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RESEARCH ARTICLE

Effect of melatonin on ripening of 'Pearl' guava fruit at ambient temperature

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ABSTRACT

In this study, fruit quality and activities of antioxidant and cell wall degrading enzymes, were investigated to explore the effect of exogenous melatonin on preservation of postharvest guava during storage at room temperature (25℃). The results showed 500 μM melatonin treatment could effectively delay the softening and yellowing of guava fruit, and maintain high soluble sugar and vitamin C content, to keep the appearance and nutrient quality. Moreover, the treatment could inhibit PPO (polyphenol oxidase) enzyme activity to prevent browning, but promote POD (peroxidase) and CAT (catalase) enzyme activities to improve the resistance of fruit during storage. Furthermore, PG (polygalacturonase) and PL (pectate lyase) enzyme activities were effectively suppressed to protect the structural integrity of the cell wall in fruit, and delay the softening process. These findings indicate, 500 μM exogenous melatonin treatment could delay the ripening of postharvest guava fruit, and present application value of preservation.

Keywords: Guava; Melatonin; Preservation; Ripening

INTRODUCTION

Guava (*Psidium guajava* L.) is one of the most popular fruit due to its high antioxidant activities and abundant nutrients (de Aquino et al., 2015). Guava is rich in vitamin C, vitamin A, soluble protein, riboflavin, fructose, glucose and dietary fiber et al. Guava also possesses reduce serum cholesterol levels and anti-hypertensive that are beneficial to human health (Murmu et al., 2018a). Moreover, guava could be processed into fruit juice, instant powder and puree, and guava seed oil could also be blended with other edible oils to improve the nutritional characteristics of edible oils (Kapoor et al., 2020). Unfortunately, guava is a typical respiratory climacteric fruit, which leads to perishing during storage (Murmu et al., 2018a). Meanwhile, guava fruits are predisposed to infection fungal pathogens and mechanical damage after harvest, which directly impact the commodity value of fruits. In addition, guava fruit are liable to chilling injury when stored below 10℃, which also lead to huge economic losses (Singh et al., 2008a).

Some studies have been performed with a view of increasing the storage period of guava fruit at present. Studies have shown that chitosan coating could improve the storage property and extend the shelf life of guava fruit (Hong et al., 2012; Silva et al., 2018). It is also reported that using an edible coating could significantly delay the loss of weight and respiration rate of guava during storage, thus delaying ripening and senescence (Martínez-Ortiz et al., 2019). Nair et al. (2018) further demonstrated that the pomegranate peel extract with chitosan and alginate could extend the storage time of guava. Besides, studies indicated that use of biopolymeric coating hydrophobized with beeswax in guava fruit could minimize the loss of chlorophyll, thus keeping postharvest quality of guava (Oliveira et al., 2018). Moreover, modified atmosphere packaging could delay the softening and senescence of guava during low temperature storage, extending the shelf life to 32 days (Murmu et al., 2018b). Although those methods could prolong the storage period of guava, exploring safe and efficient preservation methods is still a major international challenge for prolonging the storability of guava fruit.

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Melatonin (N-acetyl-5-methoxytryptamine) is one kind of multi-function molecule substances (Tan et al., 2020), which is also known as the 'brain platinum'. It has been shown that melatonin could slow down the degradation of chlorophyll and inhibit the expression of senescence-associated genes, thus delaying leaf ripening and senescence of postharvest Chinese flowering cabbage (Tan et al., 2020). Miao et al. (2020) also found that melatonin could slow down the ripening and senescence process of broccoli florets by increasing the antioxidant capacity and inhibiting the degradation of chlorophyll. Moreover, melatonin treatment could retard the quality deterioration of postharvest apple fruit by inhibiting the ethylene synthesis and enhancing the activities of antioxidant enzyme (Onik et al., 2021). Furthermore, Zheng et al. (2019) found that melatonin could maintain the nutritional and commercial value of fresh-cut pear fruit by inhibiting the expression of enzymatic browning-related genes but enhancing antioxidant capacity. Melatonin application could also increase accumulation of phenol substances in kiwifruit during storage (Wang et al., 2019b). Further results showed that the melatonin (0.1 and 1 mM) treatment could not only effectively improve the storage quality but also prolong shelf life of strawberry (Liu et al., 2018a). Therefore, melatonin may play an important role in inhibiting chlorophyll degradation and delaying fruit senescence, thus extending the storage time and maintaining the nutritional quality of fruit and vegetables.

