



## ROLE OF MELATONIN IN MAINTAINING THE FRUIT QUALITY OF EARLY GRAND PEACH DURING STORAGE

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### ABSTRACT

Postharvest deterioration significantly limits the storage life and market quality of peach fruit, leading to considerable economic losses. This study investigated the effectiveness of exogenous melatonin application in maintaining the postharvest quality and nutritional attributes of 'Early Grand' peach fruit during cold storage. Freshly harvested peaches were treated with different concentrations of melatonin (Control, 50, 100, and 150  $\mu$ M) and stored at low temperature for up to 28 days. The results demonstrated that storage duration was a primary factor driving quality changes, leading to a significant decline in titratable acidity and anthocyanin content, alongside increases in total soluble solids (TSS), pH, sugar-acid ratio, and reducing sugars. Exogenous melatonin treatment effectively modulated these postharvest physiological processes. Treatment with 150  $\mu$ M melatonin was particularly effective, significantly retaining higher titratable acidity, maintaining lower juice pH, and slowing the rate of TSS and reducing sugar accumulation compared to control fruits, indicating a suppression of respiration and a delay in ripening. Melatonin application also enhanced the retention of anthocyanins. Furthermore, melatonin treatment influenced flavor development, leading to a significantly higher sugar-acid ratio in treated fruits after extended storage, especially at 200  $\mu$ M. In conclusion, postharvest melatonin treatment, particularly at 150  $\mu$ M, extends the storage life of peach fruit by slowing senescence, preserving nutritional quality, and maintaining a favorable biochemical balance for longer shelf life.

**Keywords:** Melatonin, anthocyanins, sugar acid ratio, quality and storage.

### INTRODUCTION

Melatonin (N-acetyl-5-methoxytryptamine) is a naturally occurring bioactive compound that plays a multifunctional role in biological systems. In plants, melatonin has gained considerable attention due to its involvement in growth regulation, stress tolerance, and postharvest quality maintenance (Hardeland et al., 2012). Previous studies have demonstrated that melatonin enhances immune responses and improves antioxidant capacity in fruits by regulating reactive oxygen species (ROS) metabolism (Calvo et al., 2013; Galano et al., 2013). Plant tissues are capable of synthesizing melatonin endogenously, and its concentration varies depending on genotype, environmental conditions, and developmental stage (Byeon & Back, 2014). Owing to its amphiphilic nature, melatonin readily penetrates cellular membranes and distributes within subcellular compartments,

enabling it to participate in diverse physiological processes. In plants, melatonin has been reported to regulate flowering induction, chlorophyll biosynthesis, root regeneration, and delay leaf senescence (Tan et al., 2012; Zhang et al., 2014; Wei et al., 2015). Melatonin is also recognized as a powerful antioxidant that contributes to plant tolerance against abiotic stresses such as drought, chilling injury, and heavy metal toxicity (Posmyk et al., 2008; Wang et al., 2013; Bajwa et al., 2014). Under stress conditions, melatonin biosynthesis is enhanced, allowing plants to counteract oxidative damage by scavenging ROS directly and by stimulating antioxidant enzymes, as well as vitamins C and E (Khan et al., 2020). Furthermore, melatonin interacts with phytohormones such as gibberellic acid (GA) and abscisic acid (ABA), particularly during early developmental stages, thereby supporting plant growth and physiological stability (Zhang et al., 2014).

In postharvest, melatonin has been shown to delay senescence, maintain membrane integrity, and preserve fruit quality by suppressing chlorophyll degradation and modulating gene expression associated with stress defense pathways (Weeda et al., 2014). Considering its potential in extending shelf life and maintaining nutritional quality, the present study was conducted to evaluate the effectiveness of melatonin application on postharvest quality attributes of 'Early Grand' peach during cold storage.

## **MATERIALS AND METHODS**

### **Preparation of peach samples**

Peach fruits of the 'Early Grand' variety were harvested at the physiological maturity stage from a peach orchard. Fruits of uniform size and shape, free from blemishes, were selected. The samples were packed in corrugated boxes and transported to the postharvest laboratory on the same day of harvest.

