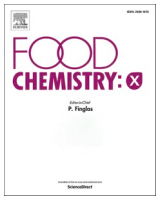
Food Chemistry: X 19 (2023) 100769

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x



# Prolong the postharvest shelf life of spinach through the antioxidative ability of melatonin

Mingying Wang [a], Jin Xu [a], Zhaoyang Ding [a,b,c,*], Jing Xie [a,b,c,*]

[a] College of Food Science and Technology, Shanghai Ocean University, Shanghai 201306, China
[b] National Experimental Teaching Demonstration Center for Food Science and Engineering, Shanghai Ocean University, Shanghai 201306, China
[c] Shanghai Engineering Research Center of Aquatic-Product Processing & Preservation, Shanghai 201306, China

ARTICLE INFO

*Keywords:*
Spinach
Melatonin
Shelf life
Antioxidative ability

ABSTRACT

Spinach is also known as Persian cuisine, it is rich in nutrients such as protein, vitamin C and minerals, and has high nutritional value. In this study, Spinach was treated with melatonin in order to prolong its shelf life. Melatonin has strong antioxidant effects as an endogenous free radical scavenger. The spinach was sprayed with 0.10, 0.20 and 0.30 mg/mL melatonin solution after harvesting, and distilled water was used as control for low temperature storage at 4 °C. The results showed that melatonin spraying Spinach delayed the degradation of chlorophyll, especially the treatment of 0.20 mg/mL melatonin was the most effective. The content of soluble sugar and soluble protein in spinach tissue was kept high, the accumulation of malondialdehyde (MDA) was reduced, and the activities of superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) were increased. These findings suggested that melatonin treatment may be a useful technique to prolong the postharvest life of spinach and improve its quality.

## Introduction

Spinach is known as Persian lettuce, red root lettuce and parrot lettuce. It is rich in carotenoids, vitamin C and abundant minerals. Because of its large leaf area and many stomata, spinach is susceptible to infection by pathogenic bacteria(Gadallah et al., 2022). In addition, spinach leaves have high water content and delicate tissues, with vigorous respiratory metabolism after harvesting(Mir et al., 2017), which can easily lose water and wilt, rot and deteriorate, seriously affecting the sensory quality and nutritional value of spinach to make its storage and preservation very difficult. There are many studies on spinach storage and preservation, such as the use of vacuum pre-cooling technology and chemical reagents(Aslam et al., 2016), but there are problems such as high costs and food safety hazards in some methods, so it is important to develop safe and low-cost methods to improve the storage and preservation of spinach for its industrial development.

Melatonin (MT) first discovered in the bovine pineal gland, also known as *N*-acetyl-5-methoxy-tryptamine, is an indole derivative of tryptophan. It is involved in circadian and photoperiodic responses in animals, which can improve sleep and treat neurasthenia, MT is allowed to be used as raw material of health food in China. While it is also signaling molecule that plays a role in immune regulation, scavenging free radicals and antioxidants, and cell protection(Guerra & Devesa, 2021). As a new, natural and safe preservative, MT has no bad smell compared with other essential oils. It can effectively maintain the postharvest quality of fruits and vegetables, slow down the aging process, and has a good application prospect. MT has the highest antioxidant effect as an endogenous free radical scavenger(D.-X. Tan et al., 2015), with higher antioxidant activity than vitamin E, vitamin C carotenoids and glutathione, etc. It has a very strong effect on the scavenging of oxygen radicals such as hydrogen peroxide, peroxyl radicals, hydroxyl radicals, singlet oxygen and superoxide anion radicals in living organisms (Gwozdzinski, Pieniazek, & Gwozdzinski, 2021). It has been reported that MT can delay degradation of chlorophyll in wheat seedlings and maize under drought stress and improve the cold resistance and salt stress tolerance of plants (Jiang et al., 2016).

Numerous studies have been reported on the application of MT for plant preservation. Liu(Liu et al., 2018) used strawberries treated with different concentrations of MT and showed by comparing the color, hardness, soluble solids content and titratable acidity of strawberry fruits that 0.1 or 1 mmol-$L^{-1}$ MT treatment significantly delayed the senescence of strawberry fruits. Luo et al. (Luo et al., 2018)used MT to treat fresh broccoli and MT treatment delayed the yellowing of broccoli by 4 days compared to the control, and the protective effect of MT

* Corresponding authors.
*E-mail addresses:* zyding@shou.edu.cn (Z. Ding), jxie@shou.edu.cn (J. Xie).

https://doi.org/10.1016/j.fochx.2023.100769
Received 22 May 2023; Received in revised form 15 June 2023; Accepted 21 June 2023
Available online 25 June 2023

treatment on vitamin C and carotenoids was significant. In addition, exogenous MT treatment can also improve cold damage symptoms in peach and tomato(Aghdam et al., 2019; Onik et al., 2021), and can suppress cold damage in strawberry, sweet cherry, and apple to delay fruit senescence by reactive oxygen levels, and also induce tomato to improve fruit disease resistance(S. Li et al., 2019), inhibit postharvest pear and banana fruit softening-related enzyme activities that maintain membrane integrity, maintaining fruit hardness and thus prolonging the storage period of fruits(Li et al., 2019).

It has been suggested that MT acts in several ways: enhancing the efficiency of mitochondrial oxidative phosphorylation, reducing electron leakage, activating antioxidant enzyme activity, directly scavenging reactive oxygen species, and enhancing the remaining antioxidant actions(Shi et al., 2018). MT may enhance enzyme activity by increasing the transcriptional levels of protective enzymes, MT also contributes to the production of antioxidants, while MT can be self-synthesized in fruits and vegetables. Other studies have pointed out that the main mechanism by which MT exerts its antioxidant protective effect is through an electron-donating reaction, providing electrons to electron-deficient molecules (Galano, Tan, & Reiter, 2011). MT is the only known antioxidant that reacts in this way, both by scavenging free radicals and by reducing some oxidized biomolecules during the electron reaction, thus maintaining their structure(Galano & Reiter, 2018).

In order to prolong the shelf life of spinach and reduce the loss of nutrients during storage. Based on the MT strong antioxidant properties, the present study was conducted to investigate the effects of different concentrations of MT on the appearance and intrinsic quality of spinach as well as its post-harvest storage and preservation by spraying spinach before and after harvest. In this study, MT was used to keep spinach fresh, and the optimum amount of compound preservative suitable for spinach storage was put forward, so as to provide technical reference and theoretical basis for spinach storage and preservation.

## Materials and methods

### *Materials*

Melatonin was purchased from Aladdin Reagent (Shanghai) Co., Ltd. Spinach was selected as the test material for this paper. The spinach was purchased from Lotus Supermarket in Shanghai in December 2022. Spinach was selected according to the requirements of freshness, uniformity of size, absence of mechanical damage, and absence of pests and diseases. Sodium chloride, calcium carbonate powder and quartz sand were purchased from Sinopharm Chemical Reagent Co., Ltd. MDA and SOD kits are from Nanjing Jiancheng Company.