To date, detailed information regarding the applications of melatonin in guava is still lacking. The objective of this study was to assess the effect of melatonin on fruit ripening of guava during storage at room temperature, and could help to develop an alternative postharvest technology for quality maintenance and shelf-life extension of guava during storage and marketing.

MATERIAL AND METHODS

Materials and treatments

Guava were bought from Fuxi fruit Wholesale Market (Guangdong, China). Guava fruits of the same size and color, and without insect, disease, or mechanical damage were selected for use in the study. In preliminary experiments, after soaking in prochloraz (0.05%) solution for 3min, guava fruits were naturally dried at room temperature. Then, fruits were randomly divided into four groups, including three groups treated with 50 μM, 200 μM and 500 μM melatonin ($C_{13}H_{16}N_2O_2$, CAS: 73-31-4, Lot # E1712039, 98% purity from Shanghai Aladdin Biochemical Technology Co., Ltd in China) for 2hr, respectively, and one group as the control treated with distilled water instead. Results indicated that 500 μM melatonin treatment for 2hr effectively prolonged the shelf life of guava fruits comparing with the other three treatments. Therefore, repeated 500 μM melatonin for 2hr treatment was selected for further study. After firmness and color determination, pulp of the equatorial line from guava fruits were sampled by liquid nitrogen frozen and stored at 40°C immediately for physiological analysis.

Firmness assay

Firmness of guava fruits was detected with a fruit firmness meter (GS-701G) at three positions around the fruit equator line with equal spacing. Nine fruits were measured each time, the average and standard deviation was further calculated to minimize variability.

Measurement of color difference

The color difference of fruit was detected in equator line, including indexes L* (luminosity), C* (saturation), and H* (chroma) by a colorimeter (CR-10, Konica Minolta). Nine fruits were measured each time, the average and standard deviation was further calculated to minimize variability.

Determination of soluble sugar content

Soluble sugars content was measured according to the method of Liu et al. (2018b) with slight modifications. Samples (0.5 g fruit tissue) were put into a centrifuge tube, followed by adding 5 ml of distilled water, then centrifuged (4,000×g, 20 min, 4°C). The volume of the supernatant was adjusted to 50 ml using distilled water, 0.15 ml of diluent was taken and the volume was adjusted to 50 ml with deionized water. The soluble sugar content was assayed with the anthrone-sulfuric acid method by using a spectrophotometer at 620 nm.

Vitamin C content analysis

The samples were measured by the molybdenum blue colorimetric method for vitamin C content (Pu et al., 2020). 1 mg/ml vitamin C solution was used as a standard. Five gram of fruit tissues was homogenized and diluted to 25 ml with oxalic acid-EDTA solution (0.05 M oxalic acid and 0.2 M EDTA). Then, centrifuged at 4,000×g for 20 min at 4°C, 6.0 ml of oxalic acid-EDTA solution was added to 4.0 ml of supernatant. Determination of mixture absorbance was followed as the same method as that for draw standard curve. The concentration of vitamin C was found by a standard curve, and the following formula was used to calculate the vitamin C content:

$$\text{Vitamin C} = \frac{m' \times V \times \text{dilusion factor}}{V \times m}$$

where m' is the vitamin C content of standard curve, V is the total volume of the aqueous extract, V_s is the volume of sample extraction solution that taken used determination and m is the weight of samples. The vitamin C content was expressed as μg/g FW.

Assay for activities of polyphenol oxidase (PPO)

PPO activity was determined by Yu et al. (2020) method with slight modifications. Fruit samples were ground to a fine powder in liquid nitrogen, 12 ml PBS (phosphate buffer saline, 0.05 M, pH 6.0) was used for extracting crude enzyme in an ice bath for 30 min, and later centrifuged (6,000×g, 15 min, 4℃), two tubes were prepared. One was mixed with 2 ml fresh enzyme extract and 1 ml catechol (0.2 M), and the other one with 2 ml inactivated enzyme extract and 1 ml PBS (0.1 M, pH6.5), both of which were equilibrated for 20 min at room temperature. Then, 0.2 ml of trichloroacetic acid (20%) were added to the mixture respectively to stop the reaction. Absorbance of the sample was read at 420 nm, and the PPO activity (U/g•min) is defined as the absorbance of per gram sample increased by 0.001 in 1 min at 420 nm.