### **Treatment of fruit samples, experimental design and storage**

At the postharvest laboratory, each fruit sample was thoroughly washed with tap water and air-dried. The fruits were randomly divided into five groups. The experiment was laid out in Completely Randomized Design (CRD) with three replications having two factors i.e melatonin and storage duration. Four groups were dipped in melatonin solutions (Sigma-Aldrich, USA) at concentrations of 50 $\mu$ M, 100 $\mu$ M, 150 $\mu$ M, and 200 $\mu$ M for five minutes, while the remaining fruits were immersed in distilled water and served as the control. All treatments were carried out under dim light conditions to prevent melatonin decomposition (Zhang et al. 2018). After treatment, the fruits were air-dried and stored at 3 $\pm$ 2°C and 70-80% relative humidity (RH) for 28 days. The middle portion of the peach flesh was used to record various parameters related to fruit quality and shelf life. Data were collected from three fruits per treatment at seven-day intervals (0, 7, 14, 21, 28 days) throughout the 28-day storage period. The mean values for each treatment were then compared with the control.

### **Data collection**

In order to evaluate the role of melatonin in the quality of early grand peach, data was recorded on the following parameters:

#### **Titrateable acidity (%)**

Percent acidity of five randomly selected fruits of each treatment will be measured as described in AOAC (1990).

##### **Procedure**

10 ml of peach juice will be taken in a beaker and quantity will be made up to 100ml. From this solution, 10 ml solution will be taken flask along with 2-3 drops of phenolphthalein as indicator. 0.1N NaOH solution will be taken in the burette and titration will be started and titration will be stopped when solution color changed to purple and the reading will be recorded at this point. Titration will be repeated thrice for each sample and titrateable acidity will be computed with the following formula:

$$\% \text{ Titratable acidity (g per 100ml)} = \frac{T \times N \times F \times 10}{D \times S} \times 100$$

T = Volume (mL) of sodium hydroxide (NaOH) titrant used to reach the endpoint.

N = Normality (N) of the sodium hydroxide (NaOH) titrant.

F = A constant representing the grams of the predominant acid equivalent to 1 mL of 1N NaOH.

D = Volume (mL) of the sample aliquot taken for titration.

S = Volume (mL) of the sample originally prepared or measured.

### Total soluble solids (TSS)

TSS of peach fruit juice will be recorded by using refractometer (Kernco, gadgets Co. Texas) for each treatment of each replication. The prism will be cleaned with distilled water and tissue paper. A drop of peach fruit juice will be placed on prism of refractometer and the reading will be noted (AOAC, 1990).

### Juice pH

Juice pH will be recorded with the help of pH meter (Modle # INOLAB pH-720) for all treatments. Juice pH meter will be normalized before using the solution. The peach juice will be taken in the beaker with the help puppet and electrode of pH meter will be dipped for 2 to 3 minutes and reading will be recorded.

### Sugar acid ratio

The total soluble solids was divided by reading of titratable acidity to get sugar to acid ratio:

$$\text{Sugar acid ratio} = \frac{\text{Total Soluble Solids}}{\text{Titratable Acidity}}$$

### Reducing sugars (%)

Reagents

Fehling-A: 34.65g of CuSO<sub>4</sub> 5H<sub>2</sub>O will be dissolved in 500ml distilled water.

Fehling-B: 173 g sodium potassium tartarate and 50g of NaOH will be taken in beaker. About 100 ml of H<sub>2</sub>O will be added and will be dissolved by striking. The solution will be transferred to 500 ml flask and volume will be made up by adding distilled H<sub>2</sub>O.

### Indicator: Methylene blue

50 g of water will be taken in the flask and after adding 0.2 grams of methylene blue and the final volume will be made up with adding more water.

### Procedure

100ml solution will be prepared in volumetric flask by dissolving 10 ml of peach fruit sample in distilled water. This solution will be taken in the burette. Conical flask having 5ml each of Fehling-A and Fehling-B solution and 0.10 ml distilled. Methylene blue will be added as indicator. Titration will started along with heating and will continue till the change of color to brick red in the flask. One drop of methylene blue will be used as indicator. Reducing sugar will be calculated as described in AOAC (1990).

### Non-reducing Sugar (%)

#### Procedure

10 ml of peach fruit juice will be taken in volumetric flask and distilled water will be added to make 100 ml solution. Then 0.20 ml solution will be taken in flask and will be added 10 ml of 1N HCl and heated up to 10minutes and finally 250ml solution will be made by adding more 1N NaOH solution. Solution will be taken in the burette and will be titrated after boiling in flask having 5ml Fehling A and 5 ml Fehling B solution along with 10 ml of distilled water and 2 to 3 drops of methylene blue.

