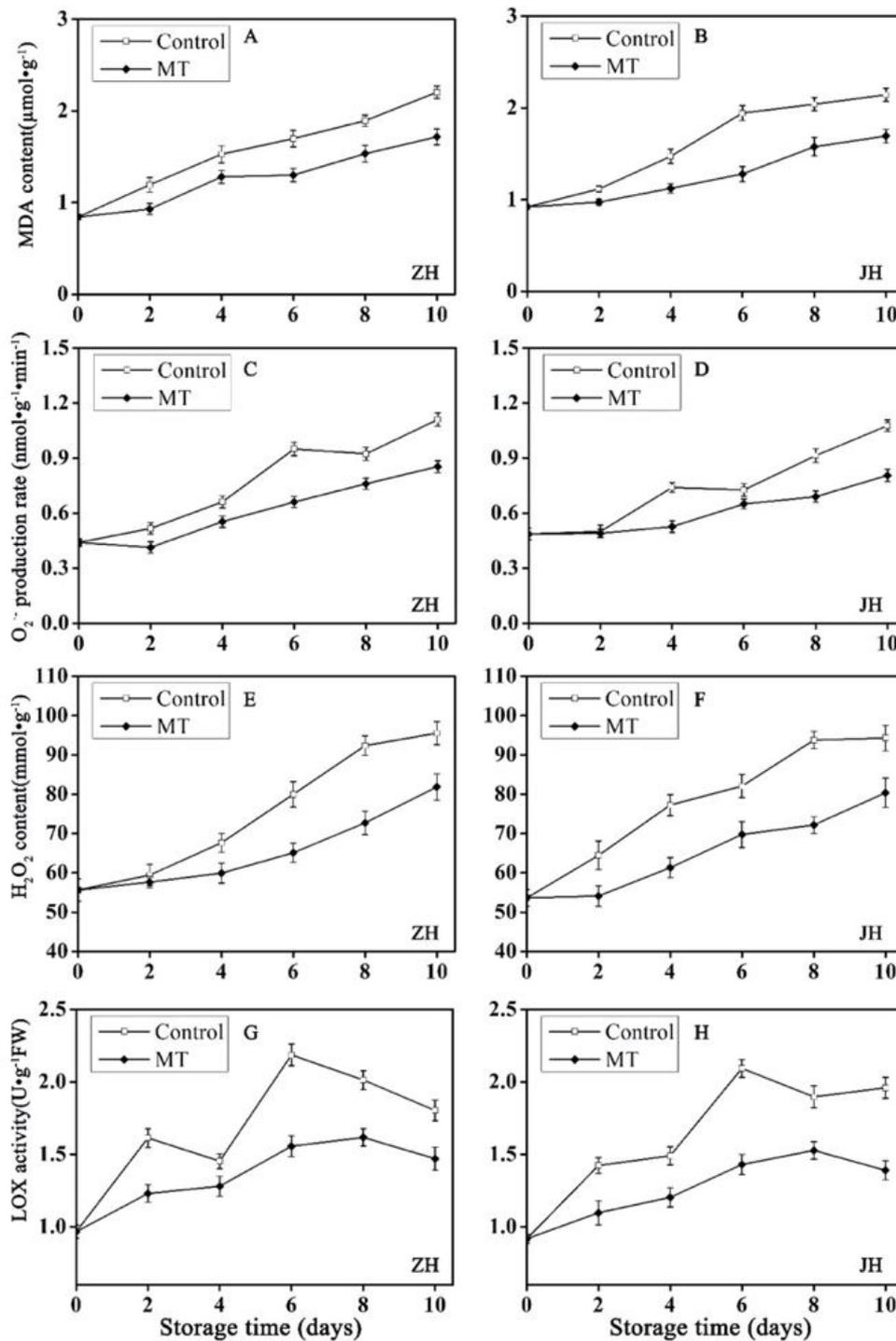


Figure 3. MDA content (A, 'ZH'; B, 'JH'), O₂^{·-} production rate (C, 'ZH'; D, 'JH'), H₂O₂ content (E, 'ZH'; F, 'JH') and LOX activity (G, 'ZH'; H, 'JH') of pitaya fruits after treatment with 0.1 mmolL⁻¹ MT during storage at (20 ± 0.5)°C for 10 days. 'ZH' and 'JH' represent pitaya cultivars of 'Zihonglong' and 'Jinghonglong', respectively. Vertical bars represent the standard errors of the means of triplicate assays.

decreases in LOX activities during the first 4 days of storage, after the initial 4 days, they tended to start increasing, reaching maximum values by day 8 in the 'JH' pitaya fruits. On the other hand, the control groups exhibited gradual increases in LOX activities, with significantly higher values than those of the MT treatment groups throughout the storage period (Figure 3g, 3h).

