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INFLUENCE OF EXOGENOUS APPLICATION OF MELATONIN ON BIOCHEMICAL CHANGES OF AVOCADO FRUIT DURING RIPENING

Vu Thi Cam Tu[1], Huynh Thi Thanh Tra[1], Hoang Duc An[1], Le Duy Thanh[1], Hoang Thi Minh Nguyet[2], Truong Thi Hue[1,*]

[1]Quy Nhon University, Binh Dinh, Vietnam
[2]Vietnam National University of Agriculture, Vietnam

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ABSTRACT

Melatonin (MT) is considered as a plant growth regulator, has extensive functions and plays a prominent role in ripening and fruit senescence. However, there has been no report regarding the influence of melatonin on biochemical changes of avocado fruit (*Persea americana* Mill.) during ripening. In this study, the cultivar of avocado fruit "034" was treated with MT at 0.1 mM and 0.5 mM to evaluate biochemical changes relating to the fruit quality during storage at 22 ^{o}C ± 1 and 75–80% relative humidity. The results showed that postharvest fruits treated with MT effectively delayed the changes in ripening parameters and that the effectiveness depends on MT concentration. Application of MT (0.5 mM, immersion for 2 hours) to "034" avocado enhanced total phenol and flavonoid accumulation and maintained titratable acidity, total soluble solids, lipid and ascorbic acid contents. Furthermore, 0.5 mM MT treatment effectively suppressed the increase of total sugar and reduced weight loss compared with the control fruit after 12 days of storage. The results indicate that the MT application could be a useful tool to maintain avocado fruit quality during the postharvest stage.

Keywords: *Persea americana*, melatonin, postharvest quality, biochemical parameter, phenolic compound.

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**Corresponding author email*: truongthihue@qnu.edu.vn

INTRODUCTION

Avocado (*Persea americana* Mill.), one of the most important tropical fruits and an excellent source of energy, containing a high amount of lipids, proteins, vitamins, fibers, antioxidants and minerals (Zhang et al., 2013; Saidi et al., 2021). Avocado fruit is also rich in bioactive compounds including vitamin E, ascorbic acid, carotenoids and soluble phenolic compounds which are highly beneficial to health (Vincent et al., 2020). Therefore, the avocado industry in the global market has been increased steadily over the past two decades, making avocado become an incredibly popular food in the daily diet in many countries.

Avocado is a climacteric fruit and thereby presents fast postharvest ripening, rapid softening and deterioration, nutritional losses; in addition, storing avocado at room temperature for 5–7 days restricts either the procedure of domestic consumption or exportation (Nguyen Minh Nam et al., 2012; Vincent et al., 2020). Avocado fruits are mainly eaten raw which means that the fruit requires effective methods to maintain its quality and extend the postharvest life. 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 biofilm application,… (Munhuweyi et al., 2020).

Melatonin (N-acetyl-5-methoxytryptamine), a derivative of the amino acid tryptophan, was discovered initially in plants in 1995. Melatonin, as an endogenous plant growth regulator with various functions in plants, such as seed germination, plant growth and development, leaf senescence; increases plant tolerance in different biotic and abiotic stress conditions; acts as an antioxidant that helps in the protection of cell membrane from damages caused by free radicals. Recent reports have shown the role of MT in the regulation of fruit ripening and senescence (Rastegar et al., 2020).

Recently, many studies have indicated that exogenous treatment of MT is an effective method in extending the postharvest life and maintaining the quality of banana (Hu et al., 2017), cherry (Miranda et al., 2020) and apple fruit (Onik et al., 2021); slowing down senescence and enhancing chilling stress tolerance in peace (Gao et al., 2016), delaying ripening and softening of postharvest mango fruit (Liu et al., 2020). However, to the best of our knowledge, there has been no report regarding the effect of melatonin on avocado fruit quality. Hence, the purpose of this study was to clarify the influence of the exogenous application of melatonin on biochemical changes in the avocado fruit during ripening. The results provide a further understanding of the mechanism of melatonin in different postharvest fruits.

MATERIALS AND METHODS

Fruit material

Avocado (*Persea americana* Mill. cv. 034) fruits at the 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 an 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 was air-dried and stored at 22 °C ± 1 and 75–80% RH. During storage, avocado samples from each treatment were randomly selected for measurement and analysis of biochemical

parameters related to commercial quality. 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.

