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Effects of melatonin treatment on the postharvest quality of strawberry fruit

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ABSTRACT

The effects of exogenous melatonin on postharvest life and quality in strawberry fruit after harvest were evaluated. To explore the optimum concentration of melatonin treatment, strawberry fruit were treated with 0, 0.01, 0.1, 1 and 10 mmol L^{-1} melatonin for 5 min and then stored at 4 °C and 90% RH for 12 d. The results showed that application of melatonin at 0.1 or 1 mmol L^{-1} was notably effective in reducing decay and weight loss of fruit. Senescence of strawberry fruit was clearly delayed by the 0.1 or 1 mmol L^{-1} melatonin treatment, as disclosed by the color, firmness, the total soluble solids content and titratable acidity of the fruit. Melatonin treatment at 0.1 or 1 mmol L^{-1} significantly reduced the accumulation of hydrogen peroxide (H_2O_2) and malondialdehyde (MDA), but increased the total phenolics and flavonoid contents, resulting in the higher antioxidant capacity. Nevertheless, melatonin treatment had a negative impact on the ascorbic acid content. The optimum concentration of melatonin for extending the postharvest life and improving the quality of strawberry fruit was 0.1 or 1 mmol L^{-1}. Moreover, melatonin treatment at 0.1 mmol L^{-1} enhanced the expression of melatonin biosynthetic genes including *FaTDC*, *FaT5H*, *FaSNAT*, and *FaASMT* and consequently increased the content of endogenous melatonin. These findings suggested that melatonin treatment may be a useful technique to extend the postharvest life and improve quality in strawberry fruit.

1. Introduction

Strawberry (*Fragaria* × *ananassa*, Duch.) fruit is one of the most commonly consumed berries both in fresh and processed forms. It is a rich source of a wide variety of nutritive compounds such as sugars, vitamins and minerals, as well as bioactive compounds such as ascorbic acid, carotenoids, phenolic compounds and folates, most of which are natural antioxidants and contribute to the high nutritional quality of the fruit (Tulipani et al., 2011; Giampieri et al., 2015). All of these compounds exert a synergistic and cumulative effect on human health promotion and in disease prevention. Strawberry fruit is a non-climacteric fruit and should be harvested at full maturity stage in order to get the maximum marketing quality. This fruit is also highly perishable, due to high respiration rate, low mechanical resistance, and high susceptibility to the pathogen attack (Hashmi et al., 2013; Neri et al., 2014). Undesirable changes observed during postharvest include desiccation, loss of flesh firmness, mechanical injury, and *Botrytis cinerea* induced decay (Charles et al., 2009; Pombo et al., 2009). Thus, there is an urgent need for reducing decay and extending the postharvest life of strawberry fruit. To date, various postharvest treatments including coating (Fan et al., 2009), chitosan coating combined with calcium treatment (Hernandez-Muñoz et al., 2008), salicylic acid treatment (Babalar et al., 2007), UV-C irradiation (Nigro et al., 2000; Charles et al., 2009; Pombo et al., 2011), and ultrasonic treatment (Cao et al., 2010) have been applied into postharvest preservation of the strawberry fruit. Nevertheless, some of these methods are not commercially reasonable due to low customer preference or need for verifying the effectiveness. The use of different fungicides is probably the most commonly used method to control postharvest decay, but it leaves residues that have potential risks to humans and the environment. Thus, it is urgent to develop new and effective methods to extend postharvest life and improve quality of strawberry fruit.

Melatonin (*N*-acetyl-5-methoxytryptamine) is an endogenously produced indoleamine in all plant species (Reiter et al., 2015). As a healthy ingredient contained in the diet, many fruits and vegetables, including tomato, apple, cherry, banana, and strawberry provide natural melatonin (Stürtz et al., 2011; Feng et al., 2014; Sun et al., 2015). As a safe and beneficial indoleamine, melatonin acts not only as a signaling molecule for enhancing the resistance of plants to biotic and abiotic stresses, but also as a powerful free-radical scavenger and has a direct antioxidant activity (Tan et al., 1993; Arnao and Hernández-Ruiz, 2015). Recently, exogenous melatonin treatment has been tested as an

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effective postharvest treatment to promote ripening and improve quality of tomato fruit (Sun et al., 2015), delay postharvest senescence and increase chilling tolerance of peach fruit (Cao et al., 2016; Gao et al., 2016), attenuate postharvest decay and maintain nutritional quality of strawberry fruit (Aghdam and Fard, 2017), and attenuate postharvest physiological deterioration of cassava storage roots (Ma et al., 2016). However, little information is available regarding the effects of melatonin as a postharvest treatment on the postharvest life and quality of strawberry fruit.

Therefore, the aim of the present study was to assess the effects of postharvest melatonin treatment on the postharvest life and quality of strawberry fruit during storage at 4 °C. This study may promote the application of melatonin on the postharvest quality in strawberry fruit as well as other fruits and vegetables in the future.

2. Materials and methods

2.1. Strawberry fruit handling and treatment

Fresh strawberry (*Fragaria* × *anannasa* cv. Hongyan) fruit at commercial ripeness (75% red stage of ripening) were harvested from a greenhouse in Hefei, China. Healthy fruit of uniform size and free from apparent disease or injuries were selected, and immediately transported to the laboratory. The strawberry fruit were rinsed in tap water before treatment and then immersed in solution for 5 min at 20 °C. For melatonin treatment, 1200 fruit were selected and grouped into 5 lots (240 fruit per lot) for the following treatments in triplicate (80 fruit per replicate). The five solutions made for treatments were: control (distilled water), M0.01 (0.01 mmol L^{-1} melatonin), M0.1 (0.1 mmol L^{-1} melatonin), M1 (1 mmol L^{-1} melatonin), and M10 (10 mmol L^{-1} melatonin). Following immersion, the fruit were dried in air at room temperature for approximately 30 min. Then, all fruit were stored at 4 °C and 90% RH for 12 d. 20 fruit of each replicate were randomly taken at 3, 6, 9 and 12 d after treatment for measurements of decay incidence, severity of decay, weight loss, color, firmness and total soluble solid contents, and then the rest of the fruit were immediately frozen in liquid nitrogen and stored at −80 °C until use.

