





Research Article

A Melatonin Treatment Delays Postharvest Senescence, Maintains Quality, Reduces Chilling Injury, and Regulates Antioxidant Metabolism in Mango Fruit

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The effects of an exogenous application of various concentrations and dipping duration of melatonin (MT) treatment on postharvest senescence, quality, chilling tolerance, and antioxidant metabolism of mango fruit cv. “Dashehari” were examined. Fruits were treated with three concentrations of MT (50, 100, or 150 μM), each applied for three times (60, 90, or 120 min), followed by storage at $5 \pm 1^\circ\text{C}$. The MT concentration of 100 μM with a dipping duration of 120 min was efficient in reducing the chilling injury and maintaining the quality of mango fruit for 28 d. Effects of this treatment were due to its effectiveness in reducing metabolic activity, specifically, respiration rate and ethylene production, resulting in higher firmness, titratable acidity, and ascorbic acid content and lower weight loss, total soluble solids, pH, and total soluble solid: acidity ratio. Moreover, it maintained a higher concentration of total phenolics and total flavonoids, as well as antioxidant capacity (2,2-diphenyl-1-picrylhydrazyl and cupric reducing antioxidant power assays), as compared to other treatments. This was further confirmed with higher activities of antioxidant enzymes superoxide dismutase and catalase and membrane stability (according to a lower malondialdehyde content and lipoxygenase activity). Thus, our data show that a 100 μM MT administered for 120 min appears to be the most appropriate treatment to maintain the quality of mango fruits stored at chilling temperatures.

1. Introduction

Mango (*Mangifera indica*) is one of the most nutritive and commonly consumed fruits in tropical and subtropical regions. Its attractive color and aroma, rich flavor, and high nutritional value have made some authors refer to it as the “king of fruits” [1]. Mango is an excellent source of bioactive compounds and nutrients like minerals, provitamin A, carotenoids, ascorbic acid, phenolic compounds, and various

other antioxidants [1]. Mango is a climacteric fruit that ripens quickly at ambient temperature, due to its high respiration rate and ethylene production, consequently having a short shelf life of only 5 to 6 days under this condition. Seasonal availability further restricts its marketability to only a few days, although this period can be extended with low-temperature storage at around 13°C . However, the temperature must be carefully regulated, since signs of chilling injury (CI) begin to develop if fruits are

stored at $<13^{\circ}\text{C}$ [2]. The major signs of CI include peel discoloration in the form of darkening or blackening, pitting, and abnormal ripening, while their severity becomes more prominent when fruits are transferred to room temperature after low-temperature storage [3].

Various studies have shown that CI in mango can be alleviated to some extent and with some constraints. For example, treatments of chitosan and polyamine spermidine [4] and nitric oxide [5] resulted in low ethylene and CO_2 production but also induced poor color development. Ethrel [6], low-temperature conditioning [7], and methyl jasmonate [8] have resulted in increased ripening, while limited CI control has been reported with treatments of oxalic and salicylic acids [9].

Melatonin (N-acetyl-5-methoxytryptamine, MT) has the same auxin function as the hormone indole-3-acetic acid, due to their similarity in biosynthetic pathways [10]. MT has been shown to regulate the stress response in plants through its endogenous accumulation [11], increasing enzymatic and nonenzymatic antioxidant system [12], and promotion of γ -aminobutyric acid (GABA) shunt pathway activity [13]. MT also stimulates the activity of the oxidative pentose phosphate pathway [14], decreases biosynthesis of ethylene and cell wall degradation [15], and maintains higher nitric oxide, polyamine, and proline concentrations [16], all of which mitigate the development of CI signs.

Exogenous MT applications have been recently studied in order to determine their effects on postharvest physiological characteristics and shelf life of various fresh commodities. For example, Hu et al. [17] report that a postharvest immersion treatment of MT (0.05 to 0.5 mM) on banana fruit suppressed ethylene biosynthesis and hydrolysis of starch, findings that were associated with delayed ripening. Zhai et al. [15] observed that an MT (0.1 mM) immersion treatment suppressed the expression of *PcACS1*, *PcACO1*, and *PcPG* in pear fruit, which also delayed ripening and decreased occurrence of physiological issues. There are a couple of opposing perceptions to those acquired from bananas and pears. For instance, Sun et al. [18] revealed that exogenous MT treatment at 0.05 mM induced the ethylene climacteric and advanced ethylene flagging transduction in tomatoes. In nonclimacteric grapes, the use of exogenous MT (0.1 mM) on unripe berries promoted aging by increasing the endogenous ethylene levels [19]. These contrasting discoveries suggest that the effects of MT on ripening and senescence are likely to vary among various agricultural commodities.

To the best of our knowledge, only a few comparable studies are available in mango fruit. For example, a 1 mM MT treatment preserved certain postharvest quality parameters, by inhibiting phenolic oxidation and stimulating cellular integrity during cold storage of about a month at 15°C [20]. Application of MT (0.5 mM for 1 h) to “Guifei” mangoes effectively delayed the changes in ripening parameters like firmness, pulp color, β -carotene levels, total soluble solids (TSS), titratable acidity (TA), and respiration rate. MT delayed climacteric ethylene production and 1-aminocyclopropane-1-carboxylic acid (ACC) levels in mango fruit during storage at 25°C . However, to the best of

our knowledge there is no study found with regard to the optimization of MT concentration and impact on CI, postharvest physiology, quality, and shelf life of mango fruit stored at a cold temperature of $5 \pm 1^{\circ}\text{C}$.

The objective of the present study was to find out the optimum concentration and dipping duration applied to mango cv. “Dashehari” stored at $5 \pm 1^{\circ}\text{C}$, which can delay senescence, maintain postharvest quality, alleviate CI, and exert effects on its antioxidant metabolism. The assessment of the best combination of concentration and dipping duration was decided on the basis of postharvest physiological, quality, and ripening parameters, enzymatic and nonenzymatic antioxidant activities, and effects on signs of CI.

2. Materials and Methods

2.1. Fruits and Treatment. Mango cv. “Dashehari” fruits at physiological maturity but unripened stage (full shoulder development, firmness 29 ± 1 Newton at the time of harvest, however, data presented here is after 3 d of shelf life) were hand-harvested, from an orchard of a local producer in Sonapat, Haryana, India. Fruits were transported within 1 h after harvest to the postharvest laboratory via cargo vehicle, under controlled temperature (20°C). Total 1200 fruits were used, which were free from apparent defects and injuries and of homogenous size and maturity. They were disinfected by dipping in a 1% (v/v) sodium hypochlorite solution for 2 min, rinsed with distilled water, shade-dried, and then randomly divided into 10 groups ($n = 120$). Fruits from the first group were dipped in distilled water (control), while the remaining ones were treated with different MT concentrations (50, 100, or $150 \mu\text{M}$) and dipping durations (90, 120, or 150 min), under the low light condition at $25 \pm 1^{\circ}\text{C}$. The range of melatonin concentration around $100 \mu\text{M}$ was decided as per the previous studies done by Zhai et al. [15], Cao et al. [21], Gao et al. [22], Aghdam et al. [23], and Zhu et al. [24]. The dipping duration was considered in the range of 1 and 2 h in combination with the above-mentioned MT concentrations. Therefore, on the basis of previous studies of Hu et al. [17], Cao et al. [21], Aghdam et al. [23], and the recent study done with mango fruit by Liu et al. [25], we have chosen three dipping durations for exogenous MT treatment. In brief, the remaining groups were dipped in $50 \mu\text{M}$ for 90 min (T1), $50 \mu\text{M}$ for 120 min (T2), $50 \mu\text{M}$ for 150 min (T3), $100 \mu\text{M}$ for 90 min (T4), $100 \mu\text{M}$ for 120 min (T5), $100 \mu\text{M}$ for 150 min (T6), $150 \mu\text{M}$ for 90 min (T7), $150 \mu\text{M}$ for 120 min (T8), and $150 \mu\text{M}$ for 150 min (T9). After removal from distilled water or MT solution, fruits were air-dried for 2 h at room temperature and immediately stored at $5 \pm 1^{\circ}\text{C}$ (relative humidity 85–90%). Weight and color measurements of fruits from each group were taken at 7 d intervals after being moved out of cold storage. All other parameters were measured at 7 d intervals, followed by a 3 d shelf life at room temperature of 25°C .

2.2. Chilling Injury (CI) Index. CI index was determined as described by Concellón et al. [26] with some modifications. The symptoms considered for CI index assessment include

discoloration of the skin, greyish scald, poor flavor, or pitting in mangoes. CI index was assessed based on the injured area on a scale of 0 to 5 where 0 = no injury, 1 = 1 to 20%, 2 = 21 to 40%, 3 = 41 to 60%, 4 = 61 to 80%, and 5 = 81 to 100%. CI index was expressed as percent (%) and calculated in triplicate (where each replicate consists of 10 fruits) according to the following formula:

$$\text{CI index} = \frac{\sum (\text{CII rank score} \times \text{number of fruits in each rank scale})}{\text{total number of fruits observed}} \quad (1)$$

2.3. Ethylene Production and Respiration Rate. An airtight glass container (800 mL) was used to quantify ethylene production, which was measured in the container's head-space by piercing the probe of an ethylene analyzer (Bio-consevacion, Spain) into it. The measurements of the apparatus range from 0 to 100 ppm through the septum fixed to the container lid. Ethylene production was measured after 2 h at 20°C in triplicate ($n=3$) and was expressed as $\text{nmol kg}^{-1} \text{ s}^{-1}$ by using the following formula:

$$\text{ethylene production} = \frac{\text{ethylene produced} \times \text{head space volume}}{\text{fruit weight (kg)} \times \text{time (s)}} \quad (2)$$

Respiration rate was measured in triplicate ($n=3$). The assessment was done by piercing the probe of an autogas analyzer (PBI Dansensor, Denmark) into the container described for ethylene production, through the rubber septum fixed on the lid of the container. Measurements were taken after 2 h at 20°C, and production of CO_2 was expressed in $\text{nmol kg}^{-1} \text{ s}^{-1}$ by using the following formula:

$$\text{respiration rate} = \frac{\text{CO}_2 \text{ produced} \times \text{head space volume}}{\text{fruit weight (kg)} \times \text{time (s)}} \quad (3)$$

2.4. Physicochemical Evaluations

2.4.1. Weight Loss. Weight loss was calculated as the difference between the initial and final weight of fruit (10 fruit) at weekly intervals. The results were expressed in terms of percentage (%) of initial fresh weight.

2.4.2. Firmness. Fruit firmness was measured using a texture analyzer (TA.HDplus, Stable Micro Systems, UK) equipped with a 2 mm diameter probe, which was set to a pretest speed of 1.5 mm s^{-1} , test speed of 0.5 mm s^{-1} , and posttest speed of 10 mm s^{-1} . Measurements were taken to a penetration depth of 5 mm at two opposite points in the equatorial region in triplicate, where each replicate consists of 3 fruits. Results were expressed in Newton (N).