Measurement of peroxidase (POD) activities

POD activity was measured using the method described by Zhang et al. (2015) with slight modifications. 2.0 g of flesh sample was ground to a fine powder in liquid nitrogen and added with 10 ml of pre-cool PBS (0.05 M, containing 4 % PVP, pH 7.0), then extracted in an ice bath for 20 min. After that, centrifuged at 8,000×g for 20 min at 4℃, and the supernatant was collected as the crude enzyme. 2.6 ml of PBS, 0.1 ml of guaiacol solution and 0.1 ml of H_2O_2 (46%) were mixed in a colorimetric tube. In advance, then 0.2 ml enzyme extract were added to the mixture to start the reaction. POD activity was determined immediately from the beginning of reaction. The increase in absorbance at 470 nm over 7 min was measured every 1 min, and the POD activity (U/g•min) is defined as the absorbance of per gram sample increased by 0.01 in 1 min at 470 nm.

Assay for activities of catalase (CAT)

CAT activity was determined by Zhang et al. (2015) method with slight modifications. Extraction of crude enzyme of CAT was followed as the same method as that for POD. The reaction mixture contained 3 ml of H_2O_2 (0.01 M) and 0.1 ml of the extract, 3 ml of H_2O_2 (0.01 M) and PBS (0.05 M, pH 7.0), and the reference solution included. The reduce in absorbance at 240 nm over 6 min was measured every 1 min, and the CAT activity (U/g•min) is defined as the absorbance of per gram sample reduced by 0.001 in 1 min at 240 nm.

Determination of polygalacturonase (PG)

The PG activity was determined using the method of Wang et al. (2020) with slight modifications. A standard curve was generated using glucose solution (1 mg/ml). 5.0 g of flesh sample was ground to a fine powder in liquid nitrogen, extracted with 10 ml of pre-cool ethyl alcohol solution (95%) at 4℃, whereafter centrifuging at 6,000×g for 20 min at 4℃. The sediment was added with 5 ml of pre-cool ethyl alcohol (80%), and extracted under mild conditions for 10 min at 4℃. Then, mixture was centrifuged under the above conditions, the sediment was added with 5 ml of acetic acid-sodium acetate buffer (50 mM, pH 5.5) and extracted under mild conditions for 20 min at 4℃. After centrifuging (6,000×g, 20 min, 4℃), the supernatant was collected for enzyme assays. Two copies of 1 ml of acetic acid-sodium acetate buffer (50 mM, pH 5.5) and 0.5 ml polygalacturonic acid solution (1%) were added to two test tubes, respectively. Then, one was mixed with 0.5 ml enzyme extract, the other with 0.5 ml inactivated enzyme (boiling at water bath for 10 min), and the mixtures were equilibrated in a 37℃ water bath for 1 hr. After that, 1.5 ml 3,5-Dinitrosalicylic acid reagent was added rapidly, and kept in a boiling water bath for 5 min. The volume of the solution was adjusted to 20 ml using distilled water, absorbance of the sample was read at 540 nm. PG activity (mg/g•hour) is defined as the amount of galacturonic acid produced by catalyzing the hydrolysis of polygalacturonic acid of per gram sample over 1hr at 37℃.

Pectin lyase (PL) activities assay

PL activity was assayed to the basis of the method of Ortiz et al. (2011) with slight modification. 6.0 g of flesh sample were ground to a fine powder in liquid nitrogen, mixed with 20 ml of Tris-HCL buffer (500 mM, pH 8.0, containing 1 M NaCl solution), whereafter centrifuged at 10,000×g for 15 min at 4℃. The supernatant was used for the enzyme assays. Two copies of extracting solution were added to two test tubes, added with 2.0 ml of pectin solution (0.5%), and the mixtures were equilibrated in a 40℃ water bath for 5 min. After that, one was mixed with 0.5 ml of enzyme extract, the other with 0.5 ml of inactivated enzyme extract (boiling at water bath for 10 min). 0.5 ml of mixture was added separately to two tubes, and 4.5 ml of HCl solution (0.01 M) was added, respectively. Absorbance of the sample was read at 235 nm. PL activity (U/g•min) was defined as the absorbance of per gram sample increased by 0.01 in 1 min at 235 nm.