2.2. Determination of decay incidence and weight loss

Decay incidence of strawberry fruit was the number of fruit showing decay symptoms (rot, lesions or visible fungal growth) relative to the total number of fruit and expressed in percentage (%). The severity of decay was evaluated by observing the decay area on the fruit surface using a 1–5 scale: 0, healthy fruit; 1, 1–20% fruit surface infected; 2, 21–40% fruit surface infected; 3, 41–60% fruit surface infected; 4, 61–80% fruit surface infected; 5, ≥ 81% fruit surface infected and showing sporulation according to Aghdam and Fard (2017). Each treatment contained 3 replicates with 20 fruit per replicate, and the experiment was repeated 3 times. Weight of each fruit was measured following the treatment at day 0 and at the different sampling day. Weight loss was expressed as the percentage loss of the initial weight.

2.3. Determination of color, firmness, total soluble solids content and titratable acidity

Fruit external color was measured using a chromometer (Chroma Meter WSC-S, Shanghai Precision and Scientific Instrument Co. Ltd., Shanghai, China) to obtain L^*, a^* and b^* values by the CIE color system. L^* value represents lightness or darkness, a^* value represents redness or greenness (−greenness to +redness) and b^* value represents blueness or yellowness (−blueness to +yellowness). The a^* and b^* values were converted to hue angle ($H = \tan^{-1}\frac{b^*}{a^*}$) and chroma ($C = \sqrt{a^{*2} + b^{*2}}$). Firmness of fruit was measured by performing a penetration test with a 5 mm cylindrical probe on the skin of whole fruit using a TA-XT2i texture analyzer (Stable Micro Systems, Guildford, UK). The penetration depth was 10 mm and the speed of the probe was 1.0 mm s^{-1}. Firmness was defined as the maximum penetration force (N). Total soluble solid (TSS) content of fruit was measured with a portable refractometer (WYT-32, Quanzhou Optical Co. Ltd., China). Result was expressed in percentage (%). The titratable acidity was measured titrimetrically with 0.1 mol L^{-1} NaOH solution using an automatic titrator (Mettler Toledo V20 volumetric titrator). The result was expressed as g of citric acid per kg of fruit fresh weight basis.

2.4. Determination of ascorbic acid

Ascorbic acid was determined by the phenolindo-2,6-dichlorophenol (DPIP) solution titration procedure (Jagadeesh et al., 2011). Frozen fruit sample was added to 20 mL of buffer solution containing 4 g L^{-1} of anhydrous sodium acetate and 1 g L^{-1} of oxalic acid. The fruit extract was titrated with a calibrated DPIP solution and the titration was repeated using standard ascorbic acid solution (0.10 g L^{-1} ascorbic acid in buffer solution) in place of the fruit extract. Result was expressed as g kg^{-1} of fruit fresh weight basis.

2.5. Determination of hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) contents

The H_2O_2 and MDA contents were measured by using the method described by Ma et al. (2016) with a slight modification. For H_2O_2 content assay, frozen fruit samples were homogenized with 1 mL of 0.1% cold trichloroacetic acid (TCA) solution and then incubated in an ice bath for 10 min. After centrifugation at 12 000 rpm for 15 min at 4 °C, 0.5 mL supernatant was mixed with 0.5 mL of 10 mmol L^{-1} potassium phosphate buffer (pH 7.0) and 1 mL of 1 mol L^{-1} potassium iodide, and the absorbance was measured at 390 nm in a UV/VIS spectrophotometer (Genesys 10S, ThermoFisher). The H_2O_2 content was calculated using H_2O_2 as a standard and expressed as μmol g^{-1} of fruit fresh weight basis.

For MDA content assay, 0.1 g fruit sample was homogenized with 0.9 mL of 10% cold TCA and then incubated in an ice bath for 10 min. After centrifugation at 12 000 rpm for 15 min at 4 °C, 50 μL supernatant was mixed with 1 mL of 10% TCA-containing 0.67% thiobarbituric acid. Then the mixture was incubated at 95 °C for 20 min and centrifuged at 12 000 rpm for 10 min at 4 °C. The absorbance was measured at 450, 532 and 600 nm. The MDA content was calculated using the formula and expressed as nmol g^{-1} of fruit fresh weight basis: MDA content (nmol g^{-1}) = $6.45 \times (OD_{532} - OD_{600}) - 0.56 \times OD_{450}$.

2.6. Determination of total phenolics and flavonoid contents

Total phenolics and flavonoid contents were measured according to the method of Toor and Savege (2005), with little modifications. For the total phenolics assay, the ethanol extract of fruit was added to 2.0 mL of 1 mol L^{-1} Folin-Ciocalteau reagent. After mixing thoroughly for 5 min, 1.5 mL of 7.5% Na_2CO_3 solution was added into the mixture. The mixtures were then incubated for 1 h at room temperature in the dark and the absorbance was measured at 765 nm. Total phenolic content was expressed as g of gallic acid per kg of fruit fresh weight basis.

For total flavonoid assay, the ethanol extract of fruit was mixed with 0.3 mL of 5% $NaNO_2$. After 5 min, 0.3 mL of 10% $Al(NO_3)_3$ was added and after 5 min, 1.5 mL of 1 mol L^{-1} NaOH was added to the mixture and adjusted to 4 mL with distilled water. The absorbance was measured at 510 nm and the result was expressed as g of rutin per kg of fruit fresh weight basis.

2.7. Determination of antioxidant capacity

2.7.1. 2,2-Di-(4-tert-octylphenyl)-1-picrylhydrazyl (DPPH·) scavenging capacity assay

DPPH scavenging capacity was determined according to Nakajima et al. (2004) with minimal modifications. Fruit sample extract was added to 1 mmol L^{-1} DPPH in methanol. The resulting reaction mixture was well shaken and allowed to stand at room temperature for 30 min, and then the absorbance was measured at 517 nm. The percent of inhibition of DPPH· was calculated as follows:

$$\text{DPPH·scavenging capacity}(\%) = \frac{Ac\text{-}As}{Ac} \times 100$$

where A_C is the absorption of the DPPH control solution against the blank of solvent, and A_S is the absorption of the fruit extract against the blank of solvent.

2.7.2. $ABTS^{\cdot+}$ scavenging capacity assay

The antioxidant capacity of strawberry fruit in the reaction with ABTS radical cation ($ABTS^{\cdot+}$) was determined according to Re et al. (1999) with a slight modification. $ABTS^{\cdot+}$ was performed by the reaction of 7 mmol L^{-1} $ABTS^{\cdot+}$ solution with 2.45 mmol L^{-1} potassium persulfate. The mixture was left to stand at room temperature in the dark for 16 h before use. The $ABTS^{\cdot+}$ solution was diluted to obtain the absorbance of 0.7 ± 0.02 at 734 nm as the test reagent. Then the fruit sample solution was mixed with $ABTS^{\cdot+}$ solution and after 6 min incubation, the percentage decrease of the absorbance at 734 nm was calculated. The calculation method of inhibition was the same as DPPH radical scavenging capacity.