### *Methods*

#### *Material treatment*

Fresh Spinach was washed with distilled water and the washed spinach was randomly divided into four groups as control and melatonin-treatment groups. The control group was sprayed with 200 mL of distilled water and the control group was sprayed with 200 mL 0.10, 0.20 and 0.30 mg/mL of melatonin solution. After air drying at 25 °C for 60 min, the spinach was individually bagged in unsealed polyethylene bags and then stored at 4 °C for 12 days. During the chilling process, several spinach leaves were randomly taken from each group at 2-day intervals to determine the indicators.

#### *Determination of quality indicators*

Determination of color: The color of spinach leaves evaluation was performed using a colorometer (Minolta, Osaka, Japan, model-NH310). The instrument was aligned with a black and white standard and observed experimental data for L *, a * and b *.

Determination of weight loss: using the weighing method.

Weight loss rate (%) = (initial mass of spinach - mass of spinach after each storage period)/initial mass of spinach × 100.

Determination of optical structures: The cut leaf sections were immediately immersed in distilled water and covered with coverslips. Bright-field imaging was performed on a microscope (Axio Vert.A1) with a 10x objective.

Sensory quality analysis was recorded using characteristics based on: muscular tissue, smell, and appearance. The evaluation team consisted of 10 people with rich experience in the sensory evaluation of each sample reasonably during the experiment. The full score of the three indicators is 10. The specific evaluation criteria are shown in Table 1.

#### *Determination of physiological and biochemical indicators*

Chlorophyll determination: Wash and dry the sample, take 0.5 g of the green leaf part, avoid the stem and large leaf veins, add 1–2 mL of extract (95% ethanol) with a small amount of quartz sand and calcium carbonate to the mortar, grind into a homogenate, then add an appropriate amount of extract and continue grinding until the tissue is white. Filter, moisten the filter paper with the acetone solution, wash the Spinach leaf residue, wash all the chlorophyll on the filter paper into a volumetric flask, fix the volume to 50 mL with the extraction solution, and shake well. The absorbance was measured at 652 nm, and then the chlorophyll content in the sample was calculated according to the following formula: Calculation of chlorophyll (mg/g).

Chlorophyll content (mg/g) $=\frac{D_{625}\times V}{W\times 1000}$ (1).

Where $D_{652}$ denotes the absorbance of the extract at 652 nm; V denotes the volume of chlorophyll extract, (mL); W denotes the weight of fresh spinach (g).

The vitamin C content of spinach samples was determined by the 2, 6-dichloroindophenol titration method. Samples were taken for determination on the day of harvesting and on days 2, 4, 6, 8, 10 and 12. The sample was weighed about 1 g, placed in a mortar and poured through a funnel with about 0.5 mL of 2% oxalic acid.

The sample was poured into a 10 mL volumetric flask through a funnel, and the mortar and rod were rinsed with 2% oxalic acid, and the washings were poured into the flask together with 2% oxalic acid and filtered. Take 1 mL of standard ascorbic acid solution into a small conical flask and titrate with 2, 6-dichloroindophenol until pink and the color does not fade within 30 s as the end point. Calculate the number of mg of 1 mL dye equivalent to ascorbic acid. Take 10 mL of sample filtrate in a small conical flask and titrate with calibrated. The sample was titrated with calibrated 2, 6-dichloroindophenol and a blank was made at the same time. The amount of vitamin C in the sample was calculated according to the following formula.

**Table 1**
Sensory-evaluation project.

| | Tissue | Smell | Appearance |
|---|---|---|---|
| 10 | Excellent shape, firm and smooth stems, flat leaves and no wilting and rot | Spinach is inherently fragrant, fragrant and odorless | Fresh and shiny, green and full, without browning |
| 8 | The shape is good, the stem is still firm and smooth, and the leaves are flat | Spinach has an inherent smell and no peculiar smell | The freshness is good, but the color is a little poor, and there is no yellowing and browning |
| 6 | The shape is general, the stems are soft and smooth | No fragrance, slightly peculiar | Not too fresh, part of the color is dim |
| 4 | Leaf edges rarely curl and wilt | Have a rotten smell | Not too fresh, yellow part |
| 2 | The vegetable body is slightly softened, the leaves are curled and wilted | The rotten smell is strong | The color is dull, partly yellow and brown |
| 0 | The vegetable body is severely softened, with obvious curly leaves and many wilts | Have a bad smell of corruption | The yellowed part has an area of ≥ 30% and is dull |

Vitamin C content of the sample (mg/g) $=\frac{(V_0-V_1)\times A}{B}\times\frac{Z}{X}\times 100$ (2).

Where $V_0$ represents the number of m L of dye used to titrate the sample; $V_1$ the number of mL of dye used to titrate the blank; A the number of mg of ascorbic acid equivalent to 1 mL of dye, mg/mL; B represents the mass of the product, g; Z represents the total volume of the sample solution after fixing, mL; X represents the volume of solution drawn during the titration of the sample, mL.

CAT enzyme activity was performed according to Periyar Selvam with some modifications (Sellamuthu et al., 2013). Analysis of the reaction mixture consisted of sodium phosphate buffer, $H_2O_2$ and spinach extract (50 μL), after which hydrogen peroxide decomposition was observed at 240 nm (Multiskan GO microplate spectrophotometer, Finland). Enzyme activity was reported as units per milligram of protein (U/mg protein). One unit represents the amount of catalase required to convert U/mg protein (Perumal, Nambiar, Sellamuthu, & Emmanuel, 2021).

POD enzyme activity was analyzed by following the method of Jiang with minor modifications reported by Periyar Selvam (Sellamuthu et al., 2013). The spinach extracts were mixed with a buffer solution containing sodium phosphate solution and guaiacol and incubated at 20 °C for 5 min. Subsequently, $H_2O_2$ was added and the increase in absorbance at 460 nm was measured for 120 s (Multiskan GO microplate spectrophotometer, Finland). One unit of enzyme activity was expressed as the number of enzymes causing a change in absorbance per milligram of protein (U/mg protein). For each treatment, three enzyme assays were performed for each sample.

Soluble sugars, soluble solids content, MDA, and SOD were measured using kits. The contents soluble sugars were calculated as milligrams contained in each gram of fresh tissue (mg/g); MDA content was calculated as the content in each milligram of protein (nmol/mg);

*Data processing*

One-way analysis of variance (ANOVA) was performed using SPSS 20.0 (SPSS, Chicago, Illinois, USA). Results were expressed as mean ± standard deviation (SD). All experiments were performed in at least three independent trials.