Statistical analysis

All assays were performed at least in triplicate and results are presented as means ± SE. All statistical analyses were completed with SPSS version 25.0 (IBM Corp., New York, USA). Duncan's test with one-way analysis of variance (ANOVA) for pairwise comparison was used to determine significant differences at a confidence level of 5% with 'storage time' as fixed factor. Figures were drawn by Sigmaplot version 14.0 (Systat Software, Inc. USA). Were the data analyzed by analysis of variance (ANOVA) and means compared by some test (Duncan, Tukey)? Also, it is lacking the statistical model of the experiment. Other doubt is regarding to the 'storage times' that should be consider as fixed or block factor in the statistic model.

RESULTS AND DISCUSSION

Melatonin participates widely in plant development and stress resistance (Kong et al., 2020). Wang et al. (2019a) demonstrated that exogenous melatonin could delays ripening and senescence of sweet cherries, and the effects of melatonin treatment on the preservation of sweet cherries increases with increasing concentrations. However, in different concentrations treatments including 0.01, 0.1, 1 and 10 mM, the 0.1 or 1 mM melatonin treatment was more effective in delaying ripening and senescence of strawberry fruit (Liu et al., 2018a). So, preservation effects of melatonin may differ in fruits. In this study, fruit firmness decreased rapider in control in comparison with melatonin (50, 200 and 500 μM) treatments, and the firmness of fruits treated with 500 μM melatonin was higher than the other two during the whole storage (Fig. 1a). It could be seen that the inhibited decline of firmness in fruits by 500 μM melatonin treatment was significantly. Fig. 1b further displayed the luminosity (L*) values of fruits treated with melatonin (50–200 and 500 μM) maintained lower levels as compared to control, with a higher saturation (C*) (Fig. 1c) and chroma (H*) values (Fig. 1d). Thus, melatonin treatment could be effective in preserving color parameters of postharvest guava fruits during storage at room temperature. Moreover, 500 μM melatonin treatment was found to be the most effective in delaying the increase of L* value and the decrease of H* value. In addition, appearance characteristics were important for evaluating the quality of fruit commodities. Fig. 2 showed that no significant differences in color of guava peel in both control and 500 μM melatonin treatment before 3 days. However, control fruits began to turn yellow and accumulate brown spots after 6 days storage, while the peel of 500 μM melatonin treatment turned yellow slightly, and brown spots were also significantly less than that of control after 15 days. So, 500 μM melatonin treatment could inhibit the yellowing and browning of guava fruits. In previous studies, Zhai et al. (2018) found that 100 μM melatonin treatment could delay the decline of pear fruit firmness during storage, thus keeping fruit commercial quality. Wu et al. (2021) reported that 100 μM melatonin treatment could effectively reduce the decline of chlorophyll content in broccoli during storage. Li et al. (2018) also found 100 μM melatonin treatment improves the capacity of cold-resistant in tea plants, and chlorophyll contents increased significantly in tea leaves after melatonin treatments. In addition, 0.5 mM melatonin treatment resulted in delayed accumulation of ABA (abscisic acid) through decreasing activity of a key ABA biosynthetic