2.8. Determination of melatonin content

The melatonin content of strawberry fruit was determined according to the method of Sun et al. (2015) with a slight modification. A total of 2 g fruit sample was homogenized in 4 mL methanol, and then ultrasonicated for 35 min at 45 °C. After centrifugation at 12 000 rpm for 15 min at 4 °C twice, the supernatants were collected and dried under nitrogen gas. Samples were dissolved in 2 mL of 5% methanol and transferred to an Oasis® HLB extraction cartridge (Waters Corporation, Ireland) for the purification of melatonin. Then the melatonin content was analyzed by HPLC-ESI–MS technologies (HPLC-LTQ Orbitrap XL ETD, Thermo Fisher). The separation was performed on Hypersil Gold C18 column (100 mm × 2.1 mm, 3 μm particle size, Thermo) at a flow-rate of 0.2 mL min^{-1} and injection volume was 5 μL. The mobile phase A (0.1% formic acid, v/v) and a mobile phase B (methanol), using a gradient program as follows: 40% B (5 min), from 40 to 80% B (3 min), 80% B isocratic (7 min), from 80 to 40% (2 min), 40% B (3 min). MS-ESI was used in positive mode with a capillary voltage of 35 V. Nitrogen was used as sheath gas at flow rates of 20 arb, and as Aux gas at 5 arb. The scan range was 220–260 *m/z* and the capillary temperature was 275 °C.

2.9. RNA extraction and real-time quantitative PCR

The fruit samples stored at −80 °C were grounded into powder using liquid nitrogen, and total RNA was extracted using Plant RNA Extraction Kit (Takara, Dalian, China) according to the instruction of the manufacturer. Then, the qualified RNA was used for cDNA synthesis by Takara PrimeScript RT reagent Kit (Takara, Dalian, China). Real-time quantitative PCR was performed using the cDNA, and the gene-specific primers used were presented in Table 1. Real-time quantitative PCR was performed using SYBR® Premix Ex Taq™ (Tli RNaseH Plus; Takara, Dalian, China) in Bio-Rad CFX96 Real-Time PCR Detection System (Bio-Rad, Hercules, CA). The PCR conditions were as follows: 95 °C for 30 s followed by 40 cycles of 95 °C for 5 s and 60 °C for 30 s, the melting cycle was form 70 °C to 95 °C and melting curve analyses of amplification products were performed at the end of the PCR reaction. The expression level was normalized by the internal control gene β-actin, using the $2^{-\Delta\Delta C_T}$ method.

2.10. Statistical analysis

All statistical analyses were performed using SAS software. The data were presented as means ± standard deviations (SD) and analyzed by one-way analysis of variance (ANOVA). Differences at $P < 0.05$ were considered to be significant.

3. Results and discussion

3.1. Effect of melatonin treatment on decay incidence, severity of decay and weight loss in strawberry fruit

Results in Fig. 1 showed that decay was observed for the first time after 9 d of storage in the control and 0.01 mmol L^{-1} melatonin treated strawberry fruit, after that, decay incidence increased as storage time progressed. Melatonin treatments, except for the lower concentration of 0.01 mmol L^{-1}, significantly decreased the decay incidence and severity of decay between day 9 and 12 ($P < 0.05$). Compared with the control fruit, about 87.50% and 75.00% decreases in decay incidence were respectively noted for 0.1 and 1 mmol L^{-1} melatonin treated fruit at the end of storage, and the difference between 0.1 and 10 mmol L^{-1} melatonin treatments was significant ($P < 0.05$).

Strawberry fruit are highly susceptible for water loss due to their thin skin structure (Hernandez-Muñoz et al., 2008). Fig. 1C showed that the weight loss of control and melatonin treated fruit increased during storage. Compared to the control and 0.01 mmol L^{-1} melatonin treatment, 0.1 and 1 mmol L^{-1} melatonin treatments significantly decreased the weight loss of the strawberry fruit from day 9 to 12 ($P < 0.05$). This might be ascribed to the better skin strength properties of the strawberry fruit, which was crucial for decreasing the weight loss. A similar result was obtained by Gao et al. (2016), who showed that the decay incidence and weight loss was significantly reduced by melatonin treatment in peach fruit during storage.

3.2. Effect of melatonin treatment on color, firmness, total soluble solids, and titratable acidity in strawberry fruit

Color is one of the most important factor can affect consumer' perception of strawberry fruit. L^* value represents lightness and is useful to evaluate the brownness and darkness of strawberry fruit during storage. In this study, the average initial L^*, a^*, b^*, hue and chroma values for strawberry fruit were 60.65, 22.12, 25.44, 50.09 and 33.06, respectively (Table 2). Both untreated and melatonin treated fruit exhibited gradual decrease in L^* values of strawberry fruit as the storage progressed, indicating darkening of the fruit samples. However, melatonin treated fruit, except for 0.01 mmol L^{-1} melatonin treated fruit, were shinier (higher L^* value) than control fruit after 9 and 12 d of storage ($P < 0.05$). Similarly, hue angle of fruit decreased continuingly during storage in all fruit samples, whereas fruit treated with 0.1 and 1 mmol L^{-1} melatonin exhibited significant higher hue angle at the day 9 and 12 than that in control and other melatonin treatments ($P < 0.05$). Lower chroma value means brighter coloration of the fruit samples. As shown in Table 2, chroma values of the strawberry fruit remained almost constant during the storage and melatonin treatment did not significantly affect the chroma values in fruit ($P > 0.05$).

In this study, strawberry fruit softened gradually during storage at 4 °C (Table 3). Firmness of the initial fruit was 3.99 N and decreased during storage, but this decrease was effectively delayed by the melatonin treatment. Compared to control, 0.1, 1 and 10 mmol L^{-1} melatonin treatments significantly maintained fruit firmness after 9 and 12 d of storage ($P < 0.05$). The concentration of 0.01 mmol L^{-1} melatonin treatment had a similar effect on firmness but to a lesser extent. This

Table 1
Primers used in real-time quantitative PCR.