Measurement of weight loss

Weight loss was evaluated by weighing the avocado fruit samples before and after storage, using the analytical scale with a readability of 0.01 g.

Measurement of total soluble solids, total acidity content

The total soluble solids (TSS) content was determined according to TCVN 4414:1987, using a hand-held-refractometer at Atago (Japan). The determination of TSS is based on the refractive index of sugar content and other organic compounds and expressed in the percentage of soluble solids in the fruit juice (%). The titratable acidity is determined by titrating NaOH 0.1 N to neutralize the acid present in fruit juice containing phenolphthalein indicator (TCVN 5483:1991).

Determination of total sugar, lipid and ascorbic acid content

Total sugar content was determined by the Bertrand method based on the reduction of copper (II) oxide to copper (I) oxide which forms a red brick precipitate in an alkaline medium, from that the percentage of reducing sugar was calculated (TCVN 4594:1988). Lipid content was obtained by the Soxhlet method which is based on the solubility of lipid in an organic solvent to extract fatty acids from fruit material. The ascorbic acid content was determined by the filtration based on the reduction of iodine 0.01 N in the presence of ascorbic acid and was expressed as mg/100 g fresh weight (FW) (Pham Thi Tran Chau et al., 1998).

Determination of total phenol and total flavonoids content

0.5 g of fruit pulp were homogenized with 1.5 mL of methanol 80%, then centrifuged at 11,000 × *g* for 20 min at 4 ^{o}C and the supernatant was used to quantify total phenol and flavonoids content.

Total phenol content was measured using Folin - Ciocalteu colorimetric procedure adapted from Singleton et al. (1999) with some modifications. 0.3 mL of methanol extract and 1.2 mL Na_2CO_3 7% were added into a 15 mL Falcon tube. 1.5 mL of Folin - Ciocalteu reagent was then added and mixed well. The mixture was incubated at room temperature for 90 mins and the absorbance of the mixture was then read at 760 nm using a UV-visible spectrophotometer (CECIL-4002, England). The results were obtained using a gallic acid standard curve and expressed as mg of gallic acid equivalents per 100 g of fresh weight.

Total flavonoid content was determined by the spectrophotometric method described by Chang et al. (2002). 0.5 mL of methanol extract and 0.1 mL $AlCl_3$ were mixed with 0.1 mL CH_3COOK 1 mM and then 2.8 mL distilled water, shaken well and then allowed to stand at room temperature for 30 mins. The absorbance was read at 415 nm using a UV–vis spectrophotometer (CECIL-4002, England). The flavonoid content was calculated from the quercetin standard curve and expressed as mg of quercetin equivalents per 100 g of fresh weight (Chang et al., 2002).

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

Effect of melatonin treatment on the total soluble solids content and total acidity of the avocado fruit

It can be seen that TSS content increased rapidly with the ripening time and peaked at 10.86% after six days of harvesting in control samples (Fig. 1A). After that, TSS content decreased and reached only 4.47% after 12 days of harvesting. However, the 0.5 mM MT treated

fruits had a slower increase in TSS, taking up to 8 days to reach the maximum value (10.57%) and decreased less than that of the control treatment and 0.1 mM MT treated to fruit, reaching 5.63% after 12 days of harvesting (Table 1).

Table 1. Effect of MT treatment on TSS content and total acidity of the avocado fruit