Titration will be stopped when red brick colour appears. The amount of non-reducing sugar will be calculated as mentioned in AOAC (1990).

### **Anthocyanin content**

Anthocyanin content of peach fruit will be recorded by Zhang and Quantick (1997) method. 10 ml of 1% methanol will be added to 500 g of peach fruit juice sample and will be grinded by using pestle and mortar. This sample will be taken to centrifuge and rotate at 19,000 g for 15 min. Then 1% HCl will be added to make the volume upto 50 ml. The reading will be recorded with the help of spectrophotometer (GENESYS 10 UV-VIS, Thermo Spectronic, Rochester, USA) at 530 nm and will be calculated in mg g<sup>-1</sup> fresh weight.

### **Statistical Analysis**

The collected data regarding all parameter were analyzed for analysis of variance by using Statistix 10.0 software. Significant means were further separated by using LSD test at a 5% probability level ( $p \leq 0.05$ ). All figures were made by using MS-Excel software.

## **RESULTS AND DISCUSSIONS**

### **Titrateable acidity**

Titrateable acidity declined significantly with increasing storage duration, reflecting the consumption of organic acids during respiration. Fresh fruits exhibited the highest acidity, while fruits stored for 28 days showed the lowest values (Table 1).

Melatonin application significantly influenced acidity retention. Fruits treated with 150  $\mu$ M melatonin maintained higher acidity compared to other treatments and control fruits. The interaction effect showed that freshly harvested fruits treated with 150  $\mu$ M melatonin retained maximum acidity, whereas untreated fruits stored for 28 days exhibited the lowest acidity (Figure 1).

The decline in acidity during storage is a common physiological phenomenon, as organic acids are utilized as energy sources (Batista-Silva et al., 2018). The ability of melatonin to maintain higher acidity may be associated with its role in reducing respiration rate, consistent with findings by Liu et al. (2018).

### **Total soluble solids (TSS)**

Table 1 shows that the total soluble solids content increased significantly with extended storage duration. The highest TSS values were recorded after 28 days of storage, reflecting the conversion of complex carbohydrates into soluble sugars during ripening.

Melatonin treatments significantly influenced TSS accumulation. Fruits treated with 150  $\mu$ M melatonin exhibited higher TSS compared to control fruits, although differences among higher melatonin concentrations were not statistically significant. The interaction analysis indicated that fruits treated with 150  $\mu$ M melatonin and stored for 14 days recorded the maximum TSS, while untreated fruits stored for seven days had the lowest values (Figure 2).

Although TSS increased during storage, melatonin-treated fruits showed a comparatively slower rate of increase, indicating delayed ripening. These findings align with reports by Liu et al. (2018) and Wang et al. (2022), who observed that melatonin application reduced sugar accumulation and delayed senescence in strawberry and papaya fruits, respectively.

### **Juice pH**

Statistical analysis revealed that fruit pH was significantly influenced by melatonin concentration, storage duration, and their interaction (Table 1). A gradual increase in pH was observed with prolonged storage, indicating progressive utilization of organic acids during respiration. The pH values remained relatively stable during the initial storage period but increased markedly after 21 days of storage. Melatonin-treated fruits exhibited comparatively lower pH values than untreated fruits, particularly at higher concentrations. Fruits treated with 150  $\mu$ M melatonin maintained the lowest pH, suggesting reduced respiration activity. The interaction effect showed that fruits stored

for 28 days without melatonin treatment recorded the highest pH, whereas freshly harvested fruits treated with 150  $\mu\text{M}$  melatonin showed the lowest pH (Figure 3).

The observed increase in pH during storage can be attributed to the continuous metabolic breakdown of organic acids, which serve as respiratory substrates after harvest (Holcroft & Kader, 1999; Saltveit, 2019). Similar trends have been reported by Gao et al. (2016), who found that melatonin application suppressed respiration rate, thereby slowing pH elevation.

### Sugar acid ratio

It is clear from table 1 that sugar–acid ratio of peach fruit increased progressively with storage duration due to increased sugar content and reduced acidity. Fruits treated with higher melatonin concentrations exhibited significantly higher sugar–acid ratios, particularly at extended storage periods. The highest sugar–acid ratio was recorded in fruits treated with 200  $\mu\text{M}$  melatonin after 28 days of storage, while the lowest ratio was observed in fresh control fruits. These results suggest that melatonin application modulates the balance between sugars and organic acids during storage, influencing flavor development (Figure 4). Similar observations were reported by Liu et al. (2019), who attributed the increase in sugar–acid ratio to enhanced sugar accumulation and accelerated organic acid degradation during prolonged storage.