2.4.3. Color. Peel color was determined based on the L^* , a^* , and b^* color system, where the value of L^* represents darkness or lightness, the value of a^* represents greenness (−) or redness (+), and the value of b^* represents yellowness (+) or blueness (−). These parameters were measured using a hand-held CR-400 colorimeter (Konica Minolta Sensing Inc., Osaka, Japan), based on the CIE system. Each fruit was

measured on the equatorial axis of two opposite sides and observations were taken in triplicate with 3 fruits in each replicate. The values of a^* and b^* were used to calculate chroma and hue angle, according to the following formula:

$$\text{chroma} = (a^{*2} + b^{*2})^{1/2}, \quad (4)$$

$$\text{hue angle} = \tan^{-1} \frac{b^*}{a^*}$$

2.4.4. Total Soluble Solids (TSSs), Titratable Acidity (TA), TSS/TA Ratio, and pH. TSSs were measured in fruit pulp that was squeezed through a muslin cloth. A drop of filtered juice was analyzed using a hand-held refractometer (Atago, Japan) at 20°C and expressed as percent (%) in triplicate, with 3 fruits in each replication. The method for the determination of titratable acidity (TA) was based on the AOAC [27]. For this, 10 g of mango pulp was homogenized (IKA T18 Digital Ultra-Turrax, Cole-Parmer, India) in 100 mL of distilled water and filtered. Afterward, 10 mL of the filtrate was titrated against 0.1 N NaOH after the addition of 1% (v/v) phenolphthalein. Titration was performed until the endpoint (appearance of light pink color) was reached in triplicate ($n=3$). The TA was expressed as percent (%). The ratio of TSS-to-TA (TSS/TA) was determined by dividing the value of TSS by that of TA. The pH of mango juice was measured with a pH meter (Cyberscan, Eutech Instruments, Canada) calibrated with standard buffers of pH 7 and 4.

2.5. Ascorbic Acid. The content of ascorbic acid in mango pulp was determined according to the method of Malik and Zora [28], with minor modifications. Frozen pulp (0.5 g) was homogenized (IKA T18 Digital Ultra-Turrax, Cole-Parmer, India) with 5 mL of 6% (w/v) metaphosphoric acid (MPA) containing 0.18% (w/v) ethylene diamine tetra-acetic acid (EDTA). The homogenate was centrifuged (3–18KS, Sigma, Germany) at 10,000 g for 10 min at 4°C, and the supernatant was used for further analysis. The reaction mixture consisted of 0.1 mL supernatant solution, 1 mL of 3% (w/v) MPA, and 1 mL of 10% (v/v) Folin Ciocalteu reagent (FCR), measured at 760 nm on a spectrophotometer (Specord200plus, Analytik Jena, Germany). Results were expressed as g kg^{-1} of fresh weight (FW) in triplicate with 3 fruits in each replication, in accordance with a standard curve of ascorbic acid.

2.6. Malondialdehyde (MDA). The main product of lipid peroxidation, i.e., malondialdehyde (MDA), was measured according to the procedure described by Jincy et al. [29]. Frozen pulp (0.2 g) was homogenized (IKA T18 Digital Ultra-Turrax, Cole-Parmer, India) in 0.1% (w/v) trichloroacetic acid (TCA), followed by centrifugation (3–18KS, Sigma, Germany) at 10,000 g for 10 min at 4°C. The reaction mixture consisted of 0.3 mL supernatant and 1.2 mL of 0.5% (w/v) thiobarbituric acid (TBA) prepared in 20% (w/v) TCA and was incubated for 30 min at 95°C. After this time, the reaction was stopped by submerging the tube in an ice bath for 5 min. The cold tubes were centrifuged (3–18KS, Sigma,

Germany) at 10,000 g for 10 min at ambient temperature. The absorbance of the supernatants was measured at 532 nm with a spectrophotometer (Specord200plus, Analytik Jena, Germany), from which the nonspecific absorbance at 600 nm was then subtracted. The concentration of MDA was expressed in mM kg^{-1} FW through the mean of triplicates (each replication consists of 3 fruits), using $155 \text{ mmol}^{-1} \text{ cm}^{-1}$ as extinction coefficient.

2.7. Extraction Procedure for Total Phenolic Content, Total Flavonoid Content, and Antioxidant Activity. Fruit tissues (peel or pulp) were extracted according to Castro-Concha et al. [30], with some modifications. One gram of frozen tissue was extracted with 10 mL of 80% (v/v) ethanol, followed by centrifugation (3–18KS, Sigma, Germany) at 10,000 g for 20 min at 4°C. The clear supernatant was used as a crude extract for the determination of total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity (AA).

2.7.1. Total Phenolic Content (TPC). TPC of mango tissues (peel or pulp) was determined according to Singleton and Rossi [31], with some modifications. The reaction mixture consisted of 0.1 mL crude extract and 1 mL of 10% (v/v) FCR, to which 1 mL of 15% (w/v) sodium carbonate was added after 2 min of room temperature incubation. A subsequent 90 min incubation at room temperature was performed under dark conditions, and the absorbance was recorded at 765 nm using a spectrophotometer (Specord200plus, Analytik Jena, Germany). Results were expressed in terms of the mean of triplicates with $n = 3$, and the result was expressed as g gallic acid equivalents per kg (g GAE kg^{-1}) of FW.

2.7.2. Total Flavonoid Content (TFC). TFC of mango tissues (peel or pulp) was quantified according to the method of Chang et al. [32], with some modifications. Crude extract (0.1 mL) was added to a reaction mixture that contained 0.1 mL of 1 M potassium acetate and 10% (w/v) aluminum chloride. The volume was made up to 3 mL by adding 2.8 mL of distilled water. The solution was incubated for 45 min at room temperature under dark conditions. Absorbance was then read at 510 nm on a spectrophotometer (Specord200plus, Analytik Jena, Germany), and the results were expressed as quercetin (QE) kg^{-1} of FW, calculated using a standard curve of QE and mean obtained from triplicates ($n = 3$) used to record observation.

2.7.3. Antioxidant Activity (AA). Two *in vitro* methods were used to determine the AA of free and bound hydrophilic fractions of antioxidants present in mango fruit. AA of the free fraction was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity assay, whereas that of the bound fraction was determined using the cupric reducing antioxidant power (CUPRAC) assay. All observations were analyzed in triplicate using 3 fruits in each replication.

2,2-Diphenyl-1-Picrylhydrazyl (DPPH) Radical Scavenging Activity. The DPPH assay was performed according to the method of Larrauri et al. [33], with some modifications. The reaction mixture contained 3.9 mL of 0.1 mM DPPH and 0.1 mL of crude extract and was incubated for 30 min under darkroom and ambient conditions. The absorbance was then measured at 515 nm on a spectrophotometer (Specord200plus, Analytik Jena, Germany). Free radical scavenging activity of DPPH was expressed as a percentage and calculated using the following formula:

$$\text{DPPH} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \quad (5)$$

where control is the reaction mixture with no added sample.

Cupric Reducing Antioxidant Power (CUPRAC). The CUPRAC assay was performed according to the method of Arscott and Tanumihardjo [34], with some modifications. A 0.1 mL volume of crude extract was added to the reaction mixture, which contained 0.5 mL of 10 mM cupric chloride solution and the same volume of a 7.5 mM alcoholic neocuproine solution and 1 M ammonium acetate buffer (pH 7.0). The reaction was incubated for 30 min under dark conditions at room temperature, and its absorbance was then read at 450 nm with a spectrophotometer (Specord200plus, Analytik Jena, Germany). Results were expressed as the mass of Trolox equivalents (TE) on an FW basis, which was calculated using a standard curve of Trolox.

2.8. Enzyme Activity

2.8.1. Superoxide Dismutase (SOD). Enzyme activity of superoxide dismutase (SOD, EC 1.15.1.1) was calculated according to the method of Kono [35]. For this, 1 g of fruit tissue was homogenized (IKA T18 Digital Ultra-Turrax, Cole-Parmer, India) with 10 mL of precooled (4°C) 100 mM potassium phosphate buffer (pH 7.0), followed by centrifugation (3–18KS, Sigma, Germany) at 10,000 g at 4°C for 20 min. The assay tube contained 1.3 mL sodium carbonate buffer (50 mM, pH 7.0), 0.5 mL of 96 μM nitroblue tetrazolium (NBT), 0.1 mL of 0.6% (v/v) triton-X, and 0.1 mL of crude extract. A 0.1 mL of 20 mM hydroxylamine hydrochloride (pH 6.0) was added to start the reaction, which lasted for 2 min. Enzyme activity was calculated in triplicate, where each replicate consists of 3 fruits and activity is assessed according to the reduction of NBT, which was detected by an increase in absorbance at 540 nm spectrophotometrically (Specord200plus, Analytik Jena, Germany), and expressed as U kg^{-1} protein.

2.8.2. Catalase (CAT). The activity of catalase (CAT, EC 1.11.1.6) was measured according to Aebi [36] with some modifications in a 0.1 mL enzyme extract volume, which was obtained from 1 g of fruit tissue homogenized (IKA T18 Digital Ultra-Turrax, Cole-Parmer, India) in precooled 100 mM potassium phosphate buffer (pH 7.0). This was added to a mixture containing 0.7 mL phosphate buffer (100 mM, pH 7.0) and 0.6 mL of 150 $\text{mM H}_2\text{O}_2$. CAT activity was quantified,

according to its ability to catalyze the decomposition of H_2O_2 into the water and molecular oxygen. Results were determined through the mean obtained from triplicate ($n=3$) and observation was recorded with a decrease in absorbance at 240 nm with a spectrophotometer (Specord200plus, Analytik Jena, Germany) and expressed as U kg^{-1} protein.

2.8.3. Lipoxygenase (LOX). The activity of lipoxygenase (LOX, EC 1.13.11) was determined according to the method of Todd et al. [37], with some modifications. For this, 1 g of tissue sample was homogenized (IKA T18 Digital Ultra-Turrax, Cole-Parmer, India) in 5 mL of precooled 100 mM Tris-HCl buffer (pH 7.0), which was centrifuged (3–18KS, Sigma, Germany) at 10,000 g for 20 min at 4°C. The supernatant was collected and used as a crude extract. The assay tube contained 1 mL of standard assay mixture (40 mL of 100 mM sodium phosphate buffer with 200 μL of Tween 20 and 40 μL of linoleic acid) and 0.2 mL of crude extract. The activity of LOX is calculated with triplicate (involving 3 fruits in each replication) and was expressed as U kg^{-1} protein, where U is the amount of enzyme that changes the absorbance at 234 nm min^{-1} spectrophotometrically (Specord200plus, Analytik Jena, Germany).