3.4 Effect of MT treatment on the activities of antioxidant enzymes in pitaya fruits

The SOD activities in the MT treatment groups slowly increased in the first 4 days of the storage period but then began decreasing until the end of the period in the 'ZH' fruits, reaching a peak value of 84.0 U/g. This trend was observed in

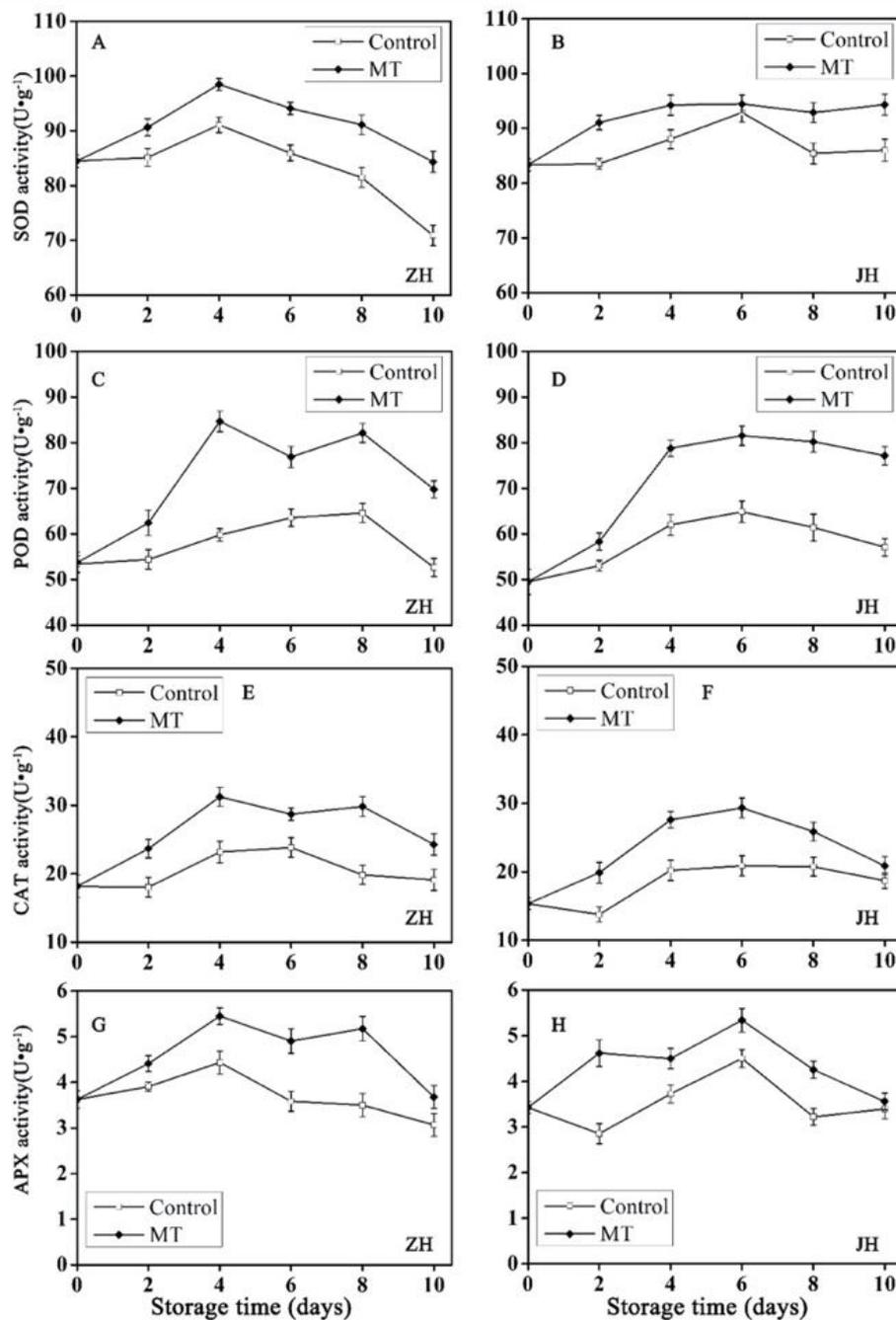


Figure 4. Activities of SOD (A, 'ZH'; B, 'JH'), POD (C, 'ZH'; D, 'JH'), CAT (E, 'ZH'; F, 'JH') and APX (G, 'ZH'; H, 'JH') of pitaya fruits after treatment with 0.1 mmolL⁻¹ MT during storage at (20 ± 0.5)°C for 10 days. 'ZH' and 'JH' represent pitaya cultivars of 'Zihonglong' and 'Jinghonglong', respectively. Vertical bars represent the standard errors of the means of triplicate assays.

both the control and MT treatment groups, with the control having significantly lower SOD activities than the MT treatment groups throughout the storage period (Figure 4a). However, in the 'JH' fruits, SOD activities fluctuated throughout the storage period, with the activities of the MT treatment groups being higher than those in the control groups (Figure 4b).

The POD activities in the pitaya fruits initially increased and then decreased throughout the storage period (Figure 4c, 4d), with the MT treatment groups having higher values compared with the control groups. The POD activities in the control and

MT treatment groups reached maximum values of 59.8 and 66.3 U/g, respectively, in the 'ZH' fruits and of 53.0 and 58.0 U/g, respectively, in the 'JH' fruits. Moreover, the MT treatment groups had significantly higher POD activities than the control groups throughout most of the storage period.

The CAT activities in the MT treatment groups of the 'ZH' and 'JH' fruits exhibited trends of initial increases throughout first 6 days of the storage period, followed by decreases until the end of the storage period. On the other hand, the CAT activities in the control groups exhibited initial declines throughout the

first 2 days of the storage period, followed by increases after 6 days, and finally, decreases until the end of the storage period. Additionally, the activities in the MT treatment groups remained higher than those of the control groups throughout most of the storage period (Figure 4e, 4f).

As shown in Figure 4g and 4h, the APX activities in the MT treatment groups were consistently higher than those in the control groups, throughout the storage period, for the 'JH' fruits. The same trend was observed in the 'ZH' fruits, except for the first 2 days. At the end of storage period, APX activities in the MT treatment groups were 3.2 U/g and 3.1 U/g for the 'ZH' and 'JM' fruits, respectively. These were higher than the activities in the corresponding control groups for the 'ZH' and 'JM' fruits by 52.9% and 51.6%, respectively.

4 Discussion

The senescence of pitaya fruits is generally associated with an increase in decay incidence and weight loss, as well as reduction in contents of total soluble solids and ascorbic acid (Nerd et al., 1999; Hoa et al., 2006; Fan et al., 2018). In the current study, dipping 'ZH' and 'JM' pitaya fruits in 0.1 mmol/L MT postharvest reduced decay incidence, weight loss, and relative membrane permeability, as well as maintained the contents of total soluble solids and ascorbic acid, throughout storage at 20°C. Therefore, the use of MT delayed the development of senescence and preserved the quality of the pitaya fruits.

These findings are similar to those of previous studies, in which exogenous MT treatment was found to increase chlorophyll content and delay senescence in apple leaves (Wang et al., 2013), as well as delay postharvest senescence in peach fruits (Gao et al., 2016). These findings were found to be associated with lowered ROS levels and enhanced activity of APX, monodehydroascorbate reductase (MDAR), dehydroascorbate reductase (DHAR), and glutathione reductase (GR) (Wang et al., 2012; Wang et al., 2013). Previous studies have also shown that indoleacetic acid (IAA) effectively delayed ripening and senescence in bananas (Vendrell, 1969) and avocados (Tingwa & Young, 1975), which had been attributed to MT and IAA having tryptophan as a common biosynthetic precursor. On the other hand, some studies have shown that 50 µM MT treatment significantly accelerated the ripening and senescence of tomato fruits postharvest (Sun et al., 2015). The discrepancy between the findings of this study and those of Sun et al. (2015), suggests that the effects of MT may vary according to species, harvest maturity, processing conditions, or treatment time.

