Hindawi
Journal of Food Biochemistry
Volume 2023, Article ID 8126640, 14 pages
https://doi.org/10.1155/2023/8126640

Research Article

Postharvest Melatonin Application Preserves Quality and Imparts Chilling Tolerance in Peaches

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Received 11 July 2023; Revised 23 September 2023; Accepted 27 September 2023; Published 4 October 2023

Academic Editor: Rana Muhammad Aadil

Peach being a climacteric fruit has a limited shelf life. Cold storage of peaches is an essential measure to maintain their quality. However, prolonged exposure to cold temperatures causes chilling injury in peaches, resulting in significant economic losses. Approximately, 40% of the produced commodity is lost in postharvest handling in which chilling injury contributes to about 25%. Melatonin can be a green solution and plays a key role in protecting peaches from chilling injury. Therefore, this experiment aimed to determine the effect of melatonin on the "Silver King" peaches by dipping them in 100 μM melatonin for 2 hours followed by storing them at chilling temperatures of $2 \pm 1^{\circ}$C and 90–95% relative humidity for 21 d. The results showed that while control fruit experienced chilling injury and poor fruit quality, increased respiration rate, and enhanced senescence, 100 μM melatonin treatment was successful in preserving the fruit quality at chilling temperatures and was associated with the delayed respiration rate and lower malondialdehyde levels, along with the preservation of certain physicochemical parameters, namely, total soluble solids, pH, fruit firmness, and titratable acidity. Moreover, a 1.5 times higher proline concentration was observed in melatonin-treated peaches when compared to that of nontreated peaches. This rise was linked to the enhanced activity of Δ^1-pyrroline-5-carboxylate synthetase and ornithine-δ-aminotransferase along with a decrease in the activity of proline dehydrogenase during 21 d of storage.

1. Introduction

Peach (*Prunus persica* L.), which belongs to the Rosaceae family, originated in China. Annual peach fruit production accounts for 15.02 million tons in China, which is nearly 61.12% of the total world's production, thus making it the world's leading producer. After China, Spain secures second position, followed by Italy, Turkey, Iran, the USA, Egypt, Chile, and India [1]. Peach is a temperate fruit that has gained worldwide attention due to its excellent commercial value, pleasing aroma, juicy texture, bright attractive colour, and abundant health benefits [2]. Peach fruit is considered a good source of proteins, minerals, soluble sugars, and dietary fibers, making it capable of providing immunity against chronic diseases such as cancer and cardiovascular diseases. Furthermore, the peach's nutritional quality is an important indicator factor that determines fruit quality [3]. However, peach fruit inevitably experiences rapid ripening, senescence, and deterioration at ambient temperature. Low-temperature storage is one of the most effective technologies for preserving fruits [4]. Unfortunately, the primary limitation of keeping peaches at low temperatures leads to the development of chilling injury (CI). CI, a physiological disorder, mainly occurs in various cultivars of peach fruit stored at a temperature between 2.2 and 7.6°C. As soon as fruits are harvested, they are kept in cold stores. Since the fresh horticultural produce is gradually subjected to cold storage, continuous cold temperatures above their freezing

point degrade the quality of the product within the course of time, and these symptoms can be clearly observed once the horticultural produce is transferred from cold storage condition to ambient conditions [5].

The development of CI symptoms in the postharvest produce is considered a complex process involving various mechanisms such as degradation of the cell wall, oxidative stress, membrane damage, and hormonal imbalance, where cell wall alteration due to a low temperature is considered the primary physiological response [6, 7]. A hypothesis was proposed in relation to the accumulation of certain toxic compounds, increased cell wall permeability, decreased state of water dynamics, metabolism imbalance, and other plant mechanisms affected due to CI [6–8].

The common CI symptoms in peaches include surface pitting, flesh mealiness (woolliness), lignification, reddening, and abnormal ripening, rendering fruit quality loss and fruit commercial value [9]. In order to alleviate CI, to date, peach fruit have been exposed to various methods involving treatment with methyl jasmonate [9], oxalic acid [10], γ-aminobutyric acid [11], salicylic acid [12], glycine betaine [13], nitric oxide followed by intermittent warming [14], jasmonic acid [15], and 24-epibrassinolide [16].

Melatonin (MT), a ubiquitous indoleamine, chemically known as N-acetyl-5-methoxytryptamine, was first discovered in 1958 in the bovine human pineal gland [17]. It has been reported to play its part in important physiological processes including fruit maturation, photoprotection, and fruit ripening. Various reports have stated the effects of MT on CI alleviation in several fruits, such as kiwifruit [18], apricot [19], tomato [20], nectarine [21], bitter lemon [22], sapota [23], bell pepper [24], mango [6–8, 11, 25], and banana [26]. MT is the most preferred signaling molecule nowadays for alleviating CI, reducing postharvest decay, maintaining quality, and extending the shelf life of fruits and vegetables. However, the literature in this context is scanty and not conclusive. To the best of our knowledge, no study reported in peach fruit on the mechanism of CI alleviation by exogenous melatonin treatment in relation to proline metabolism.

Thus, in this study, we intend (a) to analyze the effects which MT has on the concentration and metabolism of endogenous proline and (b) to understand what effect and relation endogenous proline has on chilling tolerance in peach fruit stored at $2 \pm 1^{\circ}$C of chilling temperature. The aim of the study is to build up a sound knowledge of various underlying physiological mechanisms in peach fruit through which a defense action is induced by MT in alleviating chilling tolerance.

2. Materials and Methods

2.1. Chemicals Used. Melatonin (MT), sulfosalicylic acid, ninhydrin, orthophosphoric acid, toluene, L-proline, tris-HCl buffer, magnesium chloride, phenylmethylsulfonyl fluoride (PMSF), adenosine triphosphate (ATP), monosodium glutamate, nicotinamide adenine dinucleotide phosphate (NADPH), potassium phosphate monobasic, potassium phosphate dibasic, polyvinylpolypyrrolidone (PVPP), pyridoxal phosphate, ethylenediaminetetraacetic acid (EDTA), α-ketoglutaric acid, L-ornithine monohydrochloride, perchloric acid, dithiothreitol (DTT), NAD+, bovine serum albumin (BSA), phenolphthalein, tert-butyl alcohol, trichloroacetic acid (TCA), and sodium hypochlorite solution were the analytical grade chemicals used and procured from HiMedia, India.

2.2. Fruit Selection and Treatment Application. Peach fruit variety "Silver King" was procured from the farmer's field of Rajgarh, Sirmour, Himachal Pradesh, India. "Silver King" is a chilling-sensitive variety, and chilling symptoms are showing easily. Peaches were harvested at physiological maturity but before ripening with yellow colour and just at the start of red blush tinge on the fruit skin. Fruit were transported to the laboratory within 10 h maintaining a temperature of 20°C throughout the transportation. Fruit were inspected for any bruises, blemishes, injury, disease, etc., and 400 fruit were selected with uniform maturity, colour, size, and free from any disease symptom or disorder. Fruit were divided into two groups ($n = 200$/group). Sampling was followed by disinfecting the fruit for 2 min with 1% (v/v) PVPP. The first group of peaches was again divided into five lots of 40 fruit each, and separately immersed in distilled water, used as control replicates, whereas the second group also divided into five lots comprising 40 fruit in each lot, and separately immersed in MT solution of 100 μM, was considered as treated. The immersion duration of peaches in water, as well as MT, was 2 h at $25 \pm 2^{\circ}$C in dark conditions, to prevent the degradation of MT [2]. The MT concentration (100 μM) and dipping duration (2 h) were standardized in preliminary trials. Later, the fruit were air-dried and kept at $2^{\circ}\text{C} \pm 1^{\circ}$C with 85–90% relative humidity for 21 d. Observations were recorded by removing fruit from each replication after 0, 7, 14, or 21 d of storage, in addition to a 3 d simulation period under ambient conditions (dry, clean, ventilated area at $25 \pm 2^{\circ}$C, 70–75% relative humidity). All parameters were assessed in five replicates. In addition, 10 fruit were kept separately to observe changes in colour and weight loss. After analyzing the fruit respiration and ethylene production rates and physicochemical parameters, the tissues of peach fruit were homogenized in liquid nitrogen and preserved at a freezing temperature of −80°C for further work.