## Results

*Effect of melatonin treatment on the appearance and quality of spinach*

As shown in Fig. 1, with the increase of storage time, the leaves of fresh spinach gradually turned yellow and the tops of the leaves gradually decayed, whereby the control spinach leaves showed the highest degree of decay and the best results were obtained with 0.20 mg/mL MT treatment. As shown in Fig. 2, the initial spinach cells were structurally intact, neatly arranged, relatively full, with high water content, almost unchanged cell morphology, and high cell integrity. With the increase of storage days, the spinach cell structure was significantly deformed and began to fold and curl, and the cell gap gradually increased. This indicated that the cellular integrity of spinach was damaged. The combination of cell morphology, water content, and pore space showed that 0.20 mg/mL MT treatment was the most effective, which may be due to the antioxidant effect of MT, thus maintaining cell structure(Lin et al., 2022).

During storage, spinach leaves were susceptible to yellowing, which was mainly due to the degradation of chlorophyll. The results showed that the chlorophyll content of both control and treated groups showed a decreasing trend (Fig. 3b), and on day 12, the chlorophyll content of control spinach leaves was 0.17 mg/g, while the chlorophyll content of spinach treated with 0.10, 0.20, and 0.30 mg/mL MT was 0.33, 0.53, and 0.47 mg/g. According to Fig. 1 and Fig. 3a, it was shown that with

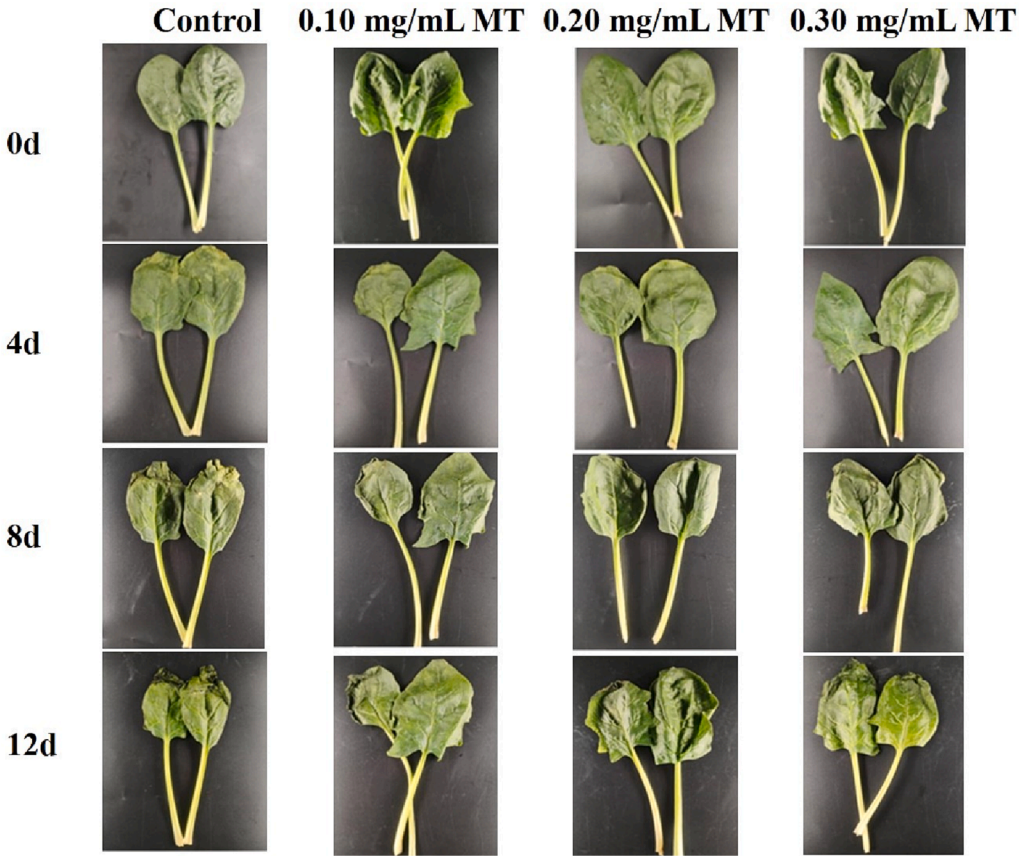



**Fig. 1.** The effect of melatonin treatment on spinach during storage at 4 °C.

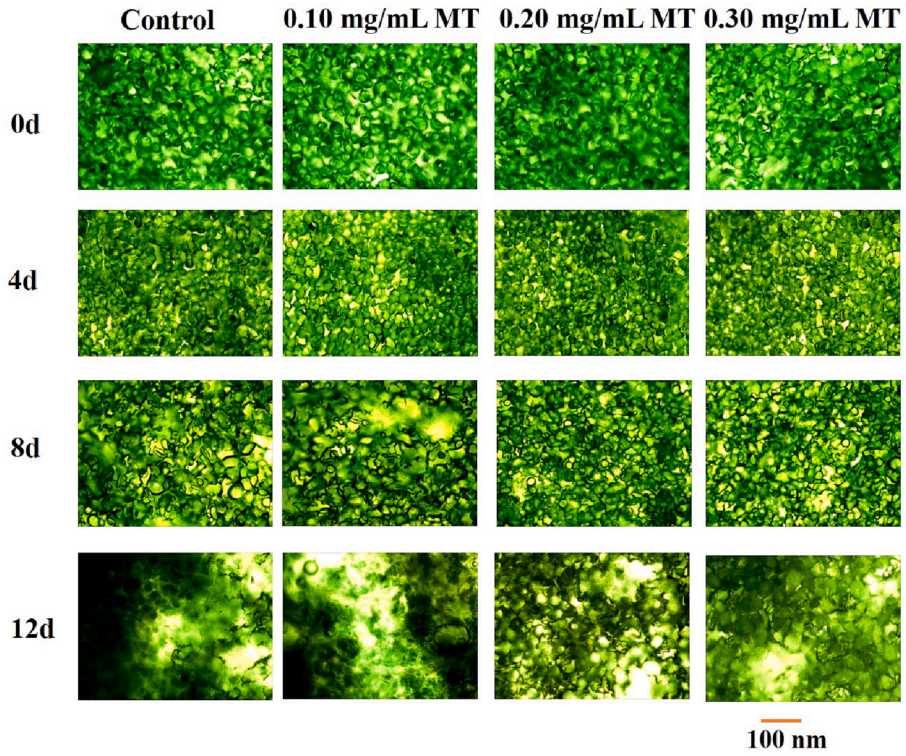



**Fig. 2.** Light microscopy images of spinach treated with different concentrations of melatonin. The scale bar for all images is 100 nm.