### Reducing and non-reducing (%)

Reducing sugar content increased significantly with storage duration, reflecting starch hydrolysis during ripening. The highest reducing sugar content was observed after 28 days of storage, while fresh fruits had the lowest values (Table 2). Melatonin-treated fruits showed comparatively lower sugar accumulation than control fruits, indicating delayed ripening. Non-reducing sugars gradually declined with storage duration, with minimal variation among melatonin treatments (Table 2). The conversion of starch into soluble sugars during fruit maturation and storage has been widely documented (Magein & Leurquin, 2000; Silva et al., 2008). Reduced sugar accumulation in melatonin-treated fruits may be associated with suppressed activity of starch-degrading enzymes, leading to delayed senescence (Hanif et al., 2020a).

### Anthocyanin ( $\mu\text{g g}^{-1}$ )

Anthocyanin concentration was significantly influenced by melatonin treatment and storage duration, while their interaction was non-significant (Table 2). Fruits treated with 150  $\mu\text{M}$  melatonin exhibited the highest anthocyanin content, whereas control fruits showed the lowest values. A gradual decline in anthocyanin concentration was observed with increasing storage duration, indicating pigment degradation over time. Anthocyanins function as important antioxidants and contribute to fruit color and nutritional value (Khoo et al., 2017; Teng et al., 2020). Melatonin application enhanced anthocyanin retention by activating antioxidant defense systems and stimulating phenylpropanoid metabolism. Similar findings were reported by Aghdam et al. (2020) and Sharafi et al. (2021), who observed improved anthocyanin accumulation in melatonin-treated fruits during storage.

**Table 1. Influence of melatonin concentration and storage duration on titratable acidity, total soluble solids (TSS), fruit pH, and sugar–acid ratio of ‘Early Grand’ peach.**

| Melatonin ( $\mu\text{M}$ ) | Titratable Acidity (%) | TSS         | pH (%)      | Sugar acid ratio |
|-----------------------------|------------------------|-------------|-------------|------------------|
| 0                           | 0.470 e                | 9.93 c      | 3.78 a      | 17.17 c          |
| 50                          | 0.560 c                | 10.34 bc    | 3.71 a      | 16.82 c          |
| 100                         | 0.640 b                | 11.01a      | 3.64 ab     | 21.25 b          |
| 150                         | 0.690 a                | 10.85 a     | 3.50 bc     | 19.39 b          |
| 200                         | 0.61 d                 | 10.72 ab    | 3.39 c      | 24.81a           |
| LSD ( $P \leq 0.05$ )       | <b>0.04</b>            | <b>0.43</b> | <b>0.19</b> | <b>2.01</b>      |

| Storage (day)                  |                |                |                |                |
|--------------------------------|----------------|----------------|----------------|----------------|
| 0                              | 0.83 a         | 9.49 d         | 3.92 a         | 11.75 e        |
| 7                              | 0.65 b         | 10.20 c        | 3.74 a         | 16.63 d        |
| 14                             | 0.56 c         | 10.82 b        | 3.54 b         | 19.74 c        |
| 21                             | 0.49 d         | 11.10 ab       | 3.43 b         | 23.51b         |
| 28                             | 0.44 e         | 11.25 a        | 3.38 b         | 27.80 a        |
| LSD ( $P \leq 0.05$ )          | <b>0.04</b>    | <b>0.21</b>    | <b>0.19</b>    | <b>2.01</b>    |
| Interaction (M×S)              |                |                |                |                |
| Significance ( $P \leq 0.05$ ) | <b>*Fig. 1</b> | <b>*Fig. 2</b> | <b>*Fig. 3</b> | <b>*Fig. 4</b> |

Values represent mean responses of peach fruits subjected to different melatonin treatments and storage periods. Means within a column sharing the same letter are statistically non-significant at  $P \leq 0.05$ . LSD values indicate least significant differences among treatments. NS denotes non-significant effects, whereas \* indicate significance at 5% probability levels.

