

EFFECT OF EXOGENOUS MELATONIN ON ANTIOXIDANT ENZYME ACTIVITIES AND MEMBRANE LIPID PEROXIDATION IN AVOCADO FRUIT DURING RIPENING

To Thi Bich Ngoc, Vo Minh Thu, Nguyen Thi Phuong Hien, Huynh Thi Thanh Tra, Hoang Duc An, Truong Thi Hue✉

Quy Nhon University, 170 An Duong Vuong Street, Quy Nhon City, Binh Dinh Province, Vietnam

✉To whom correspondence should be addressed. E-mail: truongthihue@qnu.edu.vn

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SUMMARY

Melatonin functions as a plant growth regulator, has diverse functions and plays an important role in ripening and fruit senescence. In the current study, we investigated the effect of exogenous melatonin (0.1 mM and 0.5 mM) on reactive oxygen species (ROS) metabolism, membrane lipid peroxidation and antioxidant enzyme activity of avocado (*Persea americana* Mill. cv. 034) during fruit ripening at $22^{\circ}\text{C} \pm 1$ and 75-80% relative humidity (RH). The results showed that postharvest fruits treated with 0.5 mM melatonin effectively reduced the accumulation of superoxide anion ($\text{O}_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) in the mesocarp of the fruit. In addition, melatonin treatment also significantly promoted the activities of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) in avocado. It is suggested that enhanced antioxidant enzyme activity induced by melatonin treatment may contribute to scavenge ROS and alleviating membrane lipid peroxidation in avocado fruit. The results indicate that the MT application might collectively contribute to the delay senescence and maintain postharvest quality of avocado.

Keywords: Avocado fruit, antioxidant enzyme, lipid peroxidation, melatonin, reactive oxygen species

INTRODUCTION

Avocado (*Persea americana* Mill.) is a fruit rich in lipid, protein, vitamin, fiber, antioxidants and minerals (Zhang *et al.*, 2013; Saidi *et al.*, 2021). Avocado fruit are packed with an excellent source of different bioactive compounds including vitamin E, ascorbic acid, carotenoids and soluble phenolic compounds associated with health-related benefits. However, after being harvested, avocado fruit have short storage and shelf life from 5 to 7 days at room temperature that faces difficulties in domestic and export markets (Nguyen Minh Nam *et al.* 2012; Vincent *et al.*, 2020).

Fruit ripening process is characterized by oxidation; water and nutrients are continuously consumed by transpiration and aerobic respiration, generating energy and metabolic intermediate to maintain cellular activities. This process also produces byproducts which are ROS such as $\text{O}_2^{\cdot-}$, H_2O_2 , OH ... Under normal conditions, the balance between the generation and elimination of intracellular ROS reaches a dynamic equilibrium, the intracellular ROS content is maintained at a low level without damaging the cells. However, during fruit ripening and senescence, the ROS balance is disturbed. On the other hand, avocado is a climacteric fruit which produces a large amount

of ROS followed by excessive accumulation and cytotoxic effects. If not being cleaned effectively, it will peroxidize lipids, destroy membranes and reduce nutritional value of the fruit (Tian *et al.*, 2013; Vincent *et al.*, 2020). Therefore, to combat oxidative stress, it is necessary to provide effective means to strengthen ROS defense system and reduce the oxidative damage of fruit cells (Zhang *et al.*, 2018). There are many reports in the world on postharvest preservation methods to improve avocado fruit quality such as controlled atmosphere storage, 1-MCP treatment, and biological coatings application... (Munhuweyi *et al.*, 2020). Driven by growing concerns about chemical residues and human health, safer and more effective methods still need to be adopted to ensure postharvest avocado quality.