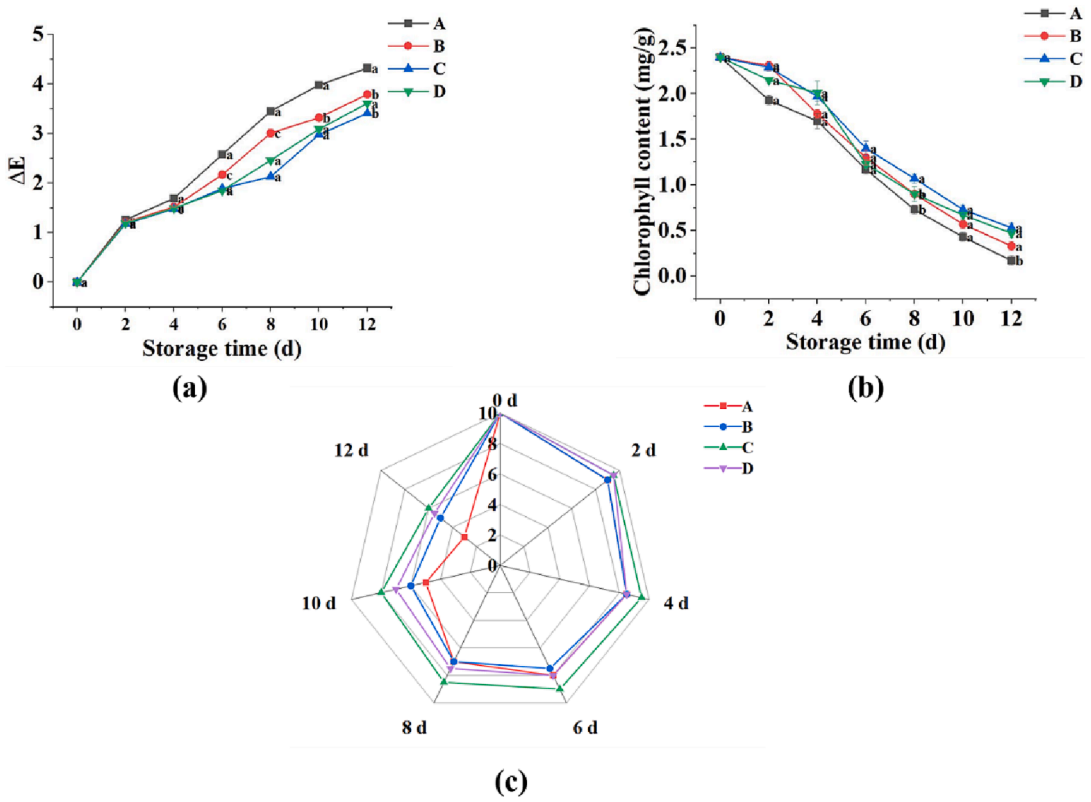



**Fig. 3.** The effect of different concentrations of melatonin treatment on the color difference(a) and chlorophyll content (b) on spinach during storage at 4 °C. (A: control group, B: 0.10 mg/mL MT, C: 0.20 mg/mL MT, D: 0.30 mg/mL MT).

the extension of the storage period, the color difference of spinach treated with 0.20 mg/mL melatonin was the least. This may also be related to the degradation of spinach chlorophyll(Jiang et al., 2016). The sensory quality of spinach gradually deteriorated and rotted during storage. At the initial stage of storage, the spinach in each group has green color, full leaves and excellent shape, and has the inherent

fragrance of spinach. As shown in Fig. 3c, the sensory score of spinach in the control group decreased rapidly from 0 to 8 days and lost its commercial value at 8 days. However, after 8 d storage, the sensory score of spinach treated with 0.20 mg/mL MT was 8.5, and the sensory score decreased slowly. This shows that 0.20 mg/mL MT treatment can keep the appearance, shape and smell of postharvest spinach and improve its commercial value.

*Effect of melatonin on antioxidant enzyme activity and malondialdehyde in spinach*

SOD can scavenge superoxide anion, generate $H_2O_2$ and $O_2$, maintain the balance of reactive oxygen metabolism and play a role in cell protection. In Fig. 4a, the overall trend of SOD activity increased in all four spinach groups during storage, promoting the increase in SOD activity in the pre-storage treatment group and delaying the decrease in SOD activity in the post-storage treatment group, with the highest activity in the 0.20 mg/mL MT treatment group by the end of storage.

Spinach POD activity increased and then decreased during storage, with significant differences appearing between the 0.20 mg/mL MT treatment and control groups (Fig. 4b). CAT catalyze the decomposition of $H_2O_2$ into water and molecular oxygen, and the trend of CAT activity in the control and treated groups was similar, both showed a gradual increase, while the highest CAT activity was observed in the 0.20 mg/mL MT treated group at 12d (Fig. 4c). As shown in Fig. 4d, the MDA content of spinach continued to increase throughout the storage period and was lower in the 0.10, 0.20, and 0.30 mg/mL treatment groups than in the control group. The effect of the 0.20 mg/mL MT treatment group was the most significant throughout the storage period, indicating that the 0.20 mg/mL MT treatment group effectively controlled the degree of cell membrane damage and thus inhibited the occurrence of cold damage symptoms.

*Analysis of water loss, vitamin C, soluble sugars and soluble solids content of spinach*

The storage quality of leafy vegetables can be reflected in the weight loss rate. As shown in Fig. 5a, the overall weight loss rate showed an increase. The Weight loss in the 0.20 mg/mL MT treatment group was significantly lower than in the control group and the 0.10 and 0.30 mg/mL MT treatment groups. From Fig. 5b, the VC content of spinach in the 0.10, 0.20 and 0.30 mg/mL MT treatment groups were significantly higher than that of the control group. Moreover, the slowest decrease in VC content was observed in the 0.20 mg/mL MT condition, with a more pronounced effect in the treatment group with 0.20 mg/mL MT concentration.

As shown in Fig. 5c, the soluble solids content of spinach in both control and treated groups gradually decreased, and after 4 d of storage, the soluble solids content of the treated group was significantly higher than that of the control group. On day 12, the soluble solids content of control spinach decreased to 2.79 mg/g, while the soluble solids content of 0.10, 0.20 and 0.30 mg/mL MT treated stems were 2.95,3.01 and 2.93 mg/g, respectively. Similarly, the soluble sugar content of spinach decreased with storage time as shown in Fig. 5d. 0.20 and 0.30 mg/mL MT treatments significantly delayed the decrease in soluble protein content of spinach.