2.9. Protein Concentration. The protein concentration of the extracts was determined according to the method of Bradford [38], with some modifications. Fruit tissue (1 g) was homogenized (IKA T18 Digital Ultra-Turrax, Cole-Parmer, India) in 10 mL of precooled 50 mM of sodium phosphate buffer, containing 2 mM of phenylmethylsulfonyl fluoride (PMSF), 2 mM of polyvinylpyrrolidone (PVPP), and 0.5 mM of MgCl_2 . The homogenate was centrifuged (3–18KS, Sigma, Germany) at 10,000 g for 20 min at 4°C, and the supernatant was used as a crude extract. A 0.1 mL of extract was then added to the reaction mixture consisting of 5 mL of Bradford's reagent. After a 5 min incubation period at room temperature, the absorbance was read at 595 nm with a spectrophotometer (Specord200plus, Analytik Jena, Germany), and protein concentration was calculated with a standard curve of bovine serum albumin (BSA) in triplicate (where each replication consists of 3 fruits).

2.10. Statistical Analysis. Experiments were performed using a completely randomized design (CRD), with three technical replicates. The data are expressed as the average of three measurements \pm standard error (SE). SPSS (version20) software was used to analyze data with a one-way analysis of variance (ANOVA) with a 0.05 probability level. The means were further separated by using the Duncan multiple range post hoc test.

3. Results

3.1. Chilling Injury (CI). The signs of CI in the form of black-brown lesions and pitting were progressive in all groups, but with different degrees of severity (Figure 1(a)). After 7 d of

cold storage ($5 \pm 1^\circ\text{C}$) plus a 3 d shelf life period at ambient conditions, most groups had apparent signs of CI, except for T5 (100 μM MT for 120 min). The first sign of CI in this group was observed at 14 d of storage, followed by 3 d shelf life under ambient conditions. However, the MT treatment significantly ($p < 0.05$) suppressed the CI index and CI incidence, with the exception of T1 (50 μM MT for 90 min) (Figure 1(a)). Among all treatments, T5 (100 μM MT for 120 min) was the most effective in reducing CI incidence (Figure 1(b)) and CI index (Figure 1(c)) by 4.16 and 4.47 times, respectively, as compared with the control.

3.2. Respiration Rate and Ethylene Production. In the control group, a climacteric rise in respiration rate (Figure 2(a)) and ethylene production (Figure 2(b)) were observed after 21 d of cold storage. The MT-treated fruits did not show such a sudden increase at this time, although a continuous rise in both parameters was apparent in MT-treated fruits throughout the observation period of 28 d. Additionally, T5 treatment (100 μM MT for 120 min) was the most effective in reducing both respiration rate and ethylene production at 28 d of observation, as compared to all other treatments.

3.3. Physicochemical Properties

3.3.1. Weight Loss. As a result of increased metabolic activity, weight loss was also progressive during the storage period (Table 1). The control group, T1 (50 μM MT for 90 min), T2 (50 μM MT for 120 min), and T6 (100 μM MT for 150 min) had weight losses of 9.23, 9.10, 9.27, and 9.20%, respectively, after 28 d whereas T3 (50 μM MT for 150 min), T7 (150 μM MT for 90 min), and T8 (150 μM MT for 120 min) had weight losses of 10.23, 10.23, and 10.00%, respectively. It is noteworthy that T5 (100 μM MT for 120 min) had the lowest weight loss among all groups, of only 7.90%.

3.3.2. Firmness. Independent of treatment, fruit firmness progressively declined during storage due to the textural changes of the fruit; however, MT-treated fruits maintained relatively higher firmness. Most notably, T5 (100 μM MT for 120 min) had significantly ($p < 0.05$) higher firmness until the end of the storage period (Table 1).

3.3.3. Color. There was a gradual increase in L^* and chroma values in the peel of all groups, during the experimental period. However, a nonsignificant ($p < 0.05$) difference was observed in both parameters at 7 d in all treated groups (Table 2). A continuous rise in hue was observed in T5 (100 μM MT for 120 min), while a nonsignificant difference ($p < 0.05$) was observed among the other treatments (Table 2).

3.3.4. Total Soluble Solids (TSS), pH, Titratable Acidity (TA), TSS/TA Ratio, and Ascorbic Acid. Results for TSS, pH, TA, TSS/TA, and ascorbic acid are shown in Table 3. The breakdown of carbohydrates that occurs during fruit

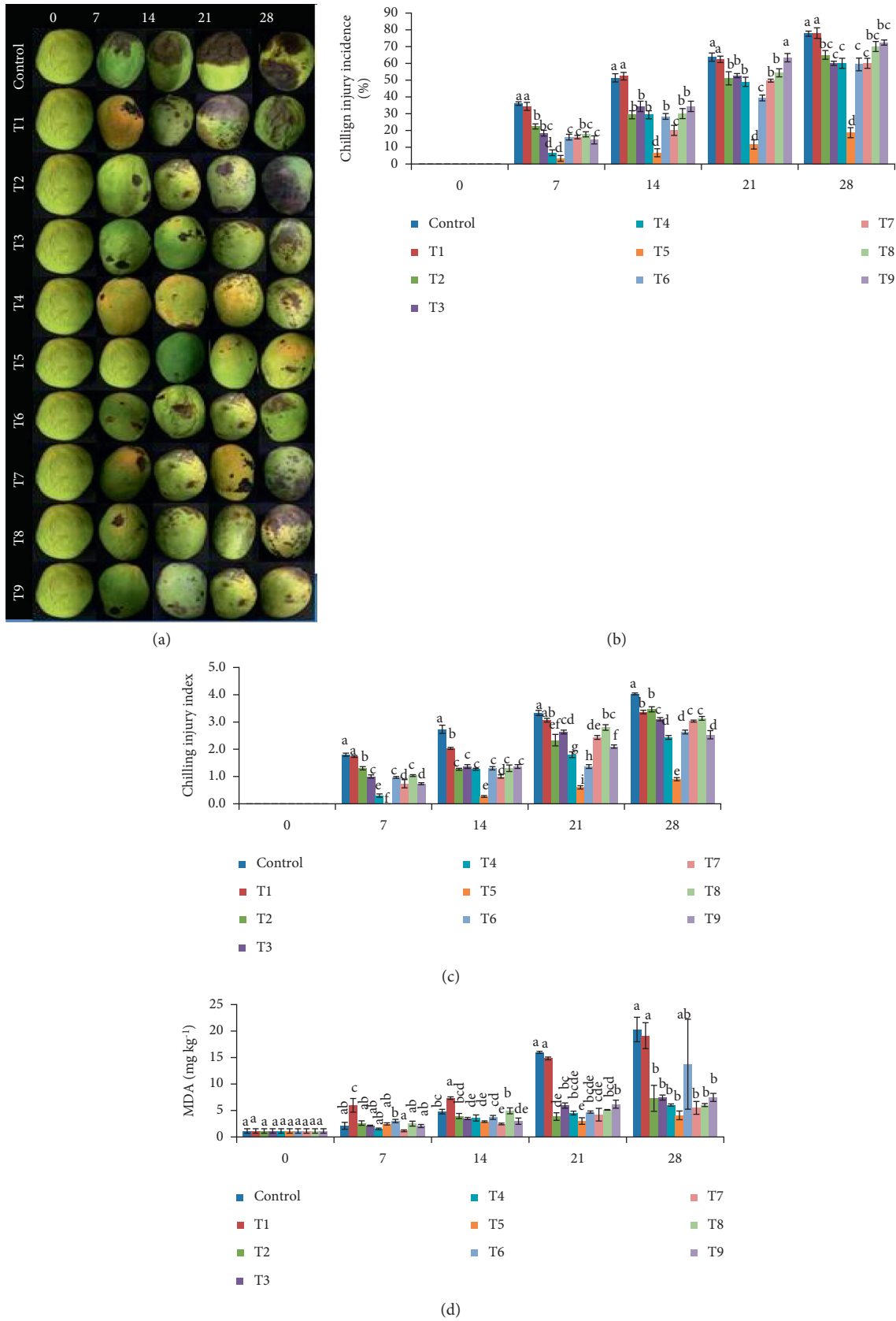


FIGURE 1: (a) Representative images, (b) chilling injury (CI) incidence, (c) CI index, and (d) malondialdehyde (MDA) of mango cv. “Dashehari” treated with melatonin (MT), followed by low-temperature storage at $5 \pm 1^\circ\text{C}$. Measurements were taken every 7 d of storage, followed by a 3 d of shelf life at room temperature. Each value is the mean of three replicates; vertical bars indicate the standard error. Different lowercase letters on the same storage period indicate a significant difference ($p < 0.05$) according to Duncan’s multiple range test. The abbreviated treatment details: 0 (control), $50 \mu\text{M}$ for 90 min (T1), $50 \mu\text{M}$ for 120 min (T2), $50 \mu\text{M}$ for 150 min (T3), $100 \mu\text{M}$ for 90 min (T4), $100 \mu\text{M}$ for 120 min (T5), $100 \mu\text{M}$ for 150 min (T6), $150 \mu\text{M}$ for 90 min (T7), $150 \mu\text{M}$ for 120 min (T8), and $150 \mu\text{M}$ for 150 min (T9).