2.3. Chilling Injury Index. The fruit of both groups were evaluated every 7 d interval of cold storage, plus 3 d at 25°C, 70–75% relative humidity after removal from cold storage. The chilling injury index (CII) was then assessed on the basis of the appearance of the fruit. A numerical scale ranging from 0 to 5 was used to analyze CI severity. CI severity including the intensity of the CI symptoms in fruit including reddening, mealiness, flesh browning, leatheriness, and flesh bleeding was recorded. The 0 score denotes no CI severity, 1 for 1–20% CI severity, 2 for 21–40% CI severity, 3 for 41–60% CI severity, 4 for 61–80% CI severity, and 5 for 81–100% CI severity on the fruit surface area showing CI symptoms [27]. Readings were taken in five replications, and CII was calculated by the following formula:

$$\text{CII} = \sum \text{rank score} * \frac{\text{number of fruit receiving the rank of chilling symptoms}}{\text{total number of fruits}}. \quad (1)$$

2.4. Respiration and Ethylene Production Rates. Measurement of ethylene production and the respiration rate was performed in five replicates as described by [28]. For measuring the rate of respiration, we used an 800 mL airtight glass jar. The ethylene analyzer (Bioconservacion, Spain) was calibrated. The peach fruit were weighed and placed inside the glass jar and closed with a rubber septum over its cap. The filled jar was kept at 20°C for the entire duration of measurement. An initial reading was taken by piercing the probe of the ethylene analyzer into the jar headspace through the septum. The final reading was taken after a duration of 2 h. After the measurement of 2 h, calculations of the time taken for ethylene production were performed and were expressed in nmol $kg^{-1}{\cdot}s^{-1}$.

A similar type of experiment was conducted for measuring the respiration rate. An autogas analyzer (PBI Dansensor, Denmark) at 20°C was used for the estimate of the respiration rate (CO_2 production rate). By inserting the probe of the autogas analyzer into the glass jars, the analyzer gave the reading of available oxygen and carbon dioxide in the gas jars. The respiration rate was measured in nmol $kg^{-1}{\cdot}s^{-1}$. The formulas used were as follows:

$$\text{ethylene production rate}\left(\frac{(\text{nmol/kg})}{s}\right) = \frac{\text{ethylene produced (nmol)}}{(\text{fruit weight (kg)} * \text{time}(s) * 1000)},$$
$$\text{respiration rate}\left(\frac{(\text{nmol/kg})}{s}\right) = \frac{\text{carbon dioxide produced (nmol)}}{(\text{fruit weight (kg)} * \text{time}(s) * 1000)}. \quad (2)$$

2.5. Physicochemical Properties

2.5.1. Weight Loss. Fruit weight loss of the fixed 10 fruit was measured every week at a 7 d interval followed by a 3 d simulation period at 25 ± 1°C. A digital weighing balance measured the difference between the final and initial fruit weight, which was expressed as a percentage (%).

2.5.2. Colour. A hand-held chromameter (CR 400, Konica, Minolta, Japan) was used to measure fruit peel colour. The same 10 fruit fixed for the weight loss assessment were used for colour measurement. The assessments were performed in five replicates and taken along the equatorial axis from two opposite sides. CIE LAB colour coordinates, L^*, a^*, b^*, C, and h°, were observed, where lightness/darkness is denoted by L^*, greenness/redness by a^*, and yellowness/blueness by b^*. Values of a^* and b^* were used for calculating chroma (C) and the hue angle (h°):

$$C = \left[\sqrt{\left(a^{*2} + b^{*2}\right)}\right],$$
$$h^\circ = \left[\frac{(b * / a *)}{\tan}\right]. \quad (3)$$

2.5.3. Firmness. For determining the firmness of the fruit, we used a texture analyzer (TA.HD Plus, Stable Microsystems, UK). The peach fruit was kept on the stationary platform of the firmness analyzer. We used a 3 mm diameter stainless steel probe at a 1.5 mm·s^{-1} pretest speed, 0.5 mm·s^{-1} test speed, and 10 mm·s^{-1} posttest speed, which pierced the fruit and determined its firmness. The measurements were recorded in five replicates and taken along the equatorial plane at two opposite points, the penetration depth being 5 mm. The readings were expressed in Newton (N).

2.5.4. Total Soluble Solids. For calculating total soluble solids (TSSs), we used the pulp of the fruit. A muslin cloth was used to squeeze the pulp out of the fruit and was followed by filtration. A filtered drop of juice was placed in a hand-held refractometer (Atago, Japan), and the readings were noted in five replicates and represented as a percentage.

2.5.5. Titratable Acidity, TSS: Acid Ratio, and pH. Determination of titratable acidity (TA) was performed according to the previously described method [29]. The fruit was squeezed using a muslin cloth to squeeze out its pulp. Its pulp (20 g) was taken, which was then homogenized in 100 mL distilled water and filtered. Titration with 0.1 N NaOH was performed on the above filtrate. Phenolphthalein (1%) was added to the above solution. Titration was performed until the endpoint was attained. The endpoint was attained by a light pink shade which resulted due to phenolphthalein. Readings were noted in five replicates, and results were expressed as a percentage. The TSS: TA ratio was measured by dividing the measurement of TSS by that of TA. For measuring the pH of the fruit juice, a calibrated pH meter (Cyberscan, Eutech Instruments, Canada) was used.

2.6. Malondialdehyde. The concentration of malondialdehyde (MDA) was determined by a procedure described earlier with slight modifications [30]. The fruit was squeezed by hand and crushed in a mortar and pestle. Liquid nitrogen

was poured onto the crushed fruit and further ground in the pestle. The finely ground tissue was stored at −80°C for subsequent analysis. For measuring the MDA content, 2 g of flesh tissue was homogenized with 10% TCA having 0.5% (w/v) thiobarbituric acid (TBA). The mixture was incubated at 100°C for 10 min, followed by immediate cooling. The supernatant was collected after centrifugation (3–18 KS, Sigma, Germany) (5,000 × g, 20 min, 4°C), and the absorbance was read at 450, 532, and 600 nm using a spectrophotometer (Specord200plus, Analytik Jena, Germany). Analysis was performed in five replicates. The concentration of MDA present in 2 g tissue was calculated in mol as converted into a per kg basis of fruit. The results were expressed in mol·kg^{-1}.

2.7. Proline Measurement. The concentration of proline in fruit tissues was measured with some modifications [31]. The fruit was squeezed by hand and crushed in a mortar and pestle. Liquid nitrogen was poured onto the crushed fruit and was further ground in the pestle. The finely ground tissue was stored at −80°C for subsequent analysis. Peach fruit tissue (1 g) was used for proline extraction. The fruit tissue was dissolved in 10 ml 3% (v/v) sulfosalicylic acid and subjected to centrifugation (3–18 KS, Sigma, Germany) (12,000 × g, 20 min, 4°C). The assay mixture contained 1 mL ninhydrin reagent and 2 mL glacial acetic acid. This mixture was subjected to 30 min boiling followed by the addition of 2 mL toluene. Its absorbance was analyzed at 520 nm using a spectrophotometer (Specord200plus, Analytik Jena, Germany). The concentration of proline present in 1 g fruit tissue was calculated in mg with respect to the standard curve of proline. The proline concentration in 1 g fruit tissue was converted into a per kg basis of fruit. The results were expressed in mg·kg^{-1}.

2.8. Proline-Metabolizing Enzymes

2.8.1. Δ^1-Pyrroline-5-carboxylate Synthetase (P5CS). Δ^1-Pyrroline-5-carboxylate synthetase (P5CS, EC 2.7.2.11/ 1.2.1.41) activity was measured in fruit tissues in five replicates, according to the method described with some modifications [32]. The fruit was squeezed by hand and crushed in a mortar and pestle. Liquid nitrogen was poured onto the crushed fruit and further ground in the pestle. The finely ground tissue was stored at −80°C for subsequent analysis. 1 g of tissue was used for the analysis. The tissue was homogenized in a refrigerated mixture of 500 mM Tris-HCl buffer having pH 7.6, 2 mM PMSF, 100 mM β-mercaptoethanol, and 10 mM magnesium chloride, followed by centrifugation (3–18 KS, Sigma, Germany) of the homogenate at 12,000 × g for 20 min. The supernatant was used as the crude extract. A 0.8 mL reaction mixture was taken, which contained 0.2 mL of crude enzyme extract, 4 mM of ATP, 76 mM sodium glutamate, and 100 mM Tris-HCl having a pH of 7.2. After adding 0.5 mM NADPH, the absorbance was recorded at 340 nm·min^{-1} using a spectrophotometer (Specord200plus, Analytik Jena, Germany). Results were defined as the decrease in absorbance by 0.001. As the above P5CS activity determined was of 1 g fruit tissue, the above value was converted into a per kg basis of fruit. The results were expressed in U·kg^{-1}·s^{-1}.

