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Effects of melatonin on blueberry fruit quality and cell wall metabolism during low temperature storage

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Abstract

Melatonin is known to influence various physiological processes, including plant ripening; however, the effect of melatonin on postharvest blueberry fruit softening is unknown. Herein, the effects of melatonin at different concentrations (100, 300, and 500 μmol L^{-1}) on the quality parameters, cell wall substances, cell-wall-degrading enzymes, and cell wall ultrastructure of blueberry fruits were investigated during low-temperature storage. Melatonin treatment was found to effectively delay the decline in fruit firmness, slow the loss of soluble solids and titratable acids, and decrease the respiration rate and decay rate of blueberry fruits. The blueberry fruits treated with 300 μmol L^{-1} melatonin exhibited the best quality. Melatonin treatment promoted anthocyanin accumulation, delayed ascorbic acid degradation, maintained a low relative membrane permeability in pulp, inhibited increases in lipoxygenase (LOX) activity, and reduced membrane lipid peroxidation. Moreover, melatonin controlled the formation of soluble pectin by inhibiting the activities of pectin methylesterase (PME), polygalacturonase (PG), cellulase (Cx), and β-glucosidase (β-Glu) while maintaining high contents of protopectin and cellulose. These results indicate that melatonin can effectively improve blueberry fruit quality and antioxidant capacity as well as and delay fruit softening by inhibiting the degradation of cell wall substances.

Keywords: blueberry fruit; melatonin; quality; cell wall metabolism.

Practical Application: Melatonin treatment was developed into a new method for blueberry fruit storage and preservation.

1 Introduction

Blueberries are popular with consumers because of their appearance, unique flavor, and nutrient richness (Chu et al., 2018). However, owing to their thin peel and juicy, soft flesh, blueberries are vulnerable to mechanical damage and pathogenic bacterial infections during storage and transportation. The resulting rapid water loss, softening, decay and, deterioration causes fruit quality to decline rapidly (Hancock et al., 2008; Zhou et al., 2014). In particular, softening is a main factor in decreasing the commodity value of blueberry fruits, thus hindering the development of the blueberry industry (Chea et al., 2019). It has been reported that blueberry fruit softening is related to the degradation of cell wall substances, such as gum, cellulose, and hemicellulose, which is mainly caused by changes in hydrolase activity (Xie et al., 2017; Wang et al., 2017a). The main hydrolases include cellulase (Cx), polygalacturonase (PG), pectin methylesterase (PME), and β-galactosidase (β-Gal) (Posé et al., 2019). During the ripening and softening of peaches (Chang et al., 2017), strawberries (Figueroa et al., 2012), and tomatoes (Rugkong et al., 2010), changes in the cell wall substances are accompanied by increases in the activities of degrading enzymes (Cx, PG, PME, and β-Gal). Moreover, the low rigidity of these fruits causes further deterioration in fruit quality and reduces the commercial value.

Melatonin (MT), a derivative of tryptophan, is widely found in fruits and vegetables such as apples, strawberries, cucumbers, and tomatoes (Feng et al., 2014; Sun et al., 2015). MT is not only an effective antioxidant but can also enhance the resistance of fruits and vegetables to biotic or abiotic stresses by neutralizing reactive oxygen species (ROS) free radicals, enhancing enzymatic or nonenzymatic antioxidant activity, and reducing lipid peroxidation (Galano et al., 2011). MT can also act as a signal molecule in various physiological processes such as regulating plant growth and development, delaying the ripening and senescence of fruits and vegetables, and enhancing the disease resistance of fruits and vegetables (Hernández-Ruiz & Arnao, 2018; Xu et al., 2019). Gao et al. (2018) found that MT treatment can promote the accumulation of total phenols and endogenous salicylic acid in peach fruits as well as delay membrane lipid peroxidation, thus reducing the occurrence of chilling injury. Hu et al. (2017) reported that MT treatment inhibits the expression of ethylene biosynthesis genes and starch degradation in bananas, which effectively delays banana ripening. In addition, MT treatment can aid in maintaining high fatty acid and ATP contents in litchi fruit, thus reducing browning (Wang et al., 2020), promote tomato ripening, thus improving storage quality (Hernández-Ruiz & Arnao, 2018; Aghdam et al.,

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2019), and delay mango ripening and softening by reducing cell wall hydrolase activity and the accumulation of water-soluble pectin (Liu et al., 2020).

However, until now, the effects of MT on the regulation of postharvest blueberry fruit softening have not been reported. The aim of this study was to investigate the effects of MT at different concentrations on fruit quality, antioxidant activity, cell wall substances, cell-wall-degrading enzyme activities, and the cell wall ultrastructure during cold storage. Treatment with 300 μmol L^{-1} MT was found to be most effective, delaying fruit softening, aiding in maintaining total soluble solids (TSS), titratable acids (TA), anthocyanins, and ascorbic acid, inhibiting increases in lipoxygenase (LOX) activity, and reducing membrane lipid peroxidation. MT also hindered destruction of the cell wall ultrastructure by inhibiting the activities of cell-wall-degrading enzymes (PG, PME, β-glucosidase (β-Gal), and Cx). These finding are expected to provide a theoretical basis for applying MT to improve the storage quality of blueberries and control fruit softening after harvest.

2 Materials and methods

2.1 Fruit materials

Blueberry fruits were picked by hand at a blueberry plantation in Majiang County, Guizhou Province, China on July 10, 2021. The picked fruits were placed in plastic baskets and immediately transferred to a room with a controlled atmosphere (approximately 15 °C) for storage. After picking, the fruits were transported by car for 3 h to a laboratory at the Guizhou Fruit Processing Engineering Technology Research Center. Blueberry fruits with no mechanical damage, no disease or insect pests, and relatively uniform fruit powder were selected for experiments.

2.2 Experimental design

The selected blueberry fruits were randomly divided into four groups. For each group, three parallel treatments were performed, with nine boxes (~ 125 g per box) used for each replicate. The four groups were (1) control group (sterile water treatment), (2) 100 μM (100 μmol L^{-1}) MT solution, (3) 300 μM (300 μmol L^{-1}) MT solution, and (4) 500 μM (500 μmol L^{-1}) MT solution. After soaking in the treatment solution for 3 min, the blueberry fruits were naturally dried at room temperature. Once dry, the initial surface moisture values of the blueberry fruits were measured on the same day. The fruits were then stored at 0 ± 0.5 °C and 85% ± 2% relative humidity for 80 d. The hardness, TSS content, decay rate, and electrical conductivity of the fruits were measured randomly every 20 d. The remaining fruits were immediately frozen in liquid nitrogen and stored at -80 °C for further analysis.