In the process of fruit senescence, ROS accumulate significantly, membrane lipid peroxidation intensifies, and permeability of cell membranes increases. Additionally, the accumulation of ROS is considered to be an important factor in the induction and promotion of senescence in fruits and vegetables (Shewfelt & Rosario, 2000). Normally, there is a dynamic balance between the production and scavenging of free radicals in plants, and a disruption of this balance causes oxygen free radicals to accumulate. When accumulation continues to occur to a certain extent, the oxidation and decomposition of unsaturated fatty acids in cell membranes by free radicals increases, resulting in damage to

the membrane structures of plants. In fruit senescence, loss of membrane integrity is strongly associated with excessive ROS accumulation, which is caused by O_2^- and H_2O_2 production, elevation of MDA content, and increased LOX activity (Wu et al., 2006; Montero-Prado et al., 2011; Yang et al., 2014). In addition, it has been previously demonstrated that MT treatment delayed senescence and inhibited the increase in MDA content, O_2^- production, H_2O_2 content, and LOX activity in peaches during the postharvest storage (Gao et al., 2016). This is consistent with our study, in which MDA content, LOX activity, O_2^- production, and H_2O_2 content were significantly reduced by MT treatment in both 'JH' and 'ZH' pitaya fruits. This suggests that MT treatment lowers the membrane lipid peroxidation of pitaya fruits, thereby enhancing their ability to resist aging-induced oxidative stress.

The metabolism of ROS is closely related to the ripening and senescence of fruits and is one of the main components of postharvest biological research (Gonzalez-Aguilar et al., 2010). SOD, POD, CAT, and APX are antioxidant enzymes that are pivotal to ROS scavenging, wherein SOD scavenges O_2^- and CAT, APX, and POD synergistically scavenge H_2O_2 (Mittler, 2002). It has been reported that the high activities of these antioxidant enzymes and their synergistic effects are involved in the inhibition of lipid peroxidation and in delaying senescence of horticultural fruits. In pitaya fruits, the senescence delay observed in lower temperatures has been attributed to the simultaneous enhancement of POD, SOD, polyphenol oxidase (PPO), phenylalanine ammonia-lyase (PAL), and CAT (Liu et al., 2013).

The current study showed that the activities of SOD, POD, CAT, and APX were simultaneously enhanced after MT treatment, as observed by the reduction in O_2^- and H_2O_2 levels in the 'ZH' and 'JH' pitaya fruits. Therefore, MT treatment enhanced the activities of antioxidant enzymes and delayed the senescence of pitaya fruits. This effect can be attributed to the maintenance of the balance of ROS metabolism, leading to an inhibition of lipid peroxidation.

Non-enzymatic antioxidants also play an essential role in preventing ROS-induced oxidative damage to cells during senescence. It has been reported that ascorbic acid is a non-enzymatic antioxidant responsible for the direct scavenging of ROS (Liu et al., 2015). Our study showed that the levels of ascorbic acid were maintained in the pitaya fruits given MT treatment and stored at 20°C. This is supported by the previously mentioned study involving MT treatment on peaches, which had similar findings (Gao et al., 2016). These studies showed that non-enzymatic antioxidants may be involved in the MT-mediated delay in senescence of pitaya fruits.

Many studies have shown that the endogenous MT contents in fruits vary according to tissue, cultivar, or species as the ripening process progresses. High levels of MT were detected in ripening tomatoes, whereas low levels were detected during non-ripening periods (Dubbels et al., 1995; Van Tassel et al., 2001). However, the MT contents in grapes were observed to gradually decrease during ripening (Murch et al., 2010). These results suggest that the MT has a complex mechanism in regulating ripening and senescence in fruits. Therefore, it is necessary to further study the effects of MT on pitaya fruits in different fruit development stages, in order to further the understanding of its effects on postharvest, aging fruits.