2.8.2. Ornithine-δ-aminotransferase (OAT). Activity of ornithine-δ-aminotransferase (OAT, EC 2.6. 1.13) was measured by homogenization of 1 g of fruit tissue, performed in a mixture of ice-cooled 50 mM potassium phosphate buffer having a pH of 7.8, consisting of 2 mM DTT, 0.2 mM pyridoxal phosphate, and 2 mM EDTA. Centrifugation (3–18 KS, Sigma, Germany) of the homogenate was performed at 12,000 × g for 20 min. The supernatant from the described process was used as the crude enzyme extract. Phosphopyridoxal (50 mM), 20 mM α-ketoglutarate, and 35 mM L-ornithine were mixed to form the reaction mixture, which was then incubated at 37°C for 15 min. After the addition of 2% ninhydrin and 3 M perchloric acid, the mixture was subjected to boiling for 20 min, centrifuged at 12,000 × g for 15 min, the precipitate obtained was dissolved in toluene, and readings were taken in five replicates, where the absorbance was read using a spectrophotometer (Specord200plus, Analytik Jena, Germany) at 510 nm. As the above OAT activity determined was of 1 g fruit tissue, the above value was converted into a per kg basis of fruit. The results were expressed in U·kg^{-1}·s^{-1} [33].

2.8.3. Proline Dehydrogenase (PDH). Proline dehydrogenase (PDH, EC 1.5.5.2) activity was measured according to the method described by Lopez-Carrion [32] with slight modifications. The liquid nitrogen ground fruit tissue was used for estimating the PDH activity. To determine, 1 g fruit tissue was taken and homogenized in a mixture of ice-cooled 0.1 M potassium phosphate buffer (pH 7.8), containing 10 mM β-mercaptoethanol and 2 mM EDTA. The above mixture was centrifuged (3–18 KS, Sigma, Germany) (12,000 × g for 20 min). The supernatant obtained after centrifugation was used as the crude enzyme extract. The reaction mixture contained 10 mM NAD^+, 0.15 M sodium carbonate-bicarbonate buffer (pH 10.3), and L-proline. The crude enzyme extract (0.2 mL) was mixed with 0.9 mL reaction mixture to initiate the reaction. Results were measured in five replicates. The above PDH activity determined was of 1 g fruit tissue, and the above value was converted into a per kg basis of fruit. The results were expressed in U·kg^{-1}·s^{-1}.

2.9. Principal Component Analysis (PCA) and Comprehensive Evaluation. The effect of MT on the storage life of peach fruit was evaluated using the principal component coefficient using R package software. It featured the eigenvalue, variance %, and cumulative variance % (Table 1). Using the values of Table 1, a scree plot was made to determine the principal component factors (Figure 1). After identifying the principal component factors, a variable correlation plot was developed (Figure 2). Furthermore, Figure 3 is developed to interpret which variables contributed the most in the extension of shelf life of MT-treated peaches.

Table 1: Loading coefficients, eigenvalues, and variance % of the components.

Components	Eigenvalue	Variance %	Cumulative variance %
Dim. 1	$9.935303e+00$	$6.623535e+01$	66.23535
Dim. 2	$2.620138e+00$	$1.746759e+01$	83.70294
Dim. 3	$1.484966e+00$	$9.899776e+00$	93.60272
Dim. 4	$4.397448e-01$	$2.931632e+00$	96.53435
Dim. 5	$2.299605e-01$	$1.533070e+00$	98.06742
Dim. 6	$1.056819e-01$	$7.045459e-01$	98.77197
Dim. 7	$8.214076e-02$	$5.476051e-01$	99.31957
Dim. 8	$4.673513e-02$	$3.115675e-01$	99.63114
Dim. 9	$2.299816e-02$	$1.533210e-01$	99.78446
Dim. 10	$1.922623e-02$	$1.281749e-01$	99.91263
Dim. 11	$8.844694e-03$	$5.896463e-02$	99.97160
Dim. 12	$2.869829e-03$	$1.913220e-02$	99.99073
Dim. 13	$1.270361e-03$	$8.469074e-03$	99.99920
Dim. 14	$1.200643e-04$	$8.004288e-04$	100.00000
Dim. 15	$1.146037e-32$	$7.640244e-32$	100.00000

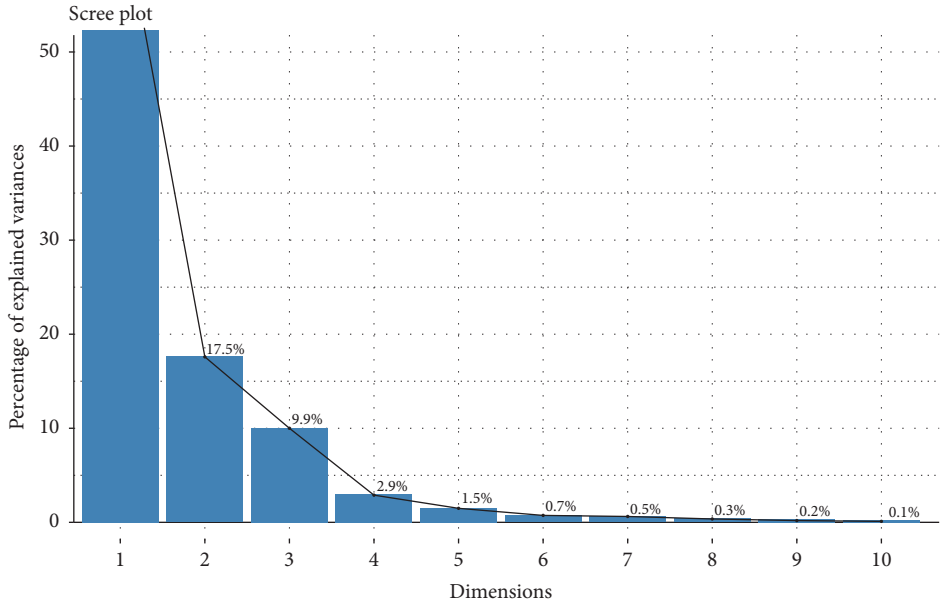

Figure 1: A scree plot of the factors studied for shelf-life extension of peaches. It shows variance shown by each factor. The horizontal axis represents dimensions, and the vertical axis represents the percentage of the explained variances.

2.10. Statistical Analysis. Experiments were performed in five replicates and based on a completely randomized design (CRD). The two-way analysis of variance (ANOVA) method was used to analyze data in statistical software SPSS (version 20.0), with $P < 0.05$ being the level of significance.

3. Results

3.1. Chilling Injury Index. The results revealed a significant difference between the CII of control and treated fruit. A higher percentage of CI severity was recorded for the control fruit from day 7 onwards on the numerical scale having ranking from 0 to 5. Mealiness, wooliness, reddening, and peel wrinkling were the main CI symptoms observed in the peach fruit related to CI. The MT-treated peach fruit started showing symptoms of CI only after 14 d of storage (Figure 4). According to the results, CII in control fruit was 66.66, 78.8, and 66.66% higher than in MT-treated on days 7, 14, and 21, respectively (Figure 5). Hence, the shelf life of the MT-treated fruit was extended and maintained up to 21 d (Figure 5). On the other hand, control peaches showed deteriorating symptoms from day 7, which may reduce their appeal for acceptability by target consumers.

3.2. Respiration and Ethylene Production Rates. One of the most important parameters for determining the metabolic activity of a fruit is its rate of respiration and ethylene production. From the results, it can be estimated that the rate of respiration and ethylene production follow a dependent pattern where no significant difference can be seen in both groups until day 7. However, the rate of respiration and ethylene production experienced a climacteric rise which was 60 and 28.57% higher in control fruit when compared to the MT-treated fruit (Figures 6(a) and 6(b)).