2.3 Determination of fruit firmness, respiration rate, and decay incidence

The fruit hardness was determined using the method of Chu et al. (2018) and other methods with slight modifications. A P/2 probe (diameter: 2 mm, measuring speed: 1.0 mm S^{-1}, measuring depth: 6 mm) was selected, and the results were expressed in N.

The fruit respiration rate was measured using a headspace analyzer (Zoneng technology Co., Ltd, Wuhan, China). For each of the three replicates in the four treatment groups, one box of fruit was placed in a sealed 2 L plastic container for 2 h. The probe was inserted into the container to determine the CO_2 content, and the respiration rate was expressed in mg CO_2 kg^{-1} h^{-1}.

The decay rate was measured according to the method of Shang et al. (2021). During the cold storage process, cryopreservation effects such as peel damage, juice outflow, and microbial infection were recorded as decay. The decay rate was expressed as the ratio (%) of decayed fruits to the total number of fruits.

2.4 Determination of TSS, TA, ascorbic acid contents

The TSS content was measured using a PAl-1 mini digital refractometer (ATAGO company, Tokyo, Japan). The TSS content was expressed as a percentage (%).

To determine the TA content, 1 g of blueberry fruit tissue was ground, and then the tissue fluid was transferred into a 100 mL volumetric flask. The volume was made up to the scale line using distilled water. After standing for 30 min, the sample was filtered and 5 mL of the filtrate was placed in a 100 mL Erlenmeyer flask. The filtrate was titrated with 0.1 mol L^{-1} NaOH to pH 8.1. The TA content was expressed as the malic acid percentage (%).

The content of ascorbic acid was determined by 4,7-diphenyl-1,10-phenanthroline colorimetry (Shang et al., 2021). The ascorbic acid content was expressed as the amount of ascorbic acid in 100g fresh weight of sample (mg $100g^{-1}$).

2.5 Sensory evaluation

The sensory evaluation was determined according to the method reported by Stavang et al. (2015) with slight modifications. Every 20 days, three boxes of fruits are randomly taken out from each treatment in the storage environment of 0 ± 0.5 °C and place at room temperature. Sensory score is carried out after the fruits recover to room temperature. The sensory group is composed of 7 evaluators. Each evaluator scores the fruit freshness, color, wax integrity, acidity, sweetness and overall taste. The scoring criteria are divided into four levels: (a) Fresh fruit, uniform color, luster, intact wax, no decay, strong sweet taste, good taste, 8-10 points; (b) Fresh fruit, uniform color, luster, intact wax, no decay and wilting, slightly sweet taste, general taste, 6-7.9 points; (c) The fruit is fresh, with uniform color, less wax, rotten and withered, light sweet taste and general taste, 2-5.9 points; (d) The fruit has poor freshness, poor color, less wax, more rotten and wilted fruit, light and tasteless fruit, poor taste, 0-1.9 points. Each evaluator writes his own score on the score sheet, and collects the score sheet after the evaluation to analyze the total score of the fruit.

2.6 Determination of LOX activity and relative membrane permeability

The LOX activity was determined using the method of Wang et al. (2017b). Weigh 5 g of sample was homogenized

with 10 mL of 50 mM phosphoric acid buffer (pH 7.0). After centrifugation at 10000 r min^{-1} for 20 min, the supernatant was collected and regarded as crude enzyme. After mixing 25 μL of 10 mmol L^{-1} sodium linoleate solution, 2.775 mL of 50 mmol L^{-1} sodium phosphate buffer (pH 7.0), and 0.5 mL of enzyme solution, the increase in absorbance at 234 nm was measured. LOX activity was expressed in U g^{-1}, where U = 0.01△OD_{234} min^{-1}.

The relative membrane permeability of the blueberry fruits was measured using a DDS-11A conductivity meter (Shanghai yidian scientific instrument Co., Ltd, Shanghai, China). Pulp (2 g) was cut into 1 mm slices with a stainless steel blade. The slices were placed in a beaker with 20 mL of distilled water for 30 min, and then the electrical conductivity (C1) was measured. Then, the beaker was placed in a boiling water bath for 15 min, and the conductivity (C2) of the solution was measured after cooling. The relative membrane permeability was expressed as the ratio of C1 to C2 (%).

2.7 Determination of cell wall polysaccharides

Soluble pectin and protopectin in the blueberry fruits were determined according to the method reported by Wang et al. (2020b). Blueberry tissue (2.0 g) was ground homogeneously and then transferred to a 50 mL centrifuge tube. After adding 25 mL of 95% ethanol, the sample was heated in a boiling water bath for 30 min, cooled to room temperature, and centrifuge at 10000 r min^{-1} for 15 min. The supernatant was discarded, 95% ethanol was added to the sediment, and the sample was heated in a boiling water bath. This procedure was repeated 3-5 times. Subsequently, 20 mL of distilled water was added to the precipitate, and the sample was maintained at 50 °C for 30 min, cooled, and centrifuged at 8000 r min^{-1} for 15 min. The supernatant was placed in a 100 mL volumetric flask. After washing the precipitate with a small amount of distilled water and centrifuging again, the supernatant was transferred to the volumetric flask, and the volume was made up to 100 mL using distilled water to obtain the soluble pectin extract solution.

The sediment was collected in a centrifuge tube, and 25 mL of 0.5 mol L^{-1} sulfuric acid was slowly added. After heating in a boiling water bath for 1 h, the sample was cooled and centrifuged at 8000 r min^{-1} for 15 min. The supernatant was transferred into a 100 mL volumetric flask and the volume was made up to the scale line with distilled water to obtain the protopectin determination solution.

A beaker containing 5 g of sample was placed in 0 °C a cold water bath. After adding 60 mL of 60% H_2SO_4, digestion was allowed to proceed for 30 min. The digested cellulose solution was transferred to a 100 mL volumetric flask and the volume was made up with 60% H_2SO_4. After shaking well and filtering, 5 mL of the filtrate was placed in a 100 mL volumetric flask and the volume was made up with distilled water to obtain the cellulose determination solution. The contents of pectin and cellulose were determined using the carbazole method and anthrone method, respectively.

2.8 Assays of PG, β-Gal, PME and Cx activities

The PG activity was determined according to the method reported by Chen et al. (2017). After adding 0.2 mL of crude enzyme solution to 3.8 mL of 0.5% polygalacturonic acid, the mixture was cooled with 5 mL of 3,5-dinitrosalicylic acid (DNS) for 30 min. After cooling, 5 mL of DNS was added to boiling water for 5 min, and then the solution was diluted to 25 mL with 0.5 mol L^{-1} sodium acetate (pH 5.5). The absorbance was measured at 540 nm. The PG activity was expressed in U g^{-1}.

