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Postharvest Exogenous Melatonin Treatment of Table Grape Berry Enhances Quality and Maintains Bioactive Compounds during Refrigerated Storage

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Abstract: Table grape berries are classified as a perishable crop that deteriorates quickly after harvest. The application of melatonin after harvest was found to be effective for retarding senescence and slowing ripening. In the current study, we tested the influence of two melatonin concentrations (50 and 100 μmol) as a postharvest application on quality, bioactive compounds, and enzyme activities of grape berries cv “Crimson” stored at 0 ± 1 °C and 90% relative humidity (RH) for 35 days. Our results indicated that melatonin application extends the shelf-life of berries by reducing weight loss and maintaining total soluble solids (TSS), titratable acidity (TA), berry adherence strength, and firmness. Melatonin treatment also reduced pectin methyl esterase (PME) and polygalactouranase (PG) enzyme activities compared to the control. Moreover, $\text{O}_2^{\bullet-}$ and H_2O_2 rates in berries were reduced by high melatonin concentration. Moreover, peroxidase (POD) and catalase (CAT) enzyme activities were increased by melatonin application. Our findings suggested using melatonin postharvest to increase shelf life and maintain quality attributes during refrigerated storage, which could be advantageous on a large scale.

Keywords: grapes; melatonin; postharvest; antioxidant; quality

1. Introduction

Table grapes (*Vitis vinifera* L.) belong to the *Vitaceae* family that cultivated globally due to their high nutritional characteristics [1]. Grape berries are high in vitamins, fiber, antioxidants, and folic acid, which help to reduce the risk of some human diseases such as blood cholesterol and hypertension [2]. The table seedless grape (Crimson) was developed by David Ramming and Ron Tarailo at the University of Fresno, CA [3,4]. Crimson berries are slightly elongated with light red berries and the color of skin varies from pink to dark pink-red. The table grape is classified as a non-climacteric fruit. However, table grape is a perishable fruit and suffers from water loss after harvest. Stem drying and browning, shatter of berries, wilting, and shrivelling causes a decrease in quality after harvest.

The senescence of fruits is an unavoidable natural process that causes several physiological and biochemical changes. A decline in color intensity, desired flavor, nutrition

levels, and shortened shelf life occur during the senescence stage [5]. Reducing the senescence processes after harvested considers the main challenge for increasing shelf-life and maintaining nutrient levels. The consumption of chemical composition by respiration, compounds oxidation, and cell wall softening are the main reasons for fruit decay after harvest [6]. The quality and shelf-life of fruits depends on their own nutritional value and exogenous factors such as temperature, humidity, and air composition [7].

Melatonin (N-acetyl-5-methoxytryptamine, MT) is an important hormone connected to many biological activities inside plant cells such as antioxidant mechanisms [8]. Melatonin is considered to be safe as reported by FDA (<https://www.fda.gov>, accessed on 1 September 2022). Additionally, it has been discovered that exogenous melatonin can help plants adapt to a variety of abiotic stresses, including drought, salt, and heavy metals [9]. Melatonin is formed in horticultural crops including grapes and plays a prominent role in crop ripening [10,11]. It has been well known that exogenous melatonin application scavenges reactive oxygen species (ROS) from fruits by enhancing the activity of antioxidant enzymes and non-enzymatic antioxidants [12–14]. In addition, exogenous melatonin treatment delayed fruit senescence [15], fruit softening [16], weight loss [17], decay rates [18], and respiration rate [19]. Therefore, the purpose of the current research was to improve the shelf life and quality of grapes by exogenous melatonin application.

