

Exogenous melatonin delays postharvest fruit senescence and maintains the quality of sweet cherries



Feng Wang^{a,b,c}, Xiaoping Zhang^d, Qingzhen Yang^{a,*}, Qifeng Zhao^d

^a Department of Life Sciences, Yuncheng University, Yuncheng, Shanxi Province 044000, PR China

^b Atopy & Health Research Institute, Jeonju University, Jeonju 55069, Republic of Korea

^c Department of Science, Technology & Industry, Yuncheng University, Yuncheng, Shanxi Province 044000, PR China

^d Research Institute of Pomology, Shanxi Academy of Agricultural Sciences, Taigu, Shanxi Province 030815, PR China

ARTICLE INFO

Keywords:

Sweet cherry

Melatonin

Senescence

Antioxidant enzyme

Antioxidant compounds

ABSTRACT

We assessed the effects of postharvest exogenous melatonin (50,100 and 150 $\mu\text{mol L}^{-1}$) on the senescence and quality of sweet cherries during storage at $0 \pm 0.5^\circ\text{C}$. Melatonin treatment decreased decay incidence, respiration rate, and weight loss. It delayed the degradation of firmness, lightness, saturation, hue angle, titratable acidity, and total soluble solids content, thus maintaining better fruit quality. Melatonin treatment inhibited increases in $\text{O}_2^{\cdot-}$, H_2O_2 , malondialdehyde content, and relative membrane permeability, while maintaining higher endogenous melatonin levels and increasing superoxide dismutase and catalase activity. Additionally, melatonin treatment enhanced the activity of antioxidant enzymes, increased the levels of ascorbic acid, and reduced glutathione levels, which are related to the ascorbate–glutathione cycle, as well as increasing the AsA:DHA and GSH:GSSG ratios. Delayed senescence in sweet cherries after exogenous melatonin treatment may be associated with high endogenous melatonin levels and increased antioxidant activity and content.

1. Introduction

Sweet cherries (*Prunus avium* L.) are a popular fruit among consumers because of their desirable taste, attractive appearance, and high nutritional value (Wang et al., 2016). Due to high respiration activity and susceptibility to mechanical damage, the fruits deteriorate quickly after harvesting even under strict cold chain management (Wang, Xie, & Long, 2014). The most common deteriorations include surface pitting, darkening of the skin, pedicel withering, flesh softening, loss of flavor, and decay (Yang, Zhang, Wang, & Zhao, 2019). Various methods, such as the use of electrolyzed water (Hayta & Aday, 2015), modified atmosphere packaging (Wang, Bai, & Long, 2015), calcium chloride solution (Wang et al., 2014), methyl salicylate (Valverde et al., 2015), acetylsalicylic acids (Giménez et al., 2014), and β -aminobutyric acid (Wang et al., 2016), delay the senescence of sweet cherries, but more effective techniques need to be identified to extend their storage period.

Melatonin (N-acetyl-5-methoxytryptamine, MT), a derivative of tryptophan, is an important indoleamine hormone that is widely found in various plant species, including apple, strawberry, tomato, cherry, cucumber, and cassava. MT plays a role in several physiological processes, such as regulating plant growth and development, delaying leaf senescence, and promoting fruit ripening (Hernández-Ruiz & Arnao,

2018). In addition, MT also increases the tolerance of plants to abiotic and biotic stresses by acting as an antioxidant that directly eliminates reactive oxygen species (ROS), activating the antioxidant system, and increasing the efficiency of other antioxidants (Debnath et al., 2019). Exogenous MT reportedly also delays postharvest senescence in broccoli (Zhu, Hu, Luo, Wu, & Li, 2018), cucumbers (Xin, Si, & Kou, 2017) and peaches (Gao et al., 2016) by reducing respiratory rates and increasing antioxidant enzyme activity, e.g., the activity of superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD). Exogenous MT upregulates the expression of genes related to antioxidant enzymes, e.g., SOD, CAT, and glutathione reductase (GR). This process contributes to a reduction in H_2O_2 levels and malondialdehyde (MDA) concentration and delays the postharvest physiological deterioration of cassava storage roots (Ma, Zhang, Zhang, & Wang, 2016). In addition, the process also delays senescence of detached apple leaves by promoting the ascorbic acid–glutathione (AsA–GSH) cycle, increasing the content of reduced substances (AsA and GSH), as well as reducing the accumulation of ROS (Wang et al., 2012). The results of these studies provide evidence regarding the involvement of MT in delaying the senescence of postharvest fruits and vegetables. Unfortunately, information regarding the effect of MT on the senescence and quality of postharvest sweet cherries is limited.

* Corresponding author.

E-mail address: yqz757575@163.com (Q. Yang).

<https://doi.org/10.1016/j.foodchem.2019.125311>

Received 3 May 2019; Received in revised form 30 July 2019; Accepted 31 July 2019

Available online 01 August 2019

0308-8146/ © 2019 Elsevier Ltd. All rights reserved.

Therefore, the present study aimed to evaluate the effects of exogenous MT on the senescence of postharvest sweet cherries and explore the possible mechanisms involved. The findings may greatly assist in the development of new postharvest treatments to extend the shelf life of sweet cherries.

2. Materials and methods

2.1. Materials and treatments

Sweet cherries (*P. avium* 'Sunburst') were sampled at 80–90% maturity by hand-plucking, together with their stalks, from a well-managed demonstration orchard in Hengshui town, Jiang County, Shanxi Province, China (lat. 35°20'19" N, long. 111°21'49" W) and transported to the Fruit and Vegetable Storage and Preservation Laboratory in a refrigerated van at 5 °C within one hour of harvesting. Sweet cherries of uniform size, uniform and bright coloration, without mechanical damage, and without indications of pest or disease infestation were selected and randomly divided into four groups with three replicates each (10 kg of fruit per replicate).

Cherries of the four groups were immediately immersed in 0 (control), 50, 100, and 150 $\mu\text{mol}\cdot\text{L}^{-1}$ MT solutions at 5 °C for 5 min. Subsamples of cherries were cold-air (5 °C) dried, transferred to plastic baskets (44 × 33 × 11 cm) with ~2.5 kg of cherries per basket, then packaged in 0.03 mm polyethylene fresh-keeping bags with a perforation ratio at 3%. The bags were loosely closed with rubber bands to allow the cherries to respiration and remain moisturized, and then stored at 0 ± 0.5 °C with a relative humidity of 90–95% for 63 d.

Forty-five fruits were removed from storage periodically to determine their respiratory rate, firmness, titratable acidity (TA), total soluble solids (TSS) content, and relative membrane permeability. The fruits were pitted, and the fruit tissue was cut into 2 mm small pieces, rapidly frozen with liquid nitrogen, and stored at –80 °C before determining their endogenous levels of MT, $\text{O}_2^{\cdot-}$, H_2O_2 , MDA, enzymatic activity, and antioxidants. One hundred fruits were used for determining the decay incidence and weight loss rate. Treatments were each replicated three times. Although the experiments were conducted over two years, similar findings were observed in both years; thus, data from only one year of experimentation are presented.

2.2. Determination of decay incidence and weight loss rate

One hundred fruits were selected to determine decay incidence and weight loss rate. The weights of the same packages were recorded on 0 d and 63 d. Decay incidence and weight loss were determined as follows:

$$\text{Decay incidence (\%)} = \frac{\text{number of fruits showing decay symptoms}}{\text{total number of fruits}} \times 100\%$$

$$\text{Weight loss (\%)} = \frac{(\text{Weight before storage} - \text{weight after storage})}{\text{Weight before storage}} \times 100\%$$

2.3. Determination of fruit quality index

2.3.1. Determination of fruit skin color

Fifteen cherries were selected to determine fruit skin color at two opposite positions around the equator of each fruit using a CR-400 Chroma Meter (Konica Minolta, Tokyo, Japan). The lightness (L^*), saturation (C^*), and hue angle (h°) were determined as described by Yang et al. (2019).

2.3.2. Determination of firmness

Fifteen cherries were selected, and their skins were removed from two opposite positions around the equator to measure fruit firmness using a TA-XT Express-v3.1 Texture Analyzer (Stable Micro System,

UK). A probe was pushed 2 mm into the fruit at 5 $\text{mm}\cdot\text{s}^{-1}$, and the peak force was taken as the firmness index (in Newtons).

2.3.3. Determination of total soluble solids content

Fifteen cherries were selected to determine TSS. The fruit tissue was homogenized in a mortar, and then homogenates were filtered through gauze. Obtained juice was used to determine TSS content with a held-held refractometer (Atago, Tokyo, Japan).