## Discussion

Both color and texture are important attributes that affect the acceptability of the product. During storage, vegetable color is very susceptible to changes(Chu et al., 2021). MT treatment significantly inhibited the increase in color difference values, indicating that MT has a delaying effect on chlorophyll degradation during decay. The effect of MT on the degradation of chlorophyll during the aging process was delayed. At the same time, MT, which is reactive oxygen species scavenger can effectively remove the excessive production of reactive oxygen species in plant cells, reduce the cell wall hydrolase activity, and

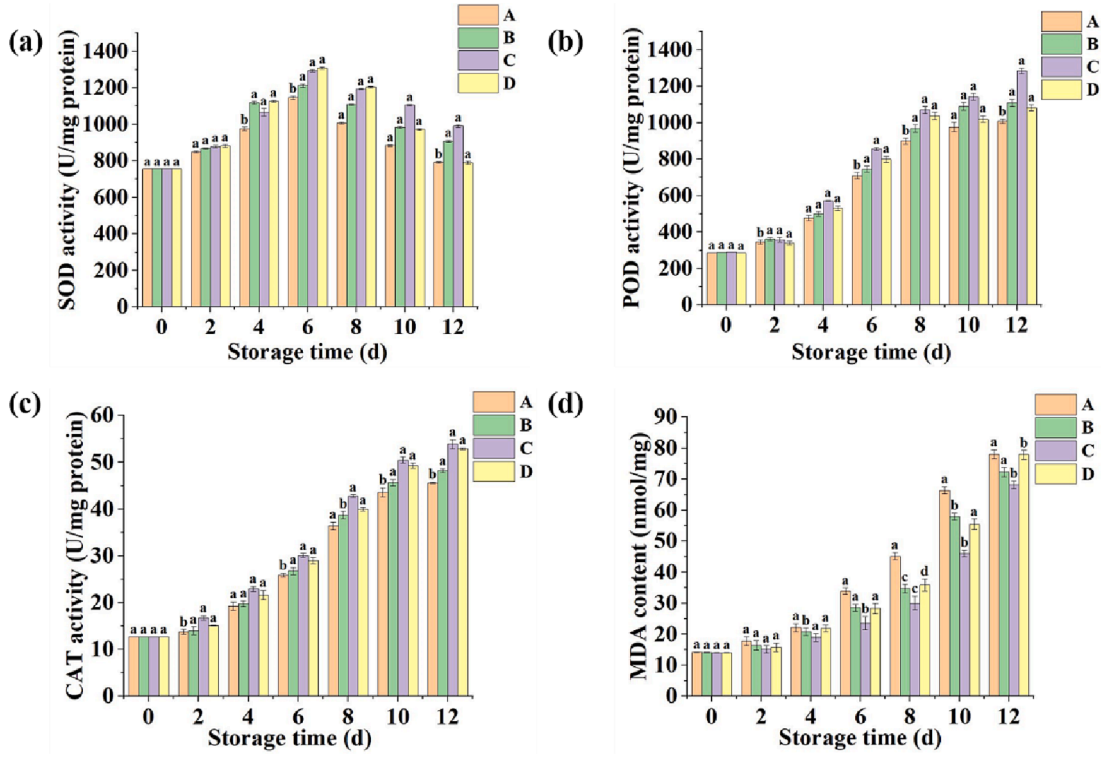



**Fig. 4.** Effect of melatonin treatment on spinach SOD (a), POD (b), CAT activity (c) and MDA content (d) during storage at 4 °C. (A: control group, B: 0.10 mg/mL MT, C: 0.20 mg/mL MT, D: 0.30 mg/mL MT).

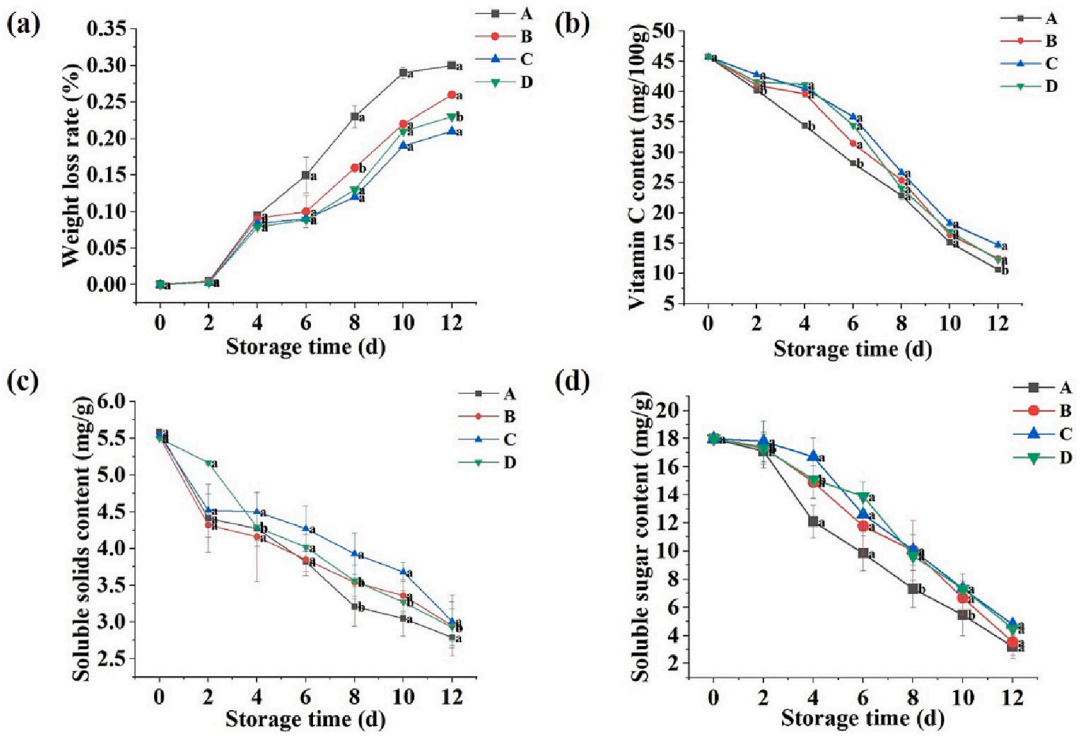



**Fig. 5.** Effect of melatonin treatment on weight loss (a), vitamin c content (b), soluble solids content (c), and soluble sugars content (d) of spinach during storage at 4 °C. (A: control group, B: 0.10 mg/mL MT, C: 0.20 mg/mL MT, D: 0.30 mg/mL MT).

thus inhibit cell wall degradation to maintain the integrity of cell structure.