Gene	Accession number.	Primer sequence (5′ to 3′)	Product size (bp)
FaActin	AB116565	F: GCTAATCGTGAGAAGATGAC R: AGCACAATACCAGTAGTACG	119
FaTDC	f.ananassa_gdr_reftransV1_0030943	F: GCTCGGTTGCTGCTGGTGATG R: CATATTGTTCCTGGCTTGACACATTGG	154
FaT5H	gene07660-v1.0-hybrid	F: CCGCAACCGCACAACTCCTAG R: AATACCGCTTGATCCTTGGCTTCAG	126
FaSNAT	f.ananassa_gdr_reftransV1_0058392	F: TCCGAACCGAATGTAATGTGGCTAC R: CCTCTGGTGGAGATGTTGATGTGTATG	135
FaASMT	f.ananassa_gdr_reftransV1_0025466	F: GCCAGTGATTCAGGAATGATGAACTTG R: GCACTATCCTGCAAGTTAGCAACAAC	188

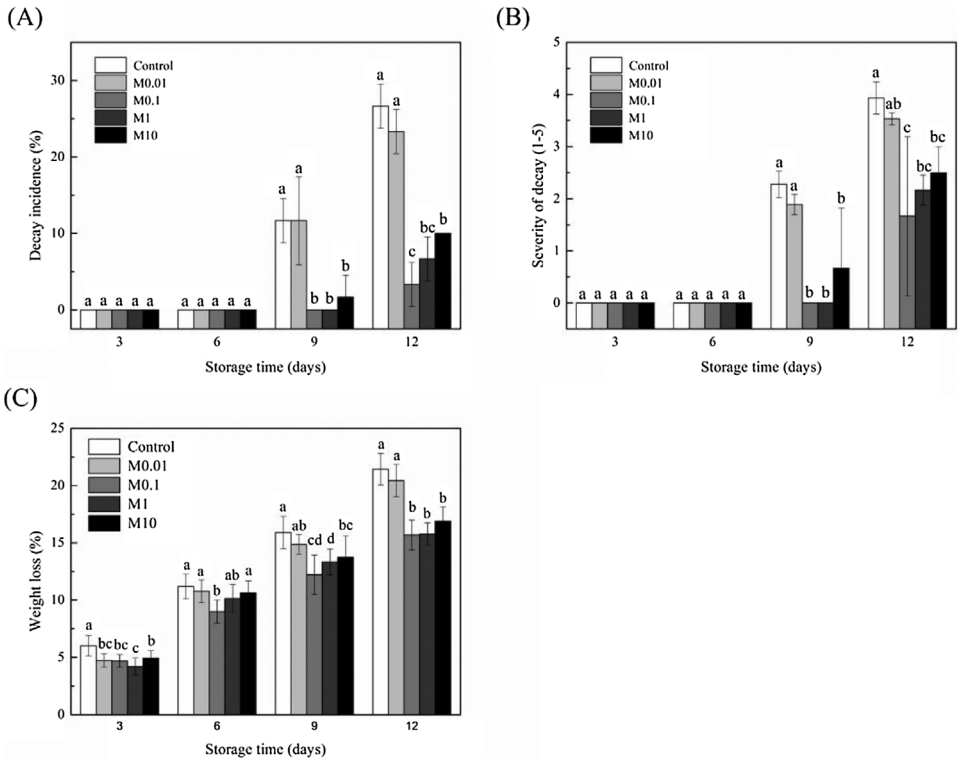

Fig. 1. Effect of melatonin treatment on decay incidence (A), severity of decay (B), and weight loss (C) of strawberry fruit during storage. Error bars represent the standard deviation of the mean of three replicates. Values with different small letters are different at $P < 0.05$.

result was consistent with Gao et al. (2016) who found that melatonin treatment significantly maintained firmness in peach fruit during storage. In contrast, Sun et al. (2015) found that application of an exogenous melatonin dipping at 50 μmol L^{-1} for 2 h markedly accelerated the softening in green mature tomato fruit. The difference responses to melatonin treatment between our study and that of Sun et al. (2015) may be due to the different ripening stages.

No significant difference in TSS content was observed between untreated and melatonin treated strawberry fruit within the first 6 d of storage ($P > 0.05$) (Table 3). Between day 9 and 12, 0.1 mmol L^{-1} melatonin treatment significantly decreased the TSS content compared to untreated and other melatonin treatments ($P < 0.05$).

As indicated in Table 3, the initial titratable acidity of the strawberry fruit was 1.13% and decreased rapidly both in untreated and melatonin treated fruit during the storage, however, this decrease was significantly delayed by 0.1 mmol L^{-1}melatonin treatment between day 6 and 9 ($P < 0.05$). However, no significant differences were found between untreated and melatonin treated fruit ($P > 0.05$). It is well known that over-ripened strawberry fruit have low levels of organic acids, which result in the loss of the fruit characteristic flavor. The relatively lower TSS content and higher titration acidity showed that 0.1 mmol L^{-1} melatonin treatment could delay the process of fruit senescence during storage at 4 °C by preventing characteristic flavor and taste of strawberry fruit.

3.3. Effect of melatonin treatment on ascorbic acid content in strawberry fruit

Ascorbic acid is one of the most important nutritional quality factors in strawberry fruit (Davey et al., 2000). As shown in Table 3, the content of ascorbic acid presented an increasing trend in both control and melatonin treated fruit during the storage period. However, melatonin treatment had a negative effect on ascorbic acid content. The average ascorbic acid content in melatonin treated fruit (0.47 g kg^{-1}) was about 11.32% lower than that in untreated fruit (0.53 g kg^{-1}) at the end of storage. On the contrary, Gao et al. (2016) have shown that the ascorbic acid content in peach fruit was significant maintained after melatonin treatment. The responded difference to melatonin treatment between the present study and that of Gao et al. (2016) was probably due to different species, maturity stages and/or to different conditions of treatment e.g. concentration and duration. Moreover, ascorbic acid in strawberry fruit could be synthesized from the D-galacturonic acid, a principal component of cell wall pectins. Pectin is a major cell wall component whose main building block, homogalacturonan and rhamnogalacturonan I, release D-galacturonic acid upon hydrolysis (Agius et al., 2003). Therefore, reduced pectin solubilization in cell walls

Table 2
Effect of melatonin treatment on color of strawberry fruit during storage.