2.3.4. Determination of titratable acidity

The TA was determined as described by Yang, Wang, and Rao (2016). Briefly, evenly mixed fruit tissue (2.0 g) was ground in a mortar, and the homogenate was flushed into a 50 mL centrifuge tube with 25 mL distilled water. After shaking well, the tube was centrifuged at 10,000 × g for 20 min. The resulting supernatant (20 mL) was transferred to a 50 mL Erlenmeyer flask and 0.1 $\text{mol}\cdot\text{L}^{-1}$ NaOH was added dropwise until the solution reached a pH of 8.1. The TA, which is an important predictor of an acid's impact on flavor, was determined as the % (mass fraction) of malic acid in the present study.

2.4. Determination of respiratory rate

The respiratory rate, expressed as $\text{mg CO}_2\cdot\text{kg}^{-1}$ fresh weight (FW) $\cdot\text{h}^{-1}$, was determined as described by Yang et al. (2013). Prior to respiratory rate measurements using a TEL-7001 Infrared CO_2 Analyzer (Telaire, Goleta, CA, USA), randomly selected cherries ($n = 45$) were sealed in a 4 L glass jar for 1.5 h.

2.5. MT identification by ultra-performance liquid chromatography-mass spectrometry

Endogenous MT was measured as described by Liu, Zheng, Sheng, Liu, and Zheng (2018), with slight modifications. The tissue of frozen cherries (4.0 g) was ground into powder using liquid nitrogen, added to 6 mL of methanol, and transferred to a centrifuge tube. The cells were lysed at 30 °C and 80 Hz for 30 min using an ultrasonic cell disrupter, followed by centrifugation at 10,000 × g and 4 °C for 15 min. Subsequently, the supernatant was dried with N_2 and the resulting powder was redissolved in 3 mL of 5% aqueous methanol. Then the supernatant was extracted by a C18 SPE cartridge (100 $\text{mg}\cdot\text{mL}^{-1}$, Agilent, USA) by adding 5 mL of methanol as activating solvent, followed by equilibration with 5 mL of ultra-pure water, loading of 3 mL of sample, and elution with 5 mL of 5% methanol solution; the flow rate was set to 1 $\text{mL}\cdot\text{min}^{-1}$. Finally, 2 mL 80% methanol solution was used for elution and to adjust the volume to 2 mL.

Melatonin was identified using ultra-performance liquid chromatography (H-Class UPLC) coupled with Xevo TQ-XS triple quadrupole mass spectrometry (MS) (Waters, Milford, MA, USA). Chromatography was performed using an Acquity UPLC BEH C18 column (2.1 × 100 mm i.d., 1.7 μm particle size) at ambient temperature. The samples were eluted with a mobile phase, which consisted of 0.1% formic acid in water (eluent A) and 0.1% formic acid in acetonitrile (eluent B), and delivered using the following gradient profile: 80% A for 0.5 min; 70% A for 1.5 min; 20% A for 3 min; 5% A for 4–6 min; 80% A for 6.5 min; and 80% A for 8 min, at a flow rate of 0.4 $\text{mL}\cdot\text{min}^{-1}$ and injection volume of 1 μL . The electron spray ionization source used in this study was operated in positive ion mode with the following working parameters: capillary voltage, 3.0 KV; cone voltage, 40 V; source temperature, 150 °C; desolvation temperature, 400 °C; desolvation gas flow rate, 800 $\text{L}\cdot\text{h}^{-1}$; cone gas flow rate, 50 $\text{L}\cdot\text{h}^{-1}$.

Detection of the ion pairs was performed by multiple reaction monitoring (MRM) mode. The m/z transition pairs (precursor ion/product ion) were 233.1/174.0 for MT. The quadrupoles were set at unit resolution. The analytical data were processed by Masslynx (Waters, USA).

2.6. Determination of endogenous MT level

Samples of the filtrate (20 μL) were analyzed by high-performance liquid chromatography (HPLC) using a system equipped with a Spherisorb C-18 analytical column (5 $\mu\text{m} \times 250.0 \text{ mm} \times 4.6 \text{ mm}$) and UV detector at 222 nm. The mobile phase was 60% methyl alcohol (v/v), had a flow rate of 1 mL min^{-1} , and a column temperature was 25 $^{\circ}\text{C}$. The MT content was quantified using the external standard method.

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

The $\text{O}_2^{\cdot-}$ production rate, expressed as $\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ FW, was measured as described by Wang and Luo (1990). Fruit tissue (3.0 g) was added to 5.0 mL of 500 $\text{mol} \cdot \text{L}^{-1}$ sodium phosphate buffer (PBS, pH 7.8, containing 1% TritonX-100, 2 $\text{mmol} \cdot \text{L}^{-1}$ EDTA, and 5% PVP). The resulting homogenate was ground in an ice bath, and then centrifuged at 12,000 $\times g$, at 4 $^{\circ}\text{C}$ for 20 min. The supernatant (2 mL) was extracted, evenly mixed with 2.0 mL of 50 $\text{mol} \cdot \text{L}^{-1}$ PBS (pH 7.8) and 2.0 mL of 1 $\text{mol} \cdot \text{L}^{-1}$ hydroxylamine hydrochloride solution, and incubated at 25 $^{\circ}\text{C}$ for 20 min. The mixture was then evenly mixed with 2.0 mL of 7 $\text{mol} \cdot \text{L}^{-1}$ 4-aminobenzenesulfonic acid and 2.0 mL of 7 $\text{mol} \cdot \text{L}^{-1}$ α -naphthylamine solution. The mixture was then incubated at 30 $^{\circ}\text{C}$ for 30 min and immediately subjected to optical density measurement at 530 nm (OD_{530}).

The H_2O_2 level, expressed as $\mu\text{mol} \cdot \text{g}^{-1}$ FW, was determined as described by Patterson, MacRae, and Ferguson (1984), with some modifications. Fruit tissue (2.0 g) was added to 3 mL of cold acetone (4 $^{\circ}\text{C}$). The homogenate was ground in an ice water bath, and then centrifuged at 11,000 $\times g$, at 4 $^{\circ}\text{C}$ for 15 min. Samples of the supernatant (1.5 mL) were mixed with concentrated HCl (0.15 mL), TiCl_4 (0.015 mL), and concentrated NH_3 (0.15 mL). The mixture was further centrifuged. The precipitate was then redissolved in 3 mL of 2 $\text{mol} \cdot \text{L}^{-1}$ H_2SO_4 and the absorbance of the solution was measured at 415 nm.

2.8. Determination of MDA level and relative membrane permeability

The MDA level, expressed as $\mu\text{mol} \cdot \text{g}^{-1}$ FW, was determined using a thiobarbituric acid colorimetric assay as described by Dhindsa, Dhindsa, and Thorpe (1981). Fruit tissue (3 g) was ground in 5.0 mL 100 $\text{g} \cdot \text{L}^{-1}$ trichloroacetic acid. The homogenate was then centrifuged at 10,000 $\times g$, at 4 $^{\circ}\text{C}$ for 20 min. The supernatant (2.0 mL) was mixed with 3.0 mL of thiobarbituric acid, incubated at 100 $^{\circ}\text{C}$ for 20 min, snap cooled, and centrifuged at 12,000 $\times g$ for 15 min. This supernatant was measured at OD_{450} , OD_{532} , and OD_{600} .

$$C(\mu\text{mol L}^{-1}) = 6.45 \times (\text{OD}_{532} - \text{OD}_{600}) \frac{0.56 \times \text{OD}_{450} V_t}{V_s \times m}$$

where, V_t represents the total volume of the sample extract, V_s represents the volume of the sample extract at the time of measurement, and m represents the sample quality.

The relative membrane permeability was measured as described by Yang et al. (2013). The peeled pulp was retrieved using a 1 cm stainless steel hole puncher and cut into 2 mm discs using a blade. Thirty discs were placed in a 50 mL small beaker, stirred slowly in 20 mL of distilled water, and allowed 30 min to reach equilibrium before measuring the electrical conductivity (C_1) using a DDS-11AT Conductivity Meter (Shanghai Leici Instrument Inc., Shanghai, China). The beaker was then incubated at 100 $^{\circ}\text{C}$ for 15 min, cooled, made up to 20 mL with distilled water, and the electrical conductivity (C_2) of the solution was measured.

$$\text{Relative membrane permeability}(\%) = \frac{C_1}{C_2} \times 100\%$$

2.9. Determination of antioxidant enzymatic activity

To determine the enzymatic activity, ~ 2 g cherry tissue and 5 mL of 100 $\text{mmol} \cdot \text{L}^{-1}$ PBS (pH 7.8, containing 5% w/v PVP, 1.0 $\text{mmol} \cdot \text{L}^{-1}$ EDTA, and 1% Triton X-100) were rapidly ground in an ice water bath, and then centrifuged at 4 $^{\circ}\text{C}$ and 10,000 $\times g$ for 25 min.