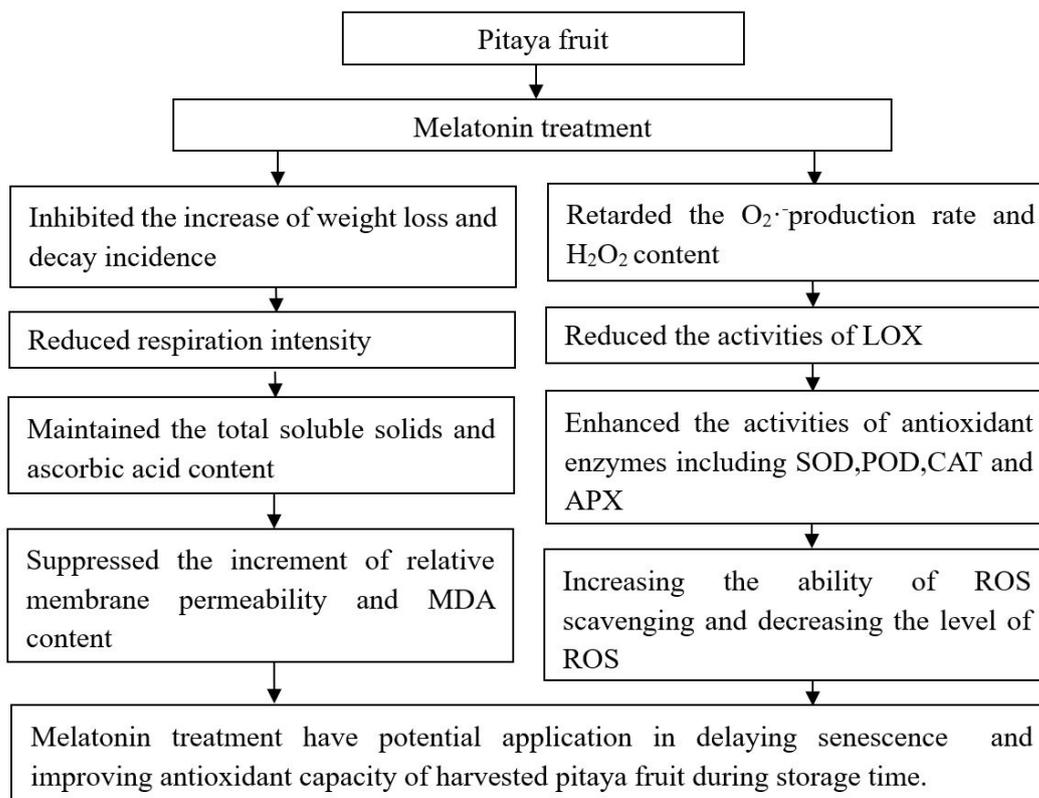


Figure 5. The probable mechanism of exogenous melatonin treatment-delayed senescence of different varieties harvested pitaya fruits through regulating ROS metabolism.

In conclusion, the application of 0.1 mmol/L MT effectively delayed senescence and maintained the quality of pitaya fruits. This effect can be attributed to its ability to mediate activities of antioxidant enzymes. Thus, we believe that MT treatment has the potential to delay senescence, as well as maintain the postharvest quality, of pitaya fruits. The probable mechanism responsible is summarized in Figure 5.

Acknowledgements

This research was funded by a grant from the National Natural Science Foundation of China (31801604) and The Science and Technology Foundation of Guizhou Province (Grant No. [2019]1007). This work was financially supported by Discipline and Master's Site Construction Project of Guiyang University by Guiyang City Financial Support Guiyang University [SY-2020].