Melatonin (N-acetyl-5-methoxytryptamine), a derivative of the amino acid tryptophan, discovered in plants in 1995 is a safe and non-toxic endogenous compound (Liu *et al.*, 2020), involved in various physiological processes such as circadian rhythms, photosynthesis, senescence, and increased plant tolerance (Arnao & Hernandez-Ruiz, 2015). Melatonin (MT) slows down fruit senescence by strengthening the antioxidant system, eliminating free radicals and protecting cell membranes (Xu *et al.*, 2019). Recently, the effect of MT on the physiological characteristics of postharvest fruit has attracted much attention. Many studies have shown that exogenous MT treatment is an effective method in extending the postharvest life and maintaining the quality of banana (Hu *et al.*, 2017), blueberries (Liu *et al.*, 2018), cherry (Miranda *et al.*, 2020), and apple fruit (Onik *et al.*, 2021); slowing down senescence and enhancing chilling stress tolerance through activating the antioxidant system (Gao *et al.*, 2016). However, to the best of our knowledge, there has been no report regarding the effect of MT application on the physiological characteristics of postharvest avocado fruit. The aim of this research was to evaluate the role of exogenous MT on ROS production, cell

membrane damage and antioxidant enzyme activity of avocado during ripening. This result will help to better understand the mechanism of MT action in different fruits.

MATERIALS AND METHODS

Fruit material

Avocado (*Persea americana* Mill. cv. 034) fruits at mature green stage were collected from a commercial orchard located at Gao commune, Pleiku city, Gia Lai province (13°53'50.9"N 107°56'51.1"E). Fruit samples were selected based on their uniformity of shape, size, weight and free of blemishes. Fruit maturity at harvest reached to a commercial grade with average fruit weight of 330.04 ± 2.0 g. Fruits were packed in cardboard boxes and then transferred to the laboratory within 6 hours after being harvested.

Fruit treatment and storage

At the laboratory, each individual fruit was rinsed with tap water, air-dried, and then randomly divided into 3 groups for 3 treatments. Fruits in two groups were immersed into MT (Bio Basic Inc., Canada) at 0.1 mM and 0.5 mM for 2 hours. The remaining fruit group immersed into distilled water served as the control. The treatments with MT were implemented under low-light conditions to avoid the decomposition of MT (Zhang *et al.*, 2018). After treatments, the fruit were air-dried and stored at $22^{\circ}\text{C} \pm 1$ and 75 - 80% RH. During storage, avocado samples from each treatment were randomly selected for analysis of ROS level, antioxidant enzyme activities and membrane lipid peroxidation. Flesh tissues were sampled from the middle part of a fruit (3 fruits of each replicate in the same formula) every 2 days during the storage period of 12 days. Each treatment for each parameter was set up in triplicate assay.

Determination of the production rate of $\text{O}_2^{\cdot-}$ and H_2O_2

The $\text{O}_2^{\cdot-}$ production rate was measured by

monitoring the nitrite formation from hydroxylamine in the presence of O_2^- (Wang and Luo, 1990). 1 g of mesocarp tissue was homogenized in 3 mL of 50 mM potassium phosphate buffer (pH 7.8) containing 1% polyvinylpyrrolidone (PVP), and then centrifuged at 10,000 rpm at 4 °C for 15 min. 1 mL supernatant was mixed with 0.1 mL of 10 mM hydroxylamine hydrochloride. The mixture was added to 1 mL of 17 mM sulfanilamide and 1 mL of 7 mM α -naphthylamine. The absorbance was recorded at 530 nm in a spectrophotometer (CECIL-4002, England). The O_2^- production rate was calculated using a standard curve of $NaNO_2$ and expressed as $\mu\text{mol}/\text{min}/\text{g}$ FW.

H_2O_2 content was measured by using the method described by Velikova (2000) with a slight modification. 0.3 g of fruit pulp samples were homogenized with 1.5 mL of 0.1% trichloroacetic acid (TCA) solution. The homogenate was centrifuged at 12,000 rpm for 15 min at 4°C and 0.5 mL supernatant was mixed with 1 mL 1 M potassium iodide. The absorbance was measured at 390 nm in a spectrophotometer (CECIL-4002, England). The H_2O_2 content was calculated using a H_2O_2 standard curve and expressed as $\mu\text{mol}/\text{g}$ of fresh weight (FW).