SOD activity, expressed as $\text{U} \cdot \text{h}^{-1} \cdot \text{g}^{-1}$ FW, was determined as described by Yang et al. (2013). The 3 mL 50 $\text{mmol} \cdot \text{L}^{-1}$ PBS reaction system (pH 7.8), which contained 130 $\text{mmol} \cdot \text{L}^{-1}$ MET solution, 0.75 $\text{mmol} \cdot \text{L}^{-1}$ nitroblue tetrazolium solution, 2 $\text{mol} \cdot \text{L}^{-1}$ riboflavin, 0.1 $\text{mmol} \cdot \text{L}^{-1}$ EDTA- Na_2 , and 0.5 mL enzyme extract, was exposed under a 4000 lx fluorescent lamp for 5 min before measuring SOD activity. The amount of enzyme that resulted in a 50% inhibition of nitroblue tetrazolium reduction was defined as one unit of SOD activity.

CAT activity, expressed as $\text{U} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ FW, was determined as described by Aebi (1984). The 3 mL 50 $\text{mmol} \cdot \text{L}^{-1}$ PBS reaction system (pH 7.0) contained 0.3 mL enzyme extract and 10 $\text{mmol} \cdot \text{L}^{-1}$ H_2O_2 . One unit of CAT activity was defined as the quantity of enzyme required to effect a 0.01 $\text{min}^{-1} \cdot \text{g}^{-1}$ fruit pulp change in the OD_{240} .

2.10. Determination of antioxidant enzyme activity and antioxidant levels in the AsA-GSH cycle

2.10.1. Determination of antioxidant enzyme activity in the AsA-GSH cycle

Cherry tissue (~ 3 g) was added to 6 mL 100 $\text{mmol} \cdot \text{L}^{-1}$ PBS (pH 7.8, containing 5% w/v PVP, 1.0 $\text{mmol} \cdot \text{L}^{-1}$ EDTA, and 1% Triton X-100) and ground in an ice water bath. The resulting homogenate was centrifuged at 12,000 $\times g$, at 4 $^{\circ}\text{C}$ for 20 min.

Ascorbate peroxidase (APX) activity, expressed as $\text{U} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ FW, was determined as described by Nakano and Asada (1981). The 3 mL 50 $\text{mmol} \cdot \text{L}^{-1}$ HEPES-KOH reaction system (pH 7.6) contained 0.3 mL enzyme extract, 0.5 $\text{mmol} \cdot \text{L}^{-1}$ AsA, 1 $\text{mmol} \cdot \text{L}^{-1}$ H_2O_2 , and 0.1 $\text{mmol} \cdot \text{L}^{-1}$ EDTA. A unit of APX activity was defined as the quantity of enzyme required to effect a 0.01 $\text{min}^{-1} \cdot \text{g}^{-1}$ fruit pulp change in the OD_{290} .

Dehydroascorbate reductase (DHAR) activity, expressed as $\text{U} \cdot \text{g}^{-1}$ FW $\cdot \text{min}^{-1}$, was measured as described by Nakano and Asada (1981). The 3 mL 100 $\text{mmol} \cdot \text{L}^{-1}$ HEPES-KOH reaction system (pH 7.0) contained 0.3 mL enzyme extract, 2.5 $\text{mmol} \cdot \text{L}^{-1}$ glutathione (GSH), and 0.2 $\text{mmol} \cdot \text{L}^{-1}$ dehydroascorbic acid (DHA). A unit of DHAR activity was defined as the quantity of enzyme required to effect a 0.01 $\text{min}^{-1} \cdot \text{g}^{-1}$ fruit pulp change in the OD_{265} .

GR activity, expressed as $\text{U} \cdot \text{g}^{-1}$ FW $\cdot \text{min}^{-1}$, was measured as described by Smith, Vierheller, and Thorne (1988). The 3 mL 100 $\text{mmol} \cdot \text{L}^{-1}$ Tris-HCl reaction system (pH 7.6) contained 0.5 mL enzyme extract, 1 $\text{mmol} \cdot \text{L}^{-1}$ EDTA, 0.1 $\text{mmol} \cdot \text{L}^{-1}$ NADPH, and 0.25 $\text{mmol} \cdot \text{L}^{-1}$ oxidized glutathione (GSSG). A unit of GR activity was defined as the quantity of enzyme required to effect a 0.01 $\text{min}^{-1} \cdot \text{g}^{-1}$ fruit pulp change in the OD_{340} .

Monodehydroascorbate reductase (MDHAR) activity, expressed as $\text{U} \cdot \text{g}^{-1}$ FW $\cdot \text{min}^{-1}$, was measured as described by Nakano and Asada (1981). The OD_{340} of the 3 mL 50 $\text{mmol} \cdot \text{L}^{-1}$ HEPES-KOH reaction system (pH 7.6), which contained 0.3 mL enzyme extract, 0.1 $\text{mmol} \cdot \text{L}^{-1}$ NADH, 0.25 $\text{mmol} \cdot \text{L}^{-1}$ AsA, and 0.3 units of ascorbate oxidase, was measured. One unit of MDHAR activity was defined as the enzyme content required to effect a 0.01 $\text{min}^{-1} \cdot \text{g}^{-1}$ fruit pulp change in the OD_{340} .

2.10.2. Determination of antioxidant levels

To determine the AsA and DHA levels, expressed as $\text{mg} \cdot \text{g}^{-1}$ FW, ~ 2.0 g fruit tissue was ground in an ice water bath with 4 mL 50 $\text{g} \cdot \text{L}^{-1}$ TCA solution. After centrifugation at 12,000 $\times g$, at 4 $^{\circ}\text{C}$ for 20 min, the supernatant was collected for the AsA and DHA assays. The AsA and DHA levels were determined as described by Kampfenkel, Van Montagu, and Inzé (1995). The 4 mL reaction system, which contained 0.2 mL extract, 0.8 mL 0.2 $\text{mol} \cdot \text{L}^{-1}$ PBS (pH 7.4), 0.8 mL 42% H_3PO_4 ,

1.0 mL 10% TCA, 0.4 mL 3% FeCl₃, and 0.8 mL 2% 2, 2'-bipyridine, was evenly mixed and incubated in a 42 °C water bath for 60 min before measuring the OD₅₂₅. The DHA level was determined by the reduction of dehydrogenated ascorbic acid to reduced ascorbic acid using dithiothreitol and *N*-ethylmaleimide. DHA levels were calculated as the differences between the initial and total AsA levels.

To determine the GSH and GSSG levels, expressed as $\mu\text{mol}\cdot\text{g}^{-1}$ FW, ~4 g fruit tissue was ground in an ice water bath with 4 mL of pre-cooled 5% TCA solution that contained 5 mmol·L⁻¹ EDTA-Na₂. The resulting homogenate was centrifuged at 12,000 × g and 4 °C for 20 min. GSH and GSSG levels were measured as described by [Castillo and Greppin \(1988\)](#). To determine the GSH level, the 3 mL reaction system, which contained 1 mL supernatant, 1 mL 100 mmol·L⁻¹ H₃PO₄ (pH 7.6), and 0.5 mL 4 mmol·L⁻¹ dithionitrobenzoic acid, was incubated at 25 °C for 10 min before the measurement of OD₄₁₂. To reduce GSSG into GSH, to obtain the total GSH, 5 U GR enzyme was added to the reaction system. GSSG levels were calculated as the difference between the initial and total GSH levels.

2.11. Statistical analysis

The experiments were conducted using a completely randomized design. The data were analyzed using one-way ANOVAs and Duncan's multiple range tests to compare multiple means. All statistical analyses were performed using SAS statistical software (version 8; SAS Institute, Cary, NC, USA). Differences were considered statistically significant at *P*-values < 0.05. Data are presented as means ± standard errors of the means (SEMs).

3. Results and analysis

3.1. Effects of exogenous MT on the decay incidence and weight loss rate

The control groups showed significantly higher decay rates (82.33%) and weight loss (following storage for 63 d, 3.97%) than the MT-treated groups (*P* < 0.05) ([Fig. 1A and B](#)). The 100 $\mu\text{mol}\cdot\text{L}^{-1}$ MT treatment resulted in the lowest weight loss (2.06%) and decay rates (25.67%), which were significantly lower than those of the 50 $\mu\text{mol}\cdot\text{L}^{-1}$ and 150 $\mu\text{mol}\cdot\text{L}^{-1}$ MT treatments (*P* < 0.05). There were no significant differences between the 50 $\mu\text{mol}\cdot\text{L}^{-1}$ and 150 $\mu\text{mol}\cdot\text{L}^{-1}$ MT treatments (*P* < 0.05).

3.2. Effects of exogenous MT on fruit quality parameters

Fruit firmness, TSS content, TA, and chromaticity (L*, C*, and h°) declined during low-temperature storage ([Table 1](#)). MT treatment not

only significantly inhibited the decline in cherry firmness, TSS, and TA, but it also maintained the L*, C*, and h° values after 63 d of storage (*P* < 0.05). The 100 $\mu\text{mol}\cdot\text{L}^{-1}$ MT treatment yielded the most effective results among all treatments. This treatment also maintained lower decay and weight loss rates. Therefore, the 100 $\mu\text{mol}\cdot\text{L}^{-1}$ MT treatment was selected to further investigate the underlying physiobiochemical mechanisms.