Days after harvest	Total soluble solids content (%)			Total acidity (%)		
	Control	MT 0,1 mM	MT 0,5 mM	Control	MT 0,1 mM	MT 0,5 mM
0	5,63 ± 0,09			0,263 ± 0,012		
2	$6,8^{a}$ ± 0,09	$6,7^{b}$ ± 0,07	$5,73^{c}$ ± 0,07	$0,245^{a}$ ± 0,018	$0,253^{a}$ ± 0,012	$0,25^{a}$ ± 0,021
4	$8,32^{b}$ ± 0,07	$8,47^{a}$ ± 0,11	$6,7^{c}$ ± 0,10	$0,193^{b}$ ± 0,012	$0,200^{b}$ ± 0,020	$0,22^{a}$ ± 0,013
6	$10,86^{a}$ ± 0,07	$10,47^{b}$ ± 0,09	$7,47^{c}$ ± 0,10	$0,092^{c}$ ± 0,009	$0,108^{b}$ ± 0,010	$0,147^{a}$ ± 0,01
8	$8,53^{c}$ ± 0,09	$8,67^{b}$ ± 0,07	$10,57^{a}$ ± 0,11	$0,070^{c}$ ± 0,008	$0,084^{b}$ ± 0,007	$0,111^{a}$ ± 0,007
10	$6,03^{c}$ ± 0,10	$6,23^{b}$ ± 0,07	$8,33^{a}$ ± 0,09	$0,066^{b}$ ± 0,006	$0,071^{b}$ ± 0,007	$0,091^{a}$ ± 0,006
12	$4,47^{c}$ ± 0,07	$4,53^{b}$ ± 0,07	$5,63^{a}$ ± 0,07	$0,056^{c}$ ± 0,007	$0,063^{b}$ ± 0,005	$0,079^{a}$ ± 0,005

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

According to Table 1, total acidity (TA) fluctuated in the opposite trend away from TSS. The total acid content of avocado fruit at harvest time (0 days) was 0.263% and decreased gradually during ripening in all experimental treatments, especially at 6 days after harvest, reaching 0.092% (control sample), 0.108% (0.1 mM sample) and 0.147% (0.5 mM sample). Melatonin treatment had no significant effect on the total acid content of avocado fruit during the first 6 days of storage. However, from day 6 to day 12 after harvest, 0.5 mM MT treated fruit had higher TA content than control fruit and 0.1 mM MT treated fruit ($P < 0.05$) (Fig. 1B).

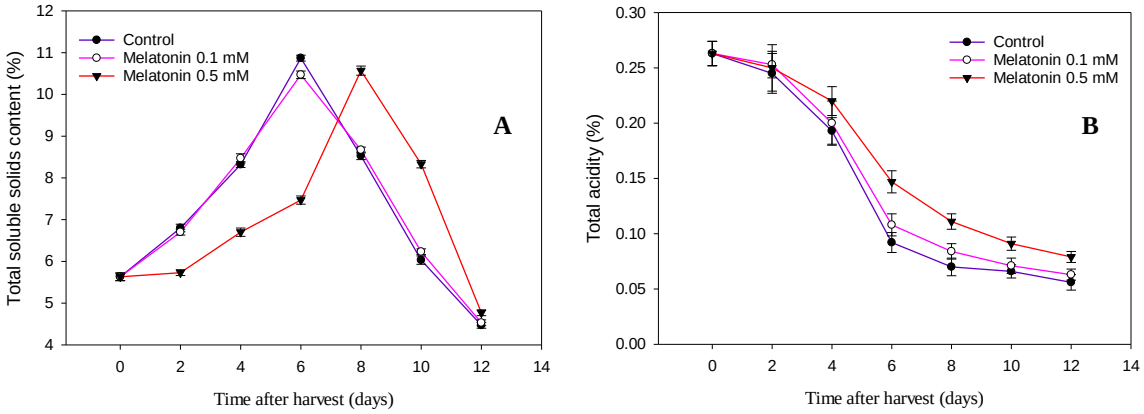

Figure 1. Effect of MT treatment on total TSS (A) and total acidity (B) of the avocado fruit during ripening

Effect of melatonin treatment on total sugar and lipid content of avocado fruit during storage

Total sugar content in control fruits and 0.1 mM treated fruits fluctuated at the same pace, increasing rapidly from day 2 to day 6 and reaching a peak value on day 8 postharvest, at 2.27% and 2.25%, respectively (Fig. 2A). Afterwards, the total sugar content decreased sharply at 10 days after harvest, by day 12 the total sugar

content was only 0.9% (control) and 0.87% (0.1 mM treatment) (Table 2). However, the 0.5 mM treated sample exhibited a slow increase in the total sugar content throughout the first days of storage, which is 1.42 times lower than control and 1.31 times lower than 0.1 mM treatment, followed by a maximum value after 10 days of harvesting (2.15%) and then by a decrease on day 12 (1.51%).