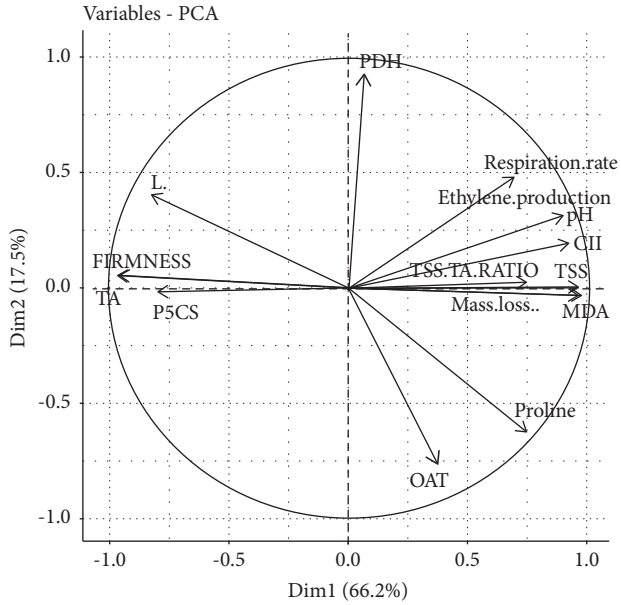

Figure 2: Correlation plot of the selected biochemical parameters determined in MT-treated peach samples during cold storage.

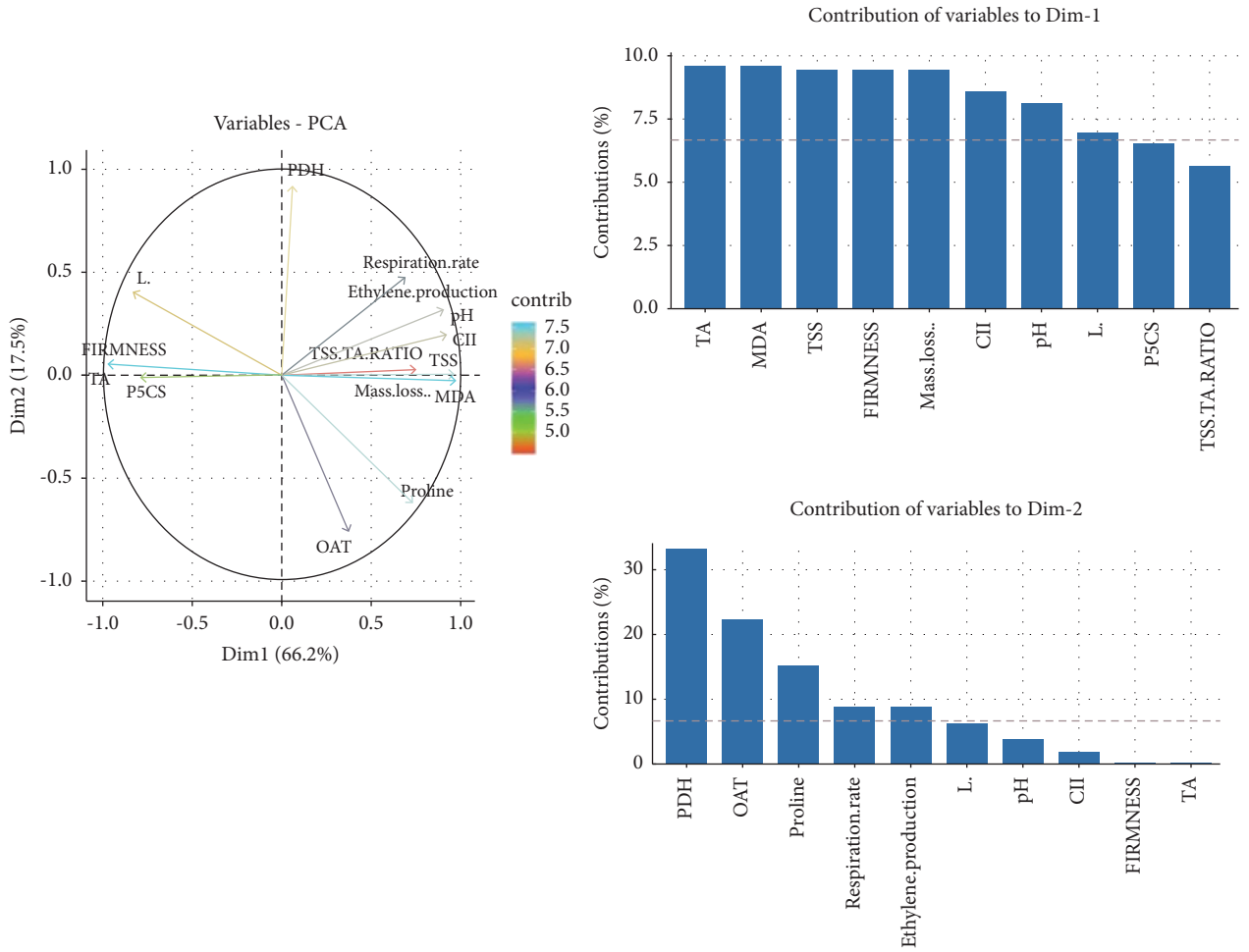

Figure 3: Contribution % of each factor to the first and second principal components, where the horizontal axis represents the parameters and the vertical axis represents the % contribution.

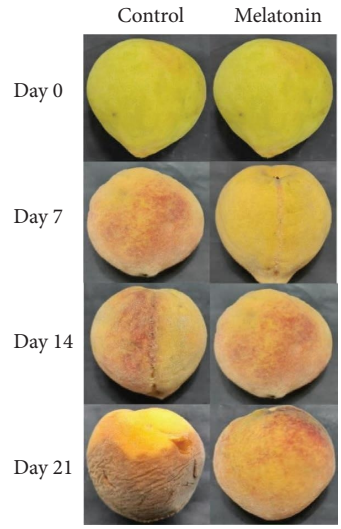

Figure 4: Effect of 0 μM (control) or 100 μM (treated) melatonin on the fruit appearance in peach fruit stored at 2 ± 1°C for 21 days followed by a simulation period of 3 days at ambient temperature.

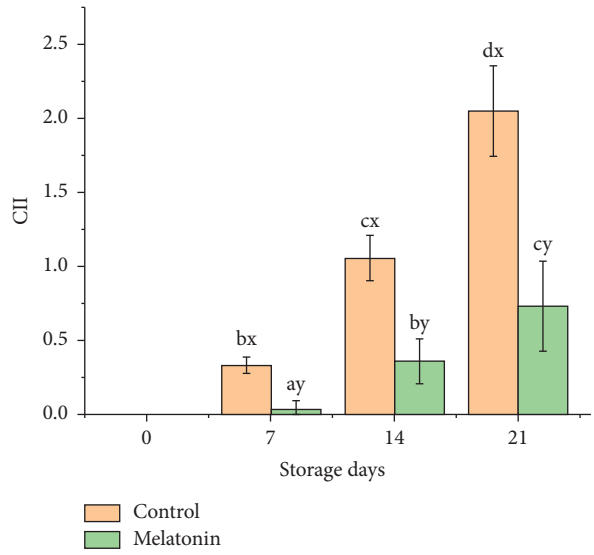

Figure 5: Effect of 0 μM (control) or 100 μM (treated) melatonin on the chilling injury index in peach fruit stored at 2 ± 1°C for 21 days followed by a simulation period of 3 days at ambient temperature. The letters a, b, and c indicated significant differences following the application of MT with 100 μM as based on Tukey's test ($p < 0.05$).

3.3. Physicochemical Properties

3.3.1. Weight Loss, Firmness, and Colour Coordinates. The metabolic and other ongoing processes lead to the loss of surface water of a fruit. On weighing the control and treated fruit every 7 d, the percentage of weight loss was more in the control fruit than in the treated one. However, throughout the storage period, no significant difference was observed among the groups (Figure 7(a)). The maturity of the fruit is determined by the firmness of the fruit. The firmness of both groups showed a decreasing trend as fruit softened with the advancement of storage duration. However, MT-treated peaches exerted less changes (Figure 7(b)). Results revealed significant differences in fruit firmness from 7 d onwards. The firmness of MT-treated fruit was found to be 8.57, 25, and 150% higher than that of the nontreated fruit. In addition to firmness, an equally important characteristic determining the quality of the fruit is its colour.