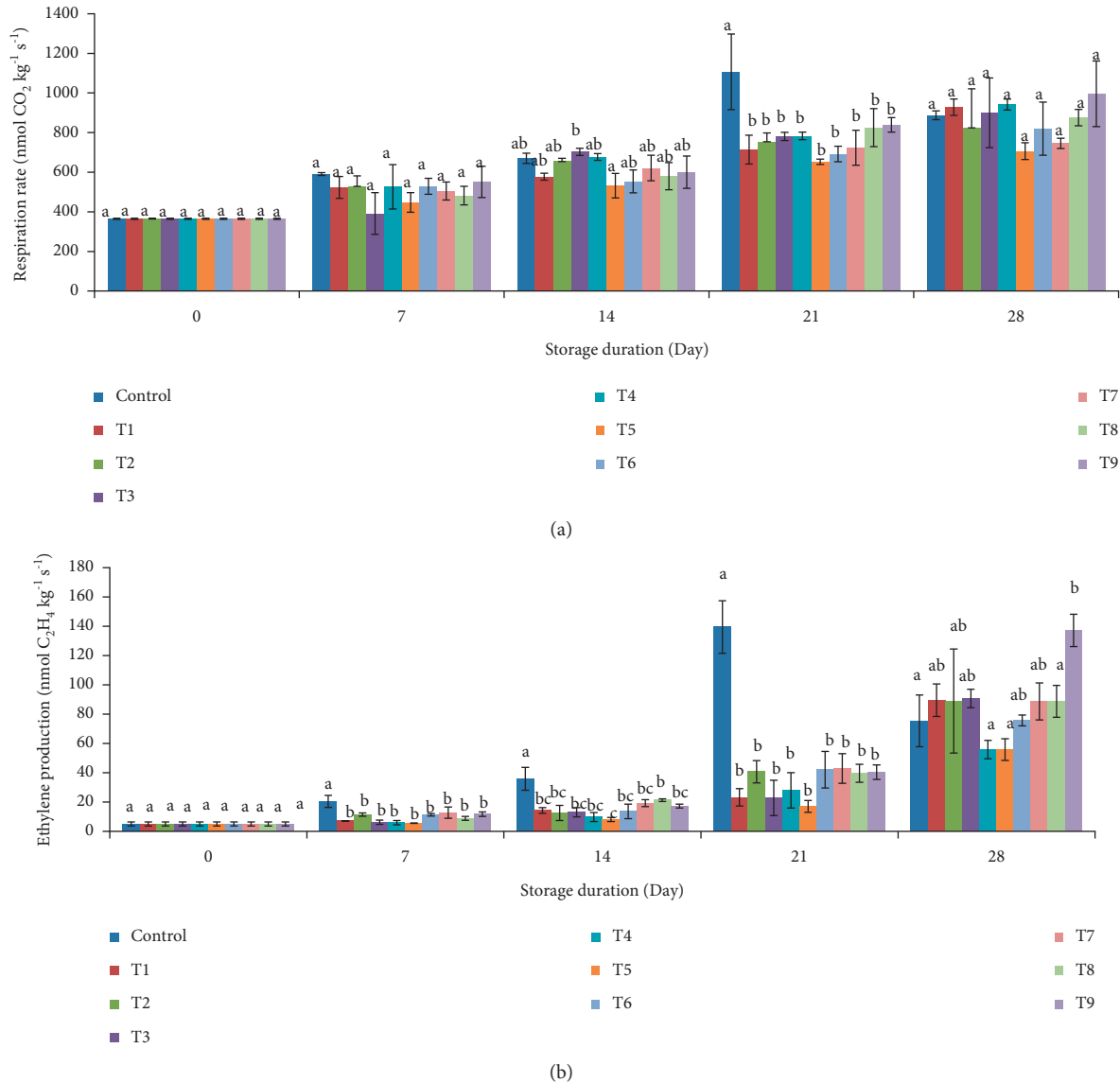


FIGURE 2: (a) Respiration rate and (b) ethylene production of mango cv. “Dashehari” treated with melatonin (MT), followed by low-temperature storage at $5 \pm 1^\circ\text{C}$. Measurements were taken every 7 d of storage, followed by a 3 d of shelf life at room temperature. Each value is the mean of three replicates; vertical bars indicate the standard error. Different lowercase letters on the same storage period indicate a significant difference ($p < 0.05$) according to Duncan’s multiple range test. The abbreviated treatment details: 0 (control), $50 \mu\text{M}$ for 90 min (T1), $50 \mu\text{M}$ for 120 min (T2), $50 \mu\text{M}$ for 150 min (T3), $100 \mu\text{M}$ for 90 min (T4), $100 \mu\text{M}$ for 120 min (T5), $100 \mu\text{M}$ for 150 min (T6), $150 \mu\text{M}$ for 90 min (T7), $150 \mu\text{M}$ for 120 min (T8), and $150 \mu\text{M}$ for 150 min (T9).

ripening resulted in an increase in TSS. T5 ($100 \mu\text{M}$ MT for 120 min) maintained its TSS value after 28 d of storage, as compared to other treatments. The advancing maturity was accompanied by a depletion of organic acids like citric acid, which resulted in a documented increase in pH, while the decline in TA was different among all treatments. The TSS/TA ratio increased with storage time, due to an increase in TSS and decrease in TA values. T5 ($100 \mu\text{M}$ MT for 120 min) group maintained a higher TA and lower TSS/TA ratio throughout the storage period. The concentration of ascorbic acid decreased in all groups during storage, although T5 (MT $100 \mu\text{M}$ for 120 min) had a higher concentration, as compared to all other groups.

3.4. Malondialdehyde (MDA). A continuous rise in MDA was apparent in all groups during the storage period. Remarkably, T7 ($150 \mu\text{M}$ MT for 90 min) and T5 ($100 \mu\text{M}$ MT for 120 min) maintained a low MDA concentration after 28 d of storage (Figure 1(d)).

3.5. Total Phenolic Content (TPC), Total Flavonoid Content (TFC), and Antioxidant Activity (AA). T5 ($100 \mu\text{M}$ MT for 120 min) had a higher TPC and TFC in both peel (Figures 3(a)–3(b)) and pulp (Figures 4(a)–4(b)), as compared to all other groups throughout the 28 d storage period. Similarly, the AA in the peel (Figures 3(c)–3(d)) and pulp

TABLE 1: Effect of melatonin (MT) treatments on weight loss and firmness of mango cv. "Dashehari" stored at 5 ± 1 °C.

Parameter	Day	Control	T1	T2	T3	T4	T5	T6	T7	T8	T9
Weight loss (%)	0	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a
	7	2.10 ± 0.06 ab	2.20 ± 0.06 abc	2.40 ± 0.06 abc	2.70 ± 0.12 c	2.00 ± 0.06 a	1.90 ± 0.22 a	2.27 ± 0.15 abc	2.53 ± 0.27 bc	2.60 ± 0.24 bc	2.27 ± 0.12 abc
	14	4.23 ± 0.15 c	4.30 ± 0.12 c	4.47 ± 0.03 c	5.23 ± 0.15 e	3.87 ± 0.07 b	3.50 ± 0.12 a	4.13 ± 0.09 bc	4.87 ± 0.07 d	4.97 ± 0.15 de	4.20 ± 0.06 c
	21	6.13 ± 0.07 a	6.17 ± 0.17 a	7.00 ± 0.12 b	7.43 ± 0.12 c	6.23 ± 0.15 a	5.77 ± 0.15 a	6.73 ± 0.15 b	7.67 ± 0.17 c	7.53 ± 0.15 c	6.90 ± 0.22 b
Firmness (N)	0	17.50 ± 0.79 a	17.50 ± 0.79 a	17.50 ± 0.79 a	17.50 ± 0.79 a	17.50 ± 0.79 a	17.50 ± 0.79 a	17.50 ± 0.79 a	17.50 ± 0.79 a	17.50 ± 0.79 a	17.50 ± 0.79 a
	7	10.50 ± 0.30 a	11.10 ± 0.48 ab	11.33 ± 0.46 ab	11.28 ± 0.20 ab	10.00 ± 0.30 a	12.67 ± 1.25b	10.68 ± 0.23 a	10.67 ± 0.91 a	10.00 ± 0.30 a	10.50 ± 0.30 a
	14	7.00 ± 0.30 a	7.07 ± 0.48 a	6.83 ± 0.21 a	7.00 ± 0.30 a	8.10 ± 0.22 b	9.17 ± 0.46 c	7.77 ± 0.23 ab	7.55 ± 0.29 ab	7.18 ± 0.21 ab	7.12 ± 0.35 ab
	21	4.83 ± 0.62 a	5.30 ± 0.61 abc	6.10 ± 0.22 abcd	6.17 ± 0.34 abcd	6.77 ± 0.15 cd	7.53 ± 0.30 d	6.57 ± 0.24 bcd	6.17 ± 0.21 abcd	4.90 ± 0.69 a	5.17 ± 0.75 ab
	28	2.83 ± 0.62 a	3.27 ± 0.38 ab	3.83 ± 0.21 abc	3.70 ± 0.36 abc	5.00 ± 0.30 c	6.45 ± 0.26 d	5.10 ± 0.22 c	4.77 ± 0.15 bc	4.00 ± 1.08 abc	3.27 ± 0.38 ab

Measurements were taken every 7 d during cold storage at 5 ± 1 °C, followed by a 3 d shelf life at room temperature. Each value is the mean of three replicates ± standard error (SE). Values with different lowercase letters on the same storage period indicate a significant difference ($p \leq 0.05$) according to Duncan's multiple range test. The abbreviated treatment details: 0 (control), 50 µM for 90 min (T1), 50 µM for 120 min (T2), 50 µM for 150 min (T3), 100 µM for 90 min (T4), 100 µM for 120 min (T5), 100 µM for 150 min (T6), 150 µM for 90 min (T7), 150 µM for 120 min (T8), and 150 µM for 150 min (T9).

TABLE 2: Effect of melatonin (MT) treatments on L^* , chroma, and hue angle of mango cv. "Dashehari" stored at $5 \pm 1^\circ\text{C}$.

