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Exogenous Melatonin as Pre- and Postharvest Application on Quality Attributes, Antioxidant Capacity, and Extension of Shelf Life of Papaya

Priyaxee Borthakur [1], Kavitha Chinnasamy [1,*], Suresh Kumar Paramasivam [2], Sivakumar Venkatachalam [1], Senthil Alagarswamy [3], Johnson Iruthayasamy [4], Elaiyabarathi Thiyagarajan [5] and Saraladevi Muthusamy [6,*]

1 Department of Fruit Science, HC and RI, Tamil Nadu Agricultural University, Coimbatore 641003, India; priyaxee@gmail.com (P.B.); sivakumarv@tnau.ac.in (S.V.)
2 Division of Crop Production and Post Harvest Technology, ICAR-NRC Banana, Tiruchirapalli 620102, India; psureshars@gmail.com
3 Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore 641003, India; senthil.a@tnau.ac.in
4 Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore 641003, India; johnson.i@tnau.ac.in
5 Department of Medicinal and Aromatic Plants, HC and RI, Tamil Nadu Agricultural University, Coimbatore 641003, India; elaiyabharathi.t@tnau.ac.in
6 Department of Plant Breeding, Swedish University of Agricultural Sciences, 23422 Lomma, Sweden
* Correspondence: ck77@tnau.ac.in (K.C.); saraladevi.muthusamy@slu.se (S.M.)

Abstract: Papaya is widely grown in tropical and subtropical climates due to its high yield potential and high returns. The vital hormone melatonin, regulating various biological processes in plants, is eco-friendly and less harmful to humans than other chemicals. This study aims to enhance the quality and antioxidant enzyme activities and lessen postharvest senescence in papaya cv. CO 8 fruits during both ambient (32 ± 2 °C and 55 ± 5% RH) and cold storage (10 ± 2 °C and 90–95% RH) as exogenous melatonin (EMT) is applied in varying concentrations. An optimum melatonin dose of 1.5 mM was applied as a pre-harvest spray 15 days before harvest and a postharvest dip proved effective in prolonging shelf life (under ambient it prolonged to day 9 and under cold storage up to 28 days) and delaying ripening and softening. Exogenous melatonin application enhanced antioxidant activity, reduced weight loss, maintained firmness, delayed ripening enzymes, and lowered ethylene and CO_2 levels. For instance, control fruits had weight losses between 7.42% and 10.09%, while fruits treated with 1.5 mM melatonin showed 5.74% and 9.06% weight loss under ambient and cold storage, respectively. In conclusion, applying EMT (1.5 mM) could be an economically viable and environmentally benign way to lessen senescence after harvest and preserve the qualities of the papaya fruit during ambient and cold storage.

Keywords: papaya; melatonin; pre-harvest; antioxidant capacity; gases evolution; postharvest storage

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1. Introduction

Papaya (*Carica papaya* L.) is a nutrient-rich fruit crop, mostly grown in tropical and subtropical climates worldwide. The production of papayas was 13.8 million metric tons in 2022 [1]. The continual bearing habit and high yield potential of this crop draw growers to cultivate it, making it commercially important. The crop is easy to grow, produces fruit in less than a year, and its cultivation is very remunerative. Papayas are climacteric fruits that ripen quickly after harvest due to a high respiration rate. Their postharvest life is severely limited by various biochemical and physical alterations, resulting in pulp softening, brown skin pigmentation, and infection caused by fungi [2]. Improper postharvest handling and storage can gravely impair the product's quality and shelf life, resulting in infections

and consumer rejection, consequently leading to large financial losses [3]. Though low-temperature storage (9–10 °C) is widely utilized to extend the postharvest life of many products, horticultural crops, particularly fruits, are susceptible to fungal decay and chilling injury [4]. Temperature, humidity, and air composition all have an ongoing impact on the nutritional value of fruits [5]. Ripe papaya fruits typically keep their freshness at 25 °C for almost a week and at 12 °C for fourteen days [6]. The majority of methods developed to prevent papaya deterioration after harvest either have high financial expenses or call for many artificial substances that are dangerous for the environment and human well-being. Thus, the requirement of an efficient and environmentally friendly method to increase papayas' shelf life is highly warranted [7].

The quality of papaya fruits during postharvest storage has been improved recently by several techniques, including using putrescine [8], ozone [9], and bioactive extracts [10], either alone or in conjunction with hot water treatment [11], gamma and UV-C irradiation [12], and edible coatings [13]. Variations in the antioxidant content and physicochemical traits of papayas during storage and postharvest ripening were documented [9]. Recently, melatonin has been used to boost fruit storage's resilience to oxidative stress, postharvest deterioration and ripening delays [14]. According to Fan et al. [15], applying melatonin to different fruits can improve their storage capability, preserve their postharvest quality features, and have safe commercial applications.