Table 2. Effect of MT treatment on total sugar and lipid content of the avocado fruit

Days after harvest	Total sugar content (%)			Total lipid content (%)		
	Control	MT 0,1 mM	MT 0,5 mM	Control	MT 0,1 mM	MT 0,5 mM
0	1,06 ± 0,17			9,87 ± 0,05		
2	$1,28^{a}$ ± 0,21	$1,25^{a}$ ± 0,20	$1,25^{a}$ ± 0,20	$10,59^{a}$ ± 0,04	$10,49^{a}$ ± 0,04	$10,34^{b}$ ± 0,05
4	$1,66^{a}$ ± 0,19	$1,60^{ab}$ ± 0,23	$1,42^{b}$ ± 0,18	$12,59^{a}$ ± 0,04	$11,10^{b}$ ± 0,05	$11,18^{b}$ ± 0,03
6	$2,10^{a}$ ± 0,15	$1,94^{a}$ ± 0,22	$1,48^{b}$ ± 0,19	$13,35^{b}$ ± 0,04	$12,87^{c}$ ± 0,04	$13,78^{a}$ ± 0,06
8	$2,27^{a}$ ± 0,16	$2,25^{a}$ ± 0,18	$1,93^{b}$ ± 0,18	$14,31^{c}$ ± 0,06	$15,07^{b}$ ± 0,04	$15,56^{a}$ ± 0,02
10	$1,08^{b}$ ± 0,17	$0,98^{b}$ ± 0,17	$2,15^{a}$ ± 0,18	$13,79^{c}$ ± 0,04	$14,39^{b}$ ± 0,04	$15,11^{a}$ ± 0,04
12	$0,90^{b}$ ± 0,17	$0,87^{b}$ ± 0,13	$1,51^{a}$ ± 0,17	$13,48^{c}$ ± 0,03	$13,83^{b}$ ± 0,04	$14,5^{a}$ ± 0,05

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

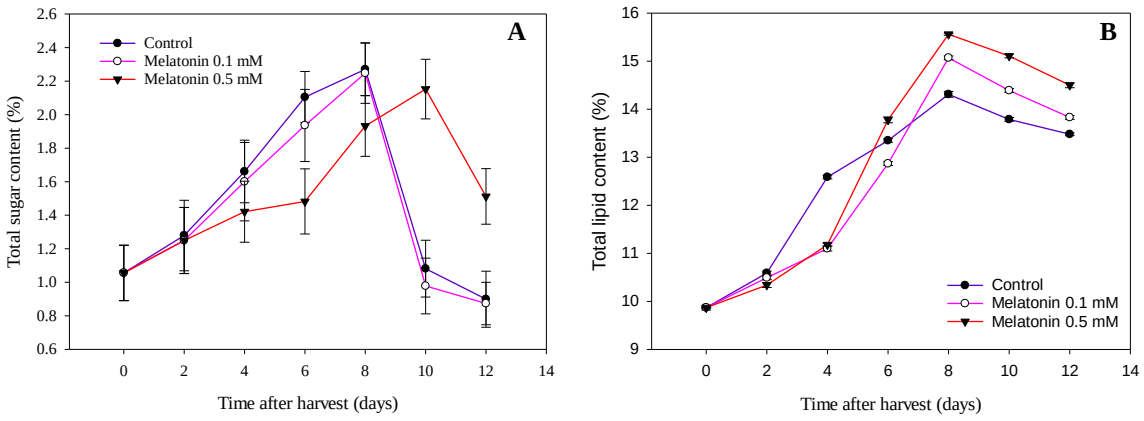

Figure 2. Effect of MT treatment on total sugar (A) and lipid content (B) of the avocado fruit

Based on the results in Table 2, it is recognized that melatonin treatment increased higher lipid accumulation compared to control treatment, reaching the value of 15.07% (0.1 mM MT treatment) and 15.56% (0.5 mM MT treatment) after 8 days of harvesting whereas the control value was 14.31% ($P < 0.05$). On the other hand, melatonin treatment was able to maintain and limit the decrease in lipid content compared to the control sample, in which the 0.5 mM MT treatment (14.5%) was more effective than 0.1 mM MT treatment (13.83%) after 12 days of harvesting (Fig. 2B).