3.3. Effects of exogenous MT on respiratory rate

The cherries showed a decline in the respiratory rate during the initial 14 d of storage, a slow increase from 15 d, and then a sharp increase in the later stages of storage ([Fig. 2A](#)). MT treatment inhibited the elevation of the respiratory rate, which showed a significantly lower respiratory rate (by 13.31% on average) compared with the control group from day 14–63 (*P* < 0.05).

3.4. Effects of exogenous MT on endogenous MT content

MT was identified using UPLC-MS analysis. The MRM chromatograms data showed that the final extraction from sweet cherry fruits ([Supplementary Fig. 1A](#)) had a similar retention time and *m/z* 233.1 > 174 to that of standard MT ([Supplementary Fig. 1B](#)), demonstrating that the final extraction from fruit contained endogenous MT.

Endogenous MT content of cherry fruits detected by HPLC increased and then decreased throughout storage ([Fig. 2B](#)). MT treatment stimulated this increase and inhibited the decrease, producing significantly higher results (by 28.37% on average) compared with the control group from day 14–56 of storage (*P* < 0.05).

3.5. Effects of exogenous MT on O₂^{·-} and H₂O₂

O₂^{·-} and H₂O₂ accumulation showed an overall increasing trend throughout storage ([Fig. 3A and B](#)). MT treatment restrained the elevation of O₂^{·-} and H₂O₂ levels, which showed significantly lower O₂^{·-} and H₂O₂ levels (by 13.94% and 21.99% on average) compared with the control group from day 7–63 (*P* < 0.05).

3.6. Effects of exogenous MT on MDA level and relative membrane permeability

Both the MDA level and the relative membrane permeability appeared to exhibit an increasing trend throughout storage ([Fig. 3C and D](#)). MT treatment significantly inhibited the elevation of the MDA levels and relative membrane permeability, showing significantly lower MDA

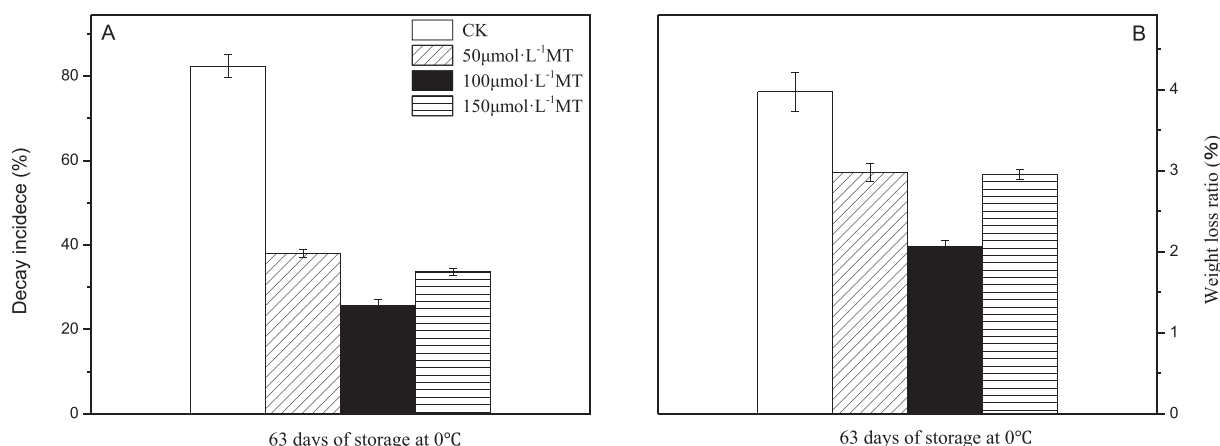
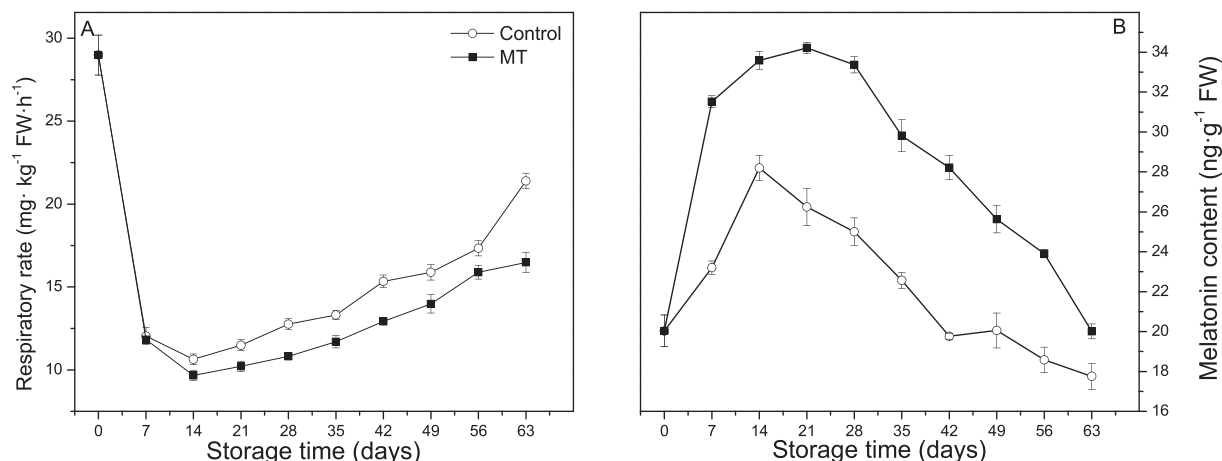
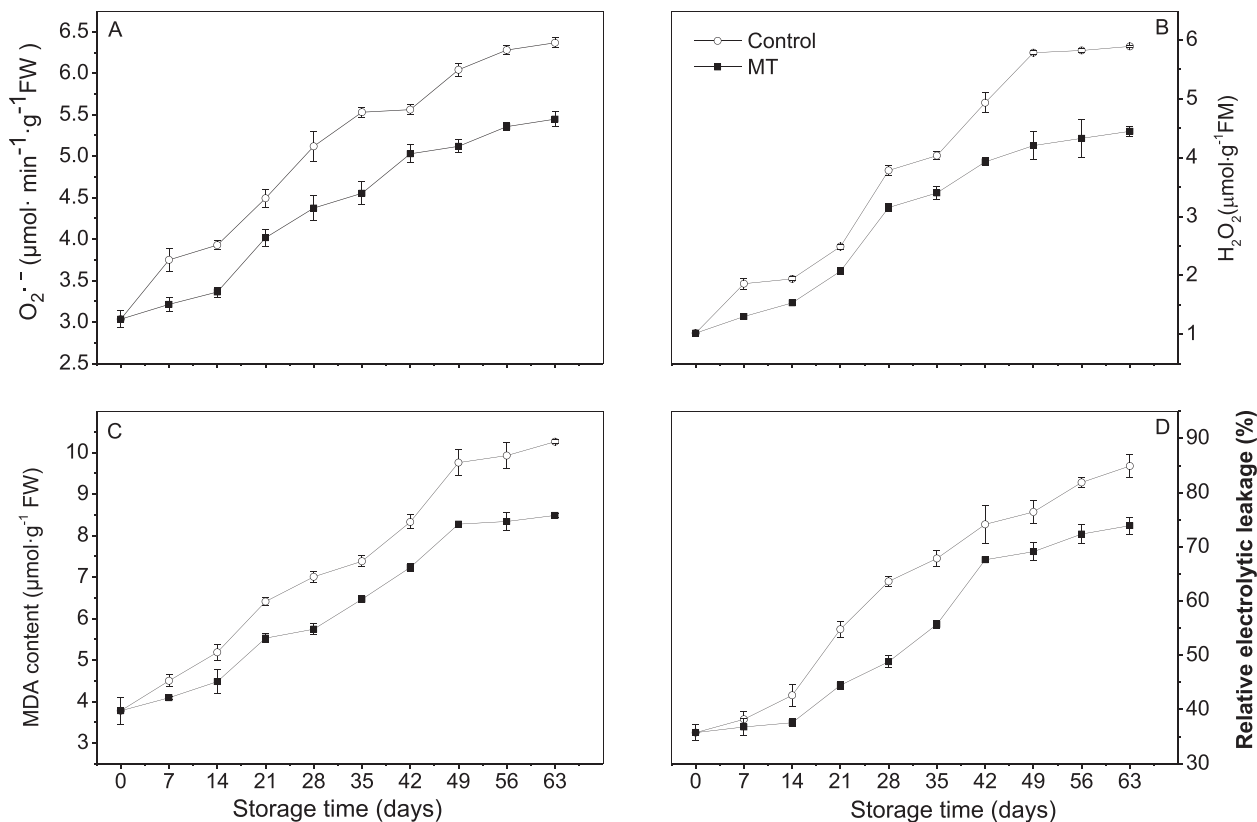


Fig. 1. (A) Decay incidence and (B) weight loss rate of sweet cherries treated with 100 $\mu\text{mol}\cdot\text{L}^{-1}$ melatonin during storage at 0 °C for 63 days. Vertical bars represent SEM (*n* = 3).