N-acetyl-5-methoxytryptamine, often known as melatonin, is an indoleamine [16] generated by various species, including fungi, bacteria, animals and plants. It is a widespread signaling molecule found in an array of plant species, and it plays a number of roles in the growth and development of plants. Many physiological activities in plants, including fruit ripening and senescence, flowering and fruit development, seed development, root development, and responses to biotic and abiotic stresses, involve melatonin [7]. In recent years, the use of melatonin applied exogenously (EMT) to postpone senescence and its impact on fruits' biochemical and antioxidant shielding systems have received much attention among researchers. EMT therapy has been applied to strawberries, peaches, and tomatoes to delay ripening and senescence, maintain quality, and increase fruit postharvest life in storage [17]. It also improved the functional properties of fruits, such as bananas, sweet cherries, and litchis [18–20]. By capturing malondialdehyde (MDA) and reactive oxygen species (ROS) levels, melatonin treatments decreased fruit senescence while increasing antioxidant activity [7]. Additionally, the membrane wall integrity of the sweet cherry fruits was preserved, and higher enzyme levels of superoxide dismutase (SOD), peroxidase (POD), glutathione reductase (GR) and catalase (CAT) were observed compared to control fruits [21]. According to Wang et al. [22], applying EMT also reduced peel browning, delayed low-temperature damage, and strengthened the antioxidant defense system, all of which helped banana fruit produce less H_2O_2 and O_2^-. Given the aforementioned benefits, EMT treatment may be a safer, more environmentally friendly option for preserving the freshness of fruits during storage than other chemical treatments. Nevertheless, the use of melatonin and its dose optimization in pre- and postharvest applications has not been comprehensively studied in papayas. Hence, this study has been formulated to examine the effect of EMT treatment in increasing shelf life by reducing postharvest senescence and delaying ripening in papayas under ambient storage and cold storage conditions.

2. Materials and Methods

2.1. Fruit Materials and Treatment

The experiment was conducted between 2023 and 2024 using var. CO 8, a dioecious variety of papaya, at the College Orchard, TNAU, Coimbatore, Tamil Nadu, India. The fruits were harvested at the color break stage, weighing 750–850 g, when yellow-colored streaks were just visible. Priorly, two separate preliminary experiments comprising (i) the pre-harvest spray and (ii) the postharvest dip of papaya fruits were performed with four different concentrations of melatonin: 0.5, 1.0, 1.5 and 2.0 mM, with a control (water spray/dip). The experiments showed that 1.0 mM and 1.5 mM of melatonin performed

better than the rest of the treatments in both pre- and postharvest applications. Therefore, in the present experiment, five different treatment combinations, viz., T_1—Control (water spray); T_2—Pre-harvest spray of 1.0 mM melatonin + Postharvest dip of 1.0 mM melatonin; T_3—Pre-harvest spray of 1.0 mM Melatonin + Postharvest dip of 1.5 mM melatonin; T_4—Pre-harvest spray of 1.5 mM melatonin + Postharvest dip of 1.0 mM melatonin; T_5—Pre-harvest spray of 1.5 mM melatonin + Postharvest dip of 1.5 mM melatonin were tested, with each treatment having ten fruits per replication to assess the role of exogenous melatonin on quality and shelf life extension in papayas. The pre-harvest spray comprising 1.0 mM, 1.5 mM melatonin, and distilled water (control) was performed on the entire fruit column in the full-bearing papaya trees 15 days prior to harvest. After 15 days, healthy and uniformly matured fruits, viz., 50, 100 and 100 numbers were harvested from papaya trees sprayed with water, 1.0 mM melatonin, and 1.5 mM melatonin, respectively. The harvested fruits were then taken to an analytical laboratory, where the fruits were retained in the ambient condition for an hour to reduce the field heat, and the postharvest treatments of 1.0 mM, 1.5 mM melatonin, and distilled water (quick dip) were applied, with each treatment consisting of 10 fruits per replication. For 30 min, the fruits were kept for air drying and stored under ambient storage conditions of 32 ± 2 °C & 55 ± 5% RH. Similarly, a second set of 250 fruits were harvested from pre-harvest sprayed trees, and after following the above procedure and applying the postharvest treatments, the fruits were stored in cold storage conditions of 10 ± 2 °C and 90–95% RH. The cold storage temperature was suggested by Wang et al. [7]. The observations were taken once at 3- and 7-day intervals for the fruits stored under ambient and cold storage conditions, respectively. There were four replications for every treatment, and each included twelve fruits. The data were recorded at every sampling point throughout the storage period.