Effect of melatonin treatment on total phenolic, flavonoid contents, ascorbic acid and weight loss of avocado fruit during ripening

The total phenol content of avocado fruit tended to accumulate gradually during theripening process, reaching the highest value of 96.08 mg GAE/100 g at 10 days after harvest (Fig. 3A). In MT treated to fruit, the variation in total phenol content was similar to that of control fruit, that was, the total phenol content elevated markedly on day 2 after harvest, thereafter increased gradually and peaked at 98.11 mg/100 g (0.1 mM) and

108.15 mg/100g (0.5 mM) on day 10 after harvest. Fruit treated with 0.5mM melatonin had a higher total phenolic content than the control at all time points of the study, from 3.12 mg GAE/100 g FW to 13.08 mg GAE/100 g FW (Table 3).

Table 3. Effect of MT treatment on total phenol and flavonoid contents of the avocado fruit

Days after harvest	Total phenol content (mg/100 g FW)			Total flavonoid content (mg/100 g FW)		
	Control	MT 0,1 mM	MT 0,5 mM	Control	MT 0,1 mM	MT 0,5 mM
0	73,98 ± 2,84			45,04 ± 1,23		
2	$80,95^{b}$ ± 3,55	$82,99^{ab}$ ± 2,23	$84,07^{a}$ ± 2,21	$46,74^{a}$ ± 1,58	$46,83^{a}$ ± 1,73	$47,92^{a}$ ± 1,70
4	$84,69^{b}$ ± 2,24	$86,48^{ab}$ ± 2,89	$89,58^{a}$ ± 3,99	$47,26^{b}$ ± 1,44	$47,95^{b}$ ± 2,21	$51,5^{a}$ ± 1,97
6	$88,72^{b}$ ± 2,71	$89,72^{b}$ ± 2,39	$96,61^{a}$ ± 2,09	$49,22^{c}$ ± 1,21	$52,81^{b}$ ± 1,00	$56,47^{a}$ ± 1,54
8	$93,12^{b}$ ± 2,85	$94,65^{b}$ ± 3,50	$101,34^{a}$ ± 2,92	$52,9^{c}$ ± 1,86	$61,47^{b}$ ± 1,11	$63,66^{a}$ ± 1,26
10	$96,08^{b}$ ± 2,46	$98,11^{b}$ ± 4,35	$108,15^{a}$ ± 2,27	$50,97^{c}$ ± 1,41	$56,75^{b}$ ± 1,00	$60,59^{a}$ ± 1,47
12	$86,93^{b}$ ± 2,63	$89,31^{b}$ ± 3,12	$100,01^{a}$ ± 2,98	$30,99^{c}$ ± 1,81	$33,26^{b}$ ± 1,75	$42,53^{a}$ ± 1,24

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

The flavonoid content also gradually increased during the first days of storage in all treatments, reached a maximum on day 8 after harvest and then decreased rapidly by the end of the storage period (Table 3). The MT treated sample also had an increased total flavonoid content compared with the control, in which 0.5 mM melatonin treatment was found to be more positive and effective than 0.1 mM, 63.66 mg QE/100 g and 61,47 mg QE/100 g, respectively whereas 52.9 mg/100 g was for the control. On the other hand, 0.5 mM MT treatment restrained the decrease in the flavonoid content during the last days of the storage process, remaining at 42.53 mg/100 g after 12 days of harvesting (Fig. 3B).

Ascorbic acid of avocado was significantly ($P < 0.05$) affected by MT treatment. Fruits treated with 0.5 mM MT maintained a higher level of ascorbic acid (4.74 mg/100 g) compared to the control (2.05 mg/100 g) at 12 days after harvest (Fig. 4A). It was observed that there was a significant difference in the rate of weight loss between the control and the MT treated fruits across the time points of analysis. Which, the control sample had the highest rate of weight loss, up to 19.82% after 12 days of harvesting, while the melatonin treated samples exhibited a much lower rate, at 17.87% (0.1 mM) and 15.23% (0.5 mM) (Fig. 4B).