Determination of membrane lipid peroxidation

The level of lipid peroxidation was determined by estimating MDA content in the fruit pulp. MDA was assayed using the method of Dhindsa *et al.* (1981) with some modification. 0.3 g flesh tissue was homogenized with 3 mL of 10% TCA and centrifuged at 12,000 rpm for 20 min at 4 °C. 1 mL of supernatant was added 3 mL of 10% TCA containing 0.67% thiobarbituric acid (TBA) and incubated at 95°C for 20 min, then cooled down on ice. The absorbance was read at 600, 532, and 450 nm in a spectrophotometer (CECIL-4002, England). The MDA content was calculated and expressed as $\mu\text{mol}/\text{g}$ FW.

Assays of antioxidant enzyme activity

Enzyme extracts were prepared by homogenizing 1 g of avocado flesh tissue in 3 mL of 50 mM phosphate buffer, pH 6.8 containing 1% PVP (Gao *et al.*, 2016), then centrifuged at 12,000 rpm for 20 min at 4 °C. The supernatants were used for studying the enzyme activity.

The SOD activity was assayed according to Dhindsa *et al.* (1981). The mixture consisted of 50 mM sodium phosphate buffer, pH 7.8, 14.5 mM methionine, 2.25 mM nitro blue tetrazolium (NBT), 30 μM EDTA, 60 μmol riboflavin and 50 μL of supernatant. The tubes were then placed below two 15 W fluorescent lamps for 15 min. SOD activity was evaluated by the capacity to inhibit the photoreduction of NBT at 560 nm and expressed as U/g FW.

The CAT activity was determined as described by Aebi *et al.* (1983). The mixture (3 mL) contains 50 mM phosphate buffer, pH 7.0, 40 mM H_2O_2 and 200 μL of enzyme extract. CAT activity was assayed by the change in absorbance at 240 nm and expressed as U/g FW.

POD activity was done by the method of Kochba *et al.* (1997). The reaction solution (3 mL) contained 0.05 M phosphate buffer solution, pH 6.8, 20 mM guaiacol, 40 mM H_2O_2 and 0.1 mL of supernatant. POD activity was measured by the change in absorbance at 470 nm, and expressed as U/g FW.

Data analysis

All data were analyzed using SAS statistical software version 8.0 (SAS Institute, USA). Experimental results were analyzed using analysis of variance (ANOVA), and Fisher's LSD, $p < 0.05$ was used for the comparison of the means of three replications.

RESULTS AND DISCUSSION

Effect of melatonin treatment on O_2^- production rate and H_2O_2 content

In higher plants, about 2% of the O_2

released during aerobic respiration can be converted to $O_2^{\cdot-}$, which is one of the major sources of ROS (Zhang *et al.*, 2013). $O_2^{\cdot-}$ can be converted to H_2O_2 under the action of SOD, then $O_2^{\cdot-}$ and H_2O_2 can be converted to OH^{\cdot} (a more toxic ROS) in the Haber-Weiss/Fenton reactions (Mittler, 2002). Excessive accumulation of ROS can promote oxidation that causes quality deterioration and postharvest senescence (Zhang *et al.*, 2018).

It was observed that the $O_2^{\cdot-}$ production rate of avocado fruit increased rapidly during

ripening and reached a peak value on the day 6 postharvest, then decreased until the end of storage in all samples. However, MT-treated fruit had a lower $O_2^{\cdot-}$ production rate than the control fruit at all study time points ($P < 0.05$), in which 0.5 mM MT treatment was more effective than 0.1 mM treatment. The highest $O_2^{\cdot-}$ production rate was 4.2 $\mu\text{mol/g/min}$ (MT 0.5 mM) and 5.28 $\mu\text{mol/g/min}$ (MT 0.1 mM) compared to the control sample at 5.94 $\mu\text{mol/g/min}$ after 6 days of harvesting (Table 1).

Table 1. Effect of MT treatment on $O_2^{\cdot-}$ production rate and H_2O_2 content of the avocado fruit during ripening.