Table 1

Effects of exogenous melatonin on firmness, TSS, titratable acidity, L*, C*, and h° of sweet cherries during storage.

Treatment	Storage time/days	Firmness/N	TSS/%	Titrateable acidity/%	L*	C*	h°
Control	0	7.44 ± 0.07 a	14.66 ± 0.33 a	1.06 ± 0.07 a	45.30 ± 0.87 a	38.59 ± 1.21 a	2.49 ± 0.01 a
	63	4.40 ± 0.05 d	9.28 ± 0.18c	0.48 ± 0.03c	27.38 ± 0.45c	21.32 ± 0.23c	1.30 ± 0.01 d
	50 µmol·L ⁻¹ MT	4.58 ± 0.08c	9.96 ± 0.12b	0.51 ± 0.01b	30.93 ± 0.28b	23.76 ± 1.00b	1.41 ± 0.01c
	100 µmol·L ⁻¹ MT	5.43 ± 0.05 a	10.98 ± 0.05 a	0.71 ± 0.01 a	34.17 ± 0.58 a	28.70 ± 0.19 a	1.78 ± 0.01 a
	150 µmol·L ⁻¹ MT	5.08 ± 0.07b	10.42 ± 0.20b	0.58 ± 0.03b	31.91 ± 0.27b	25.09 ± 0.10b	1.46 ± 0.01b

Note: Data are showed as the mean ± S.E. Values followed by different lowercase letters within each column are significantly different at $P < 0.05$.**Fig. 2.** (A) Respiratory rate, and (B) endogenous melatonin content of sweet cherries treated with 100 µmol L⁻¹ melatonin during storage at 0 °C for 63 days. Vertical bars represent SEM (n = 3).**Fig. 3.** Levels of (A) O₂^{·-}, (B) H₂O₂, (C) malondialdehyde, and (D) relative membrane permeability of sweet cherries treated with 100 µmol L⁻¹ melatonin during storage at 0 °C for 63 days. Vertical bars represent SEM (n = 3).

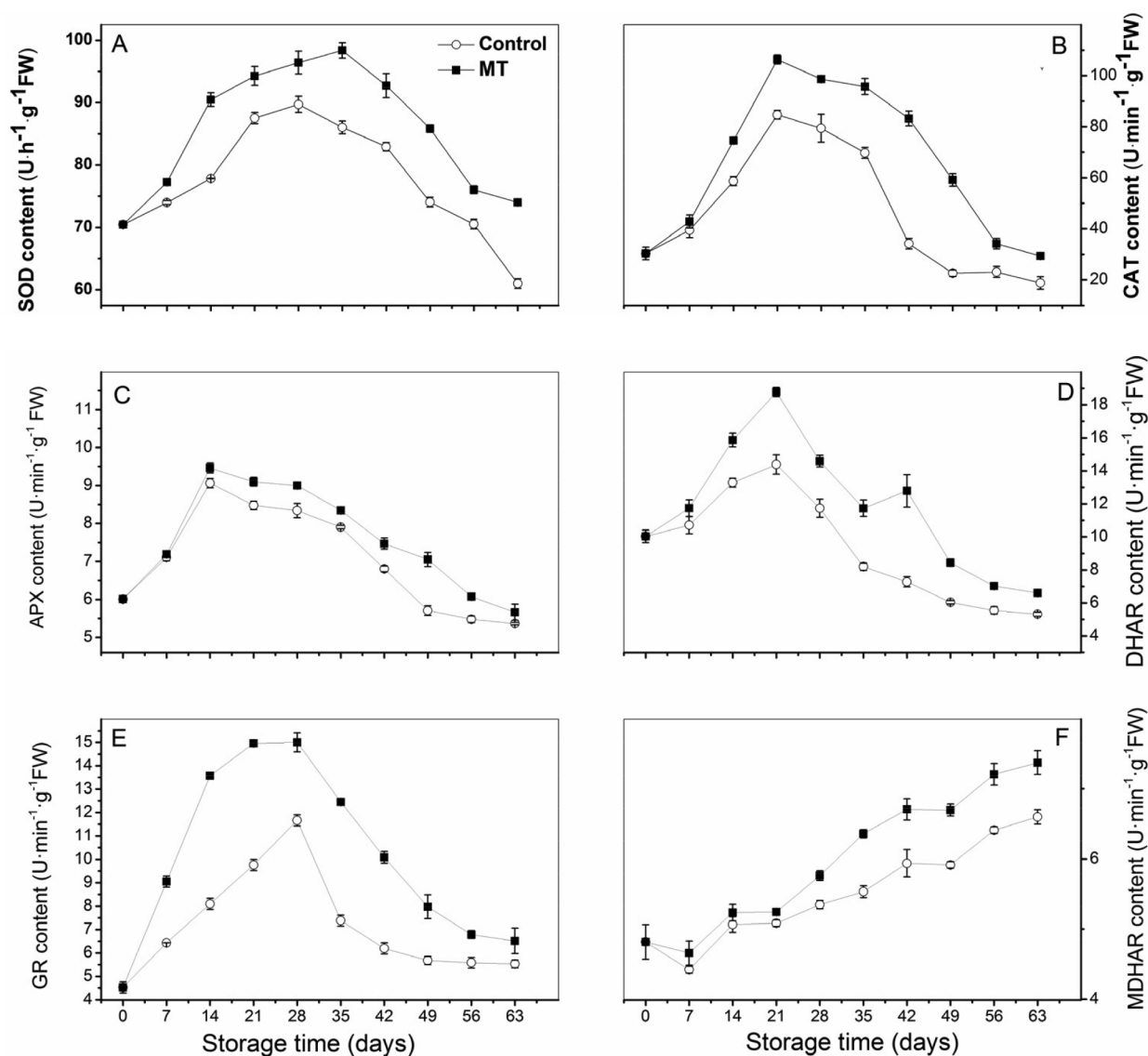


Fig. 4. Activity of (A) SOD, (B) CAT, (C) APX, (D) DHAR, (E) GR, and MDHAR (F) of sweet cherries treated with 100 $\mu\text{mol L}^{-1}$ melatonin during storage at 0 °C for 63 days. Vertical bars represent SEM (n = 3).

levels and relative membrane permeability (by 13.93% and 13.18% on average, respectively) compared with control group from day 7–63 ($P < 0.05$).

3.7. Effects of exogenous MT on antioxidant enzyme activity

The SOD activity increased and then decreased throughout the experimental storage duration (Fig. 4A). Compared with the control group, MT treatment promoted the elevation and inhibited the decline of SOD activity, which was significantly higher (by 12.89% on average) compared with the control group from day 7–63 ($P < 0.05$).

CAT activity increased rapidly in the early stage of storage and peaked on day 21 before decreasing (Fig. 4B). MT treatment stimulated this increase and inhibited the decrease, which resulted in significantly higher CAT activity levels (by 65.47% on average) compared with the control group from day 14–63 ($P < 0.05$).

3.8. Effects of exogenous MT on antioxidant enzyme activity in the AsA-GSH cycle

APX activity increased rapidly in the early stage of storage and

peaked on day 14 before decreasing (Fig. 4C). MT treatment promoted the increase and inhibited the reduction of APX and thus APX levels were significantly higher (by 9.37% on average) compared with the control group from day 14–56 ($P < 0.05$).

In the early stage of storage, DHAR activity increased rapidly, peaked on day 21, and then decreased (Fig. 4D). Treatment with MT promoted this elevation and inhibited the decline, with significantly higher values (by 35.46% on average) compared with the control group from day 14–63 ($P < 0.05$).

GR activity showed a similar trend, peaking on day 28 (Fig. 4E). MT treatment promoted GR activity and inhibited its decline. GR activity levels were significantly higher (by 47.97% on average) in the MT-treated group than in the control group from day 7–56 ($P < 0.05$).

MDHAR activity decreased slightly during the first 7 d of storage before increasing (Fig. 4F). MT treatment suppressed a decline in MDHAR activity and stimulated its increase, which was significantly higher (by 10.87% on average) compared with the control group from day 21–63 ($P < 0.05$).