References

- Arnao, M. B., & Hernández-Ruiz, J. (2007). Melatonin in plants: more studies are necessary. *Plant Signaling & Behavior*, 2(5), 381-382. <http://dx.doi.org/10.4161/psb.2.5.4260>. PMID:19704606.
- Arnao, M. B., & Hernández-Ruiz, J. (2009). Protective effect of melatonin against chlorophyll degradation during the senescence of barley leaves. *Journal of Pineal Research*, 46(1), 58-63. <http://dx.doi.org/10.1111/j.1600-079X.2008.00625.x>. PMID:18691358.
- Dhindsa, R. S., Plumb-Dhindsa, P., & Thorpe, T. A. (1981). Leaf senescence correlated with increased levels of membrane permeability and lipid peroxidation and decreased levels of superoxide dismutase and catalase. *Journal of Experimental Botany*, 32(1), 93-101. <http://dx.doi.org/10.1093/jxb/32.1.93>.
- Dionísio, A. P., Carvalho-silva, L. B., Vieira, N. M., Wurlitzer, N. J., Pereira, A. C., Borges, M. F., Garruti, D. S., & Araujo, I. S. (2020a). Antioxidant and prebiotic effects of a beverage composed by tropical fruits and yacon in alloxan-induced diabetic rats. *Food Science and Technology (Campinas)*, 40(1), 202-208. <http://dx.doi.org/10.1590/fst.34518>.
- Dionísio, A. P., Silva, M. F. G., Carioca, A. A. F., Adriano, L. S., Abreu, F. A. P., Wurlitzer, N. J., Pinto, C. O., & Pontes, D. F. (2020b). Effect of yacon syrup on blood lipid, glucose and metabolic endotoxemia in healthy subjects: a randomized, double-blind, placebo-controlled pilot trial. *Food Science and Technology (Campinas)*, 40(1), 194-201. <http://dx.doi.org/10.1590/fst.38218>.
- Du, D. D., Zhong, R. B., & Huang, Y. H. (2018). Effects of three kinds of fungicides on postharvest physiology and quality of pitaya fruits. *Baoxian Yu Jiagong*, 18(1), 14-20. <http://dx.doi.org/10.3969/j.issn.1009-6221.2018.01.003>.
- Dubbels, R., Reiter, R. J., Klenke, E., Goebel, A., Schnakenberg, E., Ehlers, C., Schiwwa, H. W., & Schloot, W. (1995). Melatonin in edible plants identified by radioimmunoassay and by high performance liquid chromatography-mass spectrometry. *Journal of Pineal Research*, 18(1), 28-31. <http://dx.doi.org/10.1111/j.1600-079X.1995.tb00136.x>. PMID:7776176.
- Fan, P., Huber, D. J., Su, Z. H., Hu, M. J., Gao, Z. Y., Li, M., Shi, X. Q., & Zhang, Z. K. (2018). Effect of postharvest spray of apple polyphenols on the quality of fresh-cut red pitaya fruit during shelf life. *Food Chemistry*, 243, 19-25. <http://dx.doi.org/10.1016/j.foodchem.2017.09.103>. PMID:29146327.