Storage time (days)	Control	M0.01	M0.1	M1	M10
L^*					
0	60.65 ± 3.82*	60.65 ± 3.82	60.65 ± 3.82	60.65 ± 3.82	60.65 ± 3.82
3	54.62 ± 3.60 b	56.92 ± 2.57 ab	59.09 ± 0.08 a	57.31 ± 1.27 ab	56.33 ± 0.26 ab
6	54.37 ± 2.75 a	53.82 ± 2.48 a	56.23 ± 3.33 a	55.33 ± 3.02 a	54.63 ± 2.89 a
9	51.41 ± 2.56 b	51.85 ± 3.03 ab	54.39 ± 2.13 a	54.36 ± 2.82 a	54.18 ± 2.64 a
12	46.85 ± 0.57 b	46.86 ± 0.61 b	53.05 ± 0.86 a	54.34 ± 0.83 a	52.96 ± 0.55 a
a^*					
0	22.12 ± 1.05	22.12 ± 1.05	22.12 ± 1.05	22.12 ± 1.05	22.12 ± 1.05
3	27.46 ± 2.20 a	27.38 ± 2.42 a	25.78 ± 1.81 a	25.70 ± 2.26 a	26.64 ± 1.03 a
6	27.48 ± 2.02 a	26.89 ± 1.84 a	26.01 ± 2.09 a	25.96 ± 2.11 a	27.26 ± 2.03 a
9	30.39 ± 2.52 ab	30.68 ± 2.01 a	28.66 ± 2.46 ab	28.27 ± 2.42 b	29.93 ± 2.70 ab
12	32.43 ± 2.77 a	33.03 ± 2.11 a	29.47 ± 2.70 b	29.62 ± 2.33 b	32.24 ± 2.47 a
b^*					
0	25.44 ± 1.73	25.44 ± 1.73	25.44 ± 1.73	25.44 ± 1.73	25.44 ± 1.73
3	24.44 ± 1.71 a	24.75 ± 2.25 a	25.15 ± 1.25 a	24.58 ± 2.07 a	24.37 ± 2.18 a
6	23.44 ± 1.95 a	22.98 ± 1.98 a	23.84 ± 2.09 a	23.76 ± 1.80 a	24.04 ± 1.49 a
9	22.71 ± 1.84 b	22.95 ± 1.68 b	24.81 ± 1.71 a	24.32 ± 1.90 ab	24.07 ± 1.64 ab
12	20.46 ± 1.88 bc	19.81 ± 1.64 c	23.16 ± 1.93 a	23.14 ± 2.17 a	21.65 ± 1.36 ab
Hue					
0	50.09 ± 3.29	50.09 ± 3.29	50.09 ± 3.29	50.09 ± 3.29	50.09 ± 3.29
3	41.75 ± 2.25 a	42.12 ± 2.75 a	44.42 ± 3.18 a	43.78 ± 2.36 a	42.49 ± 3.04 a
6	40.61 ± 2.67 a	40.62 ± 3.04 a	42.57 ± 2.78 a	42.54 ± 3.31 a	41.47 ± 3.08 a
9	36.91 ± 2.79 b	36.84 ± 2.23 b	40.90 ± 2.51 a	40.70 ± 2.86 a	39.17 ± 2.78 ab
12	32.38 ± 2.89 b	31.04 ± 2.54 b	38.21 ± 3.37 a	38.07 ± 2.11 a	33.62 ± 2.96 b
Chroma					
0	33.06 ± 1.75	33.06 ± 1.75	33.06 ± 1.75	33.06 ± 1.75	33.06 ± 1.75
3	35.55 ± 2.41 a	35.07 ± 2.31 a	35.80 ± 2.22 a	35.76 ± 2.64 a	36.28 ± 2.68 a
6	36.55 ± 2.12 a	36.06 ± 1.79 a	36.25 ± 1.75 a	37.16 ± 2.28 a	37.24 ± 1.56 a
9	37.10 ± 1.80 a	38.07 ± 1.70 a	37.76 ± 2.07 a	37.29 ± 1.61 a	37.72 ± 2.00 a
12	38.49 ± 2.00 a	38.62 ± 1.54 a	37.59 ± 2.83 a	38.06 ± 2.25 a	38.28 ± 1.60 a

* Data correspond to the means ± standard error of three independent replicates. Different small letters in the same line show significant difference ($P < 0.05$) within the same storage period.

Table 3
Effect of melatonin treatment on firmness, total soluble solids content, titratable acidity, and ascorbic acid content of strawberry fruit during storage.

Storage time (days)	Control	M0.01	M0.1	M1	M10
Firmness (N)					
0	3.99 ± 0.25*	3.99 ± 0.27	3.99 ± 0.25	3.99 ± 0.25	3.99 ± 0.25
3	3.27 ± 0.21 a	3.35 ± 0.25 a	3.46 ± 0.20 a	3.48 ± 0.23 a	3.44 ± 0.19 a
6	3.08 ± 0.55 a	3.13 ± 0.11 a	3.16 ± 0.11 a	3.13 ± 0.27 a	3.12 ± 0.78 a
9	2.60 ± 0.12 c	2.77 ± 0.14 bc	3.04 ± 0.14 a	3.05 ± 0.16 a	2.95 ± 0.21 ab
12	2.22 ± 0.17 b	2.29 ± 0.11 b	2.55 ± 0.14 a	2.63 ± 0.15 a	2.58 ± 0.18 a
Total soluble solids content (%)					
0	8.11 ± 0.26	8.11 ± 0.26	8.11 ± 0.26	8.11 ± 0.26	8.11 ± 0.26
3	8.47 ± 0.30 a	8.38 ± 0.20 a	8.37 ± 0.24 a	8.54 ± 0.34 a	8.39 ± 0.32 a
6	8.60 ± 0.31 a	8.79 ± 0.32 a	8.66 ± 0.32 a	8.82 ± 0.26 a	8.77 ± 0.31 a
9	9.31 ± 0.31 ab	9.35 ± 0.22 ab	8.85 ± 0.33 c	9.12 ± 0.31 b	9.44 ± 0.29 a
12	9.74 ± 0.30 ab	9.51 ± 0.28 ab	9.16 ± 0.27 c	9.25 ± 0.25 b	9.48 ± 0.22 a
Titratable acidity (% citric acid)					
0	1.13 ± 0. 01	1.13 ± 0. 01	1.13 ± 0. 01	1.13 ± 0. 01	1.13 ± 0. 01
3	1.04 ± 0. 01 a	1.06 ± 0. 03 a	1.07 ± 0. 02 a	1.05 ± 0. 01 a	1.08 ± 0. 01 a
6	0.91 ± 0. 01 b	0.91 ± 0. 02 ab	0.92 ± 0. 01 a	0.92 ± 0. 01 ab	0.92 ± 0. 01 ab
9	0.74 ± 0. 01 b	0.75 ± 0. 03 b	0.79 ± 0. 02 a	0.77 ± 0. 01 ab	0.78 ± 0. 01 ab
12	0.65 ± 0. 01 a	0.66 ± 0. 02 a	0.68 ± 0. 01 a	0.66 ± 0. 02 a	0.68 ± 0. 01 a
Ascorbic acid content ($g\,kg^{-1}$)					
0	0.25 ± 0.01	0.25 ± 0.01	0.25 ± 0.01	0.25 ± 0.01	0.25 ± 0.01
3	0.32 ± 0.01 a	0.31 ± 0.02 a	0.26 ± 0.01 b	0.30 ± 0.01 a	0.27 ± 0.02 b
6	0.37 ± 0.02 a	0.36 ± 0.01 ab	0.31 ± 0.01 c	0.35 ± 0.01 b	0.31 ± 0.01 c
9	0.44 ± 0.01 a	0.44 ± 0.01 a	0.40 ± 0.01 b	0.42 ± 0.01 b	0.40 ± 0.01 b
12	0.53 ± 0.02 a	0.50 ± 0.01 b	0.47 ± 0.01 c	0.47 ± 0.01 c	0.45 ± 0.01 c

* Data correspond to the means ± standard error of three independent replicates. Different small letters in the same line show significant difference ($P < 0.05$) within the same storage period.