Days after harvest	Production rate of $O_2^{\cdot-}$ ($\mu\text{mol/g FW/min}$)			H_2O_2 content ($\mu\text{mol/g FW}$)		
	Control	0.1 mM MT	0.5 mM MT	Control	0.1 mM MT	0.5 mM MT
0	1.46 \pm 0.15			21.22 \pm 0.42		
2	2.68 \pm 0.17 ^a	2.49 \pm 0.15 ^b	2.07 \pm 0.16 ^c	31.72 \pm 0.92 ^a	30.46 \pm 0.85 ^b	30.02 \pm 0.79 ^b
4	3.83 \pm 0.18 ^a	3.24 \pm 0.25 ^b	2.63 \pm 0.14 ^c	39.21 \pm 1.26 ^a	36.07 \pm 0.78 ^b	34.01 \pm 1.04 ^c
6	5.94 \pm 0.15 ^a	5.28 \pm 0.16 ^b	4.20 \pm 0.22 ^c	71.77 \pm 1.02 ^a	63.24 \pm 1.02 ^b	42.18 \pm 0.69 ^c
8	5.48 \pm 0.19 ^a	4.92 \pm 0.17 ^b	3.86 \pm 0.16 ^c	58.78 \pm 0.92 ^a	52.11 \pm 1.04 ^b	37.79 \pm 1.25 ^c
10	5.05 \pm 0.19 ^a	4.33 \pm 0.18 ^b	2.65 \pm 0.17 ^c	51.32 \pm 0.89 ^a	50.05 \pm 1.13 ^b	34.83 \pm 0.92 ^c
12	4.59 \pm 0.25 ^a	4.09 \pm 0.18 ^b	2.51 \pm 0.16 ^c	48.69 \pm 0.77 ^a	49.04 \pm 0.78 ^a	32.09 \pm 1.14 ^b

Noted: Different letter within a row represents significant differences at the 95% confidence level.

Table 1 shows that the fluctuation of H_2O_2 content is similar to the $O_2^{\cdot-}$ production rate, which increased continuously from 0 days to 6 days, rapidly increased from day 4 to day 6, and followed by a decline. The MT treatment reduced H_2O_2 content significantly compared with the control, the largest decrease was in the 0.5 mM MT-treated fruit, and 41.23% reduction after 6 days of harvesting (Fig. 1B). In our study, the content of $O_2^{\cdot-}$ and H_2O_2 increased rapidly during ripening, indicating that there is a trend similarity of change between respiration intensity (unpublished data) and ROS ($O_2^{\cdot-}$ and H_2O_2) production, suggesting a possible dependence of ROS generation on climacteric respiration in avocados. In climacteric fruit, a burst of ROS typically occurs in concert with the raise in respiration rate during ripening

period, increasing ROS participates in the synthesis of ethylene through stimulating the activation of aminocyclopropanecarboxylate oxidase (ACO), which could in turn accelerate ripening and senescence in fruit (Dong *et al.*, 2021). This result is consistent with the study on mango, the H_2O_2 content in the controls gradually increased during ripening, reached a peak value at 12 days and then decreased until 15 days (Dong *et al.*, 2021). $O_2^{\cdot-}$ production rate in control peach fruit increased gradually as storage time progressed (Gao *et al.*, 2016). Previous studies have shown that fruit cultivars differing in respiratory rates may also differ in their oxidative behaviour during fruit ripening because electron transport during respiration is one of the major sources contributing to the ROS production (Apel and Hirt, 2004). The

excessive accumulation of ROS might promote oxidative damage to trigger quality deterioration

and senescence of harvested fruit (Zhang *et al.*, 2018).