2.2. Determination of Weight Loss and Fruit Firmness

Percentage loss in weight (PLW) or weight loss (%) was calculated as the difference between the initial weight and the weight at the time of measurement. Two fruits from each treatment per replication were taken, and the loss in weight was studied at intervals of 3 days and 7 days under both ambient and cold storage conditions. Fruit firmness was measured using the Fruit Hardness Tester (Fruit Hardness Tester 1122, Bestone Industrial Ltd., Hong Kong, China).

2.3. Determination of Ripening Enzymes (Pectin Methylesterase, Polygalacturonase, Amylase and Cellulase)

The procedure described by Srivastava and Dwivedi [23] was used for the extraction of all enzymes. A 2 g pulp sample was taken and homogenized with 15 mL of phosphate buffer (0.2 M, pH 7.0) containing cysteine–HCl (0.2 M) and EDTA (0.2 M). The sample was then centrifuged at 15,000 rpm for 20 min for all enzymatic analyses.

The activity of pectin methyl esterase (PME) was measured following the method suggested by Hagerman & Austin [24], where 1 mL of pectin (0.01%), 0.2 mL of sodium chloride (0.15 M), and 0.1 mL of bromothymol blue (0.01%) were added in a test tube, and the initial reading and the decrease in absorbance after 3 min were recorded at 620 nm absorbance and represented in milliequivalents of COOH released per minute per gram of fresh weight of the sample.

For polygalacturonase, the method described by Gayathri & Nair [25] was used, where the absorbance was read at 540 nm, and the activity was expressed in µg/min/g of fresh weight of the sample. A mixture of 0.2 mL of sodium acetate buffer (0.2 M), 0.1 mL of sodium chloride (2 M), 0.3 mL of polygalacturonic acid (1%) and 0.4 mL of enzyme extract was added to a test tube and incubated for 1 h at room temperature. The reaction was terminated by adding 1 mL of Di Nitro Salicylic acid (1%) and placing it in a hot water bath.

The procedure by Bernfeld [26] was adopted for measuring the amylase, which was expressed in µg/min/g of fresh weight of the sample. One mL of substrate (0.5% potato starch), 0.5 mL of phosphate buffer (0.2 M), and 1 mL of enzyme extract were incubated at

37 °C for 1 h. The reaction was terminated by adding 1 mL of Di Nitro Salicylic acid (1%), and absorbance was read at 540 nm.

A mixture consisting of 1 mL of carboxymethyl cellulose, 1 mL of sodium acetate buffer (0.2 M), and 1 mL of enzyme extract was incubated at 37 °C for 60 min. The reaction was stopped by keeping in a water bath for 5 min and adding 1 mL Di Nitro Salicylic acid (1%), and absorbance was taken at 540 nm. This method of cellulase estimation was provided by Li et al. [27] and is expressed in µg/min/g fresh weight of the sample.

2.4. Determination of Antioxidant Enzymes (POD, CAT, SOD)

The activity of peroxidase was measured by taking 3.5 mL of phosphate buffer (0.2 M), 0.2 mL of enzyme extract, and 0.1 mL o-Dianisidine (1 mg/mL) in a test tube and adding 0.2 mL of hydrogen peroxide (0.1 M) to initiate the reaction. The enzyme kinetics were read at 430 nm using a spectrophotometer for 3 min at 30 s intervals and expressed in ΔA/min/g of fresh weight of the sample [28].

Aebi [29] provided the procedure for estimating catalase, in which a mixture of 1.5 mL of phosphate buffer (0.2 M), 50 µL of enzyme extract, 1.5 mL of distilled water, and 0.5 mL of hydrogen peroxide (12.5 mM) was used to initiate the reaction. The decrease in absorbance was read at 240 nm for 1 min at a 30-s interval and expressed in activity/min/g of fresh weight of the sample.

For superoxide dismutase, the method provided by Beauchamp & Fridovich [30] was used. For this procedure, 1 mL of assay mix (27 mL sodium phosphate buffer, 1 mL NBT, 1.5 mL methionine, 0.75 mL of Triton x-100 solution), 0.1 mL enzyme extract and 0.03 mL of riboflavin were used. The reduction of nitroblue tetrazolium by superoxide radicals to blue-colored formazan was measured at 560 nm and expressed as a unit/mg protein.