The fruit colour is expressed via L^*, a^*, b^*, hue angle (h°), and chroma (C). L^* denotes the lightness in fruit, where the negative value means the fruit has lightness in fruit and the positive value depicts darkness. The L^* value decreased in storage and showed an irregular pattern, whereas it is worth mentioning that MT-treated fruit showed delayed darkening as the values moved towards positive at a slower rate. a^* depicts redness and greenness of fruit, where the positive value depicts redness and the negative value depicts greenness. At 0^{th} day of storage, both groups show negative values of a^* denoting greenness. With the commencement of cold storage, peaches of both groups start becoming red; however, it is to be noted that MT-treated peaches show lower a^* values than the control groups on 14^{th} and 21^{st} day, denoting lower fruit redness. b^* denotes yellowness and blueness of fruit, where the positive b^* value means yellowness and the negative value means blueness. MT-treated peaches showed a more positive value than the control group, depicting more fruit yellowness. The C value tells us whether the fruit is bright or dull by having a positive and negative value, respectively. h° showed an increasing pattern in both groups, its value reaching a maximum on day 7 (Table 2).

3.3.2. Total Soluble Solids, Titratable Acidity, TSS: Acid Ratio, and pH. An increasing trend was recorded for TSS with progression in storage duration in both control and treated fruit (Figure 8(a)). TSS was 1.9, 17.19, and 14.67% higher in the nontreated fruit than in the MT-treated one. A decreasing trend in TA was observed in both treated and control fruit. MT-treated fruit showed significantly 25.71%, 75%, and 75.2% higher TA on days 7, 14, and 21, respectively (Figure 8(b)). The TSS: TA ratio was found to be increased in both control and treated fruit in storage, denoting the increase in maturity (Figure 8(c)). Significant differences in the TSS: TA ratio could be observed only after 7 d of storage, where the ratio was 23.08, 88.89, and 11.91% lower in treated fruit on days 7, 14, and 21, respectively. MT treatment was successful in reducing the TSS: TA ratio, thus delaying maturity and enhancing shelf life. Organic acids are constantly utilized in various other internal processes, leading to a decrease in the pH of the fruit juice. pH showed an increasing trend from day 7 onwards, being 21.43, 26.01, and 20% higher in control than in the treated fruit on 7, 14, and 21 days of storage, respectively (Figure 8(d)).

3.4. Malondialdehyde. Results showed a significant difference between the control and treated groups from day 14 onwards. MDA content was 26.32% and 17.39% lower in the MT-treated fruit on days 14 and 21, respectively, when compared to the control (Figure 9).

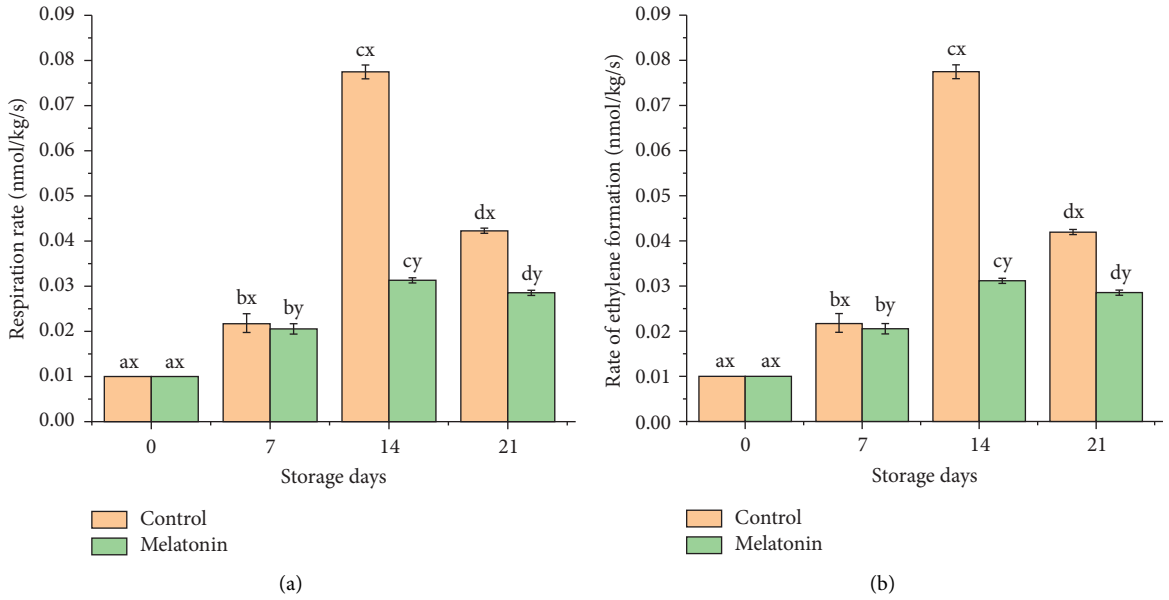

Figure 6: Effect of 0 μM (control) or 100 μM (treated) melatonin on the (a) respiration rate and (b) ethylene production rate in peach fruit stored at 2 ± 1°C for 21 days followed by a simulation period of 3 days at ambient temperature. The letters a, b, and c indicated significant differences following the application of MT with 100 μM as based on Tukey's test ($p < 0.05$).

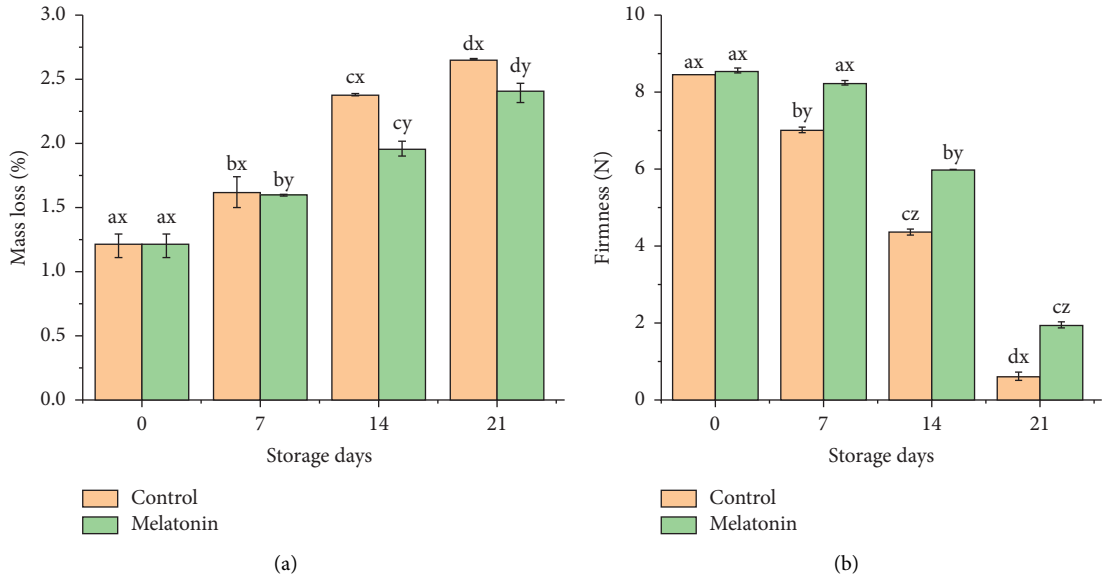

Figure 7: Effect of 0 μM (control) or 100 μM (treated) melatonin on the (a) weight loss and (b) firmness of peach fruit stored at 2 ± 1°C for 21 days followed by a simulation period of 3 days at ambient temperature. The letters a, b, and c indicated significant differences following the application of MT with 100 μM as based on Tukey's test ($p < 0.05$).