2. Materials and Methods

2.1. Experiment Design and Treatments

Crimson seedless grape fruits were purchased from private farm at El Qattah, Giza Governorate, Egypt. Clusters were harvested at a ripe stage (full berry size and 19% TSS) in mid-September free from mechanical damage, insect damage and any outer decay. Clusters were randomly divided into three groups of similar color, size and form, then clusters were immersed in M0 (control, distilled water), M50 μmol ($50 \mu\text{mol L}^{-1}$ melatonin) or M100 μmol ($100 \mu\text{mol L}^{-1}$ melatonin) for 30 min at $22 \text{ }^\circ\text{C}$ (Figure 1). The use of previous concentrations depends on previous study [17]. Each cluster was left until its surfaces dried at room temperature at $25 \pm 5 \text{ }^\circ\text{C}$, then packed into a punnet (polyethylene plastic package dimensions of $21 \text{ cm} \times 14 \text{ cm} \times 8 \text{ cm}$) of $500 \pm 30 \text{ g}$. Each group (treatment) included 5 replicates, and each replicate contained 5 punnets (25 punnets per treatment). All punnets were stored at $0 \pm 1 \text{ }^\circ\text{C}$ and 90% RH for 35 days. For every 7 days of storage duration, punnets were weighted to calculate % weight loss (storage times were 0, 7, 14, 21, 28, and 35 days). Approximately 10 g of samples were randomly taken from punnets to measure the chemical compounds levels and enzymes activities.

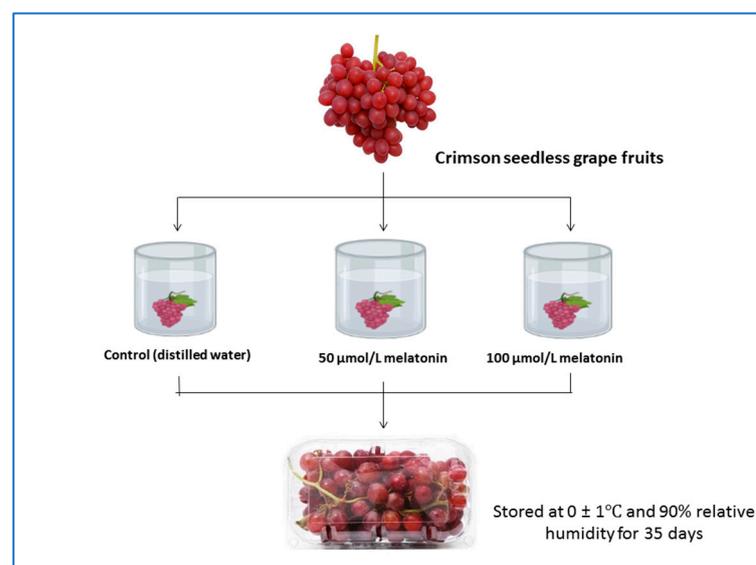


Figure 1. Treatments schema and the concentrations of melatonin.

2.2. Fruit Quality

To determine total soluble solids (TSS), a hand refractometer model HR-110 was used and the results were presented as a percentage (%) of the juice. The titratable acidity (TA) (%) was measured by titrated 10 mL of the juice to pH 8 using sodium hydroxide (0.1 N) and phenolphthalein (1%) according to AOAC [20]. TA was calculated as mg of tartaric acid per 100 mL of juice. TSS/ acid ratio was determined by divided TSS values on TA values.

To measure firmness, the fruit texture analyzer model “GS-15, serial No. FTA2, UP Umweltanalytische Produkte GmbH” was used and expressed as Newton. According to Abd Elwahab et al. [21], adherence strength (g) was measured by using a scale and force meter (Shatilons instrument). The relative membrane permeability % (RMP) was measured as described previously by Ashraf et al. [22]. In brief, 0.5 g of samples were cut and mixed with 20 mL of distilled water in test tubes. Then, tubes were vortexed for 5 s and the solution was measured for initial electrical conductivity (EC₀). After that, the tubes were kept at 4 °C for 24 h and assayed for EC₁. To determine EC₂, the samples were autoclaved at 120 °C for 20 min. The results of RMP (%) was calculated by the following formula:

$$\text{Relative permeability (\%)} = ((\text{EC}_1 - \text{EC}_0) / (\text{EC}_2 - \text{EC}_0)) \times 100 \quad (1)$$

2.3. Enzyme Activity

1.0 g of berries peduncles were taken and extracted as described by Lee and Macmillan [23] to measure the enzyme activity of pectin methyl esterase (PME) and polygalactouranase (PG) enzymes. The reaction mixture for the two enzymes consisted of 2.0 mL pectin solution, 0.5 mL bromothmol blue + 1.5 mL water. The pH was adjusted to 7.5. The initial absorbance at 620 nm for the PME enzyme and 445 nm for PG enzyme of the mixture was measured vs. water. After that, 20 µL of PME or PG enzyme substrates were added to initiate the reaction. The absorbance at 620 and 445 nm was recorded. The initial reaction rate was linear for 3 minutes. The enzyme activity was recorded as described previously [24].