2.5. Determination of Ethylene, CO_2 Evolution Rate and Textural Characteristics

The best treatment obtained from the above observation, i.e., the combination of the pre-harvest spray and the postharvest dip of 1.5 mM melatonin, was selected for further studies. Fruits under ambient storage that had only the control (T_1) and the fruits treated with the pre-harvest spray of 1.5 mM melatonin + postharvest dip of 1.5 mM melatonin were used for the study of gas evolution and texture attributes. Using an ethylene meter (Model: ETHAN Monitor, Make: Bioconservacion, Barcelona, Spain), the amount of ethylene gas was measured. The sample to be tested for ethylene gas was placed inside a sealed zip-lock bag. The ethylene gas analyzer was turned on once the device's needle was placed inside the zip-lock bag. The meter continuously showed the ethylene gas in parts per million. Once the measurement had stabilized, the final ppm levels of ethylene gas were recorded. The O_2 and CO_2 analyzer (Model: PBI- Dansensor, Make: CheckPoint II portable gas analyzer O_2 CO_2, Tendering Pacific Limited, Swaston, Cambridge, UK) was used to measure the CO_2 levels. The standard gas (AO_2 CiTicel) was used to calibrate the instrument. The equipment's needle was placed inside the ziplock bag. The built-in pump would then suck gas through the needle for ten seconds after pressing the start button. The pump automatically shuts off after this time, and the outcome was provided as a percentage.

Textural attributes, such as hardness, adhesiveness, resilience, cohesion, springiness, and chewiness, are measured using a TA-XT Plus texture analyzer (Stable Micro Systems, Surrey, UK) with Exponent Connect software (Version 8). A 2 mm cylindrical probe penetrated the fruit at 0.5 mm/s with a 10 kg load cell, and the pre- and post-test speeds were set to 1 mm/s and 10 mm/s, respectively. The fruits were positioned on the loading platform so that the longitudinal axis of the penetrating rod met the fruit precisely in the middle.

2.6. Statistics

The experiment was carried out using a completely randomized design (CRD); a one-way analysis of variance (ANOVA) was performed to compare the means, and the significantly different treatments were determined by the Least Significant Difference (LSD)

analysis of ANOVA. The statistical analysis was performed in R software using R studio (version 4.3.1 (2023-06-16 Universal C Runtime)) with suitable packages. Spearman's correlation analysis was used to understand the relationship among different quality and enzymatic parameters. The significance of data was determined at $p = 0.05$.

3. Results

3.1. Influence of EMT Treatment on Physiological Loss in Weight and Fruit Firmness

Papaya cv. CO 8 fruits treated with different concentrations of melatonin showed a significant difference in their physiological weight loss, as shown in Table 1. Regardless of the treatments, there was considerable physiological weight loss from the day of the treatment until the last day of storage. The highest weight loss was recorded in control fruits (T_1) at 7.42%, followed by a combined application of the pre-harvest spray of 1.0 mM and the postharvest dip of 1.5 mM of melatonin, and the lowest weight loss, 5.74% of PLW, was observed from the pre-harvest spray and the postharvest dip of 1.5 mM melatonin under ambient storage condition on the 9th day. Similarly, under cold storage conditions, the highest physiological weight loss of 10.09% was recorded in control fruits (T_1). It was on par with the pre-harvest spray of 1.0 mM and postharvest dip of 1.0 mM melatonin (9.90%), and the lowest of 9.06% was recorded in the pre-harvest spray of 1.5 mM and the postharvest dip of 1.5 mM melatonin on 28th day under cold storage condition.

Table 1. Effect of combination of pre-harvest and postharvest application of melatonin on physiological loss in weight (%) in papaya cv. CO 8.

Storage Period / Treatment	Ambient			Cold			
	3 Days	6 Days	9 Days	7 Days	14 Days	21 Days	28 Days
T_1	1.73 ^{b}	4.67 ^{a}	7.42 ^{b}	1.77 ^{c}	5.05 ^{a}	6.84 ^{a}	10.09 ^{a}
T_2	1.78 ^{a}	3.99 ^{c}	6.07 ^{c}	2.12 ^{a}	4.48 ^{b}	6.72 ^{a}	9.90 ^{ab}
T_3	1.82 ^{a}	4.48 ^{b}	6.85 ^{a}	2.43 ^{b}	3.99 ^{c}	6.06 ^{b}	9.70 ^{bc}
T_4	1.57 ^{c}	3.14 ^{d}	5.94 ^{c}	1.63 ^{d}	3.70 ^{d}	5.48 ^{c}	9.62 ^{c}
T_5	1.04 ^{d}	2.61 ^{e}	5.74 ^{d}	1.62 ^{d}	3.59 ^{e}	5.32 ^{c}	9.06 ^{d}
SE (d)	0.02	0.07	0.07	0.03	0.04	0.09	0.09
CD ($p \leq 0.05$)	0.04	0.14	0.16	0.06	0.08	0.19	0.21

Significant differences at $p \leq 0.05$ are shown by the mean values having different superscript letters. Data is accumulated from the mean of four replications. Treatment details: T_1—Control; T_2—Pre-harvest spray of 1.0 mM Melatonin + Postharvest dip of 1.0 mM Melatonin; T_3—Pre-harvest spray of 1.0 mM Melatonin + Postharvest dip of 1.5 mM Melatonin; T_4—Pre-harvest spray of 1.5 mM Melatonin + Postharvest dip of 1.0 mM Melatonin; T_5—Pre-harvest spray of 1.5 mM Melatonin + Postharvest dip of 1.5 mM Melatonin.