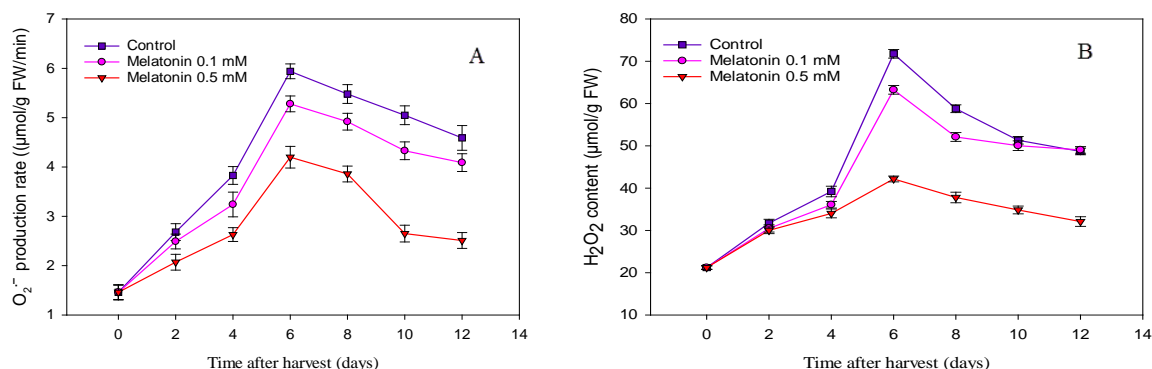


Figure 1. Effect of MT treatment on O₂⁻ production rate (A) and H₂O₂ content (B) of the avocado fruit during ripening.

In this study, the MT treatment significantly reduced H₂O₂ content and O₂⁻ production rate in avocado fruit during ripening, which is similar to the study on blueberry as observed by 17.54% reduction of the O₂⁻ production rate compared to the control at the end of storage period, the H₂O₂ accumulation increased by 14.17% while the control was 23.87% (Shang *et al.*, 2021). The result on strawberry also showed that the H₂O₂ content in 0.1 or 1 mM MT treated fruit remained lower than that of untreated fruit during the whole storage. After 12 d of storage, 0.1 and 1 mM MT treatment resulted in about 27.43% and 25.86% decreases of H₂O₂ content in strawberry fruit as compared to control, respectively. ROS accumulation in MT-treated fruit was reduced due to the inhibition of respiratory intensity and the enhancement of the antioxidant system (Ge *et al.*, 2019). These data indicated that the MT treatment could increase ROS scavenging ability and decrease ROS accumulation in avocado mesocarp, which might alleviate avocado mesocarp cell membrane oxidative damage.

Effects of melatonin treatment on membrane lipid peroxidation in ripening avocado

ROS modify the side chains of unsaturated

fatty acids in membrane lipid, initiating oxidative chain reactions and leading to lipid peroxidation, which damages membranes (Tian *et al.*, 2013). MDA, as the final oxidation product, has been used as an indicator of lipid peroxidation (Dhindsa *et al.*, 1981).

As shown in Table 2, the MDA content of avocado fruit significantly increased from 0 days to 8 days after harvest, peaked at 32.37 µmol/g, and then decreased slowly throughout the remaining days of storage. In the MT treatment, it was observed that the MDA content was different from that of the control, that was, the MDA content increased from 0 days to 4 days, and then decreased sharply on the day 6, decreasing by 37.91% (MT 0.1 mM) and 60.32 % (MT 0.5 mM), reach a peaked value at day 8 and decreased slightly at the end of storage period. The MT-treated fruit had a much lower MDA content than the control, the greatest decrease was exhibited after day 6 of harvesting at 12.53 µmol/g (MT 0.1 mM) and 7.17 µmol/g (MT 0.5 mM) compared with the control at 28.96 µmol/g (P < 0.05) (Fig. 2A).

The increase in ROS contents (Fig. 1) is consistent with increases in membrane lipid peroxidation, indicated by the accumulation of MDA contents in avocado fruit (Fig. 2).

The results on citrus fruit showed that the enhanced MDA level and loss of membrane integrity in fruit under cellular oxidative stress during the postharvest senescence process is usually associated with excessive accumulation of H₂O₂ and oxidative stress burst (Nie *et al.*, 2020). Compared to that of the control fruit, the MT-treated fruits maintained a lower level of MDA content showing that the MT-treatment in avocado was effective in maintaining the membrane integrity, which might thereby contribute to delaying ripening and senescence (unpublished data). These results are similar to the study by Onik (2021) that 1 mM MT treatment on apple reduced the MDA content by 32% and 46%, respectively, on day 28 and day 56 of the storage period. These findings are supported by the study on strawberry fruit (Liu *et al.*, 2018), compared

to untreated fruit, 0.1 or 1 mM MT treatment resulted in respectively 45.48% and 39.26% decreases of MDA content in fruit at the end of storage. It was indicated that MT treatment could enhance the capacity of strawberry fruit to resist the senescence-induced oxidative stress, and then delayed postharvest senescence of fruit. Similar observations have shown that peroxidation of membrane lipid is limited by MT treatment during postharvest storage of cherry (Miranda *et al.*, 2020), blueberry (Shang *et al.*, 2021), mango (Dong *et al.*, 2021). Melatonin can reduce lipid peroxidation by enhancing the activity of ROS-scavenging enzymes (Mittler, 2002), modulating the cell membrane lipid metabolism such as increasing unsaturated fatty acids and mediating the breakdown of membrane phospholipids (Dong *et al.*, 2021).