Parameter	Day	Control	T1	T2	T3	T4	T5	T6	T7	T8	T9	
L^*	0	38.29 ± 1.70 a	39.82 ± 2.54 a	35.77 ± 0.73 a	39.33 ± 3.29 a	38.74 ± 3.44 a	38.19 ± 1.46 a	39.55 ± 2.23 a	37.68 ± 1.28 a	38.01 ± 0.41 a	38.16 ± 1.32 a	
	7	39.61 ± 1.66 a	40.79 ± 1.69 a	35.79 ± 0.55 a	39.98 ± 1.55 a	40.36 ± 3.20 a	41.02 ± 2.00 a	41.02 ± 2.00 a	40.24 ± 1.38 a	38.26 ± 1.16 a	39.63 ± 0.71 a	40.14 ± 1.61 a
	14	46.42 ± 2.05 a	46.23 ± 0.57 a	42.82 ± 2.70 a	46.75 ± 1.74 a	46.35 ± 3.61 a	47.35 ± 1.33 a	47.35 ± 1.33 a	49.25 ± 2.63 a	46.45 ± 0.55 a	45.91 ± 0.88 a	47.33 ± 2.02 a
	21	47.09 ± 1.12 a	46.81 ± 0.51 a	44.73 ± 0.35 a	47.40 ± 2.62 a	46.91 ± 3.79 a	49.24 ± 2.61 a	49.24 ± 2.61 a	49.78 ± 3.23 a	47.10 ± 2.10 a	48.45 ± 0.99 a	47.33 ± 1.37 a
	28	47.92 ± 1.15 a	51.08 ± 1.31 a	45.65 ± 1.55 a	49.65 ± 1.29 a	47.07 ± 1.88 a	49.77 ± 1.92 a	49.77 ± 1.92 a	50.83 ± 3.02 a	47.41 ± 2.77 a	48.72 ± 0.99 a	49.12 ± 2.02 a
Chroma	0	19.61 ± 1.23 ab	22.43 ± 1.02 a	17.90 ± 0.60 b	21.05 ± 2.09 ab	19.22 ± 2.57 ab	20.15 ± 1.27 ab	19.92 ± 0.61 ab	19.33 ± 0.94 ab	20.26 ± 0.63 ab	21.05 ± 1.01 ab	
	7	20.93 ± 0.38 ab	20.90 ± 0.46 ab	18.55 ± 0.29 a	21.41 ± 1.16 ab	22.09 ± 1.47 b	22.82 ± 1.24 b	20.62 ± 1.44 ab	20.30 ± 0.43 ab	20.71 ± 0.84 ab	20.87 ± 0.90 ab	
	14	27.19 ± 2.52 a	37.09 ± 0.59 b	33.19 ± 2.70 b	38.43 ± 1.82 b	37.37 ± 3.06 b	36.53 ± 1.12 b	36.52 ± 1.63 b	36.07 ± 0.36 b	35.59 ± 0.98 b	33.42 ± 2.12 b	
	21	33.15 ± 1.71 ab	32.98 ± 1.60 ab	29.42 ± 1.36 a	32.19 ± 2.04 ab	36.25 ± 3.65 ab	38.46 ± 1.96 b	35.70 ± 2.15 ab	33.96 ± 2.83 ab	33.70 ± 1.80 ab	35.26 ± 1.56 ab	
	28	33.01 ± 1.29 ab	35.56 ± 2.39 ab	32.62 ± 1.36 ab	36.24 ± 0.92 ab	30.31 ± 2.86 a	33.72 ± 2.06 ab	37.59 ± 2.06 b	32.18 ± 2.61 ab	31.79 ± 1.67 ab	36.82 ± 1.27 b	
Hue	0	55.58 ± 0.65 ab	57.27 ± 1.38 ab	54.85 ± 0.20 a	57.37 ± 0.66 ab	57.77 ± 0.85 b	55.31 ± 0.81 ab	56.99 ± 1.04 ab	56.26 ± 1.25 ab	56.60 ± 0.48 ab	56.82 ± 0.45 ab	
	7	55.61 ± 0.38 ab	56.48 ± 0.65 ab	54.41 ± 0.26 a	56.37 ± 1.06 ab	56.62 ± 0.96 ab	57.08 ± 0.74 ab	58.41 ± 0.49 b	56.56 ± 1.65 ab	56.89 ± 0.74 ab	57.02 ± 0.98 ab	
	14	60.11 ± 0.72 a	60.73 ± 0.36 a	61.58 ± 2.64 a	62.58 ± 1.15 a	62.30 ± 1.25 a	62.13 ± 0.86 a	62.35 ± 1.45 a	62.46 ± 0.39 a	61.00 ± 0.85 a	61.32 ± 0.89 a	
	21	60.45 ± 0.97 ab	62.19 ± 0.33 abc	61.70 ± 0.61 abc	62.32 ± 1.07 abc	60.80 ± 1.30 ab	63.58 ± 0.80 bc	63.54 ± 0.79 bc	59.93 ± 1.45 a	60.06 ± 0.93 a	64.43 ± 1.47 c	
	28	59.90 ± 1.22 a	62.32 ± 1.29 ab	60.65 ± 0.86 ab	62.02 ± 0.56 ab	63.23 ± 1.31 b	63.46 ± 1.20 b	62.31 ± 1.14 ab	62.61 ± 0.21 ab	62.68 ± 1.01 ab	62.91 ± 0.27 ab	

Measurements were taken every 7 d during cold storage at $5 \pm 1^\circ\text{C}$, followed by a 3 d shelf life at room temperature. Each value is the mean of three replicates \pm standard error (SE). Values with different lowercase letters on the same storage period indicate a significant difference ($p < 0.05$) according to Duncan's multiple range test. The abbreviated treatment details: 0 (control), 50 μM for 90 min (T1), 50 μM for 120 min (T2), 50 μM for 150 min (T3), 100 μM for 90 min (T4), 100 μM for 120 min (T5), 100 μM for 150 min (T6), 150 μM for 90 min (T7), 150 μM for 120 min (T8), and 150 μM for 150 min (T9).

TABLE 3: Effect of melatonin (MT) treatments on total soluble solids (TSS), pH, titratable acidity (TA), TSS/TA ratio, and ascorbic acid of mango cv. "Dashehari" stored at 5 ± 1 °C.

Parameter	Day	Control	T1	T2	T3	T4	T5	T6	T7	T8	T9
TSS (%)	0	14.50 ± 0.52 a	14.50 ± 0.52 a	14.50 ± 0.52 a	14.50 ± 0.52 a	14.50 ± 0.52 a	14.50 ± 0.52 a	14.50 ± 0.52 a	14.50 ± 0.52 a	14.50 ± 0.52 a	14.50 ± 0.52 a
	7	16.83 ± 0.17 a	16.83 ± 0.62 a	15.17 ± 0.62 ab	15.17 ± 0.62 ab	15.50 ± 0.30 ab	15.50 ± 0.79 ab	15.50 ± 0.79 ab	15.83 ± 0.46 ab	15.50 ± 0.79 ab	15.83 ± 0.46 ab
	14	19.17 ± 0.46 a	18.50 ± 0.30 ab	16.83 ± 0.35 cde	16.33 ± 0.35 cde	16.50 ± 0.30 ab	16.83 ± 0.17 cde	16.83 ± 0.17 cde	17.83 ± 0.46 bc	17.17 ± 0.17 cde	17.33 ± 0.35 cde
pH	21	22.83 ± 0.75 a	23.17 ± 0.75 a	20.33 ± 0.35 ab	19.17 ± 0.46 c	19.17 ± 0.46 c	16.83 ± 0.46 d	19.17 ± 0.46 c	19.50 ± 0.30 c	19.17 ± 0.46 c	20.50 ± 0.30 bc
	28	25.00 ± 0.60 a	24.83 ± 0.17 a	22.33 ± 0.91 ab	22.17 ± 0.75 b	22.17 ± 0.75 b	18.17 ± 0.46 c	22.33 ± 0.91 b	22.50 ± 0.30 b	22.33 ± 0.91 b	22.83 ± 0.46 b
	0	4.80 ± 0.16 a	4.80 ± 0.16 a	4.80 ± 0.16 a	4.80 ± 0.16 a	4.80 ± 0.16 a	4.80 ± 0.16 a	4.80 ± 0.16 a	4.80 ± 0.16 a	4.80 ± 0.16 a	4.80 ± 0.16 a
	7	5.57 ± 0.24 a	5.20 ± 0.32 ab	5.00 ± 0.12 ab	5.13 ± 0.39 ab	4.77 ± 0.40 b	4.87 ± 0.19 ab	4.93 ± 0.09 ab	5.03 ± 0.09 ab	5.03 ± 0.09 ab	5.03 ± 0.15 ab
	14	6.07 ± 0.19 a	5.43 ± 0.49 a	5.23 ± 0.15 b	5.37 ± 0.31 ab	5.03 ± 0.24 b	4.93 ± 0.18 b	5.23 ± 0.15 b	5.27 ± 0.09 b	5.23 ± 0.12 b	5.20 ± 0.16 b
	21	6.10 ± 0.22 a	5.77 ± 0.52 ab	5.67 ± 0.25 ab	5.67 ± 0.25 ab	5.10 ± 0.22 b	5.03 ± 0.15 b	5.33 ± 0.25 ab	5.43 ± 0.24 ab	5.37 ± 0.31 ab	5.33 ± 0.28 ab
	28	6.17 ± 0.21 a	5.90 ± 0.48 ab	5.93 ± 0.31 ab	6.00 ± 0.12 a	5.57 ± 0.12 ab	5.10 ± 0.10 b	5.77 ± 0.15 ab	5.43 ± 0.36 ab	5.57 ± 0.24 ab	5.63 ± 0.36 ab
TA (%)	0	0.65 ± 0.02 a	0.65 ± 0.02 a	0.65 ± 0.02 a	0.65 ± 0.02 a	0.65 ± 0.02 a	0.65 ± 0.02 a	0.65 ± 0.02 a	0.65 ± 0.02 a	0.65 ± 0.02 a	0.65 ± 0.02 a
	7	0.33 ± 0.02 a	0.35 ± 0.04 a	0.37 ± 0.06 a	0.40 ± 0.02 a	0.42 ± 0.07 ab	0.58 ± 0.02 b	0.42 ± 0.08 ab	0.44 ± 0.06 ab	0.42 ± 0.08 ab	0.37 ± 0.02 a
	14	0.19 ± 0.02 a	0.26 ± 0.05 ab	0.30 ± 0.09 ab	0.33 ± 0.02 ab	0.37 ± 0.05 b	0.51 ± 0.02 c	0.33 ± 0.02 ab	0.40 ± 0.06 bc	0.35 ± 0.04 b	0.33 ± 0.02 ab
	21	0.09 ± 0.02 a	0.23 ± 0.06 ab	0.23 ± 0.06 ab	0.30 ± 0.02 bc	0.23 ± 0.05 ab	0.44 ± 0.02 c	0.23 ± 0.02 ab	0.26 ± 0.09 ab	0.28 ± 0.07 b	0.19 ± 0.05 ab
	28	0.04 ± 0.00 a	0.04 ± 0.00 a	0.05 ± 0.00 a	0.04 ± 0.00 ab	0.05 ± 0.00 ab	0.07 ± 0.00 b	0.04 ± 0.01 a	0.04 ± 0.01 a	0.04 ± 0.01 a	0.04 ± 0.00 a
	0	0.65 ± 0.02 a	0.65 ± 0.02 a	0.65 ± 0.02 a	0.65 ± 0.02 a	0.65 ± 0.02 a	0.65 ± 0.02 a	0.65 ± 0.02 a	0.65 ± 0.02 a	0.65 ± 0.02 a	0.65 ± 0.02 a
	7	22.28 ± 1.38 a	22.28 ± 1.38 a	22.28 ± 1.38 a	22.28 ± 1.38 a	22.28 ± 1.38 a	22.28 ± 1.38 a	22.28 ± 1.38 a	22.28 ± 1.38 a	22.28 ± 1.38 a	22.28 ± 1.38 a
TSS/TA	14	52.14 ± 4.47 a	49.84 ± 7.97 ab	43.89 ± 7.47 ab	39.29 ± 1.89 ab	38.86 ± 8.64 ab	24.90 ± 0.63 b	40.28 ± 9.78 ab	36.81 ± 4.07 ab	39.29 ± 6.42 ab	42.70 ± 2.71 ab
	21	107.14 ± 18.53 a	76.35 ± 12.27 ab	72.30 ± 27.52 ab	52.14 ± 4.63 bc	45.24 ± 6.21 bc	32.23 ± 1.02 c	52.02 ± 3.58 bc	47.51 ± 8.74 bc	50.32 ± 5.77 bc	53.57 ± 3.80 bc
	28	275.00 ± 63.12 a	113.02 ± 28.55 b	108.10 ± 29.09 b	67.86 ± 5.66 b	90.48 ± 21.63 b	38.27 ± 2.93 b	83.93 ± 9.37 b	93.25 ± 28.91 b	83.33 ± 30.87 b	122.62 ± 26.57 b
Ascorbic acid (g Kg ⁻¹)	0	0.49 ± 0.02 a	0.49 ± 0.02 a	0.49 ± 0.02 a	0.49 ± 0.02 a	0.49 ± 0.02 a	0.49 ± 0.02 a	0.49 ± 0.02 a	0.49 ± 0.02 a	0.49 ± 0.02 a	0.49 ± 0.02 a
	7	0.24 ± 0.02 ab	0.29 ± 0.06 ab	0.31 ± 0.02 ab	0.19 ± 0.02 ab	0.22 ± 0.05 a	0.38 ± 0.07 b	0.30 ± 0.08 ab	0.29 ± 0.01 ab	0.27 ± 0.02 ab	0.34 ± 0.06 ab
	14	0.22 ± 0.04 ab	0.21 ± 0.03 ab	0.24 ± 0.02 ab	0.17 ± 0.06 a	0.19 ± 0.01 ab	0.33 ± 0.04 b	0.25 ± 0.07 ab	0.19 ± 0.05 a	0.23 ± 0.02 ab	0.24 ± 0.05 ab
	21	0.13 ± 0.02 a	0.14 ± 0.02 a	0.20 ± 0.02 a	0.13 ± 0.02 a	0.13 ± 0.02 a	0.16 ± 0.02 a	0.13 ± 0.02 a	0.13 ± 0.03 a	0.17 ± 0.04 a	0.17 ± 0.02 a
28	0.09 ± 0.04 a	0.10 ± 0.03 a	0.12 ± 0.03 a	0.11 ± 0.03 a	0.12 ± 0.02 a	0.13 ± 0.03 a	0.08 ± 0.02 a	0.10 ± 0.03 a	0.10 ± 0.04 a	0.08 ± 0.03 a	