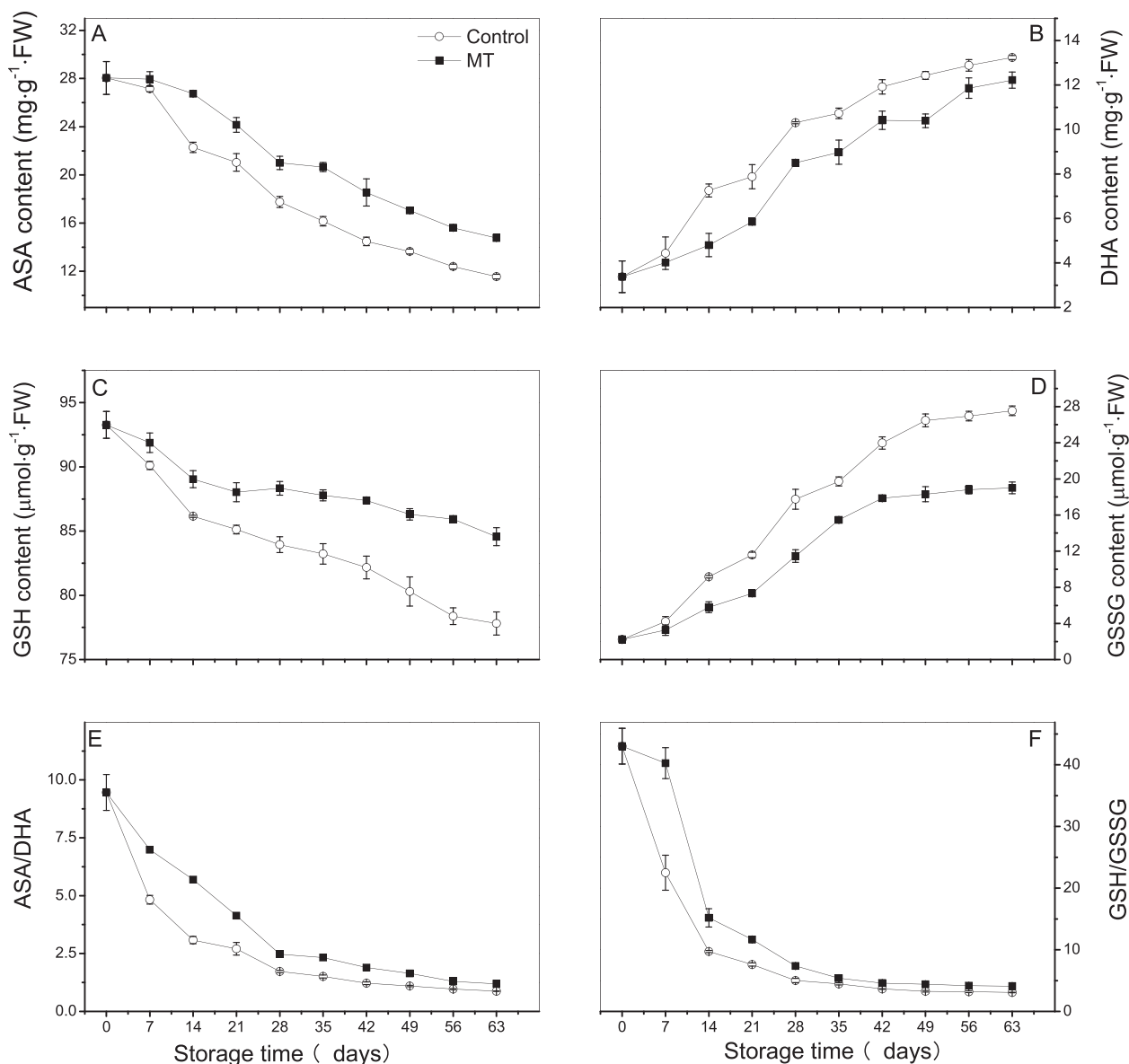


Fig. 5. Levels of (A) ASA, (B) DHA, (C) GSH, (D) GSSG, (E) AsA:DHA, and (F) GSH:GSSG of sweet cherries treated with 100 $\mu\text{mol L}^{-1}$ melatonin during storage at 0 °C for 63 days. Vertical bars represent SEM (n = 3).

3.9. Effects of exogenous MT on antioxidant levels in the AsA-GSH cycle

A decreasing trend was observed in the AsA levels throughout storage (Fig. 5A). MT treatment inhibited this decrease, producing significantly higher values (by 21.53% on average) compared with the control group from day 14–63 ($P < 0.05$).

An increasing trend was observed in the DHA levels (Fig. 5B). MT treatment inhibited this increase, producing significantly lower results (by 12.98% on average) compared with the control group from day 14–63 ($P < 0.05$).

A decreasing trend was observed in the GSH levels throughout storage, with a rapid decline in the early stage, followed by a slow decline in the middle and later stages (Fig. 5C). MT treatment inhibited this decline, with significantly higher values (by 5.96% on average) compared with the control group from day 14–63 ($P < 0.05$).

An increasing trend was observed in the GSSG levels throughout storage, with a rapid increase in the early stage, followed by a slow increase (Fig. 5D). Treatment with MT inhibited this increase, producing significantly lower values (by 33.49% on average) compared with the control group from day 7–63 ($P < 0.05$).

The AsA:DHA and GSH:GSSG ratios showed decreasing trends throughout storage, with rapid declines in the early and (for AsA:DHA) middle stages, followed by a less rapid decline (Fig. 5E and F). The ratios of AsA:DHA and GSH:GSSG in the MT-treated cherries were relatively higher than those in the control group, and the differences were significant from day 7–63 ($P < 0.05$).

4. Discussion

In the sweet cherry industry, senescence and quality deterioration, which are mainly characterized by a loss of color, softening, and diminished flavor, have seriously restricted their industrialization (Yang et al., 2019). In this study, we found that exogenous MT decreased the decay and weight loss rates (Fig. 1A and B) and inhibited the respiratory rate of sweet cherries (Fig. 2A). MT treatment also improved firmness, as well as increasing the levels of TSS and TA, and the L^* , C^* , and h^* values of cherry skin (Table 1). These findings demonstrate that exogenous MT applications may lead to improved preservation via a mechanism involving the inhibition of respiration. Consequently, by reducing the degradation rate of nutrients (such as soluble sugars and

TA), postharvest senescence is ultimately delayed and fruit quality is maintained. Similar findings have been reported for broccoli (Zhu et al., 2018), cucumber (Xin et al., 2017), and peaches (Gao et al., 2016).

Under normal circumstances, the generation and elimination of intracellular ROS reaches a state of dynamic equilibrium, and the intracellular ROS content is maintained at low levels that do not cause damage to the cells. However, as senescence progresses, the balance of ROS is disrupted, and its accumulation may promote the production of certain biomacromolecular substances as well as membrane lipid peroxidation. These responses lead to the destruction of cell membrane structure and function, which induces physiological disorders in the cell metabolism and further promotes senescence. In addition, excessive ROS may lead to ROS stress. ROS stress could induce up-regulation of plant melatonin biosynthesis genes (*TDC*, *T5H*, *SNAT*, *COMT*, and *ASMT*) and ultimately increase endogenous MT content (Arnao & Hernández-Ruiz, 2019). Increases in endogenous MT levels have been shown to contribute to the elimination of ROS, alleviate the peroxidation of membrane lipids, and help to maintain cell membrane integrity in plants. MT and its downstream metabolites, i.e., *N*-acetyl-*N*-formyl-5-methoxykynurenamine, *N*-acetyl-5-methoxykynurenamine, and cyclic 3-hydroxymelatonin, are excellent free-radical scavengers. These MT cascades can scavenge up to 10 free radicals, with a 2–4 times greater capacity for the detoxification of oxidized molecules than any single classical free-radical scavenger (Paredes, Korkmaz, Manchester, Tan, & Russel, 2009).

In the present study, the endogenous MT content increased at the early storage stage (Fig. 2B), which is consistent with the exogenous MT treatment of sweet cherry fruit on tree and may have been caused by the physical infiltration of exogenous MT (Tijero, Muñoz, & Munné-Bosch, 2019). Furthermore, this increase in endogenous MT content at the early storage stage paralleled the accumulation of $O_2^{\cdot-}$ and H_2O_2 (Fig. 3A and B). These results indicate that ROS stress may induce endogenous MT content increase. Similar findings have been reported in two sweet cherry cultivars, 'Hongdeng' and 'Rainier', during the gradual lignification of endocarp and until it completely hardened (Zhao et al., 2013). The increase in MT content in the two sweet cherry cultivars may be related to the ROS-induced up-regulation of plant tryptophan decarboxylase gene (*PaTDC*). This is consistent with the redox network model (the MT–ROS–reactive nitrogen species triangle), according to which ROS stress may upregulate MT synthase gene and thereby promote melatonin synthesis (Arnao & Hernández-Ruiz, 2019). Besides, an increase in endogenous MT content (Fig. 2B) in MT-treated cherries was observed to coincide with lower levels of $O_2^{\cdot-}$, H_2O_2 (Fig. 3A and B), MDA, and relative membrane permeability (Fig. 3C and D). These results, therefore, corroborate previous reports on strawberry (Liu et al., 2018) and cassava (Ma et al., 2016), and indicate that exogenous MT might act as a powerful antioxidant that delays the senescence of sweet cherries.