**Table 2. Effect of melatonin application and cold storage duration on reducing sugars, non-reducing sugars, and anthocyanin content of peach fruit**

| Melatonin ( $\mu\text{M}$ )    | Reducing sugars (%) | Non-reducing sugars (%) | Anthocyanin ( $\mu\text{g g}^{-1}$ ) |
|--------------------------------|---------------------|-------------------------|--------------------------------------|
| 0                              | 2.46 a              | 4.29                    | 113.20 c                             |
| 50                             | 2.49 a              | 4.26                    | 114.96 bc                            |
| 100                            | 2.25 b              | 4.22                    | 117.44 bc                            |
| 150                            | 2.25 b              | 4.20                    | 124.35 a                             |
| 200                            | 2.42 ab             | 4.23                    | 119.91 ab                            |
| LSD ( $P \leq 0.05$ )          | <b>0.21</b>         | <b>NS</b>               | <b>5.76</b>                          |
| Storage (day)                  |                     |                         |                                      |
| 0                              | 1.8 d               | 4.77 a                  | 124.67 a                             |
| 7                              | 2.1 c               | 4.53 b                  | 124.08 a                             |
| 14                             | 2.5 b               | 4.32 bc                 | 117.72 b                             |
| 21                             | 2.6 b               | 4.21 c                  | 115.76 b                             |
| 28                             | 2.8 a               | 3.37 d                  | 107.62 c                             |
| LSD ( $P \leq 0.05$ )          | <b>0.21</b>         | <b>0.47</b>             | <b>5.76</b>                          |
| Interaction (M×S)              |                     |                         |                                      |
| Significance ( $P \leq 0.05$ ) | <b>*Fig. 5</b>      | <b>*Fig. 6</b>          | <b>NS</b>                            |

Values represent mean responses of peach fruits subjected to different melatonin treatments and storage periods. Means within a column sharing the same letter are statistically non-significant at  $P \leq 0.05$ . LSD values indicate least significant differences among treatments. NS denotes non-significant effects, whereas \* indicate significance at 5% probability levels. NS indicates non-significant variation among treatments.

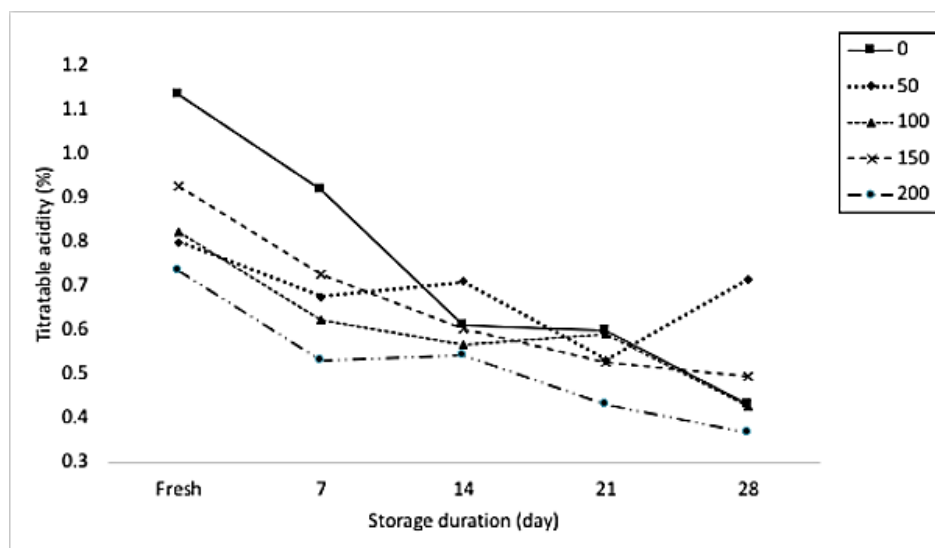


**Figure 1.** Changes in titratable acidity of peach fruit subjected to different melatonin concentrations during cold storage.

