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Effects of melatonin treatment on maintenance of the quality of fresh-cut pitaya fruit

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Abstract

The present work investigated the effects of different concentrations of melatonin (0, 50, 100, and 200 μmol L^{-1}) on the quality and antioxidant activity of fresh-cut pitaya (*Hylocereus undatus*) fruits. Results revealed that melatonin treatments significantly increased the firmness, total soluble solids, and titratable acidity of post-harvest fruits, and inhibited the rate of weight loss. When compared with the control treatment, the application of melatonin maintained higher contents of vitamin C and total phenolics in fresh-cut pitaya fruits during storage. Melatonin also decreased cell membrane electrolyte leakage and polyphenol oxidase activity, with 100 μmol L^{-1} melatonin treatment showing the best effects. In addition, 100 μmol L^{-1} melatonin significantly increased the activities of the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), and peroxidase (APX), thus decreasing the accumulation of H_2O_2 and O_2^- during storage. Collectively, these findings indicate that melatonin treatment could contribute to delaying the ripening and senescence of fresh-cut pitaya fruits, and has potential application in the preservation of fresh-cut pitaya fruits during storage.

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Introduction

Pitaya is a highly nutritious tropical and subtropical fruit that contains considerable amounts of betanidin and plant proteins that are rarely found in other fruits (Wu *et al*., 2006; Ong *et al*., 2014). Moreover, pitaya fruit is also a good source of water-soluble dietary fibres, minerals, and vitamins. The consumption of pitaya fruits has been associated with the decrease in cardiovascular diseases, which is attributable to its strong free radical scavenging activities and anti-cancer properties (Dembitsky *et al*., 2011).

With the rapid modernisation of society, healthy, nutritious, and convenient foods are becoming increasingly popular (Toivonen and Brummell, 2008). Fresh-cut fruits are generally supplied to the catering industry or directly to consumers after having been subjected to a series of handling processes such as grading, selection, cleaning, peeling, slicing, and simple packaging (Soliva-Fortuny and Martín-Belloso, 2003; Rico *et al*., 2007). However, when maintained in refrigerated storage, fresh-cut pitaya fruits can become water-soaked and translucent, and readily rots, thereby affecting their quality and nutritional value (Hodges and Toivonen, 2008).

Melatonin, also known as *N*-acetyl-5-methoxytryptamine, is an endogenous indoleamine present in a range of plant tissues (Dubbels *et al*., 1995; Reiter *et al*., 2015). Previous studies have indicated that melatonin treatment could decrease chlorophyll degradation and delay leaf senescence (Arnao and Hernandez-Ruiz, 2009), and also delay the senescence of peach fruits. These effects could be ascribed to a decrease in respiration, and increase in the activities of superoxide dismutase (SOD), peroxidase, catalase (CAT), and other associated enzymes (Gao *et al*., 2016). Similarly, studies on cassava have also revealed that melatonin could increase the activities of CAT and SOD, and decrease the accumulation of H_2O_2, thereby delaying the deterioration of fruit quality (Ma *et al*., 2016). However, the effects of melatonin treatment on the quality of fresh-cut pitaya fruits during storage are yet to be reported. Therefore, the present work aimed to assess the effects of different concentrations of melatonin (0, 50, 100, and 200 μmol L^{-1}) on fresh-cut

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pitaya fruits, by analysing their firmness, rate of weight loss, total soluble solids, titratable acidity, vitamin C (V_C) and phenolic contents, cell membrane electrolyte leakage, and polyphenol oxidase (PPO) activity. The present work also examined the cumulative contents of H_2O_2 and O_2^-, and the activities of SOD, CAT, and ascorbate peroxidase (APX), which are associated with reactive oxygen species production and antioxidant activity in fresh-cut pitaya fruits. Collectively, our findings indicated that melatonin treatment could effectively enhance the storage quality of fresh-cut pitaya fruits, and will provide a theoretical basis for improvements in their storage and shelf life.

Materials and methods

Plant material

The pitaya (*Hylocereus undatus* cv. Jinghonglong) fruits used in the present work were grown in a commercial orchard in Guangling County, Guizhou Province, China. The fruits were harvested by hand, 28 days after pollination, and immediately transported to the Laboratory at Guiyang University. Only fruits devoid of mechanical injury, pests or diseases, and of similar colour and size, were selected for experimental purposes.

The fruits were peeled, and the pulps at the top and bottom were removed. The fruits were then cut into approximately 1-cm-thick pieces, and randomly allocated to one of four groups, each containing 200 fruits. Fruit pieces in the four groups were soaked in 0, 50, 100, or 200 μmol L^{-1} melatonin, respectively, for 2 min, after which they were packed separately in fresh-keeping boxes, and stored at 4°C. The cut fruits were stored for a period of 8 d, during which time samples were collected for analysis at 2-day intervals. Each treatment was conducted in triplicate.