MT is an intrinsic antioxidant substance in plants. In this study, MT was selected for treatment of spinach under low temperature storage conditions. The experimental results showed that MT treatment inhibited leaf chlorophyll degradation. The color change of green leafy vegetables was closely related to the yellowing rate and chlorophyll content. Exogenous MT has been reported to slow down chlorophyll degradation in leaves, broccoli, and wheat leaves(F. Wang et al., 2019; H. Wang et al., 2021). Compared with the control group, MT treatment at 0.20 mg/mL maintained relatively good color, delayed the onset of yellowing and chlorophyll content, maintained better appearance of spinach, which significantly delayed the aging of spinach. As excess melatonin is harmful to post-harvest fruits, high concentrations can negatively affect the quality of fruits and vegetables(Aghdam & Fard, 2017). Tan et al (X.-l. Tan et al., 2020) used 100 μmol/L MT to soak cabbage to delay the degradation of chlorophyll. It has been reported that exogenous MT treatment delayed chlorophyll degradation in apple isolated leaves, postharvest cycads degradation of chlorophyll(P. Wang et al., 2012). It can be seen that MT treatment can effectively delay the chlorophyll content of plant leaves.

POD can catalyze the oxidation of phenolics by $H_2O_2$ to produce quinones(Dong et al., 2022). It has been demonstrated that MT treatment of plants can reduce plant oxidative damage under stress by increasing the activity of plant POD oxidative damage under stress(Yang et al., 2022). Similar results were obtained for maize treated with MT, which removed excess $H_2O_2$ through the MT scavenging cascade and promoted the expression and activity of antioxidant enzyme genes such as POD and CAT, significantly reducing reactive oxygen species levels (Chen et al., 2018). And treatment of cassava with MT protected cassava from oxidative stress(Bai et al., 2020). MT also significantly reduced malondialdehyde and electrolyte leakage levels in senescing leaves (Ahmad et al., 2020). MDA content responded to the level of cell membrane peroxidation. MDA is the end product of peroxidation of cell membrane lipids, and the level of MDA content of spinach during storage can reflect the degree of aging of fruit tissues to a certain extent.

MT is the most powerful antioxidant among known endogenous free radical scavengers. Because of its high lipophilicity MT can also act as an intracellular free radical scavenger due to its high lipophilicity and can also cross the cytoplasm into the nucleus to exert antioxidant effects due to its partial hydrophilicity (Kruk, Aboul-Enein, & Duchnik, 2021). Studies have shown that MT can directly scavenge free radicals such as $H_2O_2$, and NO in living organisms(Yong et al., 2021). MT has the ability to directly scavenge reactive oxygen species outside, thus achieving enhanced antioxidant capacity of plants.

The enzymatic antioxidant system is the main way to control the production of ROS, which regulates the extent of lipid peroxidation. SOD, CAT and POD are the key antioxidant enzymes that scavenge ROS. MT treatment can induce disease resistance in spinach by consistently increasing the activity of POD, SOD, and CAT. MT treatment up-regulated the antioxidant enzyme activity and reduced $O^{2-}$ and $H_2O_2$ levels in spinach, thus maintaining the balance of ROS metabolism, reducing lipid peroxidation, and delaying the aging of spinach(Duan et al., 2022).

Fruits and vegetables rot after harvest. Postharvest diseases or senescence of fruits and vegetables produce a lot of active oxygen, which leads to lipid peroxidation and postharvest decay(Huang et al., 2021). MT is mainly used as a powerful free radical scavenger, which can remove excess active oxygen in postharvest fruits and vegetables by increasing the contents of antioxidant enzymes, non-enzymatic antioxidants and enzymes related to oxidative protein repair. Subsequently, the contents of hydroxyl radicals and hydrogen peroxide are reduced, and the degree of membrane lipid peroxidation is reduced, thus protecting cells from oxidative damage and prolonging shelf life (Xu, Chen, & Kang, 2019).

The soluble sugar and soluble solids contents are an important index for determining the storage characteristics of fruits and vegetables, as well as the main factor for judging the quality of postharvest vegetables. Spinach contains 70% to 90% water, which is fresh and tasty, and contains many nutrients such as VC, minerals, sugars, organic acids and

crude fiber. VC is one of the parameters of nutritional quality of spinach. three concentrations of melatonin treated spinach showed significantly higher VC content than the control. The results of this experiment may be attributed to the difference in the effect of MT on VC metabolism in spinach. This indicated that the pre-harvest spraying of MT significantly improved the quality of spinach. Zahed et al(Zahedi et al., 2020) showed good effects of weekly MT sprays on soluble solids, total acidity, VC and sugars in strawberry fruits; Miao et al (Miao, Zeng, Zhao, Wang, & Wang, 2020) maintained high VC content using MT treatment on broccoli. Similar results were obtained in the present study, where MT treatment maintained high levels of soluble sugars, soluble solids and VC, resulting in better nutritional quality of spinach.

The content of chlorophyll is mainly limited by chlorophyllase, and MT reduces the activity of chlorophyllase(Santos, 2004). MT affected the color, chlorophyll degradation and chloroplast structure of spinach during storage. MT treatment can delay the decline of chlorophyll content in spinach and maintain a more complete chloroplast. At the same time, MT also inhibited the activities of chlorophyll catabolic enzymes, such as chlorophyllase (CLH) and red chlorophyll catabolic reductase (RCCR)(Song et al., 2023).

MT reduces color change and maintains titratable acidity. It can also improve the activities of glutathione reductase, ascorbic acid peroxidase and dehydroascorbic acid reductase in jujube, and increase the contents of ascorbic acid and dehydroascorbic acid(Tang et al., 2020). MT reduces color change and maintains titratable acidity. It can also improve the activities of glutathione reductase, ascorbic acid peroxidase and dehydroascorbic acid reductase in jujube, and increase the contents of ascorbic acid and dehydroascorbic acid. Besides, MT has been proved to inhibit the activities of pectin methyl esterase, cellulase and β -glucosidase and the production of water-soluble pectin, and keep the content of water-insoluble pectin, thus delaying the softening and spoilage of spinach, and improving the quality of spinach during storage(Cao et al., 2021).

## Conclusions

This study evaluated the effect of different concentrations of MT on the quality of fresh spinach. The results showed that MT as an antioxidant effectively extended the shelf life of spinach and maintained the quality of spinach under the storage temperature at 4 °C. The spinach treated with MT showed lower weight loss rate and color difference, and maintained higher activities of soluble sugar, soluble solid, VC, chlorophyll content and antioxidant enzymes, especially in the treatment of 0.20 mg/mL MT. Therefore, MT treatment may be a useful technique to prolong the postharvest life of spinach and improve its quality.

## Funding

This research was funded by Shanghai Agriculture Applied Technology Development Program, China (Grant No.T20210401).

## CRediT authorship contribution statement

**Mingying Wang:** Writing – original draft. **Jin Xu:** Conceptualization, Methodology. **Zhaoyang Ding:** Writing – review & editing. **Jing Xie:** Writing – review & editing.

## 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.

## Data availability

Data will be made available on request.