To determine the antioxidant enzyme activity, 0.2 g of the samples were homogenized in precooled 50 mM phosphate buffer (pH 7.8) (1.6 mL) and centrifuged undercooling (4 °C) at 12,000 × g for 20 min and supernatant was collected. The method described by Tao et al. [25] was used to determine the activity of peroxidase (POD, EC 1.11.1.7). To measure the catalase activity (CAT, EC 1.11.1.6), the method of Dhindsa et al. [26] was used.

2.4. Determination of O₂^{•−} Production Rate

The production rate of O₂^{•−} was measured as described previously by He et al. [27] with minor modification. Briefly, 0.2 g of samples were macerated in 2 mL of 50 mM phosphate buffer (pH 7.8) and then centrifuged under cooling (4 °C) at 12,000 × g for 20 min. After that, 0.5 mL of phosphate buffer and 0.1 mL of 10 mM hydroxylamine hydrochloride were added to 0.5 mL of supernatant and kept at room temperature for 30 min. After incubation, 1 mL of 7 mM naphthylamine and 1 mL of 17 mM sulfanilamide were incorporated into the mixture solution and incubated for an additional 30 min. The absorbance of the samples was measured at 530 nm.

2.5. H₂O₂ Measurement

The methods of Velikova et al. [28] were used to measure the hydrogen peroxide (H₂O₂) concentration with minor modifications. 0.5 g of samples were homogenized in 3 mL of tri-chloroacetic acid (TCA) (1%) (*w/v*). Then centrifuged (10,000 rpm) under cooling at 4 °C for 10 min. Then 0.75 mL of the supernatant was mixed with 0.75 mL of 10 mM K-phosphate buffer (pH 7.0) plus 1.5 mL of KI (1M). By comparing a sample's absorbance at 390 nm to a reference calibration curve, the concentration of H₂O₂ was calculate. A standard curve plotted in the range of 0 to 15 nmol mL^{−1} was used to calculate the concentration of H₂O₂.

2.6. Statistical Analysis

The data was submitted for statistical variance analysis. To compare means, the Tukey test was used. To demonstrate the differences between treatments, a one-way ANOVA test was used. SAS's analysis of variance package was used to statistically analyse the data.

3. Results

Weight Loss, TSS, Titratable Acidity, and TSS: Acids Ratio

As expected, weight loss of grape berries increased with increasing storage periods in the control and treated berries (Figure 1A). However, compared to the control, the grape berry treated with 50 or 100 μmol melatonin L^{-1} was effective in reducing weight loss after 7 days till the end of the refrigerated storage (Figure 2A). Additionally, the difference between the two concentrations was not significant. TSS increased in all treatments until 21 days of storage and then decreased in the control treatment while melatonin treatments were constant (Figure 2B). There were no significant differences between treatments until 21 days of storage. After 28 and at the end of storage time, both melatonin concentrations showed higher TSS than the control without any difference between them.