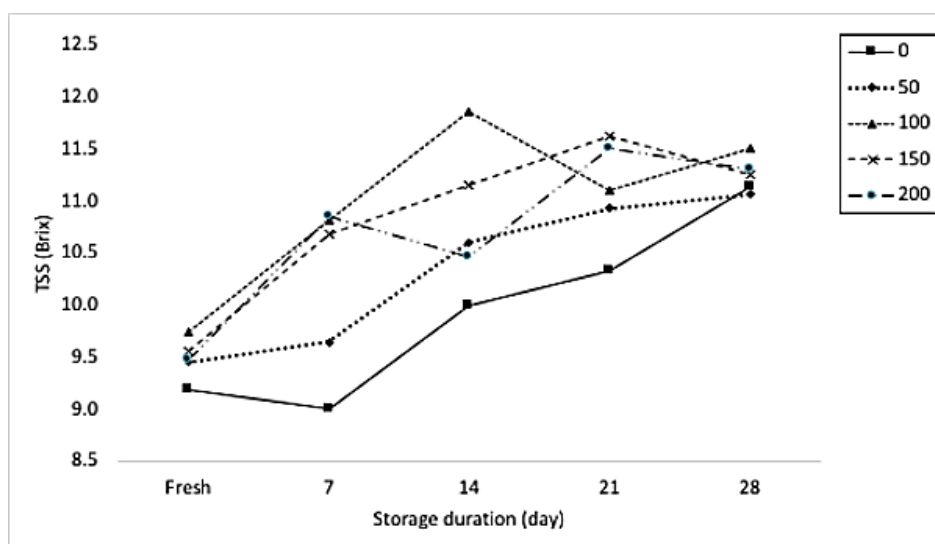


**Figure 2.** Variation in total soluble solids (TSS) of peach fruit as influenced by melatonin treatments over storage time.