3.5. Proline Content, P5CS, OAT, and PDH Enzyme Activity. An increase in proline concentration was observed both in the control as well as treated fruit with the advancement of storage duration. However, MT application resulted in 13.27, 31.43, and 17.5% higher proline accumulation on days 7, 14, and 21, respectively. This trend is related to CII. A significant difference was observed on days 7, 14, and 21 between control and MT-treated fruit (Figure 10(a)). The activity of P5CS expressed in $U \cdot kg^{-1} \cdot s^{-1}$ was enhanced by MT treatment. A significant change was observed in the fruit on days 14 and 21 when MT-treated fruit experienced 26% and 37.93% higher P5CS enzymatic activity (Figure 10(b)). OAT expressed in $U \cdot kg^{-1} \cdot s^{-1}$ was observed to increase in control as well as in MT-treated groups; however, MT-treated fruit recorded 31.03, 51.67, and 50% higher OAT enzymatic activity on days 7, 14, and 21, respectively (Figure 10(c)). MT-treated fruit showed significant changes in the PDH enzymatic activity expressed in $U \cdot kg^{-1} \cdot s^{-1}$, which was 59.25, 59.37, and 31.43% lower in the treated fruit than in the control. Thus, MT was capable of decreasing the PDH activity in the peach fruit (Figure 10(d)).

Table 2: Effect of 0 μM (control) or 100 μM (treated) melatonin on the colour parameters in peach fruit during storage at 2 ± 1°C for 21 days followed by a simulation period of 3 days at ambient temperature.

Day	Melatonin treatment (μM)	L^*	a^*	b^*	h°	C
0	0	68.35 ± 0.98^{ax}	-2.37 ± 3.27^{a}	31.32 ± 3.27^{a}	85.67 ± 0.71^{a}	31.41 ± 0.91^{a}
	100	66.20 ± 0.68^{ay}	-2.35 ± 3.35^{a}	31.17 ± 2.27^{a}	85.68 ± 0.15^{a}	31.26 ± 0.32^{a}
7	0	62.43 ± 1.44^{bx}	-1.17 ± 3.35^{bx}	29.78 ± 3.13^{bx}	87.75 ± 1.29^{bx}	29.80 ± 1.65^{bx}
	100	60.24 ± 1.0^{by}	-1.89 ± 1.17^{by}	31.00 ± 1.27^{by}	86.5 ± 1.78^{by}	31.06 ± 2.1^{by}
14	0	61.31 ± 1.99^{cx}	4.58 ± 2.04^{cx}	22.87 ± 2.07^{cx}	78.67 ± 1.44^{cx}	23.32 ± 2.1^{cx}
	100	58.71 ± 0.61^{cy}	2.34 ± 1.56^{cy}	27.65 ± 1.21^{cy}	85.16 ± 1.04^{cy}	27.75 ± 2.6^{cy}
21	0	55.36 ± 1.8^{dx}	6.76 ± 2.71^{dx}	20.97 ± 1.52^{dx}	72.13 ± 0.23^{dx}	22.03 ± 1.22^{dx}
	100	57.91 ± 1.46^{dy}	4.67 ± 1.23^{dy}	25.43 ± 1.11^{dy}	79.59 ± 0.71^{dy}	25.86 ± 1.45^{dy}

L^* denotes lightness/darkness, a^* denotes redness/greenness, b^* denotes yellowness/blueness, h° denotes the hue angle, and, C denotes chroma; letters a, b, and c indicate significant differences as based on Tukey's test ($p < 0.05$).

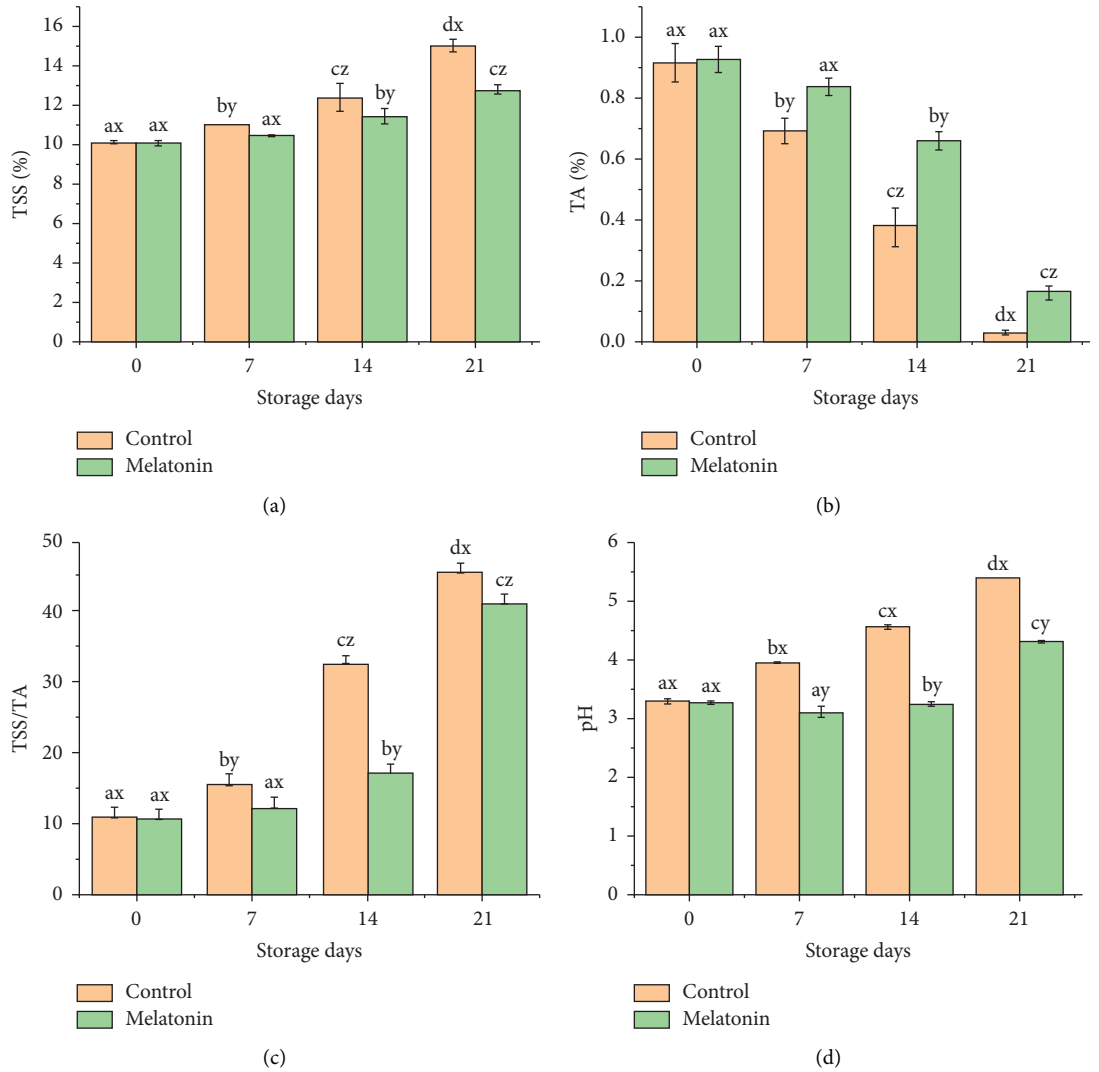

Figure 8: Effect of 0 μM (control) or 100 μM (treated) melatonin on (a) total soluble solids, (b) titratable acidity, (c) TSS/TA ratio, and (d) pH in peach fruit stored at 2 ± 1°C for 21 days followed by a simulation period of 3 days at ambient temperature. The letters a, b, and c indicated significant differences following the application of MT with 100 μM as based on Tukey's test ($p < 0.05$).

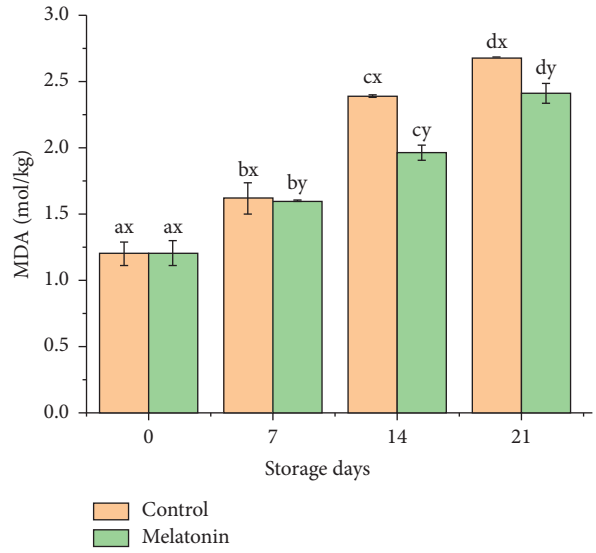

Figure 9: Effect of 0 μM (control) or 100 μM (treated) melatonin on the malondialdehyde concentration in peach fruit stored at 2 ± 1°C for 21 days followed by a simulation period of 3 days at ambient temperature. The letters a, b, and c indicated significant differences following the application of MT with 100 μM as based on Tukey's test ($p < 0.05$).