Table 2. Effect of MT treatment on MDA content and superoxide dismutase activity of the avocado fruit during ripening.

Days after harvest	MDA content ($\mu\text{mol/g FW}$)			SOD activity (U/g FW)		
	Control	0.1 mM MT	0.5 mM MT	Control	0.1 mM MT	0.5 mM MT
0	12.36 \pm 0.49 ^a			29.55 \pm 1.91 ^a		
2	21.07 \pm 0.63 ^a	16.05 \pm 0.63 ^b	16.75 \pm 0.62 ^c	71.45 \pm 2.97 ^c	74.91 \pm 2.98 ^b	79.03 \pm 2.01 ^a
4	24.73 \pm 0.52 ^a	20.18 \pm 0.67 ^b	18.07 \pm 0.53 ^c	48.46 \pm 1.98 ^b	51.23 \pm 1.92 ^a	51.77 \pm 1.98 ^a
6	28.96 \pm 0.94 ^a	12.53 \pm 0.76 ^b	7.17 \pm 0.50 ^c	71.38 \pm 2.48 ^b	65.86 \pm 1.72 ^c	81.23 \pm 2.70 ^a
8	32.37 \pm 1.20 ^a	24.71 \pm 1.25 ^b	19.29 \pm 0.93 ^c	45.89 \pm 1.86 ^c	55.58 \pm 2.70 ^b	62.74 \pm 2.20 ^a
10	26.61 \pm 0.78 ^a	20.35 \pm 0.94 ^b	17.20 \pm 0.69 ^c	36.04 \pm 1.65 ^b	37.31 \pm 1.50 ^b	57.24 \pm 2.41 ^a
12	10.19 \pm 0.66 ^a	9.59 \pm 0.66 ^a	8.56 \pm 0.56 ^b	11.85 \pm 1.66 ^c	23.18 \pm 1.64 ^b	29.36 \pm 2.20 ^a

Noted: Different letter within a row represents significant differences at the 95% confidence level

Effects of melatonin treatment on antioxidant enzyme activity

The antioxidant system plays a vital role in quenching ROS and maintaining cellular redox homeostasis, thereby regulating senescence in plants. SOD, CAT and POD are the important antioxidant enzymes, in which SOD is the first barrier against oxidative damage, catalyzing the

reduction of O₂⁻ to H₂O₂ followed by detoxification of H₂O₂ by CAT and POD (Mittler, 2002).

SOD activity in control fruit and melatonin treatment fluctuated accordingly to similar rules, spiked at 2 days after harvesting, increased 2.42 times (control), 2.54 times (MT 0.1 mM) and 2.67 times (MT 0.5 mM), declined at 4 days,

continued to increase after 6 days and then sharply decreased until the end of ripening and storage period (Table 2). The MT-treated fruit had higher SOD activity at most of the time points of the investigation (except day 6 in the 0.1 mM MT treatment) than the control fruit ($P < 0.05$) (Fig. 2B). Recently, Onik *et al.* (2021) reported that SOD activity increased both in

control and MT treated apple fruit, where MT treated fruit maintained higher SOD activity than the control fruit at 28 days of storage. MT treated fruit showed remarkably promoted SOD activity at 56 days of storage with about 85% elevation compared to the control. Similar observations on peach (Gao *et al.*, 2016), litchi (Zhang *et al.*, 2018) were also reported.