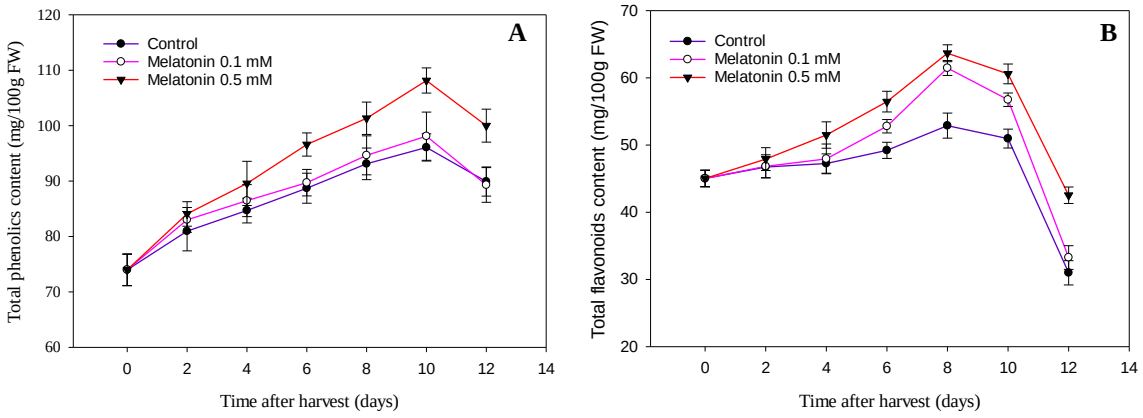

Figure 3. Effect of MT treatment on total phenol (A) and flavonoid contents (B) of the avocado fruit

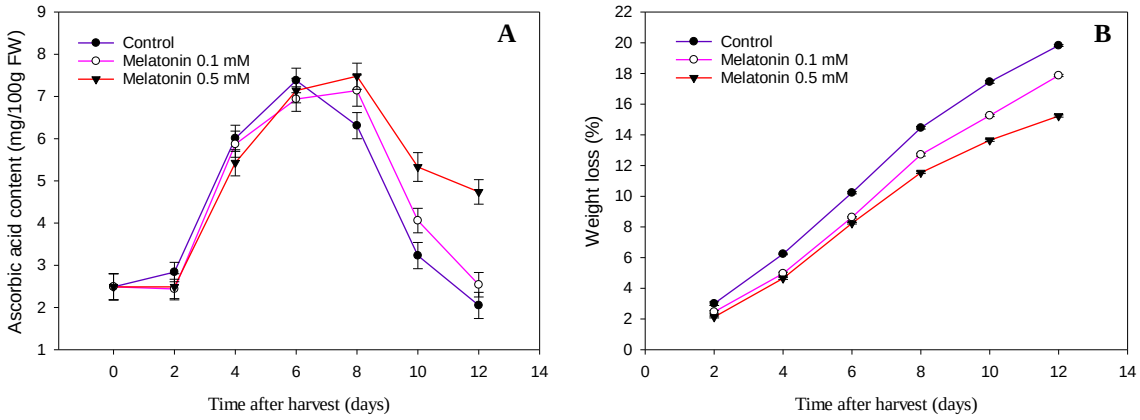

Figure 4. Effect of melatonin treatment on ascorbic acid (A) and weight loss (B) of avocado fruit during ripening

DISCUSSION

Avocado fruit is considered a fruit with a high rate of weight loss and great changes in nutritional value during ripening and storage (Aguirre-Joya et al., 2017). The results indicated that the use of exogenous melatonin could improve the 034 avocado fruit after harvest and the influence was dependent on melatonin concentration. In the present study, the 0.5 mM MT treatment for 2 hours was effective in improving the quality of 034 avocado fruit by reducing weight loss, increase in the accumulation of total phenol and total flavonoids contents. The treatment also maintained soluble solids, titratable acidity, lipid, and ascorbic acid content after 12 days of harvesting at 22 ^{o}C. The optimal MT concentration for avocado fruit during ripening observed in our study compared with those reported in jujube fruit (Tang et al., 2020) and strawberry fruit (Liu et al., 2018) was inconsistent. 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).