- Gao, H., Zhang, Z. K., Chai, H. K., Cheng, N., Yang, Y., Wang, D. N., Yang, T., & Cao, W. (2016). Melatonin treatment delays postharvest senescence and regulates reactive oxygen species metabolism in peach fruit. *Postharvest Biology and Technology*, 118, 103-110. <http://dx.doi.org/10.1016/j.postharvbio.2016.03.006>.
- Gonzalez-Aguilar, G. A., Villa-Rodriguez, J. A., Ayala-Zavala, J. F., & Yahia, E. M. (2010). Improvement of the antioxidant status of tropical fruits as a secondary response to some postharvest treatments. *Trends in Food Science & Technology*, 21(10), 475-482. <http://dx.doi.org/10.1016/j.tifs.2010.07.004>.
- Grom, L. C., Rocha, R. S., Balthazar, C. F., Guimarães, J. T., Coutinho, N. M., Barros, C. P., Pimentel, T. C., Venâncio, E. L., Collopy, I. Jr., Maciel, P. M. C., Silva, P. H. F., Granato, D., Freitas, M. Q., Esmerino, E. A., Silva, M. C., & Cruz, A. G. (2020). Postprandial glycemia in healthy subjects: which probiotic dairy food is more adequate? *Journal of Dairy Science*, 103(2), 1110-1119. <http://dx.doi.org/10.3168/jds.2019-17401>. PMID:31785881.
- Ho, T. T., Clark, C. J., Waddell, B. C., & Woolf, A. B. (2006). Postharvest quality of dragon fruit (*Hylocereus undatus*) following disinfesting hot air treatments. *Postharvest Biology and Technology*, 41(1), 62-69. <http://dx.doi.org/10.1016/j.postharvbio.2006.02.010>.
- Jiang, T. J., Feng, L. F., & Li, J. R. (2012). Changes in microbial and postharvest quality of shiitake mushroom (*Lentinus edodes*) treated with chitosan-glucose complex coating under cold storage. *Food Chemistry*, 131(3), 780-786. <http://dx.doi.org/10.1016/j.foodchem.2011.08.087>.
- Kochba, J., Lavee, S., & Spiegel-Roy, P. (1997). Difference in peroxidase activity and isoenzymes in embryogenic and non-embryogenic 'Shamouti' orange ovular callus lines. *Plant & Cell Physiology*, 18(2), 463-467. <http://dx.doi.org/10.1093/oxfordjournals.pcp.a075455>.
- Le Bellec, F., Vaillant, F., & Imbert, E. (2006). Pitahaya (*Hylocereus* spp.): a new fruit crop, a market with a future. *Fruits*, 61(4), 237-250. <http://dx.doi.org/10.1051/fruits:2006021>.
- Liu, H., Cao, J. K., & Jiang, W. B. (2015). Evaluation and comparison of vitamin C, phenolic compounds, antioxidant properties and metal chelating activity of pulp and peel from selected peach cultivars. *Lebensmittel-Wissenschaft + Technologie*, 63(2), 1042-1048. <http://dx.doi.org/10.1016/j.lwt.2015.04.052>.
- Liu, S. Z., Sun, R., Jiang, Y. L., & Hu, W. R. (2013). Effect of storage temperature on quality and senescence of pitaya. *Shipin Kexue*, 34(12), 336-340. <http://dx.doi.org/10.7506/spkx1002-6630-201312070>.
- Mittler, R. (2002). Oxidative stress: antioxidants and stress tolerance. *Trends in Plant Science*, 7(9), 405-410. [http://dx.doi.org/10.1016/S1360-1385\(02\)02312-9](http://dx.doi.org/10.1016/S1360-1385(02)02312-9). PMID:12234732.
- Montero-Prado, P., Rodriguez-Lafuente, A., & Nerin, C. (2011). Active label-based packaging to extend the shelf-life of "Calanda" peach fruit: Change in fruit quality and enzymatic activity. *Postharvest Biology and Technology*, 60(3), 211-219. <http://dx.doi.org/10.1016/j.postharvbio.2011.01.008>.
- Murch, S. J., Hall, B. A., Le, C. H., & Saxena, P. K. (2010). Changes in the levels of indoleamine phytochemicals during véraison and ripening of wine grapes. *Journal of Pineal Research*, 49(1), 95-100. <http://dx.doi.org/10.1111/j.1600-079X.2010.00774.x>. PMID:20536685.
- Narvaez-Cuenca, C., Espinal-Ruiz, M., & Restrepo-Sanchez, L. (2011). Heat shock reduces both chilling injury and the overproduction of reactive oxygen species in yellow pitaya (*hylocereus megalanthus*) fruits. *Journal of Food Quality*, 34(5), 327-332. <http://dx.doi.org/10.1111/j.1745-4557.2011.00398.x>.