In contrast to our study, Tijero et al. (2019) found that exogenous MT not only induces endogenous MT synthesis, but also increases cytokinins content and through the crosstalk with cytokinins inhibits ripening and senescence of sweet cherry fruit. Teribia, Tijero, and Munné-Bosch (2016) manifested that a variety of hormones such as abscisic acid, ethylene, gibberellins, and auxins are also involved in the regulation of ripening and senescence of sweet cherry fruit. MT regulates the expression of many elements (receptors, transcription factors, and enzymes) in the biosynthesis and catabolism of these hormones (abscisic acid, cytokinins, auxin, ethylene, gibberellins, salicylic acid, and jasmonic acid). However, these hormones cannot affect the MT levels. Thus, MT may act as a plant master regulator of many integrated responses, or as a plant hormone that regulates other plant hormones. In addition, MT can trigger downstream signal chain elements and activate responses against stressors by interacting with its receptor CAND2/PMTR1. Other studies have reported that the crosstalk associated with nitric oxide, hydrogen sulphide, and MT can delay ethylene-induced fruit ripening and senescence (Mukherjee, 2019). These results

suggested that MT inhibited maturation and senescence of fruit by crosstalk with other substances rather than by itself. Therefore, further clarification of the crosstalk with MT, hormones, nitric oxide, and H_2S may contribute to better understanding of the mechanism of MT regulation of fruit maturation and senescence.

It has been reported that an increase in antioxidant enzymes activity (SOD, POD, CAT, etc.) can alleviate membrane lipid peroxidation and delay senescence in fruits and vegetables (Gao et al., 2016). In the present study, we demonstrated that treatment with exogenous MT enhanced the activity of antioxidant enzymes (SOD and CAT) in sweet cherries (Fig. 4A and B). This is consistent with the redox network model, which states that MT increases redox enzyme activities of SOD, CAT, and APX (Arnao & Hernández-Ruiz, 2019). Increased SOD and CAT activity contributes to the elimination of $O_2^{\cdot-}$ and H_2O_2 , which may account for the lower $O_2^{\cdot-}$ and H_2O_2 levels observed (Fig. 3A and B). Meanwhile, a decrease in the $O_2^{\cdot-}$ and H_2O_2 content alleviates lipid peroxidation, which may account for the lower MDA content and relative membrane permeability (Fig. 3C and D). Similar findings have been reported for other fruits and vegetables, such as peaches (Zheng, Tian, Meng, & Li, 2007), pears (Zhai et al., 2018), strawberries (Liu et al., 2018), and eggplants (Wu et al., 2017). These results indicate that exogenous MT is able to delay senescence by maintaining the balance of ROS through upregulating the expression of genes related to antioxidant enzymes, enhancing their activity, and alleviating lipid peroxidation. Such conclusions are supported by Ma et al. (2016) and Wang et al. (2012).

The AsA-GSH cycle is an important component in the elimination of ROS. APX, DHAR, GR, and MDHAR are extremely important enzymes in the AsA-GSH redox pathway, which play crucial roles in promoting the regeneration of GSH and AsA, ensuring the normal functioning of metabolic pathways, and maintaining the balance of reduced substances in plants. APX catalyzes the oxidation of AsA into MDHA or DHA and eliminates excessive H_2O_2 free radicals from cells through the reduction of H_2O_2 into H_2O , i.e., to maintain the metabolic balance of free radicals in the cells (Sun, Li, Wang, Wu, & Wang, 2011). The conversion of GSSG into GSH in plants is catalyzed by GR. Higher levels of GR are, therefore, conducive to the maintenance of the high ratio of GSH:GSSG, which plays critical roles in the protection of organisms against oxidative damage (Castro-Mercado, Martinez-Diaz, Roman-Tehandon, & Gareia-Pined, 2009). In addition, high intracellular GSH levels also retain thiol-containing proteins or enzymes in a reduced or active state, which is important for the maintenance of normal functions as well as a high reduction potential in cells. DHAR-catalyzed GSH can also maintain a high reduction potential in cells by reducing DHA into AsA.

In the present study, exogenous MT treatment maintained high enzyme activity for APX, DHAR, GR, and MDHAR (Fig. 4C, D, E, and F), as well as high levels of AsA and GSH (Fig. 5A and C). MT treatment also maintained high AsA:DHA and GSH:GSSG ratios (Fig. 5E and F) by keeping DHA and GSSG contents at relatively low levels (Fig. 5B and D). These results indicated that exogenous MT treatment may ensure the normal functioning of metabolic pathways in the AsA-GSH cycle, maintain the balance of reduced substances, and reduce the accumulation of ROS, thereby delaying senescence in sweet cherries. Similar findings have been reported for peaches (Huan et al., 2018), pears (Zhai et al., 2018), and longans (Li et al., 2018).

Exogenous MT can promote the maturation of certain fruits and vegetables. For example, treatment with exogenous MT promoted the ripening (i.e., veraison) of grapes by significantly inducing the biochemical synthesis of ethylene and the expression of key genes in the signal transduction pathways (Xu, Yue, Bian, Zhai, & Yao, 2017). Treatment with 50 and 100 $\mu\text{mol}\cdot\text{L}^{-1}$ MT also promoted the maturation of tomatoes by activating the expression of *ACS4* and enhancing the synthesis of ethylene in green mature tomatoes (Sun et al., 2014). However, exogenous MT inhibited the ripening of sweet cherry fruits on trees (Tijero et al., 2019). These results suggest that exogenous MT plays different roles in promoting or postponing the maturation and

senescence of fruits and vegetables, depending on their type, variety, and degree of maturity. Therefore, the regulation of maturation and senescence of food crops by exogenous MT is a complex process. Future studies on the exogenous MT regulation of postharvest maturation and senescence of sweet cherries at different degrees of maturity may further clarify the mechanisms involved.

5. Conclusion

The 100 $\mu\text{mol}\cdot\text{L}^{-1}$ MT treatment was the most effective in delaying senescence in sweet cherries by reducing weight loss and decay incidence, increasing fruit firmness, TSS, and titratable acidity; delaying color development; inhibiting respiratory rates; as well as maintaining endogenous MT levels. Furthermore, the 100 $\mu\text{mol}\cdot\text{L}^{-1}$ MT treatment increased the enzyme activity of SOD, CAT, APX, DHAR, GR and MDHAR, increased the antioxidant content of AsA and GSH, and increased the AsA:DHA and GSH:GSSG ratios. MT treatment also inhibited the accumulation of $\text{O}_2^{\cdot-}$ and H_2O_2 and maintained the structural and functional integrity of cell membranes. The present study demonstrated the effectiveness of MT treatment as a preservation method for post-harvested sweet cherry fruit to delay senescence and maintain fruit quality, and thus the use of MT may have broad application prospects.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This study was supported by the Science and Technology Innovative Projects of the Key Problem of Scientific and Technical Project of Shanxi Province (20150311020-4); Shanxi Higher Education Institutions (2015182); PhD Research Startup Foundation of Yuncheng University (YQ-2016013 and YQ-2014025); Yun Cheng University Research Projects (XK-2015023); and Shanxi Key Subjects Construction (FSKSC). We thank Prof. Gang Xue-qing from School of Agriculture and Biology, Shanghai Jiao Tong University for technical guidance.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodchem.2019.125311>.