Measurements were taken every 7 d during cold storage at 5 ± 1 °C, followed by a 3 d shelf life at room temperature. Each value is the mean of three replicates ± standard error (SE). Values with different lowercase letters on the same storage period indicate a significant difference ($p < 0.05$) according to Duncan's multiple range test. The abbreviated treatment details: 0 (control), 50 μM for 90 min (T1), 50 μM for 120 min (T2), 50 μM for 150 min (T3), 100 μM for 90 min (T4), 100 μM for 120 min (T5), 100 μM for 150 min (T6), 150 μM for 90 min (T7), 150 μM for 120 min (T8), and 150 μM for 150 min (T9).

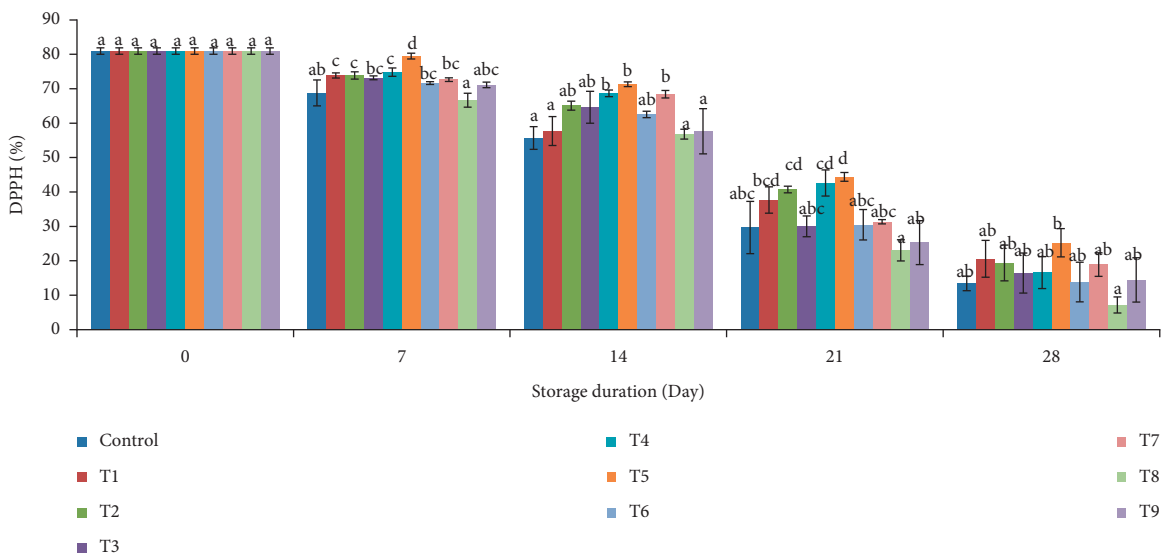
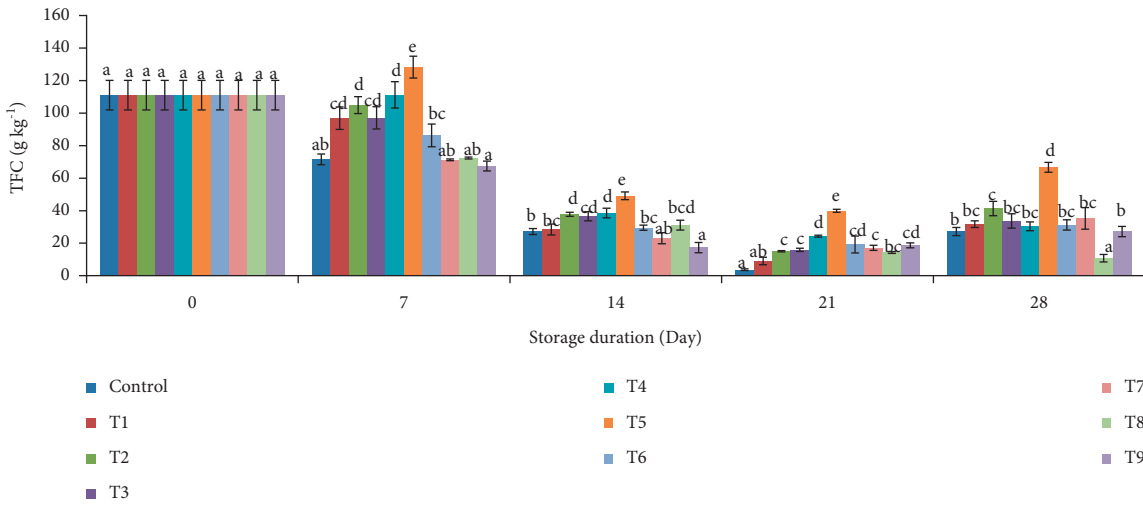
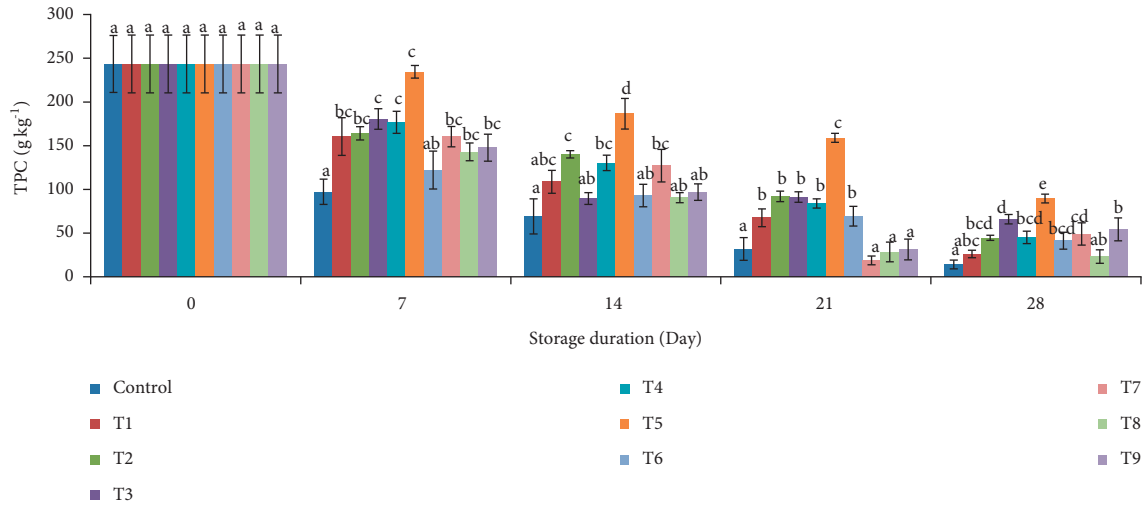


FIGURE 3: Continued.

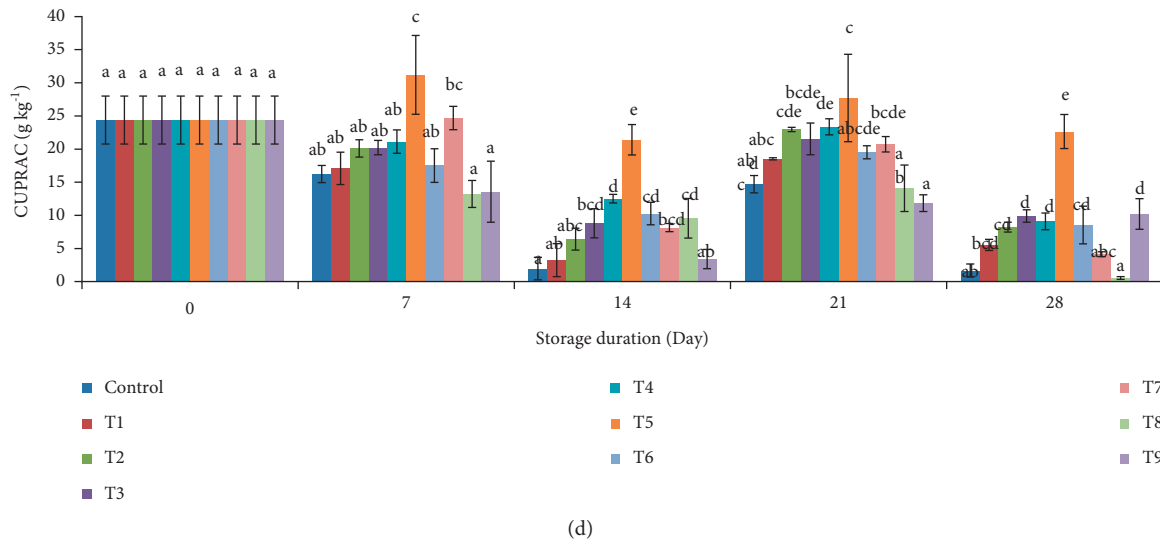


FIGURE 3: (a) Total phenolic content (TPC), (b) total flavonoid content (TFC), (c) 2,2-diphenyl-1-picrylhydrazyl (DPPH), and (d) cupric reducing antioxidant power (CUPRAC) of peel of mango cv. “Dashehari” treated with melatonin (MT), followed by low-temperature storage at $5 \pm 1^\circ\text{C}$. Measurements were taken every 7 d of storage, followed by a 3 d of shelf life at room temperature. Each value is the mean of three replicates; vertical bars indicate the standard error. Different lowercase letters on the same storage period indicate a significant difference ($p < 0.05$) according to Duncan’s multiple range test. The abbreviated treatment details: 0 (control), $50 \mu\text{M}$ for 90 min (T1), $50 \mu\text{M}$ for 120 min (T2), $50 \mu\text{M}$ for 150 min (T3), $100 \mu\text{M}$ for 90 min (T4), $100 \mu\text{M}$ for 120 min (T5), $100 \mu\text{M}$ for 150 min (T6), $150 \mu\text{M}$ for 90 min (T7), $150 \mu\text{M}$ for 120 min (T8), and $150 \mu\text{M}$ for 150 min (T9).