Firmness, weight loss, total soluble solid contents, and titratable acidity

The firmness of fresh-cut pitaya fruits was measured using a TA-XT plus physical property tester equipped with a P6 probe. The device was operated at pre-test, test, and post-test speeds of 1, 1, and 10 mm s^{-1}, respectively, with a displacement of 5 mm, trigger force of 5 g, and the maximum breaking force was used to indicate hardness. Test results were the average of 10 replicate measurements, with values being expressed in terms of kg cm^{-2}.

The rates of weight loss of the fresh-cut pitaya fruits were calculated from the weight before and after the storage period, and expressed as a percentage as compared to the initial weight.

The total soluble solid contents were measured using a hand-held refractometer (PAL-1), and expressed as a percentage.

The titratable acidity was measured using 877 Titrino plus automatic potentiometric titrator. A pulp homogenate was filtered using gauze, then 1 mL of the resulting filtrate was diluted to 100 mL with deionised water, and titrated with 0.05 mol L^{-1} NaOH solution. The volume of the alkali solution used in the titration was recorded and used to calculate the titratable acidity. This test was repeated three times.

Vitamin C and total phenolic contents

The V_C contents were measured using a method modified from that described by Ramful *et al*. (2011). Approximately, 1.0 g of fresh-cut pitaya fruit was ground, mixed with 5.0 mL of 20 g L^{-1} trichloroacetic acid solution, and centrifuged at 10,000 *g* for 15 min. Next, 1.0 mL aliquot of the resulting supernatant was added to 1.0 mL of 20 g L^{-1} trichloroacetic acid and 1.0 mL of absolute ethanol in a test tube. After mixing and shaking, 0.5 mL of 0.4% phosphoric acid-ethanol solution, 1.0 mL of 5 g L^{-1} phenanthroline-ethanol solution, and 0.5 mL of 0.3 g L^{-1} ferric chloride-ethanol solution were added to the mixture, which was then left to react at 30°C for 60 min. The absorbance of the mixture was measured in triplicate, at a wavelength of 534 nm, against a reference blank of the mixture lacking the supernatant. The V_C content in the corresponding mixture was calculated from a standard curve of the absorbance values of ascorbic acid, and the V_C contents in the fresh-cut fruit pulps were calculated and expressed as the vitamin C mass contained in a 100 g sample (mg 100 g^{-1}).

The total phenolic contents were determined using the forinol colorimetric method. Approximately 1.0 g of fresh-cut pitaya was ground and mixed with 5.0 mL of 60% ethanol. Following incubation for 2 h, the extract was centrifuged at 10,000 *g* for 15 min, with 1.0 mL of the resulting supernatant being mixed with 3.0 mL of 1.0 mol L^{-1} phenol reagent in a 25-mL stoppered tube. The mixture was shaken and allowed to stand for 5 min, after which, 6 mL of 7.5% Na_2CO_3 was added followed by dilution to 25 mL with distilled water. A sample without gallic acid served as

the control. Absorbances were read at a wavelength of 760 nm, and the analysis was performed in triplicate. The total phenol contents were calculated using gallic acid as the reference material, with the values being expressed in terms of mg 100 g^{-1} (Ramful *et al*., 2011).

Cell membrane electrolyte leakage and polyphenol oxidase activity

The cell membrane electrolyte leakage was determined using 0.5 g of fresh-cut pitaya pulp. The fruit pulp was washed with double-distilled water, and immersed in 20 mL of double-distilled water for 1 h. The conductivity of the initial solution (D1) was measured using a DDS-307 conductivity meter (Leici, China). The solution was then boiled in a test tube for 30 min, and cooled to room temperature, after which the conductivity of the solution (D2) was again determined and normalised to the conductivity of double-distilled water (D0) (Niu *et al*., 2018). Cell membrane electrolyte leakage was calculated as $\frac{D1-D0}{D2-D0} \times 100$, and expressed as a percentage.

The activity of PPO in fresh-cut pitaya fruits was determined as previously described by Zauberman *et al*. (1991) with appropriate modifications. Briefly, 15 g of pitaya fruit pulp was collected, and after thawing, 15 mL of 0.1 mol L^{-1} sodium phosphate buffer (pH = 6.8) was added. The mixture was ground in an ice bath, centrifuged (4,000 *g*, 10 min), and then the supernatant was collected for analysis. Next, 0.3 mL aliquot of the supernatant was added to 6.7 mL reaction system comprising 4.4 mL of 0.1 mol L^{-1} sodium phosphate buffer (pH 6.8) and 2.0 mL of 0.1 M sodium phosphate buffer (pH = 6.8), and the mixture was left to react at 37°C, with the change in absorbance being measured over a 14-min period at 420 nm. As a blank, we used 0.1 mol L^{-1} sodium phosphate buffer (pH = 6.8). The PPO activity was expressed as the change in absorbance per minute at 0.001 U, and expressed in terms of U g^{-1}. The analysis was repeated three times.