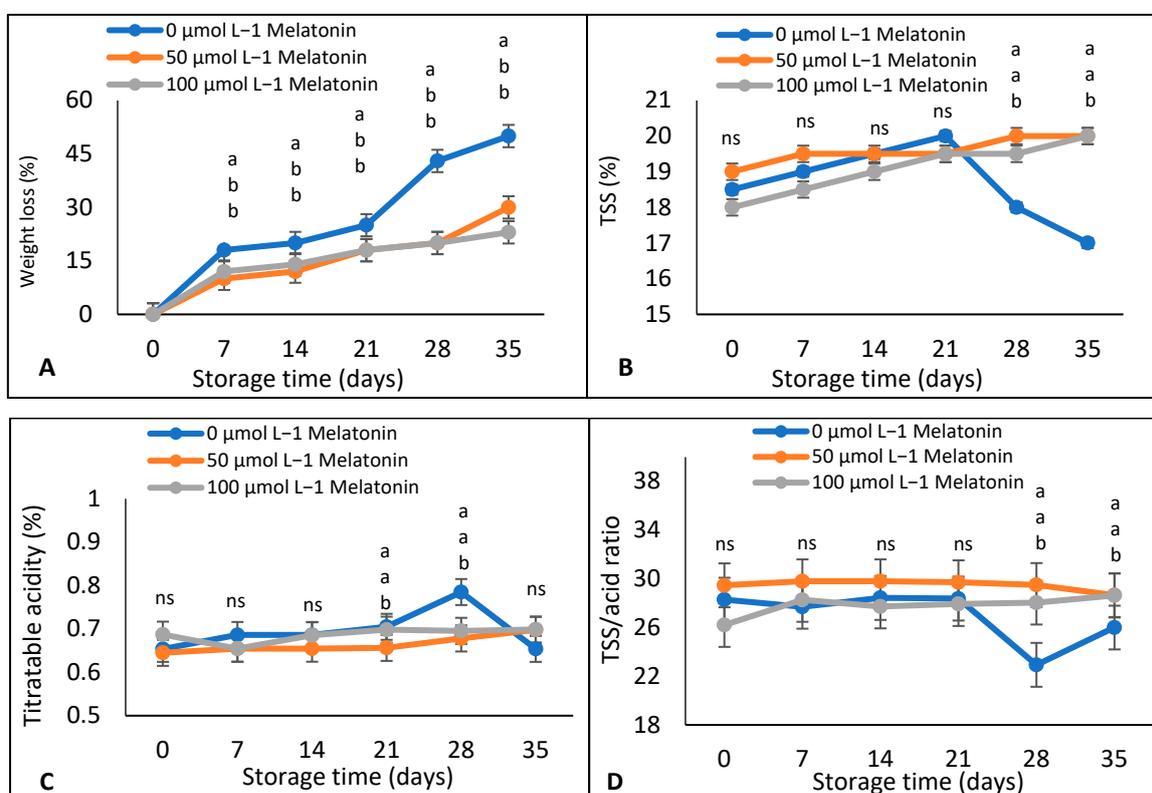


Figure 2. Effect of three concentrations of melatonin on (A) weight loss, (B) TSS, (C) titratable acidity, and (D) TSS: acids ratio of grape berries stored at 0 °C for 35 days. Values are means \pm SE from five replicates ($n = 5$). The same letter means no significant differences between the values ($p < 0.05$) according to the Tukey test.

There were no changes in TA content in grape berries until 21 days of storage (Figure 2C). However, an increase after 28 days was observed and then disappeared. There was no statistical difference between treatments until 14 days of storage. After 28 days, the lower content of TA was observed in 50 μmol melatonin L^{-1} compared to 100 μmol melatonin L^{-1} and the control. At the end of storage, no differences were observed between treatments. Regarding TSS/acid ratio, no difference was observed among all treatments until 21 days of storage (Figure 2D). After 28 and 35 days of storage, the control treatment showed the lowest ratio compared to both melatonin concentrations.

The results in Figure 3A indicated that firmness was decreased with increasing storage time. There was no significant difference observed between all treatments until 21 days of storage. In the period from 28 to 35 days of storage, both melatonin concentrations showed higher TSS content than the control. Berry adherence strength (BAS) decreased in parallel with increasing storage time (Figure 3B). The differences between treatments were not significant until 7 days of storage. However, after 14 days of storage until the end, the decreasing rate was higher in the control treatment than in either of the melatonin concentrations. Relative membrane permeability (RMP) was increased with increasing storage periods (Figure 3C). There was no significant difference recorded between treatments until 14 days of storage. However, after 21 days until the end of storage, the control treatment showed a higher RMP than both melatonin concentrations.