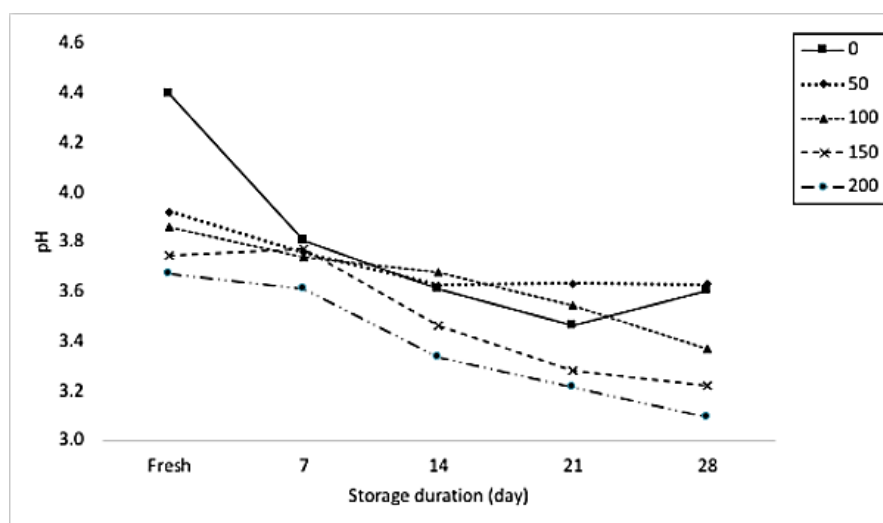


**Figure 3.** Effect of melatonin application on pH dynamics of peach fruit during cold storage.

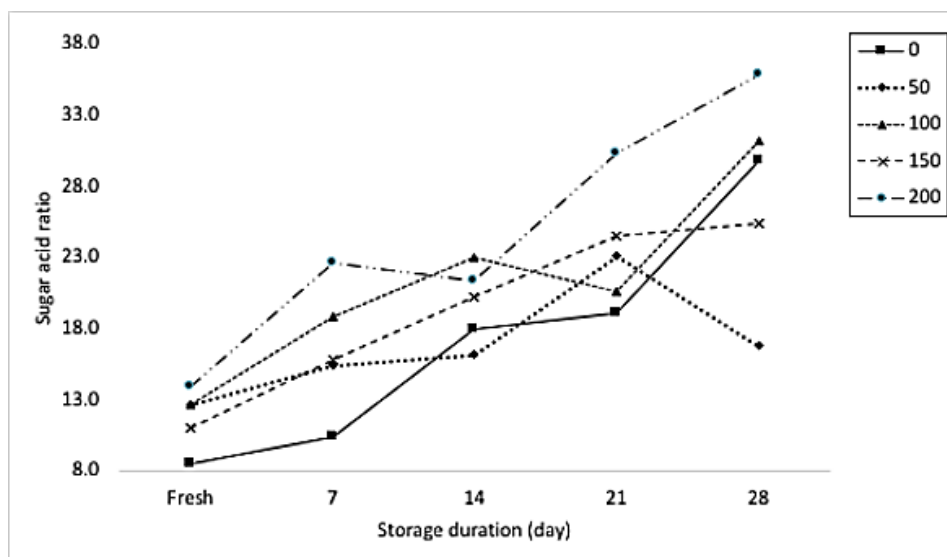


**Figure 4.** Influence of melatonin concentration and storage duration on sugar–acid ratio of peach fruit.

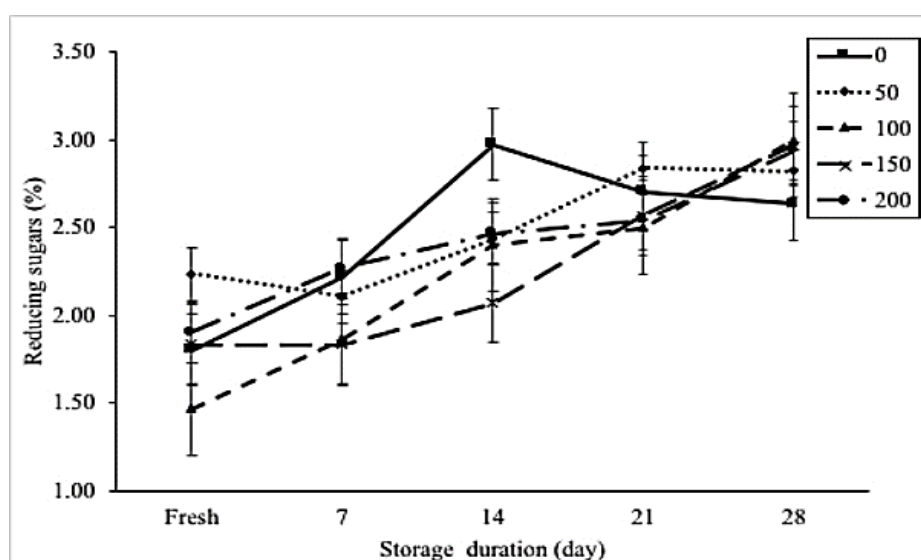


**Figure 5.** Response of reducing sugar content in peach fruit to melatonin application during storage.

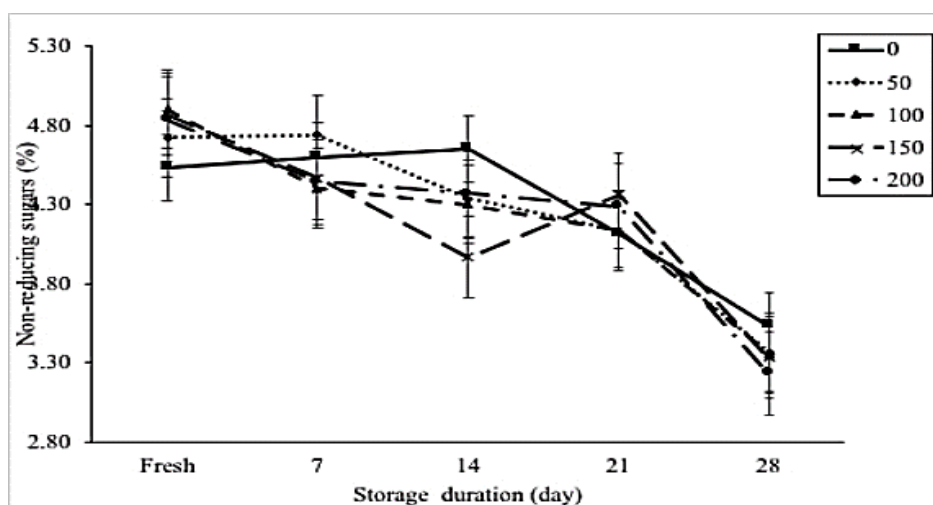


**Figure 6.** Non-reducing sugars of peach fruit as affected by melatonin during storage

## CONCLUSION

The application of 150  $\mu$ M melatonin extends the postharvest shelf life of peaches by shifting the fruit's physiology toward a state of reduced metabolic activity and enhanced stress resistance. By slowing respiration, delaying carbohydrate metabolism shifts, and activating antioxidant systems, melatonin helps maintain sensory attributes (sourness, sweetness balance) and nutritional value (anthocyanins) for a longer period. This supports its potential use as a safe, eco-friendly postharvest treatment to reduce spoilage and maintain fruit quality during storage and supply chain distribution.

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