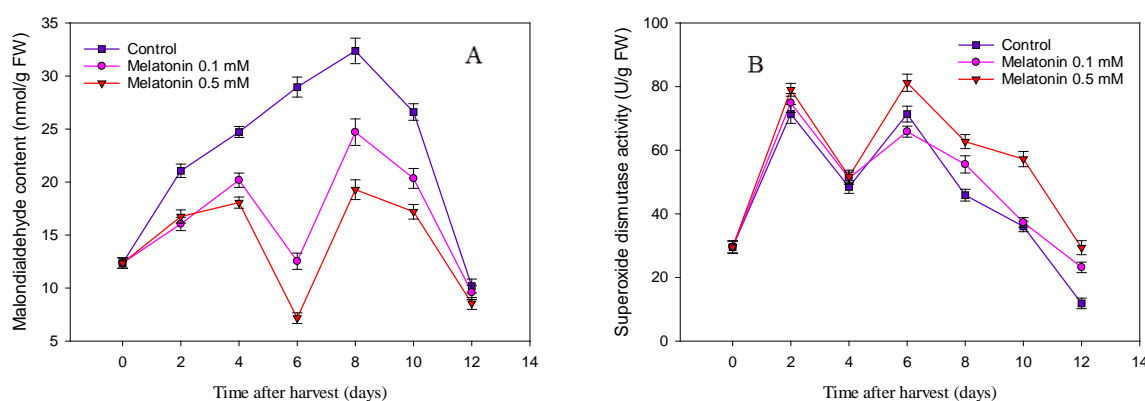


Figure 2. Effect of MT treatment on MDA content (A) and SOD activity (B) of the avocado during ripening.

From the data in Table 3, we found that the change patterns of POD and CAT activities were similar, but different from SOD, the POD and CAT activities experienced a gradual increase in the first days in all experimental treatments, reaching the highest values on day 6 before rapidly declining at the end of storage period. In present study, the POD and CAT activities of the 034 avocado gradually increased, reached a peak value at the time of climacteric respiration (after 6 days of harvesting) and decreased afterwards. These findings are consistent with studies on “Booth 7” avocado (Zhang *et al.*, 2013), POD activities in control fruit increased through 9 days, respectively, and then declined sharply following peak ethylene production to levels below those in pre-ripe fruit. The results in papaya fruit showed that CAT activity increased gradually during ripening of papaya fruit with an increase of 4 folds on 8th days of ripening as compared to control and have play a significant role to dispose of excess H_2O_2 produced during oxidative metabolism (Panday *et al.* 2013).

On the other hand, the 0.5 mM MT

treatment sample had a much increased POD and CAT activities compared to the control, especially on the 6th day after harvest, increasing 1.83 times (POD activity) and 1.21 times (CAT activity) compared with the control. However, in the final stages of storage, the POD activity in the MT treated fruits showed lower activity than the controls (Fig. 3A).

According to Gao *et al.* (2016, 2018) reports, the effect of MT on POD activity of peach fruits varied depending on the storage temperature. 0.1 mM MT treatment significantly enhanced the activity of POD in peach fruit stored at 1°C. However, 0.1 mM MT inhibited the activity of POD in peach fruit during storage at ambient conditions. In another study, Zhai *et al.* (2018) showed that the use of MT had no significant effect on the activity of peroxidase enzyme in different cultivars of pears storage at 26 °C. This might be influenced by several factors including specific fruit species and/or variety, exposure duration of MT, and storage conditions (Gao *et al.*, 2016; Liu *et al.*, 2020; Onik *et al.*, 2021).

Table 3. Effect of MT treatment on peroxidase and catalase activities of avocado fruit during ripening.

Days after harvest	POD activity (U/g FW)			CAT activity (U/g FW)		
	Control	0.1 mM MT	0.5 mM MT	Control	0.1 mM MT	0.5 mM MT
0	0.41 ± 0.022 ^a			51.66 ± 2.18		
2	0.57 ± 0.022 ^b	0.59 ± 0.025 ^b	0.63 ± 0.024 ^a	53.91 ± 2.17 ^b	55.84 ± 2.37 ^{ab}	56.16 ± 2.50 ^a
4	0.73 ± 0.016 ^b	0.71 ± 0.024 ^c	0.86 ± 0.022 ^a	59.56 ± 2.39 ^c	63.77 ± 1.71 ^b	68.81 ± 2.11 ^a
6	1.08 ± 0.026 ^c	1.25 ± 0.025 ^b	1.98 ± 0.027 ^a	80.73 ± 2.48 ^c	86.69 ± 1.49 ^b	98.03 ± 2.85 ^a
8	0.56 ± 0.027 ^c	0.63 ± 0.021 ^b	1.13 ± 0.033 ^a	67.24 ± 2.01 ^c	76.14 ± 3.75 ^b	93.07 ± 3.16 ^a
10	0.35 ± 0.024 ^a	0.31 ± 0.031 ^b	0.33 ± 0.027 ^{ab}	48.13 ± 2.26 ^c	65.04 ± 2.19 ^b	76.94 ± 2.01 ^a
12	0.31 ± 0.03 ^a	0.29 ± 0.028 ^{ab}	0.27 ± 0.029 ^b	34.91 ± 1.67 ^b	36.61 ± 2.01 ^b	50.23 ± 2.69 ^a