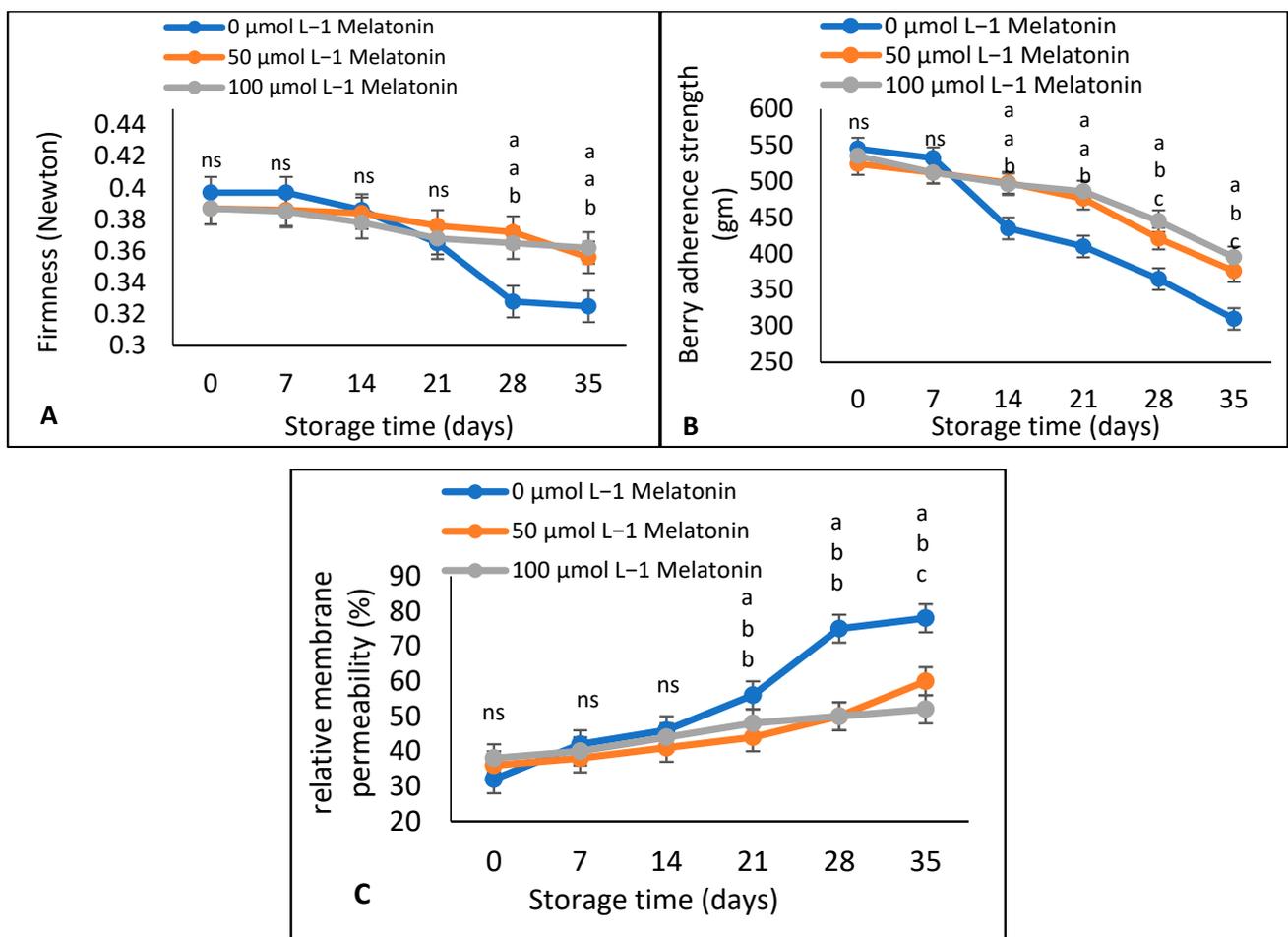


Figure 3. Effect of three concentrations of melatonin on (A) firmness, (B) berry adherence strength, and (C) relative membrane stability of grape berry stored at 0 °C for 35 days. Values are means \pm SE from five replicates ($n = 5$). Same letter means no significant differences between the values ($p < 0.05$) according to the Tukey test.