The 0.5 mM MT treatment was effective in maintaining soluble solids content of avocado fruit during storage processed at 22 ^{o}C. The change in soluble solids content is compatible with the change in total sugar content in this study. Fruit treated with 0.5 mM MT had a slower increase in total sugar content as compared to the control and 0.1 mM sample. Similarly, postharvest melatonin application reduced the conversion of starch into soluble sugars of four banana varieties with delayed ripening. Several reports have shown that melatonin can regulate sugar metabolisms, thus maintaining high soluble solids content (Tijero et al., 2019). During the ripening process, organic acids are also converted into sugars and take part in respiration, metabolism, and energy generation. 0.5 mM melatonin treatment was able to sustain effectively the total acid contents. Results of the present research are consistent with the study of Tang et al. (2020) indicating melatonin treatment of 25 µM maintained the total acid content of jujube fruit, slowing down the nutrient breakdowns and preserving fruit quality. Similar findings were also reported in peach (Gao et al., 2016) and strawberry fruit (Liu et al., 2018). Lipid, the main component in avocado flesh, has a trend in the gradual accumulation of lipid during ripening. This may be because, throughout the period of ripening, propectin (the component that binds fatty acids cells) is hydrolyzed, causing the fat cells to separate from each other and easily be broken down, leading to lipid release (Blakey et al., 2012). The treatment of melatonin at a concentration of 0.5 mM gave a better effect than 0.1 mM, maintaining the lipid content of the avocado

fruit after harvest. It can be explained that melatonin reduces the activity of degrading enzymes (polygalacturonase, cellulase), which in turn limits lipid release from fat cells and lipid oxidation that occurs in avocados.

The nutritional value of avocado fruit is also evaluated through the composition of biologically active substances and antioxidant capacity. In particular, phenol and flavonoid compounds have outstanding biological activities such as anti-inflammatory, antioxidant, and controlling cancer cells' proliferation (Aguirre-Joya et al., 2017). In addition, the phenols and flavonoids can maintain the nutritional quality of the fruit especially to affecting the flavor of the avocado pulp (Ramos-Aguilar et al., 2019). The trend toward the accumulation of total phenols and flavonoids in avocados during ripening was similar to that reported for Hass avocados (Villa-Rodríguez et al., 2011) and Booth 7 cultivar (Zhang et al., 2013). The 0.5 mM MT-treated fruit had the highest total phenol and flavonoid content and reduced the fruit quality loss of 034 cultivars during storage. Similarly, 1 mM MT-treated strawberry fruit had the highest total phenol and flavonoid content at the end of storage time at 4 ^{o}C (Liu et al., 2018). Other studies on litchi (Zhang et al., 2018), mango (Rastegar et al., 2020), grapefruit (Wang et al., 2020) also have similar results. Melatonin treatment increased the total phenol content significantly by upregulating the gene expression for important enzymes such as PAL, CHS1, CHS2 and F3H in the phenylpropanoid biosynthesis pathway of phenols and flavonoids (Zhang et al., 2016).

In addition to phenols and flavonoids, ascorbic acid is an antioxidant that can reduce fruit spoilage during storage (Rastegar et al., 2020; Wang et al., 2020). In the current study, the 0.5 mM melatonin treatment had a positive effect on the ascorbic acid content, limiting the rapid decline in the ascorbic acid content during storage. It is suggested that MT application can increase fruit resistance to oxidative stress during ripening through improvements of bioactive compounds such as ascorbic acid (Gao et al., 2016). The use of exogenous melatonin reduced the postharvest weight loss percentage of 034 cultivars, especially 0.5 mM melatonin treatment proved more effective than treatment with 0.1 mM melatonin. A positive effect of exogenous melatonin on weight loss has been reported in peach fruit (Gao et al., 2016), strawberries (Liu et al., 2018) and mango fruit (Rastegar et al., 2020). Weight loss can be attributed to the loss of surface water, mainly related to changes in cuticle composition along with aquaporin water channels as a contributing factor to water loss when the epidermis loses its integrity. Exogenous melatonin treatment increased the gene expression of CER1 (wax synthesis) and GPAT4/8 (cutin monomer) and decreased the expression of aquaporin-encoding genes in the plasma membrane during storage time (Miranda et al., 2020).

CONCLUSION

The application of 0.5 mM melatonin for 2 hours was effective, and improved the quality of avocado fruit during storage at 22 ^{o}C with 75–80% relative humidity. This effect can be attributed to an increase in the accumulation of total phenol and total flavonoids contents, reaching the highest value of 108.15 mg/100 g and 63.66 mg/100 g fresh weight, respectively; the preservation of soluble solids content, total acidity, lipid, and ascorbic acid content; limitations on the rate of increase in total sugar content and the reduction of weight loss rate (by 4.59%) compared to the control after 12 days of harvesting.

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