The β-Gal activity was determined according to the method reported by Chen et al. (2017). A mixture of 1 mL of o-nitrobenzene-β-D-galactoside and 0.1 mL of enzyme solution was placed in a water bath at 37 °C for 30 min. Then, 2 mL of 0.2 mol L^{-1} sodium carbonate solution was added, and the absorbance value was measured at 400 nm. The β-Glu activity was expressed as the amount of enzyme required to release 1 μmol nitrophenol (PNP) per minute (μmol g^{-1} min^{-1}).

The PME activity was determined according to the method reported by Ji et al. (2021) with slight modifications. A mixture of 2 mL of 0.5% pectin and 0.15 mL of 0.01% bromothymol blue was diluted to 3 mL with distilled water and kept in a water bath at 37 °C for 15 min. After adding 0.4 mL of enzyme solution, the absorbance of the mixture was measured at 620 nm for 1 min. The PME activity was expressed in U g^{-1}, where U = 0.01 min^{-1}.

The Cx activity was determined according to the method of Chen et al. (2017) with slight modifications. A mixture of 5 g of blueberry tissue and 15 mL of 50 mmol L^{-1} sodium acetate buffer (pH 4.8) was ground homogeneously and then transferred to a 50 mL centrifuge tube. After centrifuging at 10000 r min^{-1} for 15 min at 4 °C, the supernatant was collected and mixed with 2 mL of 10 g L^{-1} sodium carboxymethyl cellulose (CMC) solution. After heating at 50 °C for 30 min in a water bath, the sample was cooled and 2 mL of DNS was added. Then, the mixture was heated in a boiling water bath for 5 min, cooled, and diluted to 100 mL with distilled water. The absorbance was measured at 540 nm. The cellulose activity was expressed as the amount of enzyme required to produce 1 μg of glucose per hour (μg h^{-1} g^{-1}).

2.9 Characterization of cell wall ultrastructure

The fresh blueberry fruits, the control group after 80 d of low-temperature storage, and the MT-treated blueberry fruits were randomly sampled and observed using perspective electron microscopy. The selected blueberry fruits were cut into small pieces of 1 mm^3 with a scalpel and then immediately placed in an electron microscopy fixing solution. After fixing overnight at 4 °C, the samples were rinsed with 0.1 M phosphate buffer solution 3 times, each time for 15 min. Then, the samples were fixed in 1% osmium acid at room temperature for 7 h, dehydrated in ethanol gradient solutions (30%, 50%, 70%, 80%, 95%, and 100%) and acetone (100%, v/v) for 1 h, and embedded in epoxy resin (60 °C, 48 h). The resin block was cut into 60-80 nm ultrathin slices, which were then stained with 2% uranium acetate and 2.6% citric acid solution before imaging using a fluorescence electron microscope.

2.10 Statistical analysis

All experiments were repeated 3 times and the results are expressed as mean ± standard deviation (SD). SPSS Statistics

23 software was used to analyze the data, and Origin 2018 was used to plot the data. Significance was set at $P < 0.05$.

3 Results and discussion

3.1 Effects of MT on decay incidence, respiration rate, firmness, TSS, and TA

Figure 1 shows the appearance of the blueberry fruits after treatment and low-temperature storage for 80 d. In the control group (Figure 1A), the fruits exhibited obvious mold and decay. In contrast, less surface mold was observed on the fruits treated with MT and the degree of fruit decay was relatively low (Figure 1B-1D). After 20 d of low-temperature storage, the decay rate increased as the storage time increased (Figure 2A). During the entire storage period, MT treatment effectively inhibited fruit decay. After 40 d of low-temperature storage, the decay rate of fruits treated with 300 μM MT was significantly lower than those of other groups ($P < 0.05$). At the end of the storage period, the decay rate of the control group was 1.20, 2.30, and 1.50 times higher than those of the 100, 300, and 500 μM MT-treated groups, respectively. In addition, After 60 d of low-temperature storage, the sensory quality score of fruits treated with 300 μM MT was significantly higher than those of other groups ($P < 0.05$) (Figure 2F). Therefore, melatonin can effectively reduce fruit decay and maintain fruit appearance quality.

For both the MT-treated and control groups, the fruit firmness decreased during storage (Figure 2C). The fruit firmness of the MT-treated groups was significantly higher than that of the control group ($P < 0.05$) after low-temperature storage for 20, 40, and 80 d. Over the storage period, the fruit firmness of the control group decreased by 78.00 N, whereas those of the 100, 300, and 500 μM MT groups decreased by 72.00, 52.00, and 70.00 N, respectively. The respiration of blueberry fruits showed a peak after 20 d of storage (Figure 2B), with respiration rates of 65.81, 63.41, 61.06, and 64.73 mg $CO2\ kg^{-1}\ h^{-1}$ for the control and 100, 300, and 500 μM MT-treated groups, respectively. After 80 d of storage, the respiration rates of the fruits treated with MT were significantly lower than that of the control group ($P < 0.05$), but there were no significant differences between fruits treated with 100 and 500 μM MT ($P > 0.05$). In conclusion, MT treatment effectively delayed the decline in fruit firmness, suppressed fruit respiration, and decreased the fruit decay rate, with 300 μM MT treatment giving the best fruit quality.

The TSS content of the blueberry fruits decreased gradually during low-temperature storage (Figure 2D). At the end of the storage period, the TSS content of the fruits treated with 300 μM MT was significantly higher than that of the control group ($P < 0.05$), whereas the TSS contents of the fruits treated with 100 and 300 μM MT were not significantly different from that of the control group ($P > 0.05$). The initial TA content of 4.12% decreased as the storage time increased (Figure 2E). After 40 d of storage, the TA contents of the fruits treated with MT were significantly higher than that of the control group ($P < 0.05$). After 80 d of storage, the TA contents were 3.43%, 3.54%, 3.64%, and 3.58% for the control and 100, 300, and 500 μM MT-treated groups, respectively. Therefore, MT treatment aided in maintaining the contents of TSS and TA during low-temperature storage, and the most significant effect was observed for 300 μM MT treatment.