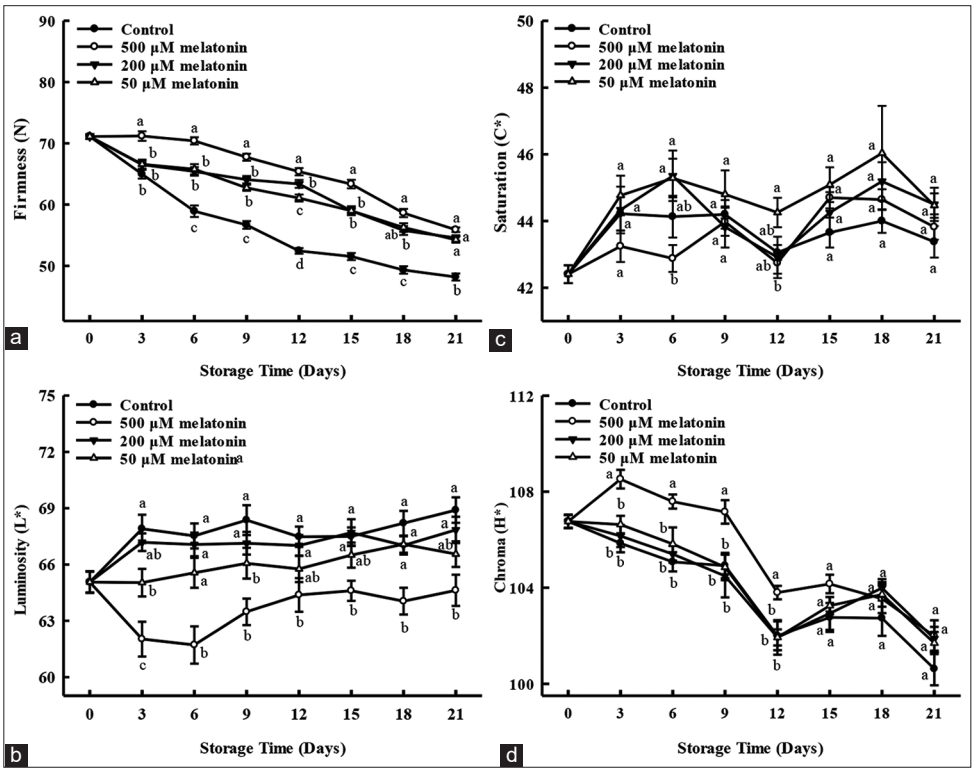

Fig 1. Effect of different treatments on (a) firmness, (b) luminosity (L*), (c) saturation (C*) and (d) chroma (H*) of guava fruit during storage 25±1℃. Values of (a) are means±SE of nonduplicate samples (n=27), Values of (b, c and d) are means±SE of nonduplicate samples (n=9), and superscript letters indicate treatments differences at the 5% significance level ($p < 0.05$) as determined by Duncan's test with one-way analysis of variance (ANOVA) with the storage time was set as blocked factor.

enzyme (9-cis-epoxycarotenoid dioxygenase, NCED), thus, attenuating chlorophyll degradation and delaying ripening and senescence of mango fruit (Liu et al., 2020). These results indicated that melatonin treatment could inhibit chlorophyll degradation in fruits and vegetables. Therefore, it was speculated that melatonin treatment could effectively delay the decline of firmness of guava fruits during storage at room temperature, and it is possible to avoid the change of fruit color parameter by regulating the expression of chlorophyll degrading enzyme gene, which might be beneficial to maintain the appearance quality of guava fruits during storage.

Postharvest guava fruits exhibit high respiratory rate due to high metabolic activities, thus contributing to its rapid perishability (Nair et al., 2018). Bashir et al. (2003) found that respiratory metabolism of guava leaded to gradual consumption of soluble sugar with the prolongation of storage period. Moreover, Hong et al. (2012) reported that losses of vitamin C and soluble sugar in guava fruits could be avoided under low temperature storage. Modified atmosphere packaging (Murmu et al., 2018b), 1-MCP treatment (Singh et al., 2008a) and biopolymeric coating hydrophobized with beeswax (Oliveira et al., 2018) could also maintain the nutritional quality of guava during storage. In this study, the content of soluble sugar showed the largest decline in both control and 500 µM treatment after 6 days storage, then followed by a small rise at 9th day. However, higher soluble sugar content was observed in 500 µM melatonin treatment after 12 days storage comparing with control fruits, which indicated 500 µM melatonin could preserve better quality for guava fruits during the late stage of storage (Fig. 3a). In addition, Fig. 3b showed that both vitamin C contents in control and 500 µM melatonin treatments increased at early stage of storage but declined thereafter. It indicated that vitamin C could be synthesized in guava fruits during storage. Furthermore, fruits treated with 500 µM melatonin contained higher content of vitamin C than control throughout the whole storage time (Fig. 3b). It is believed that vitamin C content was associated with soluble pectin content of strawberry fruit after melatonin treatment (Liu et al., 2018a). Tan et al. (2020) showed that melatonin treatment could maintain vitamin C content in posthavest fruits. And Gao et al.