After 35 days of storage, PG and PME enzyme activities were higher in the control treatment than 50 and 100 $\mu\text{mol melatonin L}^{-1}$, respectively (Figure 4A,B). However, POD enzyme and CAT enzyme activities were increased with increasing melatonin concentration (Figure 4C,D). Both $\text{O}_2^{\bullet-}$ production rate and H_2O_2 were reduced as melatonin levels increased (Figure 4E,F).

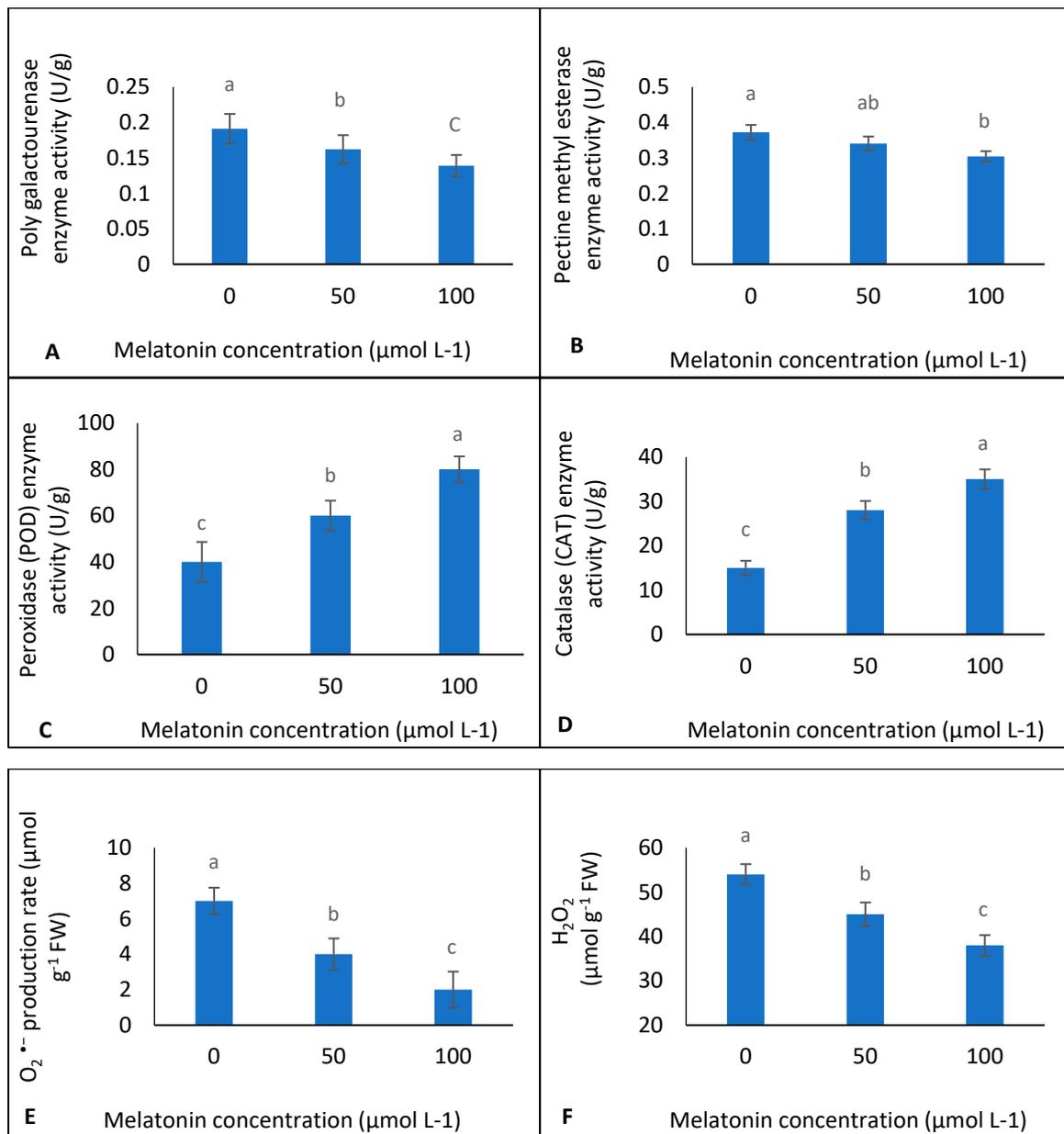


Figure 4. Effect of three concentrations of melatonin on (A) PG enzyme activity, (B) PME enzyme activity, (C) POD activity, (D) CAT activity, (E) O₂•⁻ production rate, and (F) H₂O₂ of grape berry stored at 0 °C after 35 days of storage. Values are means ± SE from five replicates (*n* = 5). Same letter means no significant differences between the values (*p* < 0.05) according to the Tukey test.