The firmness decreased with increased storability regardless of treatments and storage temperature. MT had a significant influence on firmness, which is shown in Table 2. The maximum firmness recorded under ambient storage was 6.91 kg/cm^2 on the 9th day. Under cold storage, maximum firmness was 6.37 kg/cm^2 on the 28th day in melatonin-treated fruits. The control fruits (T_1) had 6.21 kg/cm^2 and 6.00 kg/cm^2 on the last sampling days under ambient and cold storage, respectively.

Table 2. Effect of combination of pre-harvest and postharvest application of melatonin on fruit firmness (kg/cm^2) in papaya cv. CO 8.

Storage Period / Treatment	Ambient				Cold				
	0 Day	3 Days	6 Days	9 Days	0 Day	7 Days	14 Days	21 Days	28 Days
T_1	8.00 ^{ab}	7.54 ^{ab}	6.89 ^{b}	6.21 ^{b}	7.90 ^{a}	7.44 ^{ab}	7.27 ^{a}	6.91 ^{bc}	6.00 ^{b}
T_2	8.20 ^{a}	7.38 ^{bc}	6.55 ^{c}	5.83 ^{c}	7.80 ^{a}	7.29 ^{bc}	6.95 ^{b}	6.77 ^{cd}	5.54 ^{d}
T_3	7.83 ^{b}	7.23 ^{c}	6.71 ^{bc}	6.00 ^{c}	7.72 ^{a}	7.16 ^{c}	6.81 ^{b}	6.59 ^{d}	5.76 ^{c}

Table 2. *Cont.*

Storage Period Treatment	Ambient				Cold				
	0 Day	3 Days	6 Days	9 Days	0 Day	7 Days	14 Days	21 Days	28 Days
T_4	7.90 ^{ab}	7.60 ^{ab}	7.00 ^{ab}	6.77 ^{a}	8.00 ^{a}	7.55 ^{a}	7.33 ^{a}	7.17 ^{ab}	6.21 ^{a}
T_5	7.95 ^{ab}	7.71 ^{a}	7.20 ^{a}	6.91 ^{a}	7.86 ^{a}	7.60 ^{a}	7.42 ^{a}	7.26 ^{a}	6.37 ^{a}
SE (d)	0.14	0.11	0.14	0.08	0.14	0.08	0.07	0.13	0.09
CD ($p \leq 0.05$)	0.30	0.24	0.29	0.18	0.30	0.17	0.17	0.28	0.20

Mean values show significant differences at $p \leq 0.05$, which have different letters. Data were collected from the mean of four replicates. Treatment details: T_1—Control; T_2—Pre-harvest spray of 1.0 mM Melatonin + Postharvest dip of 1.0 mM Melatonin; T_3—Pre-harvest spray of 1.0 mM Melatonin + Postharvest dip of 1.5 mM Melatonin; T_4—Pre-harvest spray of 1.5 mM Melatonin + Postharvest dip of 1.0 mM Melatonin; T_5—Pre-harvest spray of 1.5 mM Melatonin + Postharvest dip of 1.5 mM Melatonin.

3.2. Effect of EMT Treatment on Ripening Enzymes

Pectin methyl esterase (PME) activity in CO 8 papaya fruits under both storage conditions increased with storage days until ripening and then decreased, as shown in Figure 1A. The PME activity of the pre-harvest spray and the postharvest dip of 1.5 mM melatonin (T_5) treated fruits significantly declined. The peak activity was delayed by more than 3 days in ambient storage (14.47 COOH/min/g on 9th day) and 7 days in cold storage conditions (17.00 m.eq. COOH/min/g on 21st day) when compared to control (T_1) fruits (16.04 m.eq. COOH/min/g on day 6 of ambient storage and 17.21 m.eq. COOH/min/g on day 21 of cold storage). The influence of MT was almost similar in both storage temperatures. The activity of the polygalacturonase enzyme under ambient and cold storage conditions is presented in Figure 1B. Melatonin significantly lowered the activity of the enzyme under both storage temperatures. The peak activity of polygalacturonase was noticed on the 6th day in control fruits (T_1) (58.33 µg/min/g), whereas fruits treated with the pre-harvest spray and the postharvest dip of 1.5 mM Melatonin (T_5) recorded peak activity on the 9th day (16.66 µg/min/g) of ambient storage. A similar trend was noticed in cold storage on the 14th (T_1 peak: 45.83 µg/min/g) and 21st day (T_5 peak: 12.16 µg/min/g), with the peak activity being lower than that of fruit in ambient storage, indicating that the activity of polygalacturonase decreased with a decrease in storage temperature.