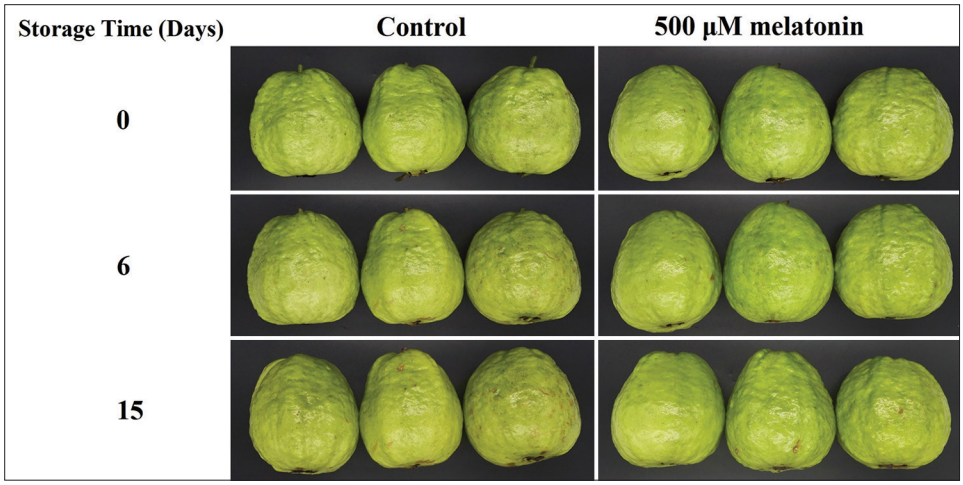

Fig 2. Effect of 500 µM melatonin treatment on appearance characteristics of guava fruit during storage at 25±1°C.

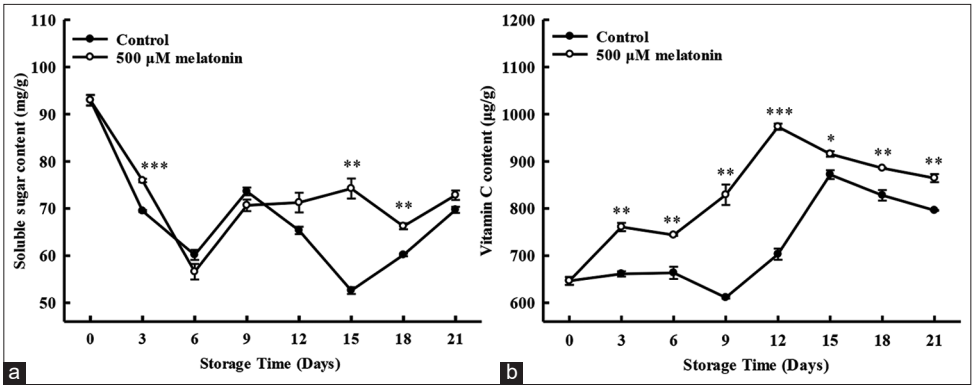

Fig 3. Effect of 500 µM melatonin treatment on (a) soluble sugar content and (b) vitamin C content of guava fruit during storage at 25±1°C. Values are means±SE of triplicate samples, and asterisks indicate treatment differences at $p < 0.05$ as determined by independent-samples T test.

(2016) also reported that melatonin treatment could delay the ripening and maintain high vitamin C content of peach fruits during storage. Therefore, it was speculated that 500 μM melatonin treatment could reduce the loss of vitamin C and inhibit the degradation of soluble sugars, thus to maintain postharvest quality of guava fruits during storage at room temperature.

Antioxidant enzymes are related to postharvest quality of fruits and vegetables (Sogvar et al., 2020). Chen et al. (2019) had shown that increase of antioxidant enzyme activity could prolong the storage time of banana fruits. Lei et al. (2018) found that the enzyme responsible for edible cultivated mushroom browning was polyphenol oxidase (PPO). And catalase (CAT) could scavenge H_2O_2 in peroxisomes (Wei et al., 2018). Peroxidase (POD) is involved in the removal of polyphenols and H_2O_2 during the late stage of storage of carrot, resulting in increased of resistance (Elsherbiny et al., 2018). In this study, PPO activity of control was higher than melatonin (500 μM) treatment of guava fruits with extended storage time (Fig. 4a). It could be concluded that 500 μM melatonin treatment could inhibit the PPO activity of guava during storage at room temperature, thus avoiding browning of peel. Fig. 4b showed that CAT activity of guava fruits treated with melatonin (500 μM) was higher than control fruits. So, it indicated that treatment of 500 μM melatonin could increase CAT activity and inhibit accumulation of H_2O_2 in guava fruit. In addition, similar to CAT, POD activity of fruits treated with 500 μM melatonin was also higher than control fruit, especially at the late period of storage (Fig. 4c). So, 500 μM melatonin treatment could be the possible reason for improvement of the resistance of guava fruits by enhancing POD activities.