- Nerd, A., Gutman, F., & Mizrahi, Y. (1999). Ripening and postharvest behaviour of fruits of two *hylocereus* species (*Cactaceae*). *Postharvest Biology and Technology*, 17(1), 39-45. [http://dx.doi.org/10.1016/S0925-5214\(99\)00035-6](http://dx.doi.org/10.1016/S0925-5214(99)00035-6).
- Niu, J. P., Hou, Z., Ou, Z. F., & Hui, W. (2018). Comparative study of effects of resveratrol, 1-MCP and DPA treatments on postharvest quality and superficial scald of 'Starkrimson' apples. *Scientia Horticulturae*, 240, 516-521. <http://dx.doi.org/10.1016/j.scienta.2018.06.037>.
- Ong, Y. Y., Tan, W. S., Mohamad, R., Sieo, C. C., & Tey, B. T. (2014). Biochemical and molecular identification of *Enterococcus* spp. from red pitaya. *Process Biochemistry*, 49(4), 563-568. <http://dx.doi.org/10.1016/j.procbio.2014.01.019>.
- Patterson, B. D., Macrae, E. A., & Ferguson, I. B. (1984). Estimation of hydrogen peroxide in plant extracts using titanium. *Analytical Biochemistry*, 139(2), 487-492. [http://dx.doi.org/10.1016/0003-2697\(84\)90039-3](http://dx.doi.org/10.1016/0003-2697(84)90039-3). PMID:6476384.
- Reiter, R. J., Tan, D. X., Zhou, Z., Cruz, M. H. C., Fuentes-Broto, L., & Galano, A. (2015). Phytomelatonin: assisting plants to survive and thrive. *Molecules (Basel, Switzerland)*, 20(4), 7396-7437. <http://dx.doi.org/10.3390/molecules20047396>. PMID:25911967.
- Shewfelt, R. L., & Rosario, D. (2000). The role of lipid peroxidation in storage disorders of fresh fruits and vegetables. *Hortscience: A Publication of the American Society for Horticultural Science*, 35(4), 575-579. <http://dx.doi.org/10.21273/HORTSCI.35.4.575>.
- Shi, H., Reiter, R. J., Tan, D. X., & Chan, Z. L. (2015). Indole-3-acetic acid inducible 17 positively modulates natural leaf senescence through melatonin-mediated pathway in Arabidopsis. *Journal of Pineal Research*, 58(1), 26-33. <http://dx.doi.org/10.1111/jpi.12188>. PMID:25324183.
- Suh, D. H., Lee, S., Heo, D. Y., Kim, Y. S., Cho, S. K., Lee, S., & Lee, C. H. (2014). Metabolite profiling of red and white pitayas (*Hylocereus polyrhizus* and *Hylocereus undatus*) for comparing betalain biosynthesis and antioxidant activity. *Journal of Agricultural and Food Chemistry*, 62(34), 8764-8771. <http://dx.doi.org/10.1021/jf5020704>. PMID:25101804.
- Sun, Q. Q., Zhang, N., Wang, J. F., Zhang, H. J., Li, D. B., Shi, J., Li, R., Weeda, S., Zhao, B., Ren, S. X., & Guo, Y. D. (2015). Melatonin promotes ripening and improves quality of tomato fruit during postharvest life. *Journal of Experimental Botany*, 66(3), 657-668. <http://dx.doi.org/10.1093/jxb/eru332>. PMID:25147270.
- Surrey, K. (1964). Spectrophotometric method for determination of lipoxidase activity. *Plant Physiology*, 39(1), 65-70. <http://dx.doi.org/10.1104/pp.39.1.65>. PMID:16655881.
- Tingwa, P. O., & Young, R. E. (1975). The effect of indole-3-acetic acid and other growth regulators on the ripening of avocado fruits. *Plant Physiology*, 55(5), 937-940. <http://dx.doi.org/10.1104/pp.55.5.937>. PMID:16659195.
- Van Tassel, D. L., Roberts, N., Lewy, A., & O'Neill, S. D. (2001). Melatonin in plant organs. *Journal of Pineal Research*, 31(1), 8-15. <http://dx.doi.org/10.1034/j.1600-079X.2001.310102.x>. PMID:11485009.
- Van To, L., Ngu, N., Duc, N. D., & Huong, H. T. T. (2002). Dragon fruit quality and storage life: effect of harvesting time, use of plant growth regulators and modified atmosphere packaging. *Acta Horticulturae*, (575), 611-621. <http://dx.doi.org/10.17660/ActaHortic.2002.575.72>.
- Vendrell, M. (1969). Reversion of senescence: effects of 2,4-dichlorophenoxyacetic acid and indoleacetic acid on respiration, ethylene production, and ripening of banana fruit slices. *Australian Journal of Biological Sciences*, 22(3), 601-610. <http://dx.doi.org/10.1071/B19690601>.
- Wang, P., Sun, X., Chang, C., Feng, F. J., Liang, D., Cheng, L. L., & Ma, F. W. (2013). Delay in leaf senescence of *malus hupehensis* by