## References

Aghdam, M. S., & Fard, J. R. (2017). Melatonin treatment attenuates postharvest decay and maintains nutritional quality of strawberry fruits (Fragaria× anannasa cv. Selva) by enhancing GABA shunt activity. *Food chemistry, 221*, 1650–1657. https://doi.org/10.1016/j.foodchem.2016.10.123

Aghdam, M. S., Luo, Z., Jannatizadeh, A., Sheikh-Assadi, M., Sharafi, Y., Farmani, B., … Razavi, F. (2019). Employing exogenous melatonin applying confers chilling tolerance in tomato fruits by upregulating ZAT2/6/12 giving rise to promoting endogenous polyamines, proline, and nitric oxide accumulation by triggering arginine pathway activity. *Food chemistry, 275*, 549–556. https://doi.org/10.1016/j.foodchem.2018.09.157

Ahmad, S., Su, W., Kamran, M., Ahmad, I., Meng, X., Wu, X., … Han, Q. (2020). Foliar application of melatonin delay leaf senescence in maize by improving the antioxidant defense system and enhancing photosynthetic capacity under semi-arid regions. *Protoplasma, 257*(4), 1079–1092. https://doi.org/10.1007/978-3-319-21197-8-2

Aslam, M., Sultana, B., Anwar, F., & Munir, H. (2016). Foliar spray of selected plant growth regulators affected the biochemical and antioxidant attributes of spinach in a field experiment. *Turkish Journal of Agriculture and Forestry, 40*(2), 136–145. https://doi.org/10.3906/tar-1412-56

Bai, Y., Guo, J., Reiter, R. J., Wei, Y., & Shi, H. (2020). Melatonin synthesis enzymes interact with ascorbate peroxidase to protect against oxidative stress in cassava. *Journal of Experimental Botany, 71*(18), 5645–5655. https://doi.org/10.1093/jxb/eraa267

Cao, S., Qu, G., Ma, C., Ba, L., Ji, N., Meng, L., … Wang, R. (2021). Effects of melatonin treatment on the physiological quality and cell wall metabolites in kiwifruit. *Food Science and Technology, 42*. https://doi.org/10.1590/fst.85421

Chen, Y. E., Mao, J. J., Sun, L. Q., Huang, B., Ding, C. B., Gu, Y., … Yuan, S. (2018). Exogenous melatonin enhances salt stress tolerance in maize seedlings by improving antioxidant and photosynthetic capacity. *Physiologia plantarum, 164*(3), 349–363. https://doi.org/10.1111/ppl.12737

Chu, Y., Wei, S., Ding, Z., Mei, J., & Xie, J. (2021). Application of ultrasound and curing agent during osmotic dehydration to improve the quality properties of freeze-dried yellow peach (Amygdalus persica) slices. *Agriculture, 11*(11), 1069. https://doi.org/10.3390/agriculture11111069

Dong, S., Dong, Y., Liu, B., Liu, J., Liu, S., Zhao, Z., . . . He, F. (2022). Guiding Transition Metal-Doped Hollow Cerium Tandem Nanozymes with Elaborately Regulated Multi-Enzymatic Activities for Intensive Chemodynamic Therapy. *Advanced Materials, 34*(7), 2107054. https://doi.org/ 10.1002/adma.202107054.

Duan, W., Lu, B., Liu, L., Meng, Y., Ma, X., Li, J., … Dong, H. (2022). Effects of Exogenous Melatonin on Root Physiology, Transcriptome and Metabolome of Cotton Seedlings under Salt Stress. *International Journal of Molecular Sciences, 23*(16), 9456. https://doi.org/10.3390/ijms23169456

Gadallah, F. M., Nevein, A., Belal, H. E., Majrashi, A., El-Tahan, A. M., El-Saadony, M. T., … El-Saadony, F. M. (2022). Nitrogen-molybdenum-manganese co-fertilization reduces nitrate accumulation and enhances spinach (Spinacia oleracea L.) yield and its quality. *Saudi Journal of Biological Sciences, 29*(4), 2238–2246. https://doi.org/10.1016/j.sjbs.2021.11.036

Galano, A., & Reiter, R. J. (2018). Melatonin and its metabolites vs oxidative stress: From individual actions to collective protection. *Journal of pineal research, 65*(1), e12514. https://doi.org/ 10.1111/jpi.12514.

Galano, A., Tan, D. X., & Reiter, R. J. (2011). Melatonin as a natural ally against oxidative stress: a physicochemical examination. *Journal of pineal research, 51*(1), 1-16. https://doi.org/ 10.1111/j.1600-079x.2011.00916.x.

Guerra, J., & Devesa, J. (2021). Melatonin Exerts Anti-Inflammatory, Antioxidant, and Neuromodulatory Effects That Could Potentially Be Useful in the Treatment of Vertigo. *International Journal of Otolaryngology, 2021*. https://doi.org/ 10.1155/2021/6641055.

Gwozdzinski, K., Pieniazek, A., & Gwozdzinski, L. (2021). Reactive oxygen species and their involvement in red blood cell damage in chronic kidney disease. *Oxidative Medicine and Cellular Longevity, 2021*. https://doi.org/ 10.1155/2021/6639199.

Huang, X., Ren, J., Li, P., Feng, S., Dong, P., & Ren, M. (2021). Potential of microbial endophytes to enhance the resistance to postharvest diseases of fruit and vegetables. *Journal of the Science of Food and Agriculture, 101*(5), 1744–1757. https://doi.org/10.1002/jsfa.10829

Jiang, C., Cui, Q., Feng, K., Xu, D., Li, C., & Zheng, Q. (2016). Melatonin improves antioxidant capacity and ion homeostasis and enhances salt tolerance in maize seedlings. *Acta physiologiae plantarum, 38*(4), 1-9. https://doi.org/ 10.1007/s11738-016-2101-2.

Kruk, J., Aboul-Enein, B. H., & Duchnik, E. (2021). Exercise-induced oxidative stress and melatonin supplementation: current evidence. *The Journal of Physiological Sciences, 71*(1), 1-19. https://doi.org/ 10.1186/s12576-021-00812-2.