In addition, breakdown of cell wall structure accelerated the ripening and senescence of fruit, which is related to two major cell wall degrading enzymes including polygalacturonase (PG) and pectin lyase (PL) in fruit tissues. Singh et al. (2008b) reported that PG could lyse pectinic acid and promote loose structure of cell wall, and the activity of PG was high in the early stage of fruit storage, but decreased gradually with extended storage time. And Figueroa et al. (2008) reported that PL also was a softening enzyme, which degraded pectin and participated in the softening process of strawberry fruits at late stage of storage. As shown in Fig. 5a, PG activity reduced gradually during storage in control fruit, however, decreased rapidly and remained at a low level during storage in 500 μM melatonin treatment. Thus, PG maybe be mainly involved in fruit softening during the early stage of storage, and 500 μM melatonin treatment could inhibit PG activity and its softening. Fig. 5b showed that PL activity of guava fruits reached the peak at day 15, thereafter, PL activity gradually decreased with extended storage time. However,

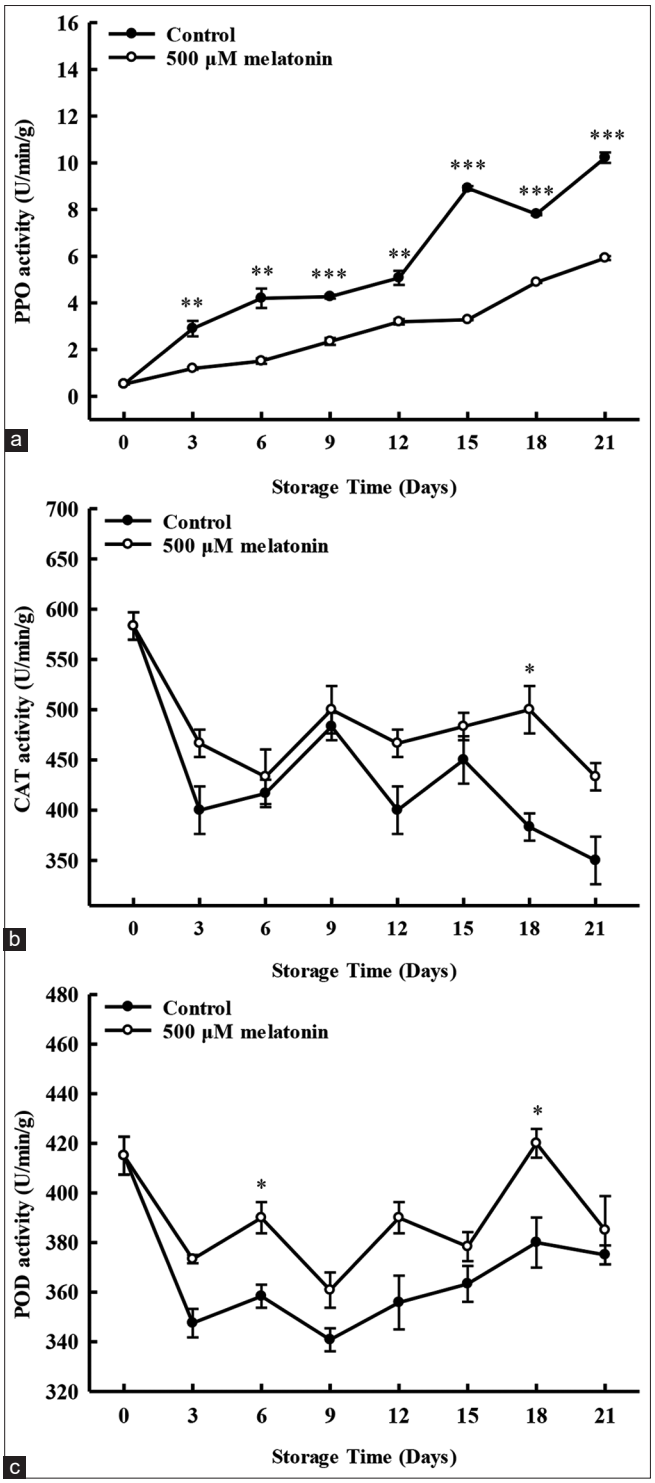

Fig 4. Effect of 500 μM melatonin treatment on (a) PPO activity, (b) CAT activity and (c) POD activity of guava fruit during storage at 25±1°C. Values are means±SE of triplicate samples, and asterisks indicate treatment differences at $p < 0.05$ as determined by independent-samples T test.

PL activity raised slowly in 500 μM melatonin treatment during storage, and significantly lower than that of control. Hong et al. (2012) had shown that treatments with chitosan coating could delay the decrease of CAT activity but induce

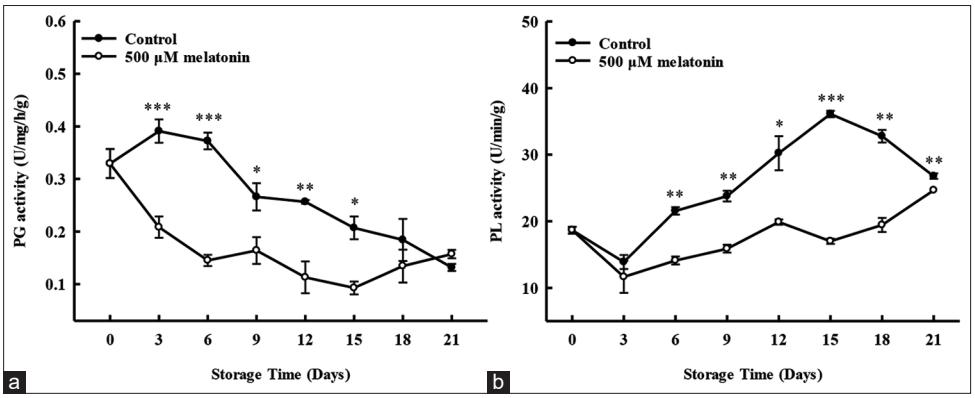

Fig 5. Effect of 500 μM melatonin treatment on (a) PG activity and (b) PL activity of guava fruit during storage at 25±1℃. Values are means±SE of triplicate samples, and asterisks indicate treatment differences at $p < 0.05$ as determined by independent-samples T test.