3.2 Effects of MT on relative membrane permeability, LOX activity, and ascorbic acid content

During the entire low-temperature storage period, the relative membrane permeability and LOX activity of the blueberry fruits

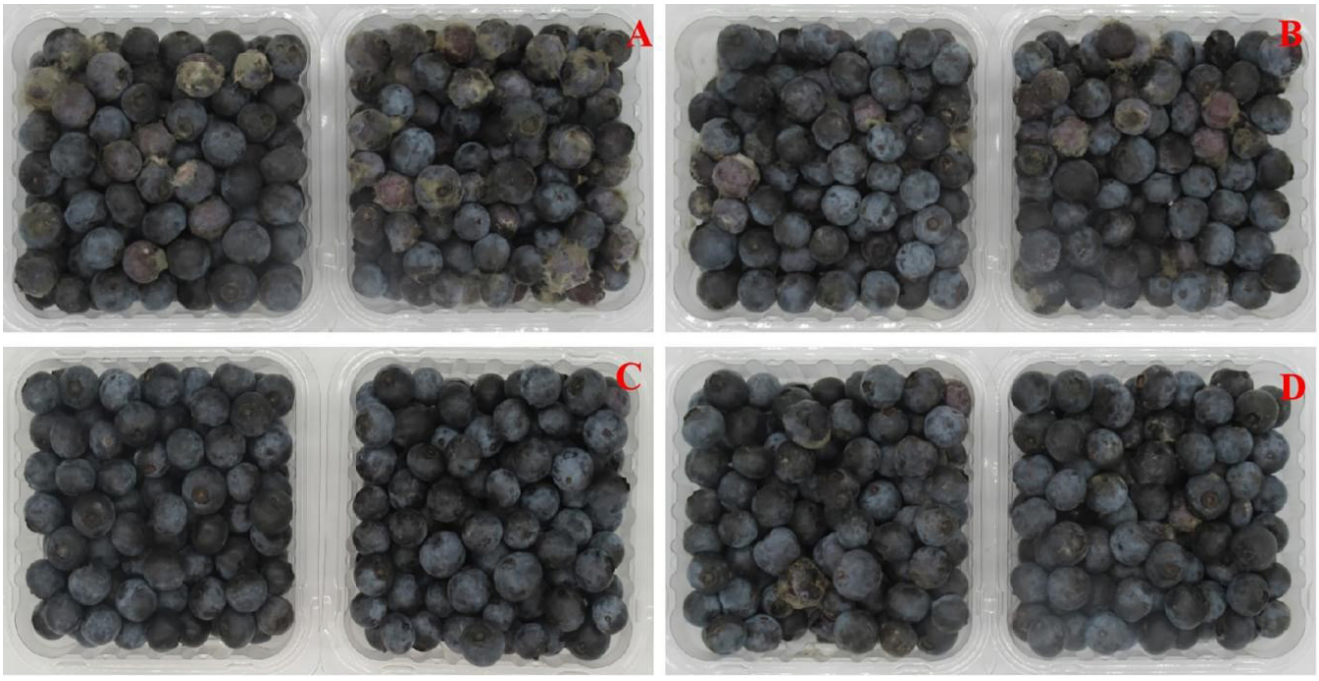

Figure 1. Appearance of blueberry fruits after 80 d of low-temperature storage. A: control; B-D: fruits treated with 100, 300, and 500 μM(μmol L^{-1}) melatonin (MT), respectively.

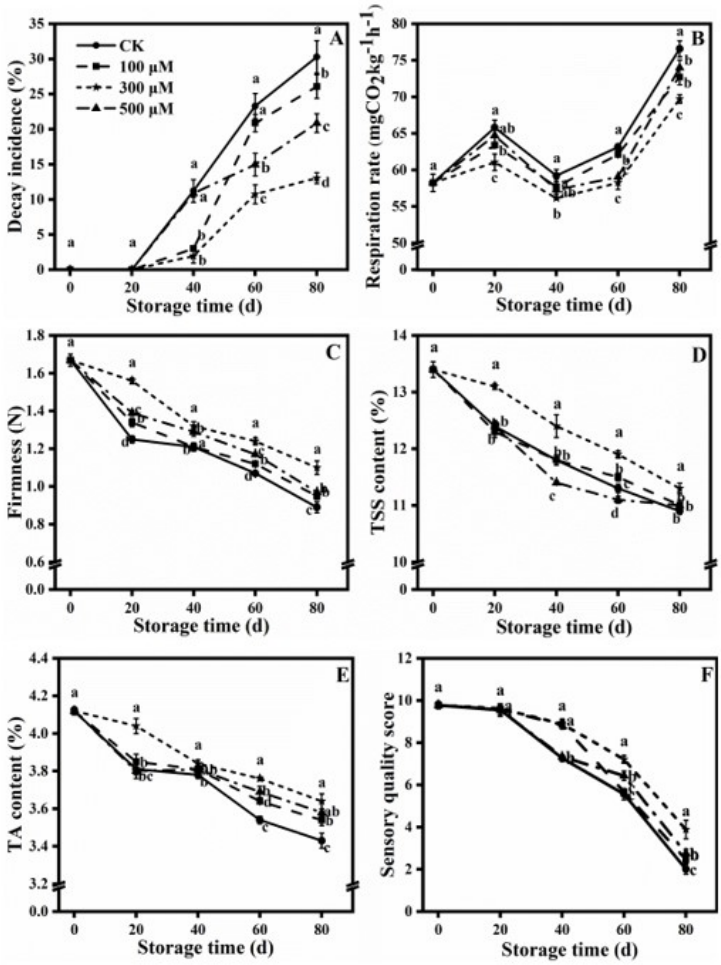

Figure 2. Effects of MT treatment on (A) decay rate, (B) respiration rate, (C) firmness, (D) total soluble solids (TSS) content, (E) titratable acids (TA) content, and (F) sensory quality score. Each data point is the mean of three replicates. Significant differences in the mean values are indicated by different letters ($P < 0.05$).

tended to increase (Figure 3A-3B). The relative membrane permeabilities and LOX activities of the MT-treated fruits were significantly lower than those of the control group ($P < 0.05$). The control and 100, 300, 500 μMMT-treated groups had relative membrane permeabilities of 75.25%, 69.16%, 65.01%, 66.05%, respectively. Furthermore, the LOX activity of the control group was 1.10, 1.20, and 1.10 times higher than those of the fruits treated with 100, 300, and 500 μM MT, respectively. The initial ascorbic acid content of the blueberry fruits was 51.57% (Figure 3C). After 80 d of low-temperature storage, the ascorbic acid content of the control group decreased by 18.89%, whereas those of the fruits treated with 100, 300, and 500 μM MT decreased by 16.64%, 14.07%, and 17.50%, respectively. Therefore, MT treatment may delay membrane lipid peroxidation in blueberry fruits during low-temperature storage.