3.6. Determining Principal Components. The scree plot (Figure 1) shows that approximately 98.06% of the information (variances) contained in the data is retained by the first five principal components. So we might want to stop at the fifth principal component.

3.7. Correlation Analysis. The correlation coefficient values between all the physiological parameters are shown in Figure 2. It is known as the correlation plot which shows the relation between all variables. Positively correlated variables are grouped together. Negatively correlated variables are positioned on opposite sides of the plot origin (opposed quadrants). From the plot, it was concluded that the respiration rate, ethylene production, pH, CII, TSS, the TSS/TA ratio, MDA, and PDH are positively correlated, whereas firmness, P5CS, OAT, proline, and mass loss are negatively correlated.

3.8. Contribution of Variables. The contributions of variables in accounting for the variability in a given principal component are expressed in percentage. Variables that are correlated with PC1 (i.e., Dim. 1) and PC2 (i.e., Dim. 2) are most important in explaining the variability in the dataset. Variables that do not correlate with any PC or correlated with the last dimensions are variables with low contribution and might be removed to simplify the overall analysis. The larger the value of the contribution, the more the variable contributes to the component. Figure 3 reveals that proline, MDA, TSS, firmness, the respiration rate, ethylene production, CII, PDH, and L^* contribute greatly to the extended shelf life of MT-treated peaches.

3.9. Principal Component Analysis. Loading values and scores in PCA enabled visualization of similarities and differences in physicochemical parameters of the studied peach samples. PCA allowed understanding which parameters contributed the most. The two components accounted for 83.70% of the variation in the dataset. The most influential parameters on first principal component 1 (PC1) accounted for 66.20% of variance (Figure 3). These parameters were MDA, CII, firmness, TSS, TA, P5CS, mass loss, and the TSS/TA ratio. The main contributions to second principal component 2 (PC2), which accounted for 17.50% of variance, were PDH, OAT, proline, the respiration rate, ethylene production, and L^*.

4. Discussion

Peach being a climacteric fruit has a limited shelf life due to which it is advisable to keep it at a cold temperature. Continuous chilling temperatures above the freezing point of the fruit cause CI, which is the main hindrance responsible for decreasing its postharvest life and quality. A decreasing trend in CI has been reported in various fruit when treated with exogenous MT. Hundred μM MT-treated pomegranate [34] and 100 or 200 μM MT-treated tomato [4] experienced alleviation in CI due to prestorage MT application. MT treatment was studied in relation to CI reduction in mango fruit and established the relationship with proline metabolism, γ-aminobutyric acid shunt pathway, fatty acid composition, and internal cell energy status [6–8, 11, 25]. Sevillano et al. [35] stated the contribution of various molecular, physiological, and hormonal mechanisms to this discrepancy. There are several factors that are responsible for chilling tolerance regulation in peach fruit, among which CO_2 is the most common. Carbon dioxide gives rise to ethylene which is an essential plant hormone responsible for ripening and senescence. Carbon dioxide acts as a cofactor for 1-aminocyclopropane-1-carboxylic (ACC) oxidase [15, 27]. 1-Aminocyclopropane-1-carboxylic (ACC) is the key enzyme for ethylene production and suppressed at cold

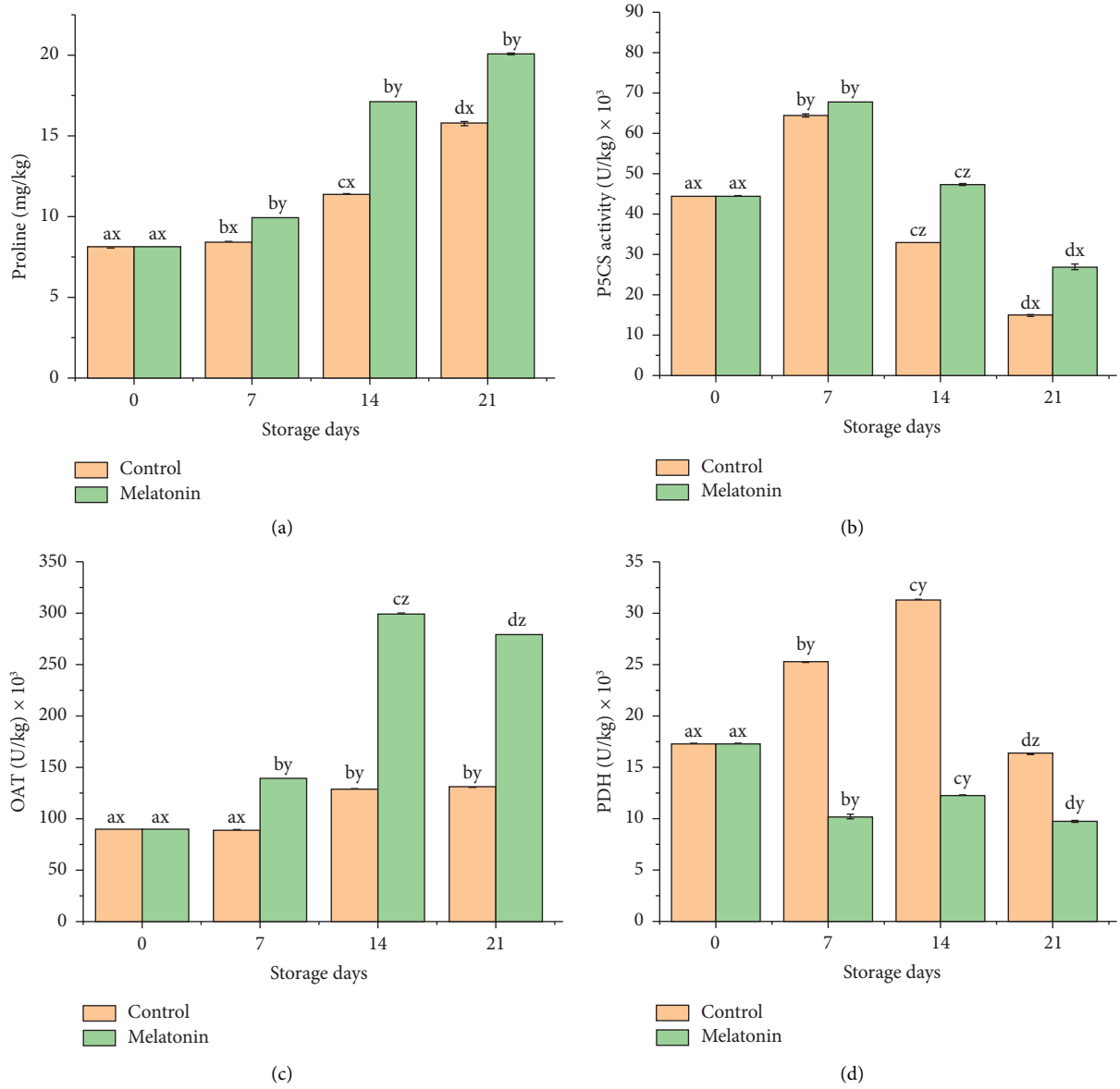

Figure 10: Effect of 0 μM (control) or 100 μM (treated) melatonin on (a) proline content, (b) Δ^1-pyrroline-5-carboxylate synthetase (P5CS), (c) ornithine-δ-aminotransferase (OAT), and (d) proline dehydrogenase (PDH) activity in peach fruit stored at 2 ± 1°C for 21 days followed by a simulation period of 3 days at ambient temperature. The letters a, b, and c indicated significant differences following the application of MT with 100 μM as based on Tukey's test ($p < 0.05$).

temperatures. Keeping the fruit at room temperature reconditions it and results in a rapid ethylene increase due to ACC production (Figure 6(b)). This, in turn, enhances the occurrence of CI [35]. Here, MT is found to delay the climacteric rise in the fruit. Thus, exogenous MT application was capable of shifting the climacteric rise.