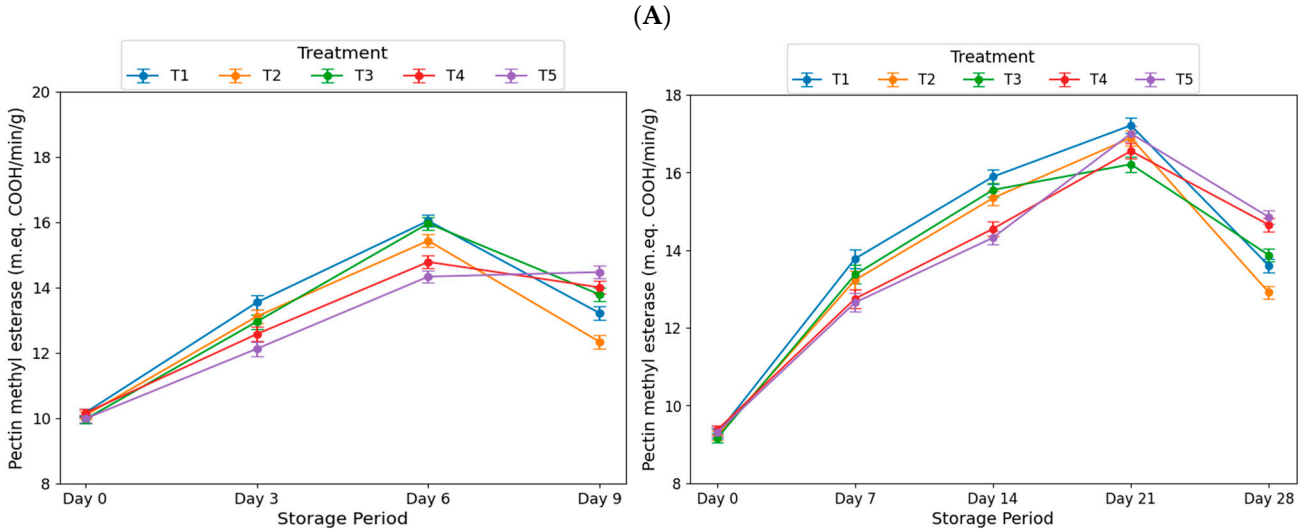

Figure 1. *Cont.*

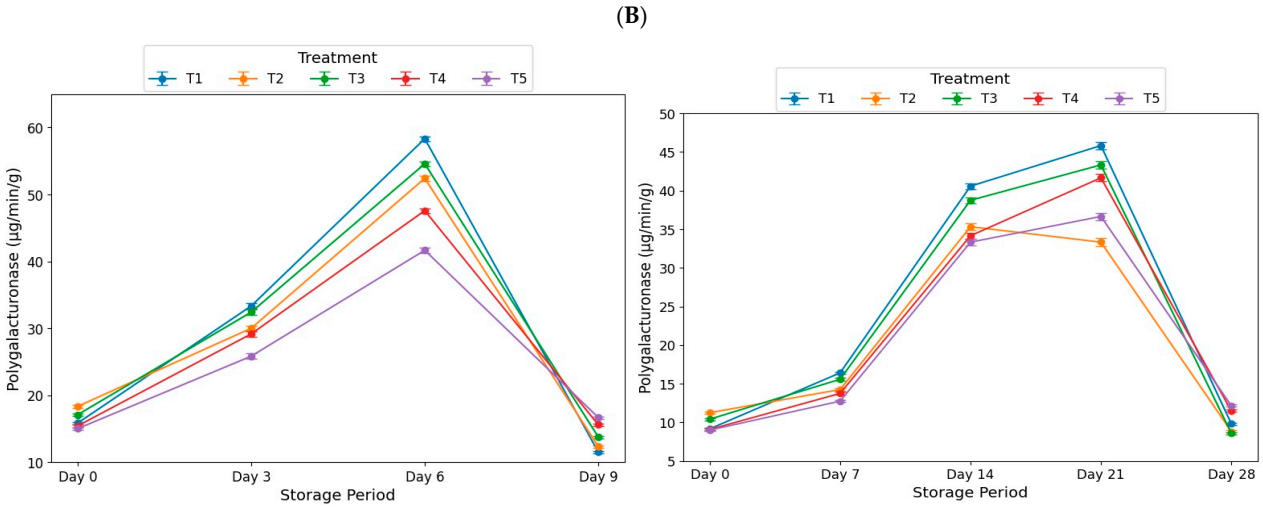

Figure 1. The effect of EMT treatment on pectin methylesterase (**A**) and polygalacturonase (**B**) activity in papaya fruits during ambient and cold storage. Collected data is calculated from the mean of four replicates, and the standard error of the means ($p \leq 0.05$) is indicated by the lines. Treatment details: T_1—Control; T_2—Pre-harvest spray of 1.0 mM Melatonin + Postharvest dip of 1.0 mM Melatonin; T_3—Pre-harvest spray of 1.0 mM Melatonin + Postharvest dip of 1.5 mM Melatonin; T_4—Pre-harvest spray of 1.5 mM Melatonin + Postharvest dip of 1.0 mM Melatonin; T_5—Pre-harvest spray of 1.5 mM Melatonin + Postharvest dip of 1.5 mM Melatonin.

The activity of amylase is shown in Figure 2A. The peak activity of amylase in ambient storage was 24.71 µg/min/g, while in cold storage, it was 25.59 µg/min/g in CO 8 papayas. The peak activity of amylase in control fruits (T_1) was observed on day 6 (23.53 µg/min/g) in ambient storage and on day 21 in fruits stored in cold storage (26.18 µg/min/g). The peak was noticed 3 days later in ambient storage (14.97 µg/min/g) and 7 days later than in controls (T_1) in cold storage (15.86 µg/min/g) in fruits dipped in the pre-harvest spray and the postharvest dip of 1.5 mM melatonin (T_5). The cellulase activity (Figure 2B) increased in the first few days and then showed a decreasing trend. The peak activity of cellulase in fruits treated with the pre-harvest spray and the postharvest dip of 1.5 mM melatonin (T_5) on day 9 (14.47 µg/min/g) in ambient storage and on day 21 (45.34 µg/min/g) showcased maximum cellulase activity on day 28 in cold storage condition. The corresponding control fruit (T_1) was 3 (79.72 µg/min/g on the 6th day) and 7 (70.00 µg/min/g on the 21st) days earlier in terms of peak cellulase activity.