H_2O_2 contents and O_2^- production

The H_2O_2 content was measured using the method described by Patterson *et al*. (1984), with appropriate modifications. Fresh-cut pitaya tissue (5 g) was homogenised in 5 mL of cold acetone, and centrifuged for 15 min at 5,000 *g* and 4°C. Next, 1 mL aliquot of the resulting supernatant was mixed with 0.1 mL of titanium sulphate (22 mM) and 0.2 mL of ammonia, and then centrifuged for 10 min (5,000 *g*, 4°C). The pellet obtained was dissolved in 3 mL of 1 M sulphuric acid, and then centrifuged for 10 min (5,000 *g*, 4°C). The production of H_2O_2 in fresh-cut pitaya fruits was calculated and expressed as mmol g^{-1} (Gao *et al*., 2016).

The measurement of O_2^- production in fresh-cut pitaya tissue (3 g) was based on the method described by Wang and Luo (1990) with appropriate modifications. O_2^- production was calculated using $NaNO_2$ as the standard, and expressed on a fresh weight basis as nmol g^{-1} min^{-1} (Gao *et al*., 2016).

SOD, CAT, and APX activities

The activities of SOD, CAT, and APX were determined as described by Gao *et al*. (2016) with appropriate modifications. For the analysis of each enzyme, approximately 2 g of fresh-cut pitaya tissue was homogenised in appropriate pre-cooling buffers (4°C). The reaction mixture used for SOD activity determination was prepared as previously described by Dhindsa *et al*. (1981), with appropriate modifications. The mixture contained 2.25 mM nitro blue tetrazolium, 50 mM sodium phosphate buffer (pH = 7.8), 30 μmol L^{-1} EDTA,14.5 mM methionine, 60 μmol L^{-1} riboflavin, 2.25 mM nitro blue tetrazolium, and 30 μL of supernatant. The SOD activity was determined at 560 nm based on the inhibition of nitroblue tetrazolium reduction, and expressed as U g^{-1}, where one unit is the amount of SOD that can inhibit the reduction of 50% nitro blue tetrazolium in 1 min (Gao *et al*., 2016).

The CAT activity was determined as described by Zhou *et al*. (2014) with appropriate modifications. Activity against H_2O_2 was determined based on a reduction in absorbance at 240 nm, with one unit of CAT activity being defined as the decomposition of 1 mM H_2O_2 per minute. The results were expressed in terms of units per gram (Zhao *et al*., 2020).

The APX activity was determined based on a reduction in absorbance at 290 nm and recorded for 5 min. One unit of APX activity was defined as the oxidation of 1 mM ascorbic acid per minute, and the results were expressed as U g^{-1} (Nukuntornprakit *et al*., 2015).

Statistical analysis

The study was based on a completely randomised design. All experiments were performed in triplicate to minimise standard error values. Values were then presented as mean ± SE. Significance analysis was conducted using SPSS version 19.0.

Multiple comparisons were performed using ANOVA in conjunction with Duncan's test, whereas pairwise comparisons were performed using Student's *t*-test. Differences were considered statistically significant at the $p < 0.05$ level.

Results

Effect of melatonin treatment on firmness, weight loss, total soluble solids, and titratable acidity of fresh-cut pitaya fruits

Table 1 shows that the firmness of fresh-cut pitaya fruits decreased over the course of the assessed storage period. During the latter period of storage, the firmness of the control treatment underwent the most rapid reduction, from 0.877 kg cm^{-2} on day 0 to 0.792 kg cm^{-2} on day 8, and over the entire storage period decreased by 9.69%. Comparatively, the firmness of fresh-cut pitaya fruits treated with 50, 100, and 200 μmol L^{-1} melatonin decreased by 7.86, 6.16, and 8.32%, respectively. Overall, the decrease in the firmness of fresh-cut pitaya fruits in the melatonin treatment groups was more pronounced as compared to that in the control group, being slowest in fresh-cut pitaya fruits subjected to the 100 μmol L^{-1} melatonin treatment.

Table 1. Effect of different concentrations of exogenous melatonin treatment on changes of quality indicators in fresh-cut pitaya fruits during 8-day storage at 4°C.