(Figures 4(c)–4(d)), as measured with the DPPH and CUPRAC assays, was also higher in T5 ($100 \mu\text{M}$ MT for 120 min).

3.6. Enzyme Activity

3.6.1. Superoxide Dismutase (SOD). SOD activity in T5 ($100 \mu\text{M}$ MT for 120 min) increased until 14 d and then decreased until the end of the storage period. This group had higher SOD activity, as compared to all other treatments (Figure 5(a)).

3.6.2. Catalase (CAT). CAT activity remained higher in T5 ($100 \mu\text{M}$ MT for 120 min). The activity of CAT in this group first showed a decline, reaching its minimum value after 14 d and again increasing after 21 d, followed by another decrease after 28 d (Figure 5(b)).

3.6.3. Lipoxygenase (LOX). An increased LOX activity was seen up to 14 d, which then declined in all groups. T5 ($100 \mu\text{M}$ MT for 120 min) maintained a low LOX activity throughout storage (Figure 5(c)).

4. Discussion

The postharvest storage of mango fruit at low temperature may be an option to ensure its extended availability, but conditions must be carefully controlled, since the development of CI is a major constrain to this practice. Previous studies have reported alleviation of CI when concentrations of MT similar to the ones used here were applied to various fruits, such as peach with $100 \mu\text{M}$ [22], tomato with 100 and

$200 \mu\text{M}$ [39], and pomegranate with $100 \mu\text{M}$ MT [40]. In line with these studies, our results showed that a $100 \mu\text{M}$ MT for 120 min treatment (T5) had a significant influence on the chilling tolerance of mango fruit. However, it should be noted that each combination of concentration and dipping duration had a particular impact on the results.

The relationship between CI and ethylene is complex, with some inconsistencies reported in various climacteric and nonclimacteric fruits [41]. For example, the severity of CI signs when fruits were reconditioned at room temperature after cold storage has been correlated with a steep rise in ethylene production [42]. In contrast, the promotion of ethylene production subsequent to an ethrel application resulted in the reduction of CI in mango fruits [43]. MT can apparently promote ethylene production in tomatoes stored at 15°C when administered at $50 \mu\text{M}$ for 2 h [18] whereas suppression of ethylene production was reported in mango when $50 \mu\text{M}$ MT was administered for 1 h [25], as well as in banana fruit at 200 and $500 \mu\text{M}$ when administered for 2 h at 25°C [17]. These contrasting results may be explained by considering MT as a regulator of two intrinsically linked processes, i.e., ripening and senescence [19]. Ripening was positively regulated, while senescence was negatively regulated with MT treatments. Such a mode of action of ethylene after MT treatment needs further investigation, but it is likely that it is dependent on cultivar, concentration, immersion duration, storage conditions, etc. Additionally, and in line with the studies of Hu et al. [17] on banana fruit and Liu et al. [25] on mango fruit, our results also show that MT treatment significantly inhibited ethylene production. The minimum ethylene production in T5 ($100 \mu\text{M}$ MT for 120 min) was correlated with minimum CI signs and maximum quality maintenance for up to 28 d (Figure 1(a)).

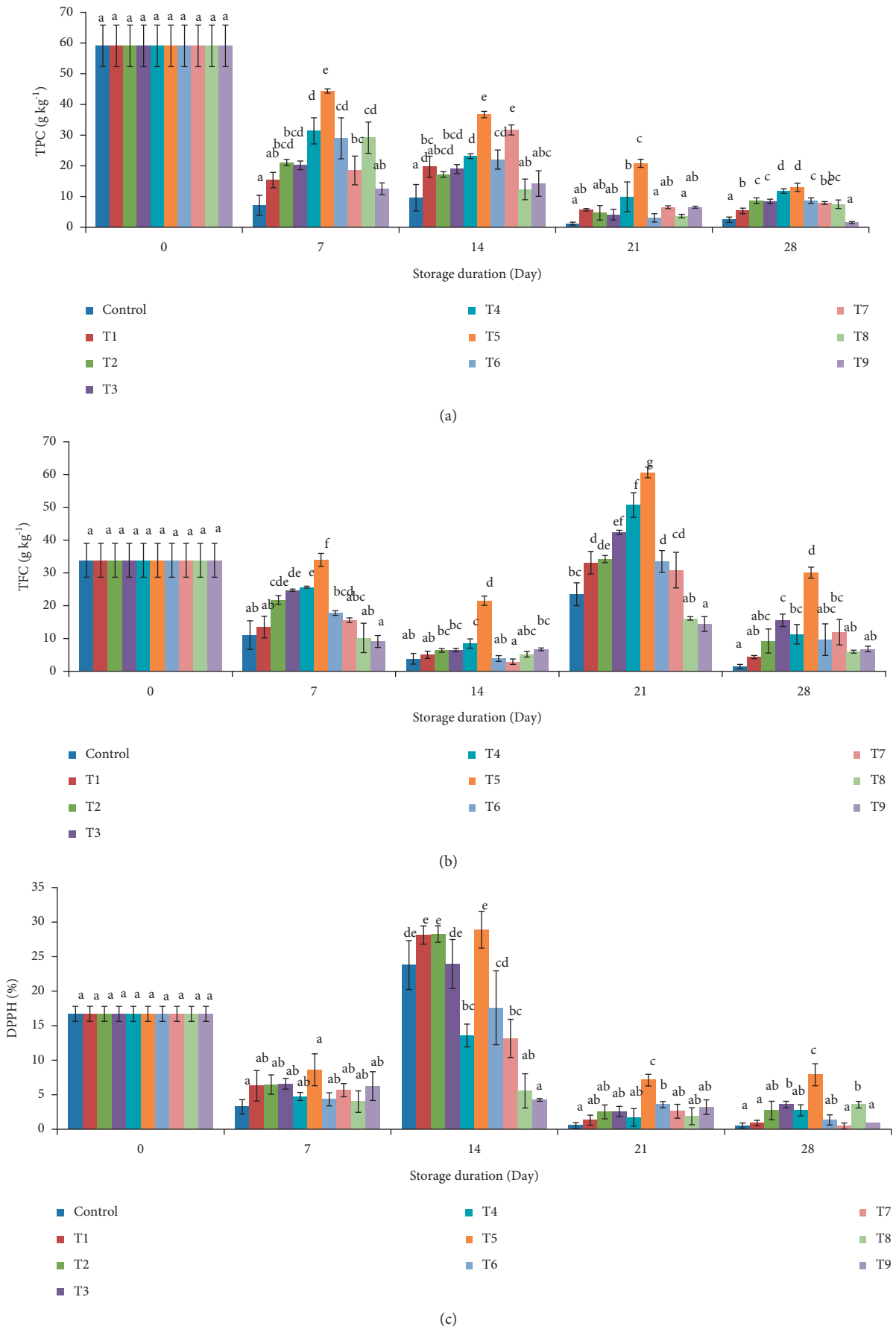


FIGURE 4: Continued.

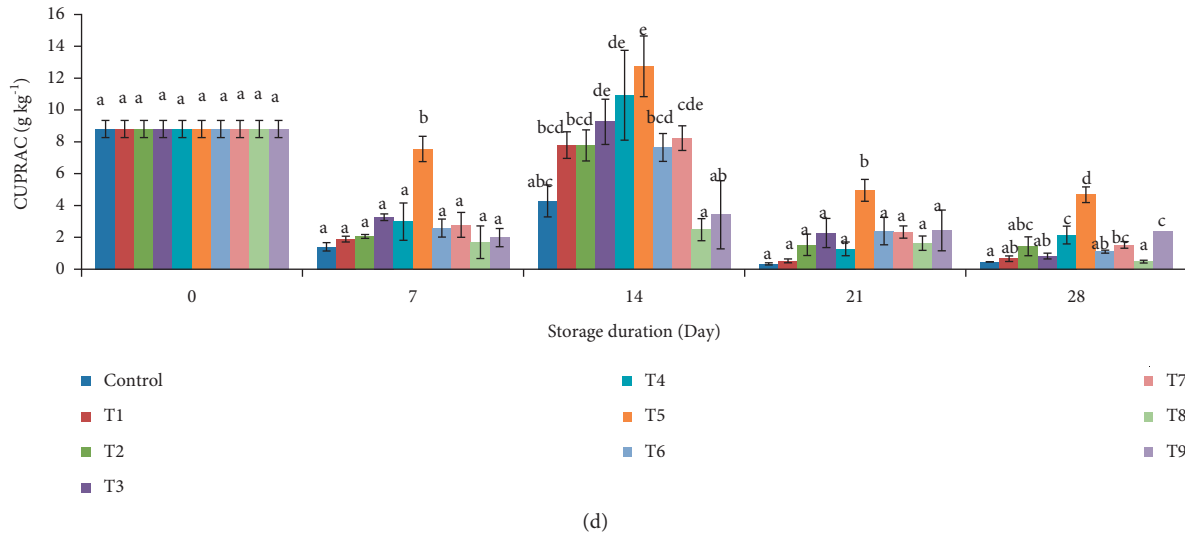


FIGURE 4: (a) Total phenolic content (TPC), (b) total flavonoid content (TFC), (c) 2,2-diphenyl-1-picrylhydrazyl (DPPH), and (d) cupric reducing antioxidant power (CUPRAC) of pulp of mango cv. “Dashehari” treated with melatonin (MT), followed by low-temperature storage at $5 \pm 1^\circ\text{C}$. Measurements were taken every 7 d of storage, followed by a 3 d of shelf life at room temperature. Each value is the mean of three replicates; vertical bars indicate the standard error. Different lowercase letters on the same storage period indicate a significant difference ($p < 0.05$) according to Duncan’s multiple range test. The abbreviated treatment details: 0 (control), $50 \mu\text{M}$ for 90 min (T1), $50 \mu\text{M}$ for 120 min (T2), $50 \mu\text{M}$ for 150 min (T3), $100 \mu\text{M}$ for 90 min (T4), $100 \mu\text{M}$ for 120 min (T5), $100 \mu\text{M}$ for 150 min (T6), $150 \mu\text{M}$ for 90 min (T7), $150 \mu\text{M}$ for 120 min (T8), and $150 \mu\text{M}$ for 150 min (T9).

The reduced activities of ACC synthase (ACS) and ACC oxidase (ACO), as well as transcription of the *MaACS1* and *MaACO1*, may explain the suppressed production of ethylene in MT-treated mangoes [17].