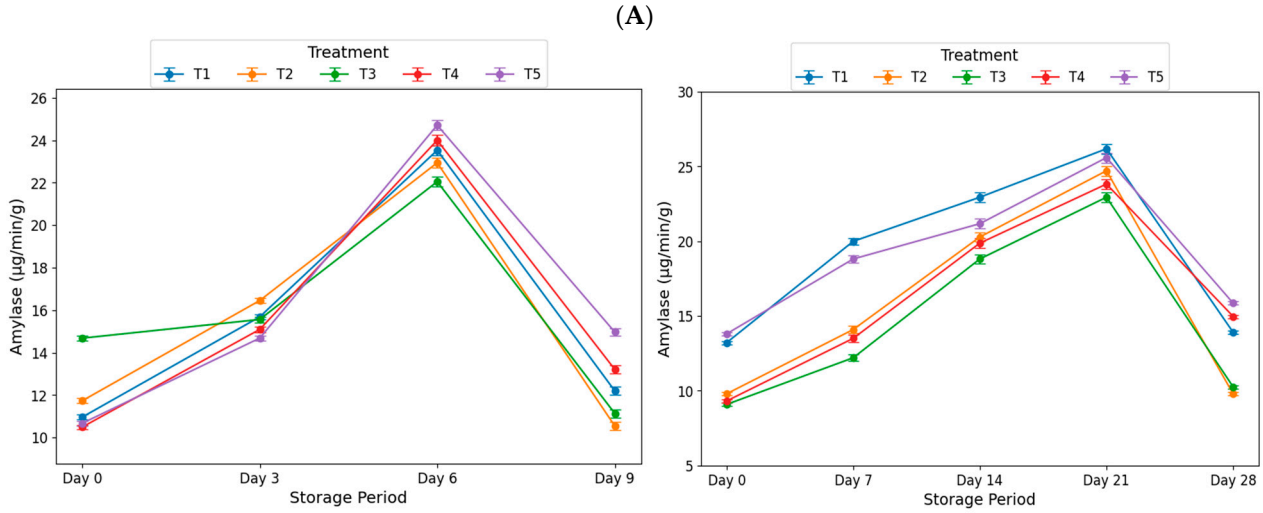

Figure 2. *Cont.*

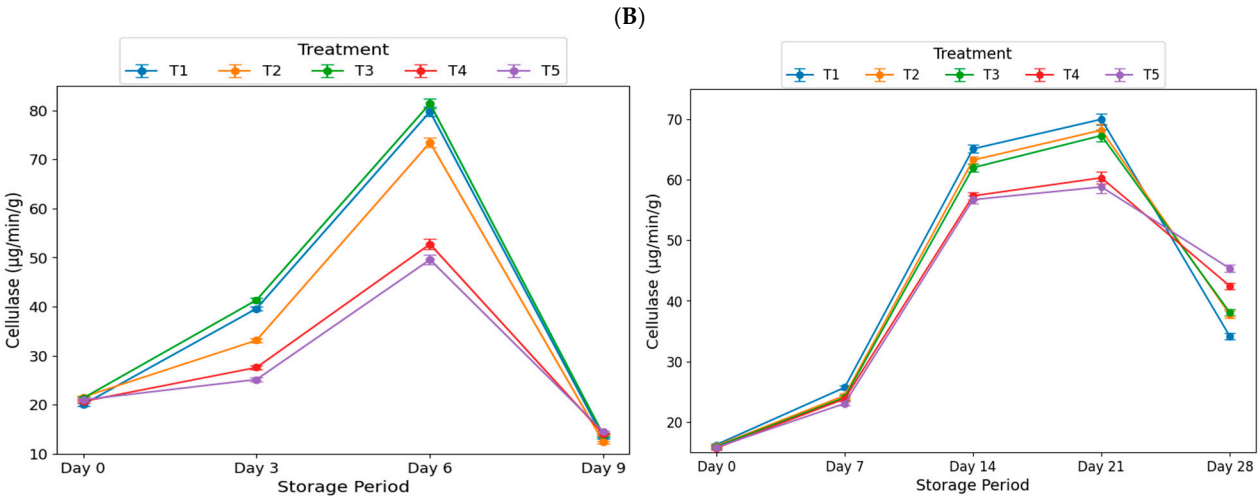

Figure 2. Impact of EMT treatment on amylase (**A**) and cellulase (**B**) activity in papaya fruits during ambient and cold storage. Four replicates' worth of data were gathered, and the lines show the standard error of the means ($p \leq 0.05$). Treatment details: T_1—Control; T_2—Pre-harvest spray of 1.0 mM Melatonin + Postharvest dip of 1.0 mM Melatonin; T_3—Pre-harvest spray of 1.0 mM Melatonin + Postharvest dip of 1.5 mM Melatonin; T_4—Pre-harvest spray of 1.5 mM Melatonin + Postharvest dip of 1.0 mM Melatonin; T_5—Pre-harvest spray of 1.5 mM Melatonin + Postharvest dip of 1.5 mM Melatonin.

3.3. Influence of EMT Treatment on Antioxidant Enzymes

As shown in Figure 3A, MT treatment significantly affected the peroxidase activity under both storage conditions. The activity of peroxidase in the pre-harvest spray and the postharvest dip of 1.5 mM melatonin (T_5) treated fruits (0.74 ΔA/min/g) was higher than in the controls (T_1) (0.61 ΔA/min/g) in CO 8 papayas on day 9 of ambient storage. Similarly, the peak activity was 0.63 ΔA/min/g in T_5 treated fruits compared to control fruits (T_1) (0.48 ΔA/min/g) on day 28 under cold storage. It was apparent from the table that the activity was lower in cold storage, indicating that peroxidase activity was temperature-dependent.