3.3 Effects of MT on soluble pectin, protopectin, and cellulose contents

Opposite trends were observed for soluble pectin and protopectin in the blueberry fruits during low-temperature storage (Figure 4A-4B). MT treatment effectively inhibited the formation of soluble pectin and the degradation of protopectin. After 60 d of storage, the soluble pectin content increased rapidly, whereas the protopectin content decreased rapidly after 40 d of storage, although the protopectin content of fruits treated with 300 μM MT decreased more slowly. After 20, 40, 60, and 80 d of storage, the soluble pectin contents of the fruits treated with 300 μM MT were 11.00%, 8.00%, 6.00%, and 13.00% lower than those of the control group, respectively, whereas the protopectin contents were 7.00%, 7.00%, 26.00%, and 16.00% higher than those of the control group, respectively. After 80 d of storage, the soluble pectin and protopectin contents of all the fruits treated with MT were significantly higher than those of the control group ($P < 0.05$).

The cellulose content decreased during low-temperature storage (Figure 4C). The cellulose contents of the MT-treated fruits were significantly higher than those of the control group after 20, 40, and 80 d of storage ($P < 0.05$). At the end of the storage period, the cellulose contents of the fruits treated with 100, 300, and 500 μM MT were 1.06, 1.15, and 1.08 times higher than that of the control group. Therefore, MT treatment can

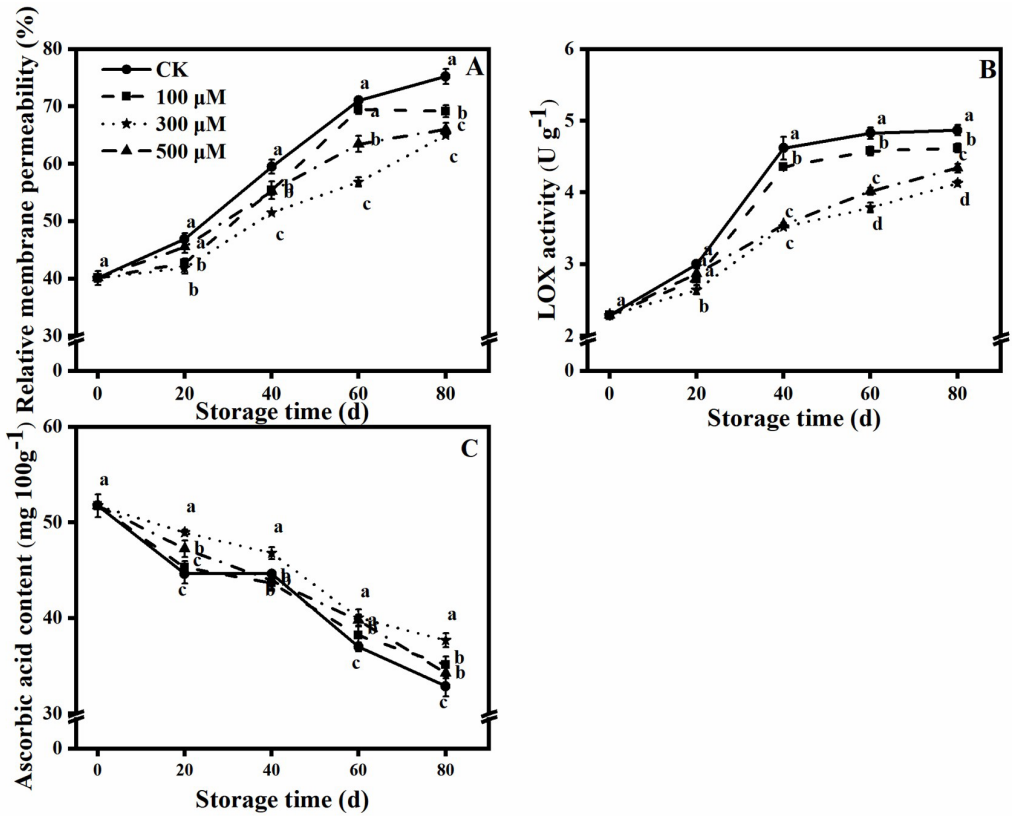

Figure 3. Effects of MT treatment on (A) relative membrane permeability, (B) lipoxygenase (LOX) activity, and (C) ascorbic acid content. Each data point is the mean of three replicates. Significant differences in the mean values are indicated by different letters ($P < 0.05$).

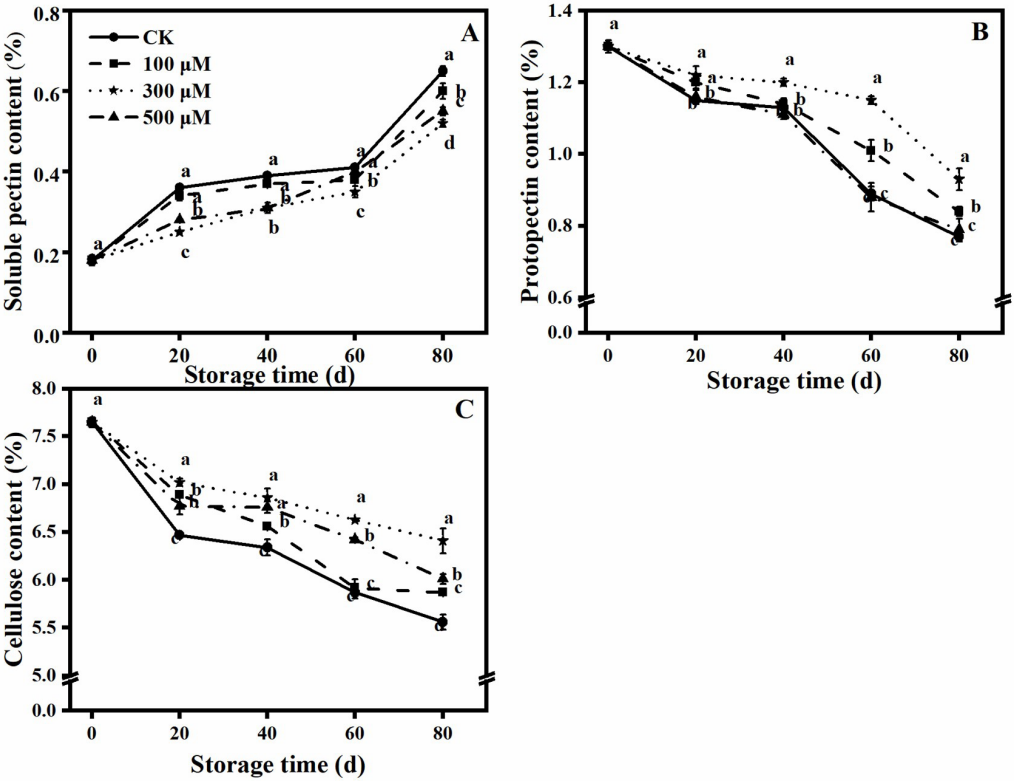

Figure 4. Effect of MT treatment on the contents of (A) soluble pectin, (B) protopectin, and (C) cellulose. Each data point is the mean of three replicates. Significant differences in the mean values are indicated by different letters ($P < 0.05$).

delay the degradation of cell wall substances, thus maintaining the stability of the cell wall structure.