Respiration rate in T5 ($100 \mu\text{M}$ MT for 120 min) was reduced, in accordance with the study of Gao et al. [12], where peach fruits were treated with $100 \mu\text{M}$ MT for 10 min. Similar to our study, a postharvest application of nitric oxide ($10, 20, \text{ and } 40 \mu\text{L L}^{-1}$) alleviated CI in mango fruit, by inhibiting respiration rate and ethylene production [5]. During ripening, carbohydrates are converted into simple sugars, which results in an increase in TSS [44]. Furthermore, consumption of organic acids by various metabolic processes leads to a decrease in TA, with a subsequent increase in pH and TSS/TA ratio. However, mango fruits in T5 ($100 \mu\text{M}$ MT for 120 min) maintained a significantly high TA and firmness, along with low weight loss, pH, TSS, and TSS/TA ratio. These results indicate that MT delayed senescence and preserved the quality of treated fruits. Our findings are therefore in accordance with previous studies done on peach [12] and pear [15], where $100 \mu\text{M}$ MT had positive effects against senescence.

A reduced activity of the enzymatic and nonenzymatic antioxidant system, progressive ripening, biotic or abiotic stress, and other conditions will lead to disruption of cellular homeostasis, which leads to oxidative stress. In line with the previous studies done by Gao et al. [12] on peach fruit and Zhai et al. [15] on pear, our data shows that T5 ($100 \mu\text{M}$ MT for 120 min) applied to mango fruits maintained significantly higher SOD and CAT activities, which apparently mitigated cellular damage due to reactive oxygen species (ROS). SOD and CAT are key players in scavenging ROS since their combined activities dismutate $\text{O}_2^{\cdot -}$ into H_2O_2 (SOD), which is

then converted into H_2O and molecular oxygen (CAT) [45]. Furthermore, MT treatment also maintained a significantly higher concentration of nonenzymatic antioxidants, i.e., ascorbic acid, TPC, and TFC. As a result of significantly higher enzymatic and nonenzymatic antioxidant systems, the total AA (DPPH and CUPRAC assays) was also higher in T5 ($100 \mu\text{M}$ MT for 120 min). This maintenance in AA is in agreement with previous results obtained by Liu et al. [46] in strawberry fruit, Zhang et al. [47] in litchi fruit, and Rastegar et al. [20] in mango fruit at a temperature of 15°C .

LOX is a lipid-oxidizing enzyme, which can contribute to the peroxidation of membrane lipids in concert with ROS. The process of lipid peroxidation will result in the formation of lipid hydroperoxides from polyunsaturated fatty acids found in the cellular membrane, thereby leading to a loss of membrane integrity [48]. This phenomenon affects not only compartmentalization but also the activities of proteins related to signaling, energy generation systems, and various other processes. MDA is an end product of lipid peroxidation and is one of the most commonly studied indicators of this process [49]. Our data showed that mango fruit treated with $100 \mu\text{M}$ MT for 120 min (T5) had a significantly lower LOX activity and MDA concentration, in comparison to all other treatments, which is in accordance with previous studies done on peach [12], pear [15], and strawberry [46].

Moreover, the plasma membrane is permeable for the entry of exogenously applied MT application into the cell and leads to enhancement of endogenous MT level [50]. Raised level of endogenous MT initiates a cascade of events (auxin, ethylene, and mitogen-activated protein kinase, MAPK) due to the signaling property of MT during stressful conditions [51]. MT activates signaling pathways namely, gibberellic acid (GA), salicylic acid (SA), and abscisic acid (ABA). These

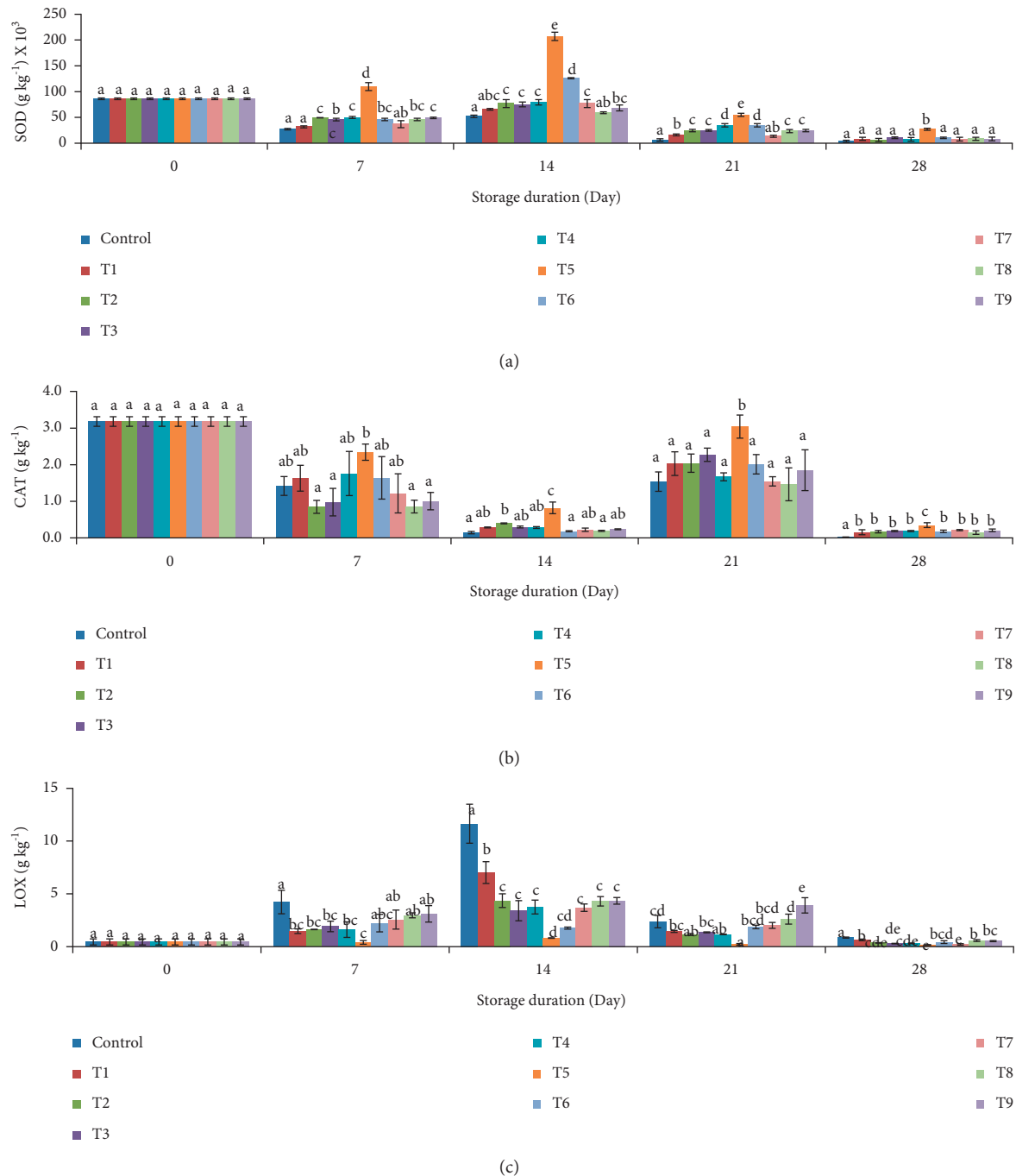


FIGURE 5: (a) Superoxide dismutase (SOD), (b) catalase (CAT), and (c) lipoxygenase (LOX) activities of mango cv. “Dashehari” treated with melatonin (MT), followed by low-temperature storage at $5 \pm 1^\circ\text{C}$. Measurements were taken every 7 d of storage, followed by a 3 d of shelf life at room temperature. Each value is the mean of three replicates; vertical bars indicate the standard error. Different lowercase letters on the same storage period indicate a significant difference ($p < 0.05$) according to Duncan’s multiple range test. The abbreviated treatment details: 0 (control), $50 \mu\text{M}$ for 90 min (T1), $50 \mu\text{M}$ for 120 min (T2), $50 \mu\text{M}$ for 150 min (T3), $100 \mu\text{M}$ for 90 min (T4), $100 \mu\text{M}$ for 120 min (T5), $100 \mu\text{M}$ for 150 min (T6), $150 \mu\text{M}$ for 90 min (T7), $150 \mu\text{M}$ for 120 min (T8), and $150 \mu\text{M}$ for 150 min (T9).

activities of MT lead to the modulation of physiological characteristics of a cell that persists in its survival under adverse situations of stress [50]. Apparently, apart from its direct antioxidative behavior, metabolites of MT (3-hydroxymelatonin, *N1-acetyl-N2-formamyl-5-methoxykynuramine*, and 2-hydroxymelatonin) also act in the removal of ROS and reactive nitrogen species (RNS) [52], which

eliminate the necessity of exact recovering pathway in order to have redox cycle accomplishment [53]. Furthermore, chloroplast and mitochondria were regarded as the site of MT synthesis in plants that relates its efficacy in free radical removal and *proficiency* in electron transport chain promotion [54], which directly related it to the higher maintenance of energy status of cell and plasma membrane stability.

However, all these discoveries are on the limited edge in postharvest fruits and vegetables in comparison with plant and animal cells. But there are studies, which have provided results for the maintenance of the antioxidant system with MT application in postharvest fruit and vegetables under stressful conditions (cold stress in the present study) as mentioned above in line with the previous studies done with tomato [39], pomegranate [40], sapota [55], strawberry [13], and peach [22] fruit. Additionally, cold stress tolerance is developed through enhanced activity of enzymes namely, H-ATPase, Ca-ATPase, and cytochrome *c* oxidase that will indirectly lead to maintenance of a high ratio of unsaturated/saturated fatty acid and thereby low MDA production [40]. Furthermore, treatment of MT has promoted the synthesis of polyamine [56], NO [23], and proline [57] in order to confer CI.

5. Conclusions

A 100 μ M melatonin treatment administered for 120 min maintained the postharvest quality parameters of mangoes cv. "Dashehari" that were cold-stored at $5 \pm 1^\circ\text{C}$ for 28 d. This was due to higher membrane integrity, which resulted from low malondialdehyde production and lipoxygenase activity. The same treatment also delayed senescence through an inhibited ethylene production and respiration rate, parameters that are closely related to the natural ripening process. Low oxidative stress was apparently due to stimulating the enzymatic and nonenzymatic antioxidant systems of mango fruit. Thus, a melatonin treatment alleviated the signs of chilling injury in mangoes. Optimization studies may be required to determine ideal concentrations and times in other fruits.

Data Availability

Data will be made available on request to the corresponding author.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors' Contributions

Renu Bhardwaj contributed to the setup of the experiment, data collection, analysis of the data interpretation, and drafting of the article. Sunil Pareek was responsible for the formalization of concept, experimental design, interpretation, reviewing the manuscript, supervision, and coordination. Sarvanan Mani assisted in performing some sets of experiments. G.A. Gonzalez-Aguilar and J. Abraham Domínguez-Avila edited the manuscript and improve the overall presentation.

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