Table 4
Effect of melatonin treatment on H_2O_2 and MDA contents of strawberry fruit during storage.

Storage time (days)	Control	M0.01	M0.1	M1	M10
H_2O_2 content (μmol g^{-1})					
0	7.84 ± 0.05*	7.84 ± 0.05	7.84 ± 0.05	7.84 ± 0.05	7.84 ± 0.05
3	17.10 ± 0.78 a	16.23 ± 0.58 a	9.02 ± 0.23 c	8.99 ± 0.54 c	11.53 ± 0.80 b
6	23.64 ± 0.63 a	18.56 ± 1.19 b	9.57 ± 0.62 d	10.76 ± 0.53 d	15.51 ± 0.99 c
9	16.56 ± 1.17 a	15.56 ± 0.93 ab	12.28 ± 0.72 d	13.45 ± 0.59 cd	14.59 ± 0.60 bc
12	13.38 ± 1.03 a	11.92 ± 0.91 b	9.71 ± 0.73 c	9.92 ± 0.23 c	12.40 ± 0.62 ab
MDA content (nmol g^{-1})					
0	19.83 ± 0.81	19.83 ± 0.81	19.83 ± 0.81	19.83 ± 0.81	19.83 ± 0.81
3	24.06 ± 1.31 a	24.04 ± 2.11 a	19.38 ± 0.76 b	20.92 ± 1.83 b	21.55 ± 1.49 ab
6	35.68 ± 1.94 a	37.78 ± 1.41 a	27.56 ± 1.17 b	28.15 ± 0.85 b	30.09 ± 2.67 b
9	53.69 ± 2.95 a	45.91 ± 2.83 b	31.89 ± 2.96 d	38.41 ± 1.45 c	39.68 ± 2.05 c
12	64.16 ± 2.04 a	51.13 ± 2.23 b	34.98 ± 0.93 e	38.97 ± 1.83 d	45.72 ± 2.01 c

* Data correspond to the means ± standard error of three independent replicates. Different small letters in the same line show significant difference ($P < 0.05$) within the same storage period.

Table 5
Effect of melatonin treatment on total phenolics and flavonoid contents of strawberry fruit during storage.

Storage time (days)	Control	M0.01	M0.1	M1	M10
Total phenolics content (g kg^{-1})					
0	4.08 ± 0.04*	4.08 ± 0.04	4.08 ± 0.04	4.08 ± 0.04	4.08 ± 0.04
3	4.33 ± 0.06 a	4.24 ± 0.04 a	4.56 ± 0.10 a	4.50 ± 0.29 a	4.52 ± 0.16 a
6	4.72 ± 0.13 c	4.90 ± 0.02 c	6.13 ± 0.34 ab	6.41 ± 0.14 a	5.84 ± 0.34 b
9	5.46 ± 0.11 c	5.51 ± 0.11 c	6.81 ± 0.25 ab	6.97 ± 0.30 a	6.39 ± 0.44 b
12	6.86 ± 0.37 c	6.72 ± 0.06 c	8.60 ± 0.10 a	8.98 ± 0.06 a	7.78 ± 0.27 b
Total flavonoids content (mg kg^{-1})					
0	0.81 ± 0.03	0.81 ± 0.03	0.81 ± 0.03	0.81 ± 0.03	0.81 ± 0.03
3	0.82 ± 0.04 a	0.84 ± 0.06 a	0.86 ± 0.04 a	0.87 ± 0.02 a	0.81 ± 0.02 a
6	1.01 ± 0.03 c	1.10 ± 0.08 bc	1.22 ± 0.07 ab	1.15 ± 0.09 ab	1.25 ± 0.03 a
9	1.17 ± 0.03 b	1.07 ± 0.07 b	1.38 ± 0.06 a	1.34 ± 0.04 a	1.35 ± 0.09 a
12	1.36 ± 0.08 b	1.41 ± 0.03 b	1.77 ± 0.07 a	1.73 ± 0.09 a	1.76 ± 0.04 a

* Data correspond to the means ± standard error of three independent replicates. Different small letters in the same line show significant difference ($P < 0.05$) within the same storage period.

Table 6
Effect of melatonin treatment on antioxidant capacity of strawberry fruit during storage.

Storage time (days)	Control	M0.01	M0.1	M1	M10
DPPH· scavenging capacity (%)					
0	82.91 ± 2.21*	82.91 ± 2.21	82.91 ± 2.21	82.91 ± 2.21	82.91 ± 2.21
3	82.74 ± 2.23 b	83.93 ± 1.07 ab	86.32 ± 1.32 a	85.64 ± 1.78 ab	86.50 ± 1.27 a
6	86.32 ± 1.07 a	86.84 ± 2.82 a	87.86 ± 0.30 a	87.35 ± 1.23 a	87.35 ± 2.09 a
9	88.20 ± 1.36 a	88.55 ± 2.82 a	90.94 ± 2.18 a	90.26 ± 2.08 a	89.74 ± 2.60 a
12	88.07 ± 2.63 b	90.94 ± 1.67 ab	92.48 ± 2.14 a	92.65 ± 1.57 a	92.23 ± 1.79 a
$ABTS^{\cdot+}$ scavenging capacity (%)					
0	68.00 ± 1.73	68.00 ± 1.73	68.00 ± 1.73	68.00 ± 1.73	68.00 ± 1.73
3	75.68 ± 1.96 b	77.27 ± 1.88 ab	79.52 ± 2.43 a	79.38 ± 2.11 ab	79.62 ± 1.15 a
6	78.70 ± 0.91 a	79.38 ± 1.67 a	80.77 ± 2.02 a	80.14 ± 1.23 a	81.39 ± 1.02 a
9	79.28 ± 0.43 b	80.62 ± 2.43 ab	82.88 ± 1.52 a	83.26 ± 1.35 a	82.21 ± 1.30 ab
12	80.96 ± 2.66 b	80.48 ± 3.28 b	86.52 ± 1.58 a	85.80 ± 1.48 a	85.08 ± 1.32 a

* Data correspond to the means ± standard error of three independent replicates. Different small letters in the same line show significant difference ($P < 0.05$) within the same storage period.

usually resulted in lower ascorbic acid. Previous study by Gao et al. (2016) has found that melatonin treatment could maintain fruit firmness and cell wall integrity during storage, which may lead to less D-galacturonic acid released from the cell walls and consequently influence the synthesis of ascorbic acid in strawberry fruit.