Treatment	Storage time (day)	Firmness (kg cm^{-2})	Weight loss (%)	TSS (%)	TA (%)
Control	0	0.877 ± 0.005	0 ± 0	12.166 ± 0.028	0.394 ± 0.003
	2	0.860 ± 0.006^{a}	0.033 ± 0.004^{a}	11.760 ± 0.026^{b}	0.384 ± 0.003^{a}
	4	0.833 ± 0.005^{a}	0.216 ± 0.035^{a}	11.556 ± 0.035^{b}	0.364 ± 0.004^{a}
	6	0.812 ± 0.003^{b}	0.430 ± 0.020^{a}	11.373 ± 0.030^{b}	0.338 ± 0.004^{b}
	8	0.792 ± 0.004^{b}	0.603 ± 0.025^{a}	11.113 ± 0.025^{b}	0.290 ± 0.003^{c}
50 μmol L^{-1}	0	0.877 ± 0.005	0 ± 0	12.166 ± 0.028	0.394 ± 0.003
	2	0.869 ± 0.003^{a}	0.028 ± 0.003^{a}	11.946 ± 0.035^{ab}	0.388 ± 0.003^{a}
	4	0.850 ± 0.005^{a}	0.163 ± 0.025^{a}	11.843 ± 0.015^{b}	0.373 ± 0.004^{a}
	6	0.827 ± 0.002^{ab}	0.310 ± 0.020^{ab}	11.483 ± 0.025^{ab}	0.349 ± 0.003^{ab}
	8	0.808 ± 0.004^{ab}	0.430 ± 0.017^{ab}	11.273 ± 0.028^{ab}	0.313 ± 0.004^{bc}
100 μmol L^{-1}	0	0.877 ± 0.005	0 ± 0	12.166 ± 0.028	0.394 ± 0.003
	2	0.871 ± 0.006^{a}	0.025 ± 0.001^{a}	12.090 ± 0.036^{a}	0.390 ± 0.002^{a}
	4	0.863 ± 0.005^{a}	0.113 ± 0.025^{a}	11.916 ± 0.025^{b}	0.382 ± 0.004^{a}
	6	0.849 ± 0.004^{a}	0.233 ± 0.015^{b}	11.726 ± 0.020^{a}	0.366 ± 0.004^{a}
	8	0.823 ± 0.005^{a}	0.366 ± 0.020^{b}	11.480 ± 0.030^{a}	0.344 ± 0.004^{a}
200 μmol L^{-1}	0	0.877 ± 0.005	0 ± 0	12.166 ± 0.028	0.394 ± 0.003
	2	0.869 ± 0.004^{a}	0.024 ± 0.002^{a}	12.040 ± 0.030^{ab}	0.392 ± 0.004^{a}
	4	0.847 ± 0.004^{a}	0.150 ± 0.010^{a}	11.876 ± 0.046^{b}	0.373 ± 0.004^{a}
	6	0.831 ± 0.005^{ab}	0.366 ± 0.015^{ab}	11.550 ± 0.026^{ab}	0.352 ± 0.001^{ab}
	8	0.804 ± 0.004^{ab}	0.493 ± 0.015^{ab}	11.323 ± 0.035^{ab}	0.328 ± 0.004^{ab}

Values are mean ± standard error (SE) of three biological replicates ($n = 3$). Means followed by different lowercase superscripts are significantly different at $p < 0.05$.

Table 1 also shows that with the progression of storage, there was an increase in the rate at which the weight of fresh-cut pitaya fruits in all four treatment groups was lost, with that in the control group being most marked, increasing from 0 to 0.60% during the assessed storage period. Comparatively, losses of 0.43, 0.37, and 0.49% were recorded for fresh-cut pitaya fruits in the 50, 100, and 200 μmol L^{-1} melatonin treatment groups, respectively, which were significantly lower than those in the control group. These observations indicated that melatonin treatment could effectively inhibit the rate of weight loss in fresh-cut pitaya fruits, with 100 μmol L^{-1} melatonin being the most effective treatment.

The total soluble solid (TSS) contents of fresh-cut fruits and vegetables reflect their sweetness index

during storage (Gonzalez-Aguilar *et al*., 2011). Table 1 shows that the TSS contents decreased with the progression of storage. On day 8 of storage, the TSS contents in the control group was 11.11%, as compared to that in the 50, 100, and 200 μmol L^{-1} melatonin groups that was notably higher, thus indicating that melatonin treatment could delay the loss of soluble solids in fresh-cut pitaya fruits during storage.

Table 1 further shows that the titratable acidity (TA) of fresh-cut pitaya fruits gradually decreased during storage. On the final day of the assessed storage period, the TA of control group fruit was 0.29%, which represented a 26.40% reduction over the storage period. In comparison, decreases of 20.56, 12.69, and 16.75% were detected in fresh-cut pitaya fruits subjected to the 50, 100, and 200 μmol L^{-1} melatonin treatments, respectively. These indicated that melatonin could delay the decrease in TA of fresh-cut pitaya fruits during storage, thus maintaining their quality. Melatonin at 100 μmol L^{-1} was again found to be the most effective treatment.