4. Discussion

Weight loss is the main challenge after harvest because it decreases the appearance and quality of horticultural crops. It has been reported that transpiration and respiration of fresh fruits and vegetables are the main reasons for water loss [29,30]. Our results and previous work indicate that melatonin application retards the loss of water during cold storage [31]. Moreover, Wang et al. [32] found that melatonin postharvest application decreases the weight loss of sweet cherry fruits. In this study, no significant difference was observed in TSS by melatonin treatments until 21 days of storage. However, at the end of storage periods, both melatonin concentrations increased TSS content compared to the control. (Figure 2B). Similar increases in TSS were also observed by exogenous melatonin application in grape berries [18,33], tomatoes [34], strawberries [35], and sweet cherries [15]; this increase in TSS could be due to melatonin reducing respiration and reducing the loss

of soluble acids [33]. Melatonin treatment decreased the loss of TA in berries after 35 days from storage (Figure 2C). Previously, Fan et al. [36] recorded higher TA levels in guava fruits treated with exogenous melatonin compared with control. Moreover, previous works reported that exogenous melatonin application reduces the loss of TA in grape berries and sweet cherries during cold storage [15,37]. The reduction of TA loss by melatonin application could be due to its role in senescence reduction [18].

Grape berry firmness is the most important quality parameter which affects the storage period. The loss of firmness in fruits and vegetables was found to be linked with the degradation and solubilization of plant cell walls by numerous enzymes such as PG and PME that response for cell wall degradation [38]. In this study, melatonin application delayed the loss of firmness (Figure 3A). In accordance with this result, a previous report showed that application of melatonin on guava fruits had higher firmness level than untreated fruits [36]. Additionally, other study indicated that melatonin application conserved the firmness of sweet cherry fruits [15]. The results in Figure 4A,B supported the hypothesis that melatonin application decreased the softening of grape berries by reducing PG and PME enzymes activities. Moreover, other work found that exogenous melatonin application decreased the activities of PG and PME enzymes in mango fruits, resulting in less softness [16]. The previous result could be related to the role of melatonin in reducing ethylene production [38]. Our results in Figure 3C showed that RMP was decreased by melatonin treatment. A possible explanation for this result could be due to the role of melatonin in mitigating the peroxidation of plant cell membrane lipids and maintaining the integrity of the cell membrane [15].

The result of this study in Figure 4C corroborates the finding of Li et al. [39], who indicated that exogenous melatonin treatment increased the activity of POD in cherry tomato fruit. POD and CAT activities were also increased in broccoli florets by exogenous melatonin application [40]. In a previous study, the application of exogenous melatonin enhanced the POD and SOD activities in sweet cherries [15]. Increasing POD and CAT activities were found to be effective for reducing the harmful effects of $O_2^{\bullet-}$ and H_2O_2 rates that decreased in our study (Figure 4E,F). It has been well known that the senescence of fruits is mainly due to membrane lipid oxidation, reactive oxygen metabolism [41], and oxidation of nucleic acids and proteins [12]. Our results in Figure 4E,F supported the hypothesis that melatonin application reduced $O_2^{\bullet-}$ and H_2O_2 rates in the berries, which resulted in lower ripening and senescence during storage [42]; these results are in agreement with some other reports that found that melatonin application significantly lowers the H_2O_2 rate in stored peach and cassava [43,44]. Moreover, Wang et al. [15] observed a decrease in $O_2^{\bullet-}$ and H_2O_2 rates in sweet cherries by melatonin postharvest application. More studies on the effect of melatonin on bioactive compounds such as chlorophylls, pigments, and vitamins should be performed [45].