the increase in POD activity, resulted in less oxidative stress of guava fruits. Moreover, ethylene signal transduction pathway was positively correlated with the expression of cell wall degrading enzyme (PG, PL) gene, which directly accelerated the ripening and softening process of fruits (Park et al., 2019). Therefore, it was speculated that 500 μM melatonin treatment could affect the activities of antioxidant enzymes and cell wall degrading enzymes of guava fruits by ethylene signal transduction pathway, thus to protect the cell membrane integrity and cell wall stability, delay the softening process and extend the shelf life of guava fruits.

CONCLUSIONS

As a final point, our results demonstrated the valuable impact of 500 μM melatonin treatment on maintaining the postharvest quality of guava fruit. 500 μM melatonin treatment could enhance antioxidant capacity, including CAT and POD activity, retain the contents of vitamin C and soluble sugar, inhibited chlorophyll degradation, and PPO activity, and stabilize cell wall structure to postpone fruit softening by inhibiting PG and PL activity during storage at room temperature. Considering the obtained results, application of 500 μM melatonin could delay the quality deterioration of guava fruit during ambient storage. 500 μM melatonin postharvest treatment could act as a promising and safe method for preserving the quality and storability of guava during ambient storage.

AUTHORS CONTRIBUTIONS

All authors contributed extensively to the work presented in this article. Y.Y. and C.X.Y.: Performed research, analyze data and wrote the manuscript. Z.L.R.: Contributed to the performance of experiment. Y.H.L.: Performed statistical analysis and reviewed the manuscript. W.F.W.: Designed research and revised the manuscript. All authors read and approved the final manuscript.

DECLARATIONS OF INTEREST

The authors declare that they have no conflicts of interest.

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