3.4 Effects of MT on PG, β-Gal, PME, and Cx activities

As shown in Figure 5A, the PG activity of the control group increased from 0 to 40 d and then decreased rapidly after 40 d of storage. In contrast, the PG activities of the MT-treated groups increased gradually from 0 to 60 d and the decreased rapidly after 60 d of storage, although a slower rate of decrease was observed for the fruits treated with 300 μM MT. At the end of the storage period, the PG activity of the fruits treated with 300 μM MT was significantly lower than that of the control group ($P < 0.05$), whereas the PG activities of the fruits treated with 100 and 500 μM MT were not significantly different ($P > 0.05$).

The β-Gal activity of the control and MT-treated groups first increased gradually and then decreased during low-temperature storage (Figure 5A). During the entire storage period, the β-Gal activities of the MT-treated fruits were significantly lower than those of the control group ($P < 0.05$), with the lowest β-Gal activities observed for the fruits treated with 300 μM MT. The β-Gal activities of the fruits treated with 300 μM MT were 12.42%, 11.37%, 10.07%, and 11.00% lower than those of the control group after 20, 40, 60, and 80 d of storage, respectively.

After MT treatment, the PME activity of the blueberry fruits increased gradually during low-temperature storage (Figure 5C), whereas the PME activity of the control group first increased and then decreased. The PME activities of the MT-treated fruits were significantly lower than those of the control group after 20, 40, and 60 d of storage ($P < 0.05$). After 80 d of storage, the PME activity of the control group was 1.01, 1.19, and 1.14 times higher than those of the fruits treated with 100, 300, and 500 μM MT, respectively.

The Cx activity increased sharply from 20 to 40 d of storage and then slowly at longer storage times (Figure 5D). During the entire storage period, the Cx activities of the fruits treated with 300 μM MT were significantly lower than those of the other groups ($P < 0.05$). In conclusion, MT treatment effectively inhibited the activity of cell-wall-degrading enzymes and further delayed fruit softening, with 300 μM MT treatment having the best effect.

3.5 Fruit cell wall ultrastructure

Before and after low-temperature storage, the changes the cell wall structure in the blueberry fruit tissue were observed using fluorescence electron microscopy (Figure 6A-6E). Before storage, the cell wall of the fresh blueberry fruit was intact, with the primary wall and intercellular layer showing high electron densities, deep colors, and dense and uniform structures (Figure 6A). For the control group after low-temperature storage for 80 d, the cell wall structure was loose, the cytoplasm was dissolved and significant swelling occurred, and the intercellular layer began to dissolve and disappear (Figure 6B). Unlike the control group, the cell walls of the MT-treated fruits were relatively intact after

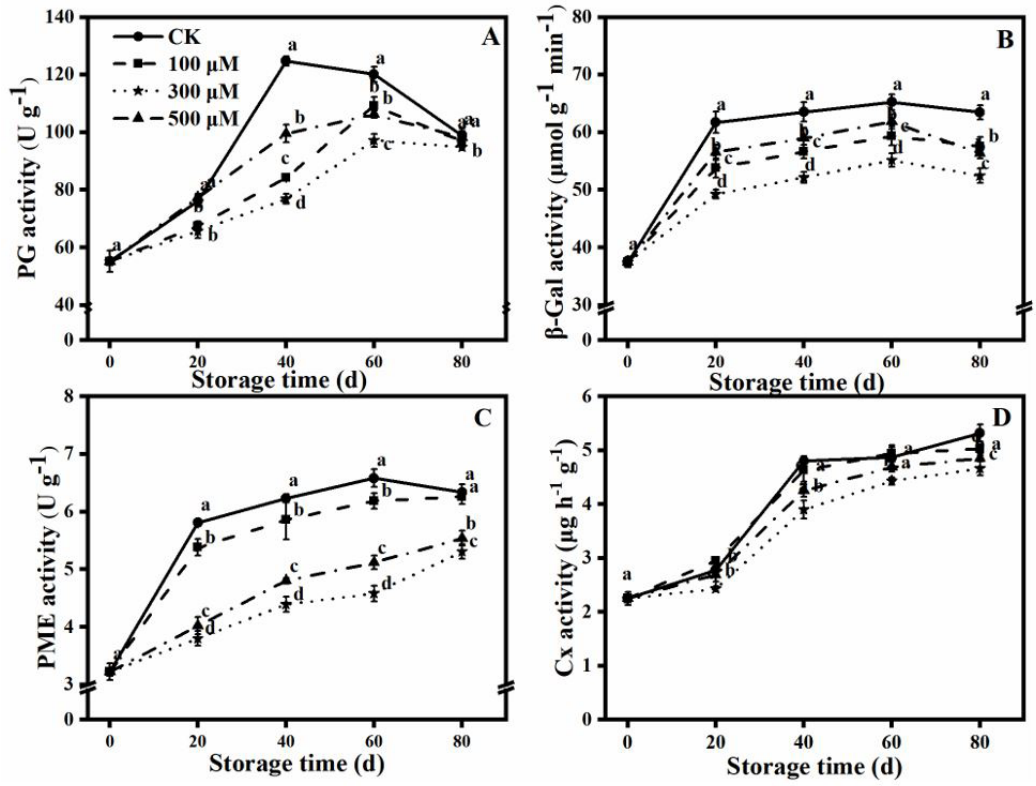

Figure 5. Effects of MT treatment on the activities of (A) polygalacturonase (PG), (B) β-glucosidase (β-Gal), (C) pectin methylesterase (PME), and (D) cellulase (Cx). Each data point is the mean of three replicates. Significant differences in the mean values are indicated by different letters ($P < 0.05$).