Li, H., Fan, Y., Zhi, H., Zhu, Y., Liu, Y., & Wang, Y. (2019). Influence of fruit stalk on reactive oxygen species metabolism and quality maintenance of peach fruit under chilling injury condition. *Postharvest Biology and Technology, 148*, 141–150. https://doi.org/10.1016/j.postharvbio.2018.10.018

Li, S., Xu, Y., Bi, Y., Zhang, B., Shen, S., Jiang, T., & Zheng, X. (2019). Melatonin treatment inhibits gray mold and induces disease resistance in cherry tomato fruit during postharvest. *Postharvest Biology and Technology, 157*, Article 110962. https://doi.org/10.1016/j.postharvbio.2019.110962

Lin, Y., Zhan, L., Shao, P., & Sun, P. (2022). Phase-change materials and exogenous melatonin treatment alleviated postharvest senescence of Agaricus bisporus by inhibiting browning and maintaining cell membrane integrity. *Postharvest Biology and Technology, 192*, Article 112009. https://doi.org/10.1016/j.postharvbio.2022.112009

Liu, C., Zheng, H., Sheng, K., Liu, W., & Zheng, L. (2018). Effects of melatonin treatment on the postharvest quality of strawberry fruit. *Postharvest Biology and Technology, 139*, 47–55. https://doi.org/10.1016/j.postharvbio.2018.01.016

Luo, F., Cai, J.-H., Zhang, X., Tao, D.-B., Zhou, X., Zhou, Q., … Ji, S.-J. (2018). Effects of methyl jasmonate and melatonin treatments on the sensory quality and bioactive compounds of harvested broccoli. *Rsc Advances, 8*(72), 41422–41431. https://doi.org/10.1039/C8RA07982J

Miao, H., Zeng, W., Zhao, M., Wang, J., & Wang, Q. (2020). Effect of melatonin treatment on visual quality and health-promoting properties of broccoli florets under room temperature. *Food chemistry, 319*, 126498. https://doi.org/ 10.1016/j.foodchem.2020.126498.

Mir, S. A., Shah, M. A., & Mir, M. M. (2017). Microgreens: Production, shelf life, and bioactive components. *Critical Reviews in Food Science and Nutrition, 57*(12), 2730–2736. https://doi.org/10.1080/10408398.2016.1144557

Onik, J. C., Wai, S. C., Li, A., Lin, Q., Sun, Q., Wang, Z., & Duan, Y. (2021). Melatonin treatment reduces ethylene production and maintains fruit quality in apple during postharvest storage. *Food chemistry, 337*, Article 127753. https://doi.org/10.1016/j.foodchem.2020.127753

Perumal, A. B., Nambiar, R. B., Sellamuthu, P. S., & Emmanuel, R. S. (2021). Use of modified atmosphere packaging combined with essential oils for prolonging post-harvest shelf life of mango (cv. Banganapalli and cv. Totapuri). *Lwt, 148*, 111662. https://doi.org/ 10.1016/j.lwt.2021.111662.

Santos, C. V. (2004). Regulation of chlorophyll biosynthesis and degradation by salt stress in sunflower leaves. *Scientia Horticulturae, 103*(1), 93–99. https://doi.org/10.1016/j.scienta.2004.04.009

Sellamuthu, P. S., Sivakumar, D., Soundy, P., & Korsten, L. (2013). Essential oil vapours suppress the development of anthracnose and enhance defence related and antioxidant enzyme activities in avocado fruit. *Postharvest Biology and Technology, 81*, 66–72. https://doi.org/10.1016/j.postharvbio.2013.02.007

Shi, Y., Fang, Y.-Y., Wei, Y.-P., Jiang, Q., Zeng, P., Tang, N., . . . Tian, Q. (2018). Melatonin in synaptic impairments of Alzheimer's disease. *Journal of Alzheimer's Disease, 63*(3), 911-926. https://doi.org/ 10.3233/JAD-171178.

Song, L., Liu, S., Yu, H., & Yu, Z. (2023). Exogenous melatonin ameliorates yellowing of postharvest pak choi (Brassica rapa subsp. chinensis) by modulating chlorophyll catabolism and antioxidant system during storage at 20° C. *Scientia Horticulturae, 311*, Article 111808. https://doi.org/10.1016/j.scienta.2022.111808

Tan, D.-X., Manchester, L. C., Esteban-Zubero, E., Zhou, Z., & Reiter, R. J. (2015). Melatonin as a potent and inducible endogenous antioxidant: Synthesis and metabolism. *Molecules, 20*(10), 18886–18906. https://doi.org/10.3390/molecules201018886

Tan, X.-L., Zhao, Y.-T., Shan, W., Kuang, J.-F., Lu, W.-J., Su, X.-G., … Chen, J.-Y. (2020). Melatonin delays leaf senescence of postharvest Chinese flowering cabbage through ROS homeostasis. *Food Research International, 138*, Article 109790. https://doi.org/10.1016/j.foodres.2020.109790

Tang, Q., Li, C., Ge, Y., Li, X., Cheng, Y., Hou, J., & Li, J. (2020). Exogenous application of melatonin maintains storage quality of jujubes by enhancing anti-oxidative ability and suppressing the activity of cell wall-degrading enzymes. *Lwt, 127*, Article 109431. https://doi.org/10.1016/j.lwt.2020.109431

Wang, F., Zhang, X., Yang, Q., & Zhao, Q. (2019). Exogenous melatonin delays postharvest fruit senescence and maintains the quality of sweet cherries. *Food chemistry, 301*, Article 125311. https://doi.org/10.1016/j.foodchem.2019.125311

Wang, H., Li, Z., Yuan, L., Zhou, H., Hou, X., & Liu, T. (2021). Cold acclimation can specifically inhibit chlorophyll biosynthesis in young leaves of Pakchoi. *BMC plant biology, 21*(1), 1-15. https://doi.org/ 10.1016/j.foodchem.2019.125311.

Wang, P., Yin, L., Liang, D., Li, C., Ma, F., & Yue, Z. (2012). Delayed senescence of apple leaves by exogenous melatonin treatment: Toward regulating the ascorbate–glutathione cycle. *Journal of pineal research, 53*(1), 11–20. https://doi.org/10.1111/j.1600-079X.2011.00966.x

Xu, T., Chen, Y., & Kang, H. (2019). Melatonin is a potential target for improving post-harvest preservation of fruits and vegetables. *Frontiers in plant science, 10*, 1388. ttps://doi.org/10.3389/fpls.2019.01388.

Yang, B., Han, Y., Wu, W., Fang, X., Chen, H., & Gao, H. (2022). Impact of melatonin application on lignification in water bamboo shoot during storage. *Food Chemistry: X, 13*, Article 100254. https://doi.org/10.1016/j.fochx.2022.100254

Yong, W., Ma, H., Na, M., Gao, T., Zhang, Y., Hao, L., … Deng, X. (2021). Roles of melatonin in the field of reproductive medicine. *Biomedicine & Pharmacotherapy, 144*, Article 112001. https://doi.org/10.1016/j.biopha.2021.112001

Zahedi, S. M., Hosseini, M. S., Abadía, J., & Marjani, M. (2020). Melatonin foliar sprays elicit salinity stress tolerance and enhance fruit yield and quality in strawberry (Fragaria× ananassa Duch.). *Plant Physiology and Biochemistry, 149*, 313–323. https://doi.org/10.1016/j.plaphy.2020.02.021