3.4. Effect of melatonin treatment on H_2O_2 and MDA contents in strawberry fruit

In control fruit, the H_2O_2 content increased rapidly and then decreased during storage (Table 4). However, the H_2O_2 content in 0.1 or 1 mmol L^{-1} melatonin treated fruit remained lower than that of untreated and 0.01 mmol L^{-1} melatonin treated fruit during the whole storage ($P < 0.05$). And the difference between 0.1 and 1 mmol L^{-1} melatonin treatment was not significant ($P > 0.05$). After 12 d of storage, 0.1 and 1 mmol L^{-1} melatonin treatment resulted in about 27.43% and 25.86% deceases of H_2O_2 content in strawberry fruit as compared to control, respectively.

As shown in Table 4, the MDA content increased continuously during storage in both untreated and melatonin treated strawberry fruit. However, it was significantly lower in the 0.1 or 1 mmol L^{-1} melatonin treated fruit than in untreated and 0.01 mmol L^{-1} melatonin

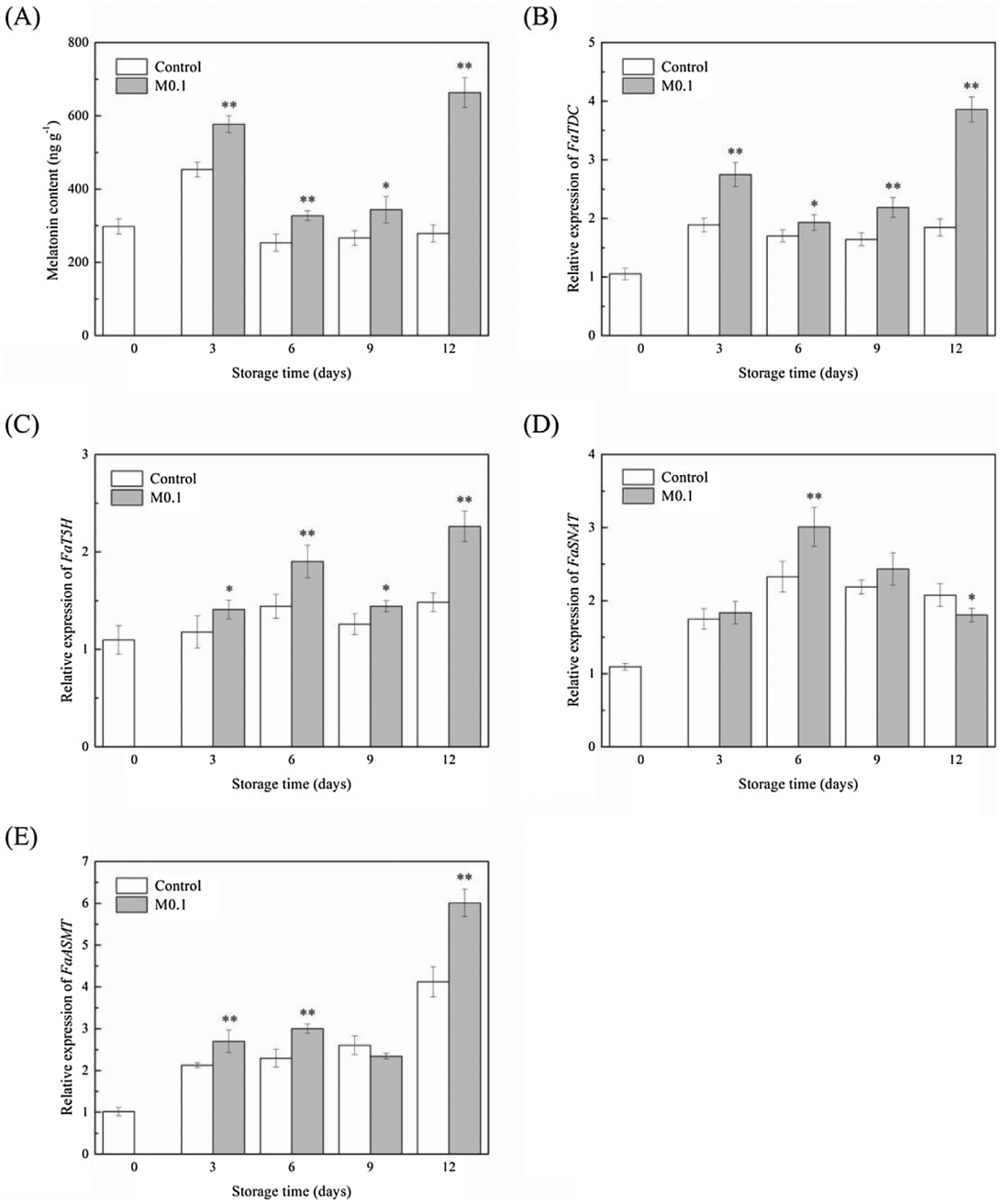

Fig. 2. Effect of melatonin treatment on endogenous melatonin content (A), and relative expression of *FaTDC* (B), *FaT5H* (C), *FaSNAT* (D), and *FaASMT* (E) in strawberry fruit during storage. Error bars represent the standard deviation of the mean of three replicates. Statistical significance of the difference was confirmed according to Duncan's multiple range test at $P < 0.05$ (∗, $P < 0.05$; ∗∗, $P < 0.01$).

treated fruit during the whole storage ($P < 0.05$). Compared to untreated fruit, 0.1 or 1 mmol L^{-1} melatonin treatment resulted in respectively 45.48% and 39.26% deceases of MDA content in fruit at the end of storage.

In this study, H_2O_2 and MDA contents in strawberry fruit were significantly decreased by the melatonin treatment. It was indicated that melatonin treatment could enhance the capacity of strawberry fruit to resist the senescence-induced oxidative stress, and then delayed postharvest senescence of fruit. Similar result was obtained by Ma et al. (2016), who found that the H_2O_2 and MDA contents were significantly lower in the cassava storage root slices treated with melatonin than in untreated slices.