- long-term melatonin application is associated with its regulation of metabolic status and protein degradation. *Journal of Pineal Research*, 55(4), 424-434. <http://dx.doi.org/10.1111/jpi.12091>. PMID:24103092.
- Wang, P., Yin, L. H., Liang, D., Li, C., Ma, F. W., & Yue, Z. Y. (2012). Delayed senescence of apple leaves by exogenous melatonin treatment: toward regulating the ascorbate-glutathione cycle. *Journal of Pineal Research*, 53(1), 11-20. <http://dx.doi.org/10.1111/j.1600-079X.2011.00966.x>. PMID:21988707.
- Wu, L. C., Hsu, H. W., Chen, Y. C., Chiu, C. C., Lin, Y. I., & Ho, J. A. (2006). Antioxidant and antiproliferative activities of red pitaya. *Food Chemistry*, 95(2), 319-327. <http://dx.doi.org/10.1016/j.foodchem.2005.01.002>.
- Wu, L., Weng, M., Zheng, H., Lai, P., Tang, B., Chen, J., & Li, Y. (2020). Hypoglycemic effect of okra aqueous extract on streptozotocin-induced diabetic rats. *Food Science and Technology (Campinas)*, 40(4), 972-978. <http://dx.doi.org/10.1590/fst.28619>.
- Yang, Z., Cao, S. F., Su, X. G., & Jiang, Y. M. (2014). Respiratory activity and mitochondrial membrane associated with fruit senescence in postharvest peaches in response to UV-C treatment. *Food Chemistry*, 161, 16-21. <http://dx.doi.org/10.1016/j.foodchem.2014.03.120>. PMID:24837916.