References

- Aebi, H. (1984). Catalase in vitro. *Methods in Enzymology*, 105, 121–126.
- Arnao, M. B., & Hernández-Ruiz, J. (2019). Melatonin: A new plant hormone and/or a plant master regulator? *Trends in Plant Science*, 24, 38–48.
- Castillo, F. J., & Greppin, H. (1988). Extracellular ascorbic acid and enzyme activities related to ascorbic acid metabolism in *Sedum album* L. leaves after ozone exposure. *Environmental and Experimental Botany*, 28, 231–238.
- Castro-Mercado, E., Martínez-Díaz, Y., Roman-Tehandon, N., & Gareia-Pined, E. (2009). Biochemical analysis of reactive oxygen species production and antioxidative responses in unripe avocado (*Persea americana* Mill var Hass) fruits in response to wounding. *Protoplasma*, 235, 67–76.
- Debnath, B., Islam, W., Li, M., Sun, Y. T., Lu, X. C., Mitra, S., et al. (2019). Melatonin Mediates enhancement of stress tolerance in plants. *International Journal of Molecular Sciences*, 20, 1040. <https://doi.org/10.3390/ijms20051040>.
- Dhindsa, R. S., Dhindsa, P. P., & Thorpe, T. A. (1981). Leaf senescence: Correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. *Journal of Experimental Botany*, 32, 93–101.
- Gao, H., Zhang, Z. K., Chai, H. K., Cheng, N., Yang, Y., Wang, D. N., et al. (2016). Melatonin treatment delays postharvest senescence and regulates reactive oxygen species metabolism in peach fruit. *Postharvest Biology and Technology*, 118, 103–110.
- Giménez, M. J., Valverde, J. M., Valero, D., Guillén, F., Martínez-Romero, D., Serrano, M., et al. (2014). Quality and antioxidant properties on sweet cherries as affected by preharvest salicylic and acetylsalicylic acids treatments. *Food Chemistry*, 160, 226–232.
- Hayta, E., & Aday, M. S. (2015). The effect of different electrolyzed water treatments on the quality and sensory attributes of sweet cherry during passive atmosphere packaging storage. *Postharvest Biology and Technology*, 102, 32–41.
- Hernández-Ruiz, J., & Arnao, M. B. (2018). Relationship of melatonin and salicylic acid in biotic/abiotic plant stress responses. *Agronomy*, 8, 33. <https://doi.org/10.3390/agronomy8040033>.
- Huan, C., An, X. J., Yu, M. L., Jiang, L., Ma, R. J., Tu, M. M., et al. (2018). Effect of combined heat and 1-MCP treatment on the quality and antioxidant level of peach fruit during storage. *Postharvest Biology and Technology*, 145, 193–202.
- Kampfenkel, K., Van Montagu, M., & Inzé, D. (1995). Extraction and determination of ascorbate and dehydroascorbate from plant tissue. *Analytical Biochemistry*, 225, 165–167 PMID: 7778771.
- Li, T. T., Wu, Q. X., Zhou, Y. J., Yun, Z., Duan, X. W., Jiang, Y. M., et al. (2018). L-cysteine hydrochloride delays senescence of harvested longan fruit in relation to modification of redox status. *Postharvest Biology and Technology*, 143, 35–42.
- Liu, C. H., Zheng, H. H., Sheng, K. L., Liu, W., & Zheng, L. (2018). Effects of melatonin treatment on the postharvest quality of strawberry fruit. *Postharvest Biology and Technology*, 139, 47–55.
- Ma, Q. X., Zhang, T., Zhang, P., & Wang, Z. Y. (2016). Melatonin attenuates postharvest physiological deterioration of cassava storage roots. *Journal of Pineal Research*, 60, 424–434.
- Mukherjee, S. (2019). Recent advancements in the mechanism of nitric oxide signaling associated with hydrogen sulfide and melatonin crosstalk during ethylene-induced fruit ripening in plants. *Nitric Oxide*, 82, 25–34.
- Nakano, Y., & Asada, K. (1981). Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant and Cell Physiology*, 22, 867–880.
- Paredes, S. D., Korkmaz, A., Manchester, L. C., Tan, D. X., & Russel, J. (2009). Reiter. Phyto-melatonin: A review. *Journal of Experimental Botany*, 60, 57–69.
- Patterson, B. D., Macrae, E. A., & Ferguson, I. (1984). Estimation of hydrogen peroxide in plant extracts using titanium (IV). *Analytical Biochemistry*, 139, 487–492 PMID, 6476384.
- Smith, I. K., Vierheller, T. L., & Thorne, C. A. (1988). Assay of glutathione reductase in crude tissue homogenates using 5, 5-dithiobis (2-nitrobenzoic acid). *Analytical Biochemistry*, 175, 408–413.
- Sun, H. P., Li, L., Wang, X., Wu, S., & Wang, X. F. (2011). Ascorbate–glutathione cycle of mitochondria in osmoprimed soybean cotyledons in response to imbibitional chilling injury. *Journal of Plant Physiology*, 168, 226–232.
- Sun, Q., Zhang, N., Wang, J., Zhang, H., Li, D., Shi, J., et al. (2014). Melatonin promotes ripening and improves quality of tomato fruit during postharvest life. *Journal of Experimental Botany*, 66(3), 657–668.
- Teribia, N., Tijero, V., & Munné-Bosch, S. (2016). Linking hormonal profiles with variations in sugar and anthocyanin contents during the natural development and ripening of sweet cherries. *New Biotechnology*, 33, 824–833.
- Tijero, V., Muñoz, P., & Munné-Bosch, S. (2019). Melatonin as an inhibitor of sweet cherries ripening in orchard trees. *Plant Physiology and Biochemistry*, 140, 88–95.
- Valverde, J. M., Giménez, M. J., Guillén, F., Valero, D., Martínez-Romero, D., Serrano, M., et al. (2015). Methyl salicylate treatments of sweet cherry trees increase antioxidant systems in fruit at harvest and during storage. *Postharvest Biology and Technology*, 109, 106–113.
- Wang, A. G., & Luo, G. H. (1990). Quantitative relation between the reaction of hydroxylamine and superoxide anion radicals in plants. *Plant Physiology Communications*, 26, 55–57.
- Wang, L., Zhang, H., Jin, P., Guo, X. F., Li, Y., Fan, C., et al. (2016). Enhancement of storage quality and antioxidant capacity of harvested sweet cherry fruit by immersion with β -aminobutyric acid. *Postharvest Biology and Technology*, 118, 71–78.
- Wang, P., Yin, L. H., Liang, D., Li, C., Ma, F. W., Yue, Z. Y., et al. (2012). Delayed senescence of apple leaves by exogenous melatonin treatment: Toward regulating the ascorbate–glutathione cycle. *Journal of Pineal Research*, 53, 11–20.
- Wang, Y., Bai, J. H., & Long, L. E. (2015). Quality and physiological responses of two late-season sweet cherry cultivars ‘Lapins’ and ‘Skeena’ to modified atmosphere packaging (MAP) during simulated long distance ocean shipping. *Postharvest Biology and Technology*, 110, 1–8.
- Wang, Y., Xie, X. B., & Long, L. E. (2014). The effect of postharvest calcium application in hydro-cooling water on tissue calcium content, biochemical changes, and quality attributes of sweet cherry fruit. *Food Chemistry*, 160, 22–30.
- Wu, X. X., Zhu, Z. W., Zhang, A. D., Xu, S., Yao, J., Zha, D. S., et al. (2017). Effects of exogenous melatonin on the growth photosynthesis and antioxidant system in eggplant (*Solanum melongena* L.) seedlings under low temperature stress. *Acta Bot Boreali-Occidentalia Sin*, 37(12), 2427–2434.
- Xin, D. D., Si, J. J., & Kou, L. P. (2017). Postharvest exogenous melatonin enhances quality and delays the senescence of cucumber. *Acta Horticulturae Sinica*, 44(5), 891–901.
- Xu, L. L., Yue, Q. Y., Bian, F., Zhai, H., & Yao, Y. X. (2017). Effects of melatonin treatment on grape berry ripening and contents of ethylene and ABA. *Plant Physiology*, 53(12), 2181–2188.
- Yang, Q. Z., Wang, F., & Rao, J. P. (2016). Effect of putrescine treatment on chilling injury, fatty acid compositions and antioxidant system in kiwifruit. *PLoS ONE*, 11, e0162159.
- Yang, Q. Z., Zhang, X. P., Wang, F., & Zhao, Q. F. (2019). Effect of pressurized argon combined with controlled atmosphere on the postharvest quality and browning of sweet cherries. *Postharvest Biology and Technology*, 147, 59–67.
- Yang, Q. Z., Zhang, Z. K., Rao, J. P., Wang, Y. P., Sun, Z. Y., Ma, Q. S., et al. (2013). Low temperature conditioning induces chilling tolerance in ‘Hayward’ kiwifruit by enhancing antioxidant enzyme activity and regulating endogenous hormones levels. *Journal of the Science of Food and Agriculture*, 93, 3691–3699.

- Zhai, R., Liu, J. L., Liu, F. X., Zhao, Y. X., Liu, L. L., Fang, C., et al. (2018). Melatonin limited ethylene production, softening and reduced physiology disorder in pear (*Pyrus communis* L.) fruit during senescence. *Postharvest Biology and Technology*, 139, 38–46.
- Zhao, Y., Tan, D. X., Lei, Q., Chen, H., Wang, Lin, Li, Q. T., et al. (2013). Melatonin and its potential biological functions in the fruits of sweet cherry. *Journal of Pineal Research*, 55, 79–88.
- Zheng, X. L., Tian, S. P., Meng, X. H., & Li, B. Q. (2007). Physiological and biochemical responses in peach fruit to oxalic acid treatment during storage at room temperature. *Food Chemistry*, 104, 156–162.
- Zhu, L. L., Hu, H. L., Luo, S. F., Wu, Z. X., & Li, P. X. (2018). Melatonin delaying senescence of postharvest broccoli by regulating respiratory metabolism and antioxidant activity. *Transactions of the Chinese Society of Agricultural Engineering*, 34(3), 300–308.