3.5. Effect of melatonin treatment on total phenolics and flavonoid contents in strawberry fruit

Changes of total phenolic and flavonoid contents in strawberry fruit were shown in Table 5. Both the control and melatonin treated strawberry fruit presented an increasing trend in total phenolics and flavonoid contents during storage at 4 °C. Strawberry fruit treated with 0.1, 1 and 10 mmol L^{-1} melatonin demonstrated significantly higher levels of total phenolics and flavonoid contents when compared to the untreated fruit ($P < 0.05$) from day 6 to 12, whereas no significant difference was observed between the control and 0.01 mmol L^{-1} melatonin treatment ($P > 0.05$). And 1 mmol L^{-1} melatonin treated fruit had the highest total phenolics content (8.98 g kg^{-1}) at the end of the storage, followed by 0.1 mmol L^{-1} melatonin treatment (8.60 g kg^{-1}). Aghdam

and Fard (2017) reported that melatonin treatment significantly increased total phenols content of strawberry fruit during storage at 4 °C, which was in accordance with our findings. Sun et al. (2016) noted that melatonin treatment up-regulated the expression of important enzymes genes in the phenylpropanoid pathway, such as *PAL*, *CHS1*, *CHS2* and *F3H*, contributing to the accumulation of total phenolics and flavonoids. Anthocyanins belong to a class of flavonoids synthesized via the phenylpropanoid pathway. Zhang et al. (2016) also reported that the higher anthocyanins accumulation in cabbage seedling in response to melatonin treatment was the result of higher expression of phenylpropanoid biosynthetic genes (*PAL*, *C4H*, *CHS*, *CHI*, *F3H*, etc.).

3.6. Effect of melatonin treatment on antioxidant capacity in strawberry fruit

In this study, two methods were used to study the effect of melatonin treatment on antioxidant capacity in strawberry fruit during cold storage (Table 6). DPPH· scavenging capacity in both untreated and melatonin treated fruit indicated an increasing trend during the whole storage period. And at the end of storage, melatonin treatments, except 0.01 mmol L^{-1} treatment, significantly increased the DPPH· scavenging capacity compared to the control ($P < 0.05$). Similar to DPPH· scavenging capacity, the $ABTS^{\cdot+}$ scavenging capacity presented an increasing trend in both control and melatonin treated fruit during the storage. After 9 and 12 d of storage, 0.1 and 1 mmol L^{-1} melatonin treatments significantly increased $ABTS^{\cdot+}$ scavenging capacity as compared to control, and 1 mmol L^{-1} melatonin treated fruit had the highest $ABTS^{\cdot+}$ scavenging capacity (86.52%) at the end of storage ($P < 0.05$). Previous study also found that melatonin treatment significantly increased the DPPH scavenging capacity in strawberry fruit during storage at 4 °C (Aghdam and Fard, 2017). These results were correlated with the total phenolics content and this positive correlation with total phenolics content and antioxidant capacity was also reported by Puerta-Gomez and Cisneros-Zevallos (2011). Based on these results, we suggested that the higher total phenolics and flavonoid contents in strawberry fruit treated with melatonin was most likely associated with the increased DPPH· and $ABTS^{\cdot+}$ scavenging capacities.

3.7. Effect of melatonin treatment on endogenous melatonin content and the expression of melatonin biosynthesis genes in strawberry fruit

On the basis of the observation that melatonin treatment delayed the senescence of strawberry fruit, we hypothesized that endogenous melatonin content changed during storage. Because 0.1 or 1 mmol L^{-1} melatonin treatment seemed to be the most effective in extending the postharvest life and improving the quality of strawberry fruit, the 0.1 mmol L^{-1} melatonin treatment was selected to further study the endogenous melatonin content along with the expression of melatonin biosynthesizing genes (Fig. 2). As shown in Fig. 2A, both untreated and melatonin treated fruit showed an increasing trend in endogenous melatonin content during the initial 3 d, then decreased in the following 3 d, and again increased towards the end of storage. Exogenous melatonin treatment significantly increased endogenous melatonin content as compared to control during the whole storage ($P < 0.05$). The result indicated that endogenous melatonin might act not only as a signaling molecule for attenuating decay, but also as a powerful antioxidant for delaying the senescence of strawberry fruit.

To further understand the effect of exogenous melatonin treatment on the endogenous melatonin accumulation in strawberry fruit, we analyzed the expression of *FaTDC*, *FaT5H*, *FaSNAT* and *FaASMT* genes, which are involved in melatonin biosynthesis. The results are shown in Fig. 2. It was possible to see that the exogenous melatonin treatment increased the endogenous melatonin content, and it is associated to the increased gene expression levels in the melatonin biosynthesis pathway. The expressions of *FaTDC* and *FaT5H* were significantly up-regulated in the melatonin treated fruit compared to the control fruit from 3 to 12 d of storage ($P < 0.05$). The expression of *FaSNAT* in melatonin treated fruit gradually increased and reached the peak after 6 d of storage and decreased thereafter. The expression of *FaSNAT* was significantly up-regulated in melatonin treated fruit after 6 d of storage ($P < 0.01$). However, at the end of the storage, the expression of *FaSNAT* in control fruit was significantly higher than that in the melatonin treated fruit ($P < 0.05$). The expression of *FaASMT* in melatonin treated fruit increased sharply between 9 and 12 d of storage and reached the peak at the end of storage. The expression of *FaASMT* was significantly up-regulated in melatonin treated fruit except at 9 d of storage ($P < 0.01$). Therefore, exogenous melatonin treatment enhanced the expression of melatonin biosynthetic genes including *FaTDC*, *FaT5H*, *FaSNAT* and *FaASMT*.

4. Conclusions

It can be concluded from this study that 0.1 or 1 mmol L^{-1} postharvest melatonin treatment was most effective in delaying senescence of strawberry fruit by reducing decay incidence, severity of decay and weight loss, delaying the color development, maintaining fruit firmness and titratable acidity, as well as decreasing the TSS, H_2O_2 and MDA content. Furthermore, 0.1 or 1 mmol L^{-1} melatonin treatment promoted the accumulation of total phenolics and flavonoid, resulting in higher antioxidant capacity. Nevertheless, melatonin treatment had a negative impact on the ascorbic acid content. Moreover, 0.1 mmol L^{-1} melatonin treatment enhanced the expression of melatonin biosynthetic genes including *FaTDC*, *FaT5H*, *FaSNAT*, and *FaASMT* and consequently increased the content of endogenous melatonin. In summary, melatonin treatment at intermediate concentration had positive effects on the postharvest life and quality of fresh strawberry fruit.

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