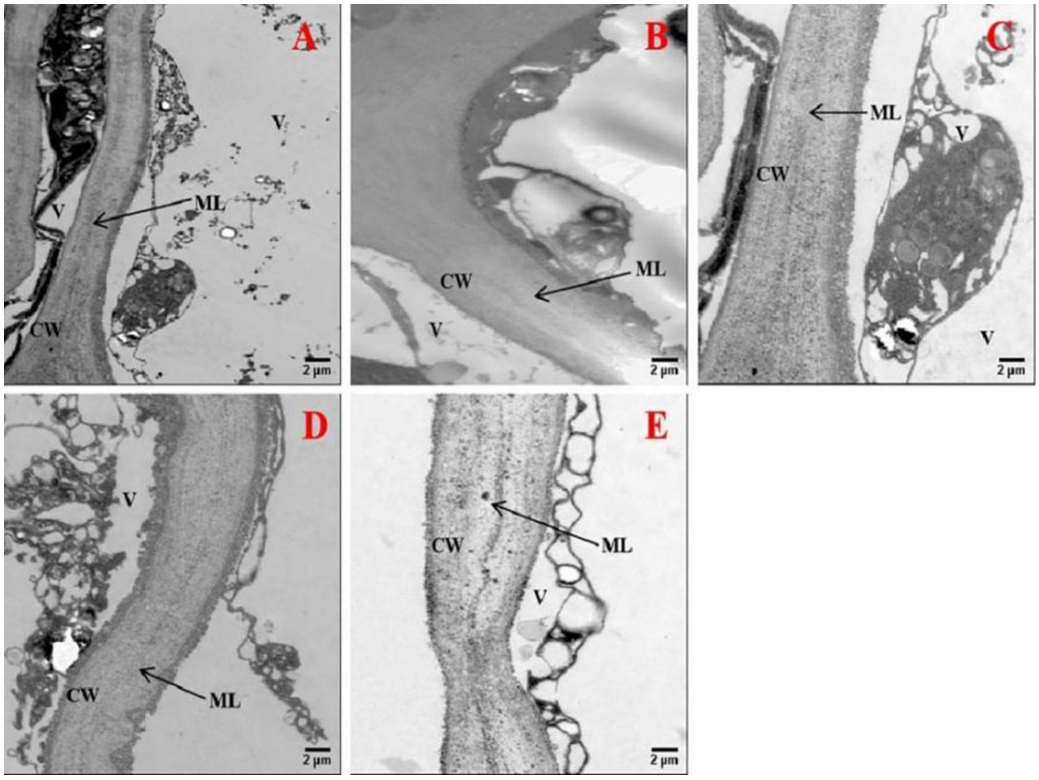

Figure 6. Ultrastructural observations of blueberry cell walls by perspective electron microscopy. (A): fresh blueberry fruit; (B): control group after low-temperature storage for 80 d; and (C-E): blueberries treated with 100, 300, and 500 μM MT, respectively, after low-temperature storage for 80 d. CW: cell wall; ML: medium adhesive layer; V: vacuole.

storage for 80 d (Figure 6C-6E). In addition, the cells of the fruits treated with 100 and 300 μM MT were relatively rich in insoluble substances (Figure 6C-6D). The cell wall of the fruit treated with 300 μM MT was denser and darker in color than that of the fruit treated with 100 μM MT. However, part of the cell wall was dissolved, indicating that the fruit had entered the senescence stage. Overall, the integrity of the cell walls in blueberry fruits during low-temperature storage was best maintained following treatment with 300 μM MT.

4 Discussion

The postharvest senescence of blueberries is usually related to a decrease in firmness, increased respiration and decay rates, and decreases in TSS and TA contents (Shang et al., 2021; Ge et al., 2018). In this study, different concentrations of exogenous MT were used to treat blueberry fruits. The results showed that MT treatment effectively inhibited the growth of mold on the fruit surface (Figure 1), delayed the decreases in fruit firmness, TSS content, and TA content, and slowed the respiratory and decay rates during low-temperature storage, Maintain fruit appearance quality (Figure 2). In particular, 300 μM MT treatment had the most significant effect. Thus, MT treatment can effectively delay the senescence and maintain the postharvest quality of blueberry fruits. These results are similar to those of previous studies, which reported that exogenous MT treatment can reduce the decay rate, inhibit ROS accumulation, and delay the senescence of peach fruits (Wang et al., 2020a), delay the ripening and softening of mangos (Liu et al., 2020), and maintain the postharvest quality of sweet cherries (Wang et al., 2019). However, other studies have found that MT treatment significantly promotes the postharvest ripening and senescence of tomato fruits (Sun et al., 2015) as well as triggering GAMYB gene expression and promoting ABA accumulation to accelerate strawberry ripening (Wang et al., 2019). The differences between these findings and the results of the current study may be related to the fruit species, harvest maturity, treatment time, and treatment methods.

During fruit senescence or adversity, active oxygen radicals accumulate in large quantities, and the metabolic balance between free radicals is destroyed, which leads to membrane lipid peroxidation. Membrane lipid peroxidation is usually related to increases in relative membrane permeability and LOX activity (Wang et al., 2020a). Some endogenous antioxidants, such as ascorbic acid and anthocyanins, can scavenge ROS and enhance the antioxidant capacity of fruits (Zhou et al., 2020). Various studies have shown that exogenous MT treatment can effectively reduce the browning of litchi fruit (Wang et al., 2020a) and chilling injury in green peppers during storage (Kong et al., 2020). These effects may be due to MT inhibiting the increase in LOX activity and the expression of related enzyme genes, thus regulating membrane lipid metabolism. For blueberry fruits, exogenous MT treatment was found to effectively delay the degradation of ascorbic acid, inhibit the increase in LOX activity, and maintain a lower level of pulp membrane permeability (Figure 3). Therefore, MT treatment can reduce membrane lipid peroxidation in blueberry fruits and reduce the degree of damage to the fruit cell membrane caused by ROS.