Effects of melatonin treatment on vitamin C and phenolic contents in fresh-cut pitaya fruits

Fresh-cut pitaya fruits are a rich source of V_c and other nutrients, and as shown in Figure 1A, with the progression of storage, there was a gradual decrease in the contents of V_c in fresh-cut pitaya fruits subjected to all four treatments. On day 8 of storage, the level of V_c in the control group was 61.62% of that measured on day 0, whereas corresponding values of 70.75, 82.20, and 77.60% were obtained for fresh-cut pitaya fruits treated with 50, 100, and 200 μmol L^{-1} melatonin, respectively. Therefore, melatonin treatment could be used to effectively delay the decrease in V_c contents of fresh-cut pitaya fruits during storage, with the effect obtained using 100 μmol L^{-1} melatonin being the most obvious.

The data presented in Figure 1B show that during storage, the total phenolic contents of fresh-cut pitaya fruits initially underwent a slow increase, peaking on day 4, after which there was rapid decrease. During this initial phase, total phenolic contents showed significant increases in response to treatment with the three different concentrations of melatonin, and these were significantly higher than that in the control group at the end of the 8-day period of storage. However, we detected no significant differences among the three melatonin treatments.

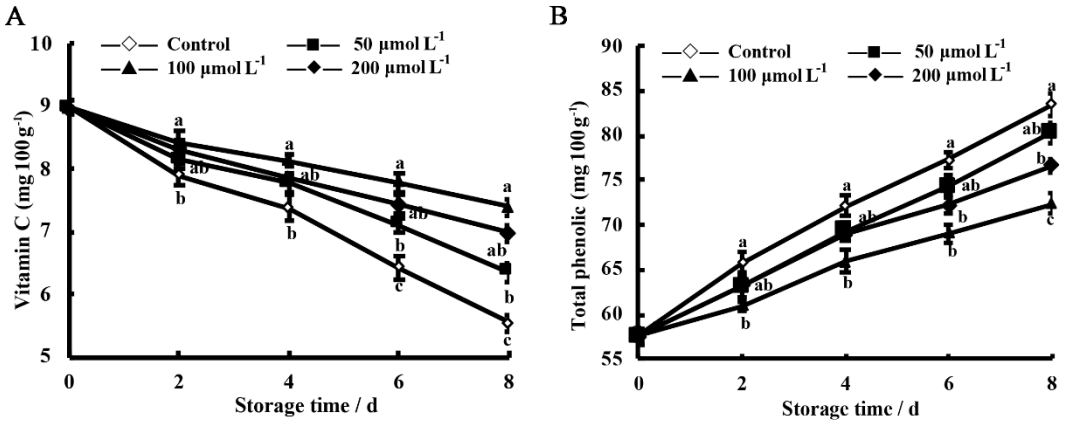

Figure 1. Effect of different concentrations of exogenous melatonin treatment on changes of vitamin C **(A)** and total phenolic **(B)** in fresh-cut pitaya fruits during 8-day storage at 4℃. Values are mean ± standard error (SE) of three biological replicates (n = 3). Means followed by different lowercase letters are significantly different at $p < 0.05$.

Effect of melatonin treatment on cell membrane electrolyte leakage and PPO activity in fresh-cut pitaya fruits

Figure 2A shows that the cell membrane electrolyte leakage increased gradually with the progression of storage, with the levels being higher in control group than in fresh-cut pitaya fruits treated with each of the three concentrations of melatonin. On day 8 of storage, the electrolyte leakage measured in the control group was 78.37%, which was 1.07-, 1.21-, and 1.14-fold higher than recorded for the 50, 100, and 200 μmol L^{-1} melatonin groups, respectively. Moreover, we detected significant differences among the four treatments. These indicated that melatonin treatment could effectively delay the increase in relative conductivity, thus

contributing to retarding the increase in cell membrane breakdown. Again, the 100 μmol L^{-1} melatonin treatment was identified as the best treatment.

As shown in Figure 2B, the PPO activities of fresh-cut pitaya fruits initially increased and subsequently decreased during storage, reaching maximum values on day 6 of storage. On day 8 of storage, the PPO activity in the control group was 41.37 U g^{-1}, whereas comparatively, the values recorded in fresh-cut pitaya fruits treated with 50, 100, and 200 μmol L^{-1} melatonin were respectively 93.62, 90, and 90.62% of the control fruit value, thus indicating that the degree of the inhibitory effect of melatonin decreased in the order of 100 μmol L^{-1} > 200 μmol L^{-1} > 50 μmol L^{-1}. Consequently, we established that melatonin treatment could significantly inhibit an increase in PPO activity, thus inhibiting the browning of fresh-cut pitaya fruits during storage.