Noted: Different letter within a row represents significant differences at the 95% confidence level

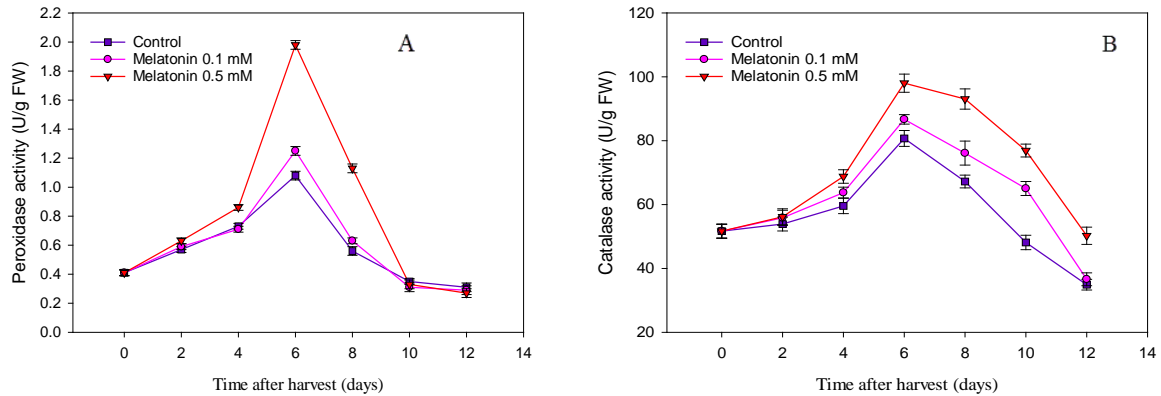


Figure 3. Effect of MT treatment on POD (A) and CAT (B) of the avocado fruit during ripening.

In general, MT treatment simultaneously activated SOD, POD and CAT enzymes with lower O₂⁻ and H₂O₂ levels in MT treated avocado, respectively, suggesting that MT can effectively reduce ROS concentration by enhancing the activity of antioxidant enzymes, contributing to the reduction of lipid peroxidation, slowing down the senescence and ripening of avocado. MT treatment eliminated excess ROS in postharvest fruit by enhancing antioxidants and enzymes involved the repair of oxidized proteins (Xu *et al.*, 2019). MT maintained ROS balance through regulating the expression of genes related to antioxidant enzymes, enhancing their activity and attenuating lipid peroxidation (Wang *et al.*, 2019; Miranda *et al.*, 2020). However, further

research should be carried out comparing different varieties, storage conditions to ensure the maximum possible positive effect of MT on avocado fruit storage. Due to the complexity of the melatonin mechanism in ripening the fruits, molecular studies are still needed to fully clarify the mechanisms involved in fruit quality maintaining after MT treatment. It is hoped that the results of this study will help to further understand the mechanism of this growth regulator in different fruits.

CONCLUSION

The application of 0.5 mM MT for 2 hours was effective, improved the antioxidant potential of avocado fruit during ripening at 22

°C with 75-80% RH. It showed the positive effects on improving the levels of ROS and reducing membrane lipid peroxidation, which attributed to protect membranes from oxidative stress damage. Furthermore, the MT treatment significantly enhanced antioxidant enzymes activity such as SOD, POD and CAT in avocado fruits during the storage. The results suggest that at optimum concentration, MT may be considered as a promising preservation approach for harvested avocado fruit.

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