Fruit softening is closely related to the degradation of pectin, cellulose, and hemicellulose in cell walls and the activity of cell-wall-degrading enzymes (Chen et al., 2017). Pectin, which is the main component of the gum layer and primary wall in plant cells, is easily degraded by PME and PG during fruit softening, thus promoting the accumulation of soluble pectin and reducing fruit hardness (Shi et al., 2019). Cellulose, a chain-like macromolecular polysaccharide linked by D-glucose molecules through β-1,4-glycosidic bonds, exists in cell walls in the form of microfibrils. Cx is a multicomponent enzyme that hydrolyzes cellulose into β-glucose (Chen et al., 2017). β-Gal is involved in the catalytic removal of galactose residues in pectin and xylan molecules during fruit softening (Wei et al., 2010). Studies on Jujuba fruit treated with MT (Tang et al., 2020) and blueberries treated with γ-radiation (Wang et al., 2017a) have revealed that the activities of cell-wall-degrading enzymes (PG, PME, β-Gal, and Cx) increase during the ripening and softening of fruits, resulting in the degradation of cell wall substances (pectin, cellulose, and hemicellulose). In addition, Zhai et al. (2018) found that MT treatment can delay the softening and senescence of pear fruits by inhibiting the transcription of PcPG and PcCel genes, which are related to cell wall relaxation and degradation. The results of the current study showed that MT treatment effectively inhibited the degradation of cell wall substances in blueberry fruits during low-temperature storage, as demonstrated by the slow decrease in the contents of protopectin and cellulose with a concomitant slow increase in the content of soluble pectin (Figure 4). These phenomena may be due to the MT-induced regulation of gene expression for cell-wall-degrading enzymes (PG, PME, β-Gal, and Cx), which would inhibit the activities of these enzymes (Figure 5). Therefore, molecular studies are needed to clarify the effect of MT on the degradation mechanism of blueberry cell walls.

Ultrastructural changes in the cell walls of blueberry fruits before and after low-temperature storage were observed using perspective electron microscopy (Figure 6). Before storage, the primary wall and middle gum layer of the blueberry fruit had dense structures. At the end of the storage period, MT treatment delayed the degradation of the fruit cell wall, thus maintain relatively high contents of insoluble substances within the cell. Because the fruit cell wall was relatively intact, the softening of the blueberry fruits during low-temperature storage was further delayed. This phenomenon was positively correlated with the delayed decline in firmness observed for MT-treated blueberry fruits (Figure 2C). This processing helps to maintain high hardness, reduce fruit decay rate and respiratory intensity, delay fruit softening, and maintain good nutritional quality and appearance quality (Figure 7). Similar results have been observed for blueberries treated with methyl jasmonate (Wang et al., 2021) and mangos treated with MT (Liu et al., 2020).

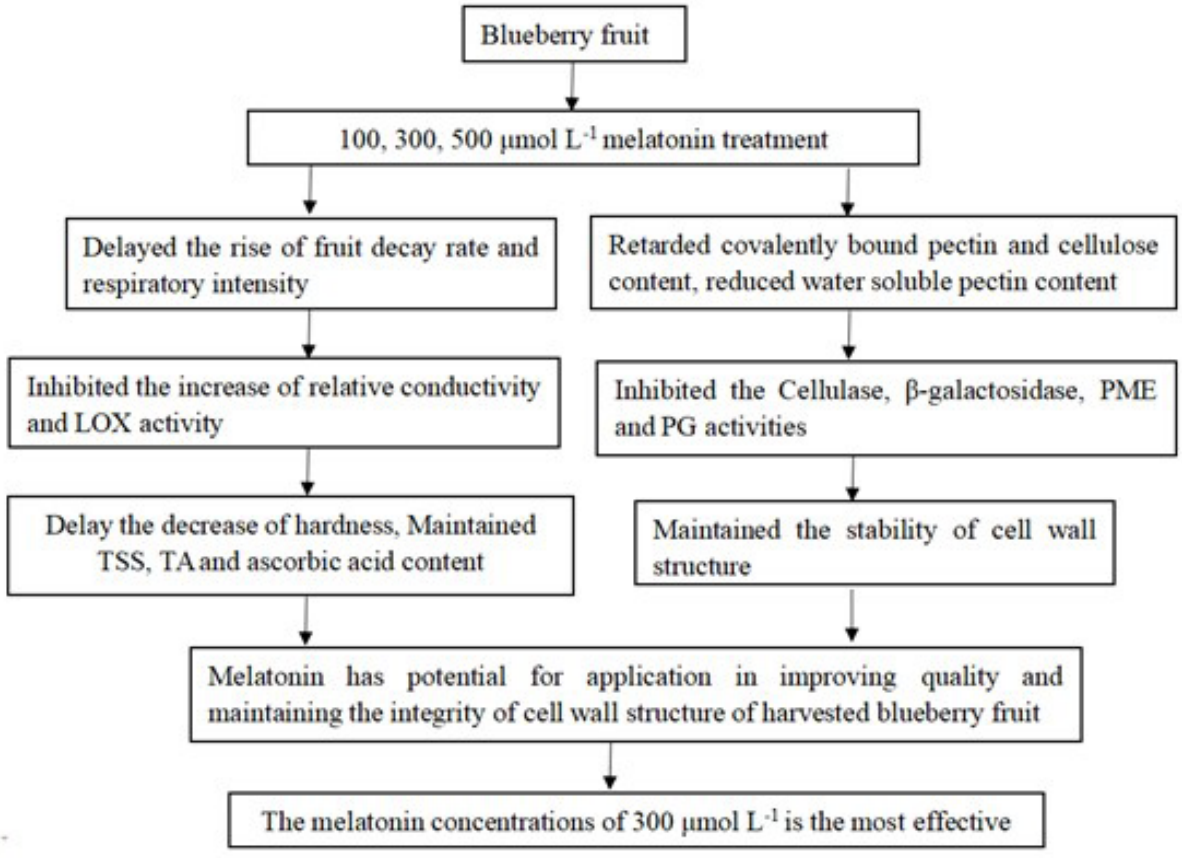

Figure 7. The possible mechanism of melatonin treatment delaying blueberry fruit softening and improving fruit quality is through regulating cell wall metabolism.

5 Conclusions

Treatment with MT at various concentrations effectively delayed the decrease in fruit firmness, decreased the respiration and decay rates, maintained the contents of TSS, TA, ascorbic acid, and fruit appearance quality, inhibited increases in the relative membrane permeability and LOX activity, and decreased the membrane lipid peroxidation of blueberries during low-temperature storage. The best effects were observed for 300 μM MT treatment. In addition, MT also retarded the degradation of cell wall substances by inhibiting the activities of cell-wall-degrading enzymes (PG, PME, β-Gal, and Cx). MT treatment also delayed the destruction of cell wall ultrastructure of blueberry fruit during low-temperature storage. These results demonstrate that MT treatment is an effective technique for maintaining blueberry fruit quality as well as delaying membrane lipid oxidation, fruit softening, and senescence after harvest. However, further research is required to clarify the molecular mechanism by which MT treatment inhibits blueberry fruit softening.

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