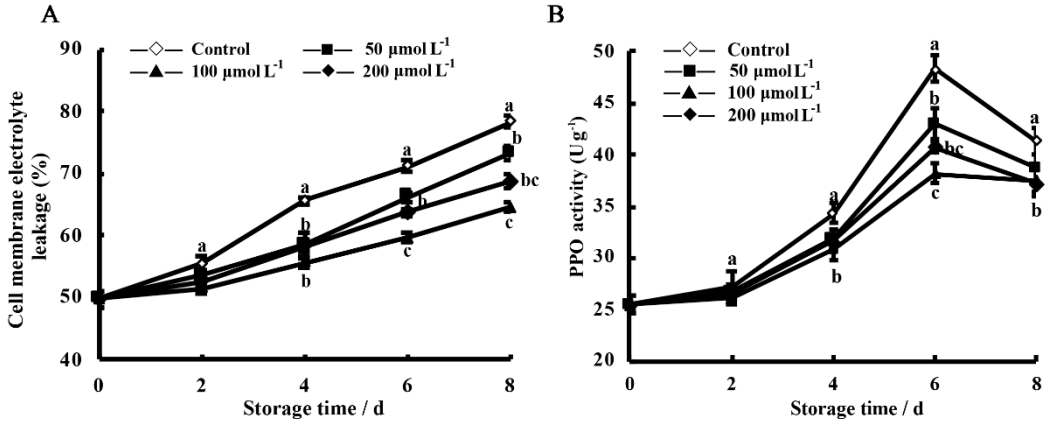

Figure 2. Effect of different concentrations of exogenous melatonin treatment on changes of cell membrane electrolyte leakage **(A)** and PPO activity **(B)** in fresh-cut pitaya fruits during 8-day storage at 4°C. Values are mean ± standard error (SE) of three biological replicates (n = 3). Means followed by different lowercase superscripts are significantly different at $p < 0.05$.

Effects of melatonin treatment on H_2O_2 contents and O_2^- production in fresh-cut pitaya fruits

Given the observed superior effect of melatonin applied at 100 μmol L^{-1} in maintaining the quality of fresh-cut pitaya fruits, we used this as a treatment in further experiments. As shown in Figure 3A, the contents of H_2O_2 in the control group increased with the progression of storage, reaching a maximum value on the final day, increasing from 19.64 mmol kg^{-1} on day 0 to 23.27 mmol kg^{-1} on day 8. Contrastingly, in response to melatonin treatment at 100 μmol L^{-1}, an increase of only 1.43% was detected at the end of the storage when compared with that recorded on the first day. Melatonin could thus be used to inhibit the accumulation of H_2O_2 in fresh-cut pitaya fruits, thereby delaying the aging process.

O_2^- production showed a single-peak trend; however, the peak value and its time of occurrence in the melatonin treatment group differed from those recorded for the control group (Figure 3B). O_2^- production in the control group fruit increased rapidly with the progression of storage, reaching a maximum value on day 4, and thereafter, underwent a gradual decrease. In contrast, O_2^- production peaked on day 6 of storage in melatonin-treated fresh-cut pitaya fruits, and was found to be significantly inhibited during the initial four days of storage. These indicated that melatonin could effectively decrease O_2^- production during the early period of storage.

Effects of melatonin treatment on SOD, CAT, and APX activities in fresh-cut pitaya fruits

The SOD activity was found to increase gradually with the progression of storage, reaching maximum values in control and 100 μmol L^{-1} melatonin groups at 42.37 and 47.08 U g^{-1}, respectively (Figure 4A). The trend in CAT activity was relatively similar to that observed for SOD, with the activities in both control and treatment groups peaking on day 6 of storage, and with activity in the melatonin-treated fresh-cut pitaya fruits being higher than that in control (Figure 4B). Consistently, APX activities in the control and melatonin treatments increased gradually, and reached a peak on day 6 (Figure 4C). At the end of the storage period, APX activity in the 100 μmol L^{-1} melatonin-treated fresh-

cut pitaya fruits was found to be 12.83% higher than the control group value of 3.74 U g^{-1}, which represented a significant difference. Thus, treating fresh-cut pitaya fruits with 100 μmol L^{-1} melatonin could significantly increase the activities of the antioxidant enzymes SOD, CAT, and APX during storage.