The synergistic effect of ripening and catalytic cell wall enzymes such as endo-1,4-β-D-glucanase, polygalacturonase, and pectin esterase contributes to fruit softening. The cell wall mainly comprises glycoproteins, cellulose, hemicellulose, and calcium pectate. Fruit firmness depends on wall-to-wall adhesion of cells. As ripening progresses, dissolution of pectin starts taking place. Enzyme pectinase breaks down pectin present in the middle lamella of the fruit cell wall and results in softening of the fruit. Thus, the significant difference in firmness between both groups contributed to the delayed respiration rate. The L^* value greatly affects the acceptability of the fruit in the market. With the commencement of storage, the ongoing biochemical processes of fruit lead to the production of enzymes, which turns the shade of fruit from light to dark. A lighter shade in MT-treated peaches denotes that MT successfully slowed down biochemical processes leading to a reduction in enzymatic production. The a^* and b^* values show that MT-treated fruits experienced lower ripening stress and remained greener/yellower when compared to the control. Throughout cold storage, MT-treated peaches experienced a higher C value than the control, denoting that

MT successfully maintained the brightness of the fruit. h° was higher in MT-treated peaches, denoting MT was successful in maintaining the yellow colour of peaches and delayed its change from yellow to green.

A lower value means the colour of the fruit is close to dark, and a higher value represents its lightness. Due to various ongoing processes, the storage of fruit leads to the development of various monosaccharides, mainly glucose, and fructose. Being a climacteric fruit, peach experiences rapid ripening and respiration, leading to the metabolization of complex carbohydrates to simple sugars. These simple sugars mainly include glucose and fructose, which contribute to an increase in TSS for both groups [6–8, 25]. Ongoing respiration leads to the depletion of organic acids, leading to the reduction in the pH of fruit juice. The decreasing trend in TA of both groups can be attributed to several metabolic processes in the fruit. As respiration occurs, it utilizes available organic acids as its substrate and contributes to TA reduction [25]. MDA is known to be the final product of lipid peroxidation and hence acts as a reliable indicator for the same [11]. Progressive fruit ripening leads to reactive oxygen species (ROS) formation, which when produced in excess enhances oxidative stress. In order to counter this deleterious process, enzymatic and non-enzymatic antioxidant systems are ramped up. ROS species are highly active, which initiate the process of lipid peroxidation, leading to the formation of various end products, such as MDA, alkanes, alcohols, epoxides, and lipid alkoxyl [8]. Lipoxygenase triggers the activity of lipid peroxidation resulting in cell membrane destabilization. Thus, a decrement in the membrane integrity of the control fruit is linked to an increment in the MDA content. These results agree with previous studies which reported positive results in CI alleviation due to low MDA activity in litchi fruit treated with 100 μM MT [36].

Proline is an amino acid that is nonpolar in nature and acts as an osmotic adjustment substance against unfavorable conditions [11]. It plays an essential role as a nonenzymatic antioxidant, molecular chaperone, energy supplier, and signaling molecule in plants. The results obtained favor previous studies which reported better stress tolerance and alleviation of CI by proline accumulation [2, 11, 37, 38]. A recent study by Aghdam et al. [39] has documented MT treatment of 100 μM for 5 min and 400 μM MT for 15 min to mitigate CI by initiating proline accumulation in tomato and litchi, respectively. Bhardwaj et al. [11] noticed 1.39 times increased proline content in 100 μM MT-treated "Langra" mangoes (for 2 h) on 28 d of storage, and it is correlated with the decrease in CI incidence. In agreement with the above studies, the current work confirms that administering peaches to 100 μM MT for 2 h results in a significant increase in proline levels. The observed CII pattern is in accordance with the proline accumulation trend. Furthermore, the evident reduction in MDA concentration, weight loss, and chilling symptoms followed by increased levels of proline in MT-treated peaches confirms the antioxidative and protective nature of amino acid, proline. P5CS is the key enzyme that plays a major role in the synthesis of proline through the glutamate pathway [11]. It is a dual function and rate-limiting enzyme, resulting in the synthesis of glutamate-derived proline. It acts as a catalyst in the formation of Δ^1-pyrroline-5-carboxylate (P5C) from the cyclization of glutamate-γ-semialdehyde (GSA) which is followed by the production of $NADP^+$. $NADP^+$ produced is reduced to NADPH by its coupling with the pentose phosphate pathway (PPP), thus ensuring an uninterrupted reducing power for various cell processes [11]. The biosynthesis pathways of phenylalanine and tryptophan are also supplied, which later leads to an increment in MT and phenol accumulation. Thus, these molecules form an important part of the plant's antioxidant system [8]. OAT through the ornithine pathway contributes to proline synthesis. In accordance with the previous studies on litchi [5], this present study confirms that exogenous MT application to fruit significantly enhances the activity of P5CS and OAT, contributing to the accumulation of proline. PDH is known as the rate-limiting enzyme as it forms glutamic acid by catalyzing proline [40]. It causes hindrance in protein accumulation. In accordance with previous studies in tomatoes [39], litchi [5], and mangoes [11], MT was successful in lowering PDH activity. Therefore, MT-treated peaches increased the P5CS and OAT activity, which in turn decreased the PDH activity, thus adding to their increased chilling tolerance.

Proline being a non-enzymatic antioxidant was found to play an important role in the mitigation of CI in peaches. MT was found to activate P5CS and OAT, initiating glutathione and ornithine pathways, which in turn suppressed the formation of PDH, which led to the increment in proline levels. The biosynthesis pathways of tryptophan and phenylalanine also contributed to MT accumulation, thus enhancing its performance. A parallel trend was obtained between the proline levels and the occurrence of CII. Proline was found to decrease the MDA content as it lowered excess ROS production, thus preventing lipid peroxidation and electrolyte leakage of the fruit cell wall. In a nutshell, MT application to fruit activated its antioxidant system, where many antioxidants including proline came into action and aided in mitigating the symptoms of chilling injury and increased chilling tolerance.

MT in peaches has been exogenously applied to elucidate many pathways, including phenolic pathways, membrane fatty acid regulation, oxidative damage pathway, sucrose metabolism, cell wall disassembly, and GABA shunt pathway; however, the proline pathway still remains unexplored. Further studies are required at the transcriptomic and genomic levels to elucidate the complex molecular network and proposed biochemical pathways of melatonin in peaches in response to chilling injury.

5. Conclusion

The present study focused on the effect of exogenous MT application on the alleviation of CI in the "Silver King" peach cultivar stored at $2 \pm 1^{\circ}$C and 90–95% relative humidity for 21 d. Melatonin-treated fruit experienced a 78.8% lower CI than the control fruit; moreover, they were recorded to experience a 60% lower respiration rate, 1.5 times higher

firmness, 17.19% lower TSS, 75.2% higher TA, 26.01% lower pH, 26.32% reduction in MDA levels, and 31.43% higher proline accumulation. P5CS, OAT, and PDH were found to be the key enzymes for proline synthesis. The partial CI tolerance efficacy of MT treatment shown in "Silver King" peach fruit might be due to proline accumulation in the fruit.

Data Availability

The data that support the findings of this study are available from the corresponding author on request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

Authors' Contributions

Hansika Sati worked on the methodology, conducted the experimental research, and wrote the original draft. Renu Bhardwaj derived the methodology. Olaniyi Amos Fawole reviewed and edited the draft. Sunil Pareek supervised the study and reviewed the draft. All the authors have read and agreed to the published version of the manuscript.

Acknowledgments

The authors are thankful to the National Institute of Food Technology Entrepreneurship and Management, Kundli, India, for providing contingencies, facilities, and support. The authors thank Sarvanan Mani, lab in-charge of Biochemistry, NIFTEM-K, for providing his constant support throughout the research work. In addition, the authors would like to acknowledge Dr. Vikas Singh at Montpellier Business School, France, for his guidance in data analysis. This work was supported by the National Institute of Food Technology Entrepreneurship and Management, Kundli, Sonipat, Haryana, India.

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