5. Conclusions

The current work emphasises the hypothesis that melatonin application after harvest markedly increases the shelf-life and storage ability of grape berries. The enhancement of grape shelf-life in response to melatonin might be linked with the activities and synthesis of cell-wall degradation enzymes including PG and PME (Figure 5). In addition, melatonin treatment enhanced POD and CAT activities, which mitigate $O_2^{\bullet-}$ and H_2O_2 rates. In conclusion, we suggest that melatonin treatment could be an effective application for preserving grape berries quality during cold storage. It is recommended to use a concentration of 100 μ mol melatonin as it increases the rate of adhesion of grape berries and thus reduces the separation of berries from the cluster, which is one of the qualities required during the export of grapes.

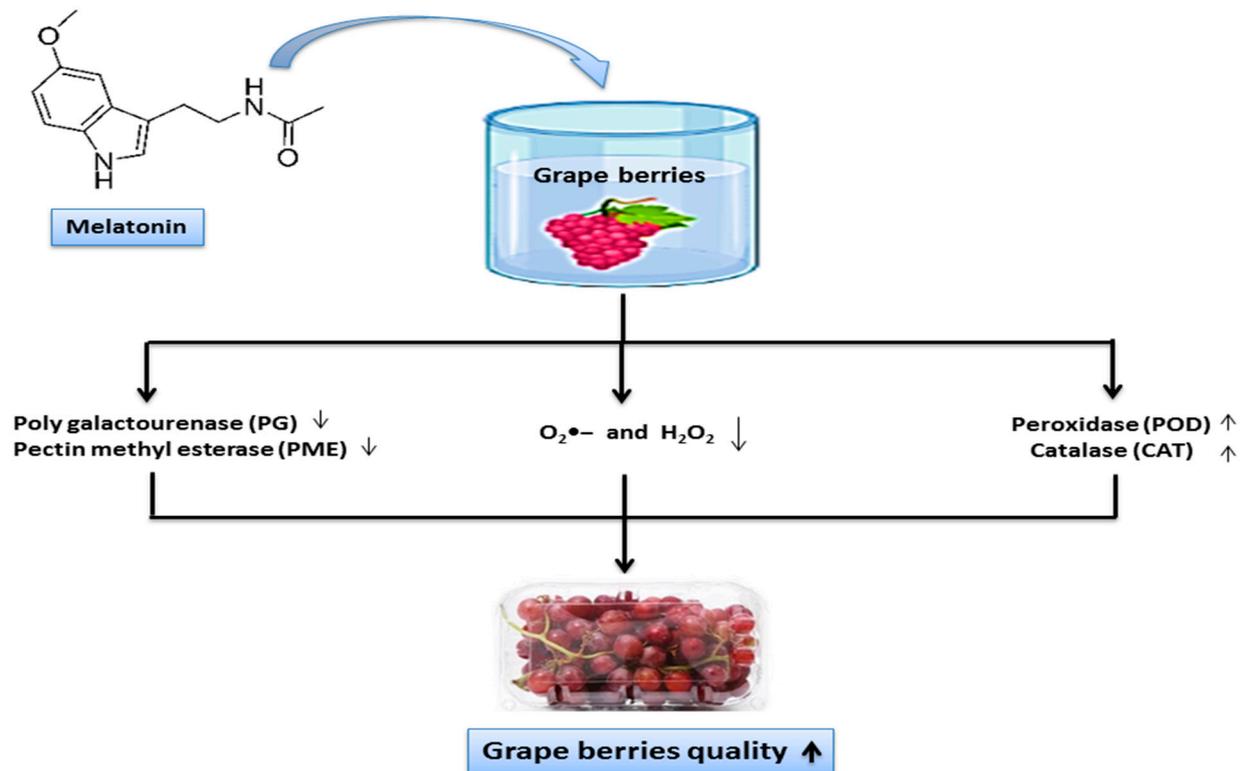


Figure 5. Simplified model for the suggested effect of melatonin on the shelf-life and storage ability of grape berries, increasing activities and synthesis of cell-wall degradation enzymes such as PG and PME, increasing activities of antioxidant enzymes (POD and CAT), which mitigate $O_2^{\bullet-}$ and H_2O_2 rates.

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