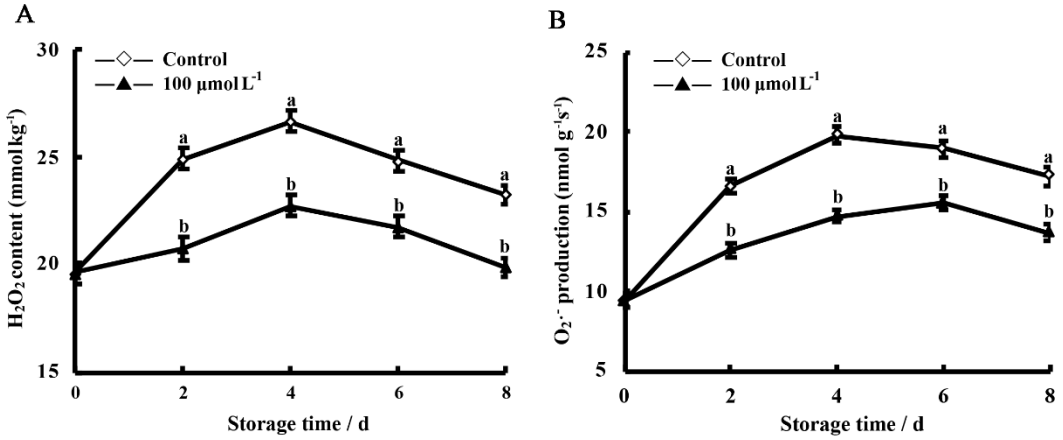

Figure 3. Effect of 100 μmol L^{-1} exogenous melatonin treatment on changes of H_2O_2 content **(A)** and O_2^- production **(B)** in fresh-cut pitaya fruits during 8-day storage at 4°C. Values are mean ± standard error (SE) of three biological replicates (n = 3). Means followed by different lowercase superscripts are significantly different at $p < 0.05$.

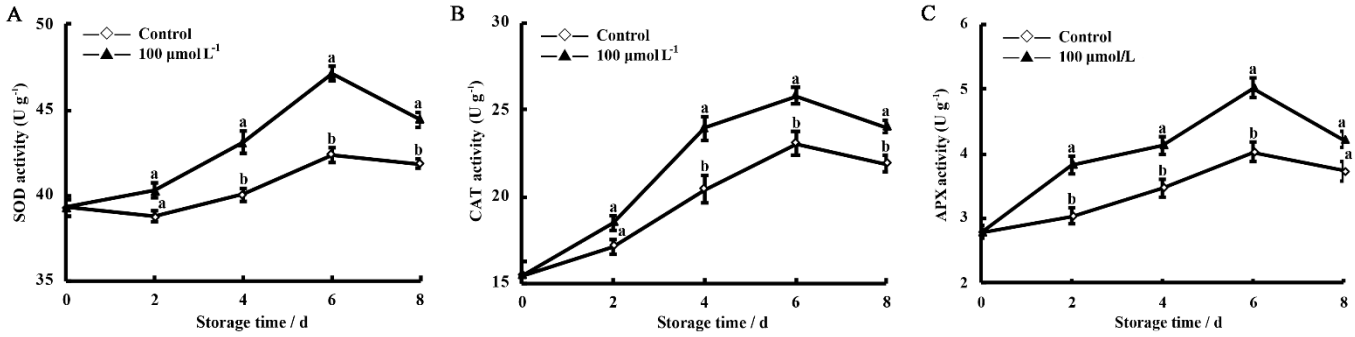

Figure 4. Effect of 100 μmol L^{-1} exogenous melatonin treatment on changes of the activities of SOD **(A)**, CAT **(B)**, and APX **(C)** in fresh-cut pitaya fruits during 8-day storage at 4°C. Values are mean ± standard error (SE) of three biological replicates (n = 3). Means followed by different lowercase superscripts are significantly different at $p < 0.05$.

Discussion

In the earlier experiments, investigations were designed to determine the optimum concentration of melatonin that effectively maintained the quality of fresh-cut pitaya fruits. The deterioration of fruit quality is invariably attributable to softening, weight loss, and changes in TA and TSS contents (Liu *et al*., 2017). Consequently, we initially examined the firmness, rate of weight loss, and TA and TSS contents of fresh-cut pitaya fruits to evaluate the effects of different concentrations of melatonin (50, 100, and 200 μmol L^{-1}) on the quality control of fresh-cut pitaya fruits during storage. As shown in Table 1, we found that there was a gradual decrease in the firmness of fresh-cut pitaya fruits, whereas there was a concomitant gradual increase in the rate of weight loss and decrease in the contents of TSS and TA with the progression of storage. When compared with control, however, treatment of fresh-cut pitaya fruits with melatonin at three concentrations was found to be effective in delaying the decrease in firmness and the rate at which weight was lost, although tended to have little effect on the TSS and TA contents of fresh-cut pitaya fruits. Moreover, we established that among the concentrations of melatonin assessed, 100 μmol L^{-1} proved to be the most effective in inhibiting weight loss and maintaining firmness. Similar results

have been obtained for other fruit products, such as sweet cherries (Bai *et al.*, 2011) and strawberries (Li *et al.*, 2018). In this regard, it has been reported that lower weight loss results in a higher turgor pressure in cells, which plays a major role in maintaining the firmness of fruits. Accordingly, the inhibition of the decrease in firmness and weight loss could be associated with the protection of the plasma membrane damage and the improvement of water-retaining property (Li *et al.*, 2018). Thus, the positive influences caused by melatonin delayed the quality deterioration of fresh-cut pitaya fruits.

Cell membrane electrolyte leakage is routinely used as a marker to indicate the integrity of membrane structures. It is typically measured to assess changes in cell membrane permeability, and consequently, the degree of damage to and resistance of the cell membrane (Ren and Li, 2013; Zhou *et al.*, 2014). In the present work, we found that the membrane electrolyte leakage in all fresh-cut pitaya fruits increased with increasing storage time, and that the application of melatonin effectively suppressed this increase, thus implying that melatonin could protect the integrity of the cellular membrane structure (Figure 2A).

Cutting or slicing will inevitably cause the tissue to suffer from wounding stress, which damages the plasma membrane, and induces the oxidative enzyme systems to react with the existing phenolic compounds, thus causing the oxidation of phenolics and the browning of tissues and accelerating the deterioration processes of fresh-cut pitaya fruits (Hodges and Toivonen, 2008). Therefore, it is important to evaluate the oxidative enzyme systems in melatonin-maintained quality of fresh-cut pitaya fruits. A further marker often assessed with respect to fruit quality is the activity of PPO, an increase in which is closely associated with the enzymatic browning of harvested fruits, which is attributable to an increase in the oxidation of phenolic compounds (Yao *et al*., 2014). In the present work, we observed that melatonin treatment could effectively inhibit the post-harvest increase in PPO activity (Figure 2B) and contributed to reducing the total phenolic contents (Figure 1B), thereby further illustrating that melatonin could maintain cellular structure, which is consistent with the findings of Cao *et al*. (2017).

In the second part of the present work, we focused on the reactive oxygen species (ROS) production and radical scavenging systems. During normal plant metabolism, free radicals are produced and scavenged in a dynamic balance; however, an imbalance in active oxygen metabolism can result in the accumulation of free radicals, which has the effect of accelerating senescence and the deterioration of produce quality. In this regard, antioxidant enzymes, including SOD, CAT, and APX play essential roles in preventing oxidative stress in cells and degradation of nucleic acids attributable to the excessive production of ROS (Xu *et al*., 2012). Stored fruits and vegetables are characterised as an accumulation of ROS (H_2O_2 and $O_2^{-\cdot}$), thereby resulting in membrane lipid peroxidation and an increase in cell membrane permeability (Mittler, 2002). However, increases in the activities of SOD, CAT, and APX can eliminate excessive ROS generation *in vivo*, thereby delaying post-harvest senescence and maintaining the quality of fruits and vegetables (Tian *et al*., 2013). In the present work, we found that melatonin treatment could increase the activities of SOD, CAT, and APX, thereby effectively suppressing the accumulation of H_2O_2 and production of $O_2^{-\cdot}$ (Figures 3 and 4) in fresh-cut pitaya fruits during storage, which is consistent with the findings previously reported by Gao *et al.* (2016) and Ma *et al.* (2016). Accordingly, our observations indicated that by enhancing the activities of antioxidant enzymes, exogenous melatonin treatment could contribute to increasing the efficacy of the antioxidant system in post-harvest fruit tissue, thereby inhibiting ROS accumulation.

Conclusion

The present work demonstrated that melatonin treatment could delay the rate of weight loss and decreases in firmness, total soluble solids, titratable acidity, vitamin C, and phenolic compounds in fresh-cut pitaya fruits during storage. Moreover, exogenously applied melatonin could decrease cell membrane electrolyte leakage and polyphenol oxidase activity, and increase the activities of the antioxidant enzymes superoxide dismutase, catalase, and ascorbate peroxidase, thus decreasing the accumulation of H_2O_2 and O_2^{-}. Collectively, these responses contributed to a delay in senescence and deterioration, thus highlighting the clear beneficial effects of melatonin treatment with respect to maintaining the quality of fresh-cut pitaya fruits during storage. Moreover, findings also indicated that the application of melatonin at a concentration 100 μmol L^{-1} would have the best preservation effect in practice.

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