The effect of the pre-harvest spray and the postharvest dip with MT on catalase activity was significant in both storage temperatures (Figure 3B). The activity of catalase increased with the advancement of ripening. Fruit applied with the combination of the pre-harvest spray and the postharvest dip of 1.5 mM melatonin (T_5) recorded 3.95 catalase activity/min/g, which was higher than that found in control fruit (3.01 catalase activity/min/g) in the papaya CO 8 cultivar on last day of ambient storage. A similar trend was also noticed in fruits stored in cold storage, where the peak enzyme activity in cold storage treated with T_5 was 2.84 ΔA/min/g, which was higher than in T_1 fruits (2.18 ΔA/min/g). It was clearly visible from the table that the activity was lower in cold storage than in ambient storage, indicating that catalase activity was temperature-dependent.

The postharvest dip of MT significantly increased the superoxide dismutase activity in papayas compared to control fruits in both ambient and cold storage (Figure 3C). The SOD activity showed an increasing trend in fruits treated with MT throughout the storage period in ambient and cold temperatures. The highest SOD activity was observed in fruit applied with the pre-harvest spray and the postharvest dip of 1.5 mM melatonin (T_5) (0.59 units/mg protein in ambient storage and 0.61 units/mg protein in cold storage) and the lowest was observed in control fruits (T_1) (0.37 units/mg protein in ambient storage and 0.47 units/mg protein in cold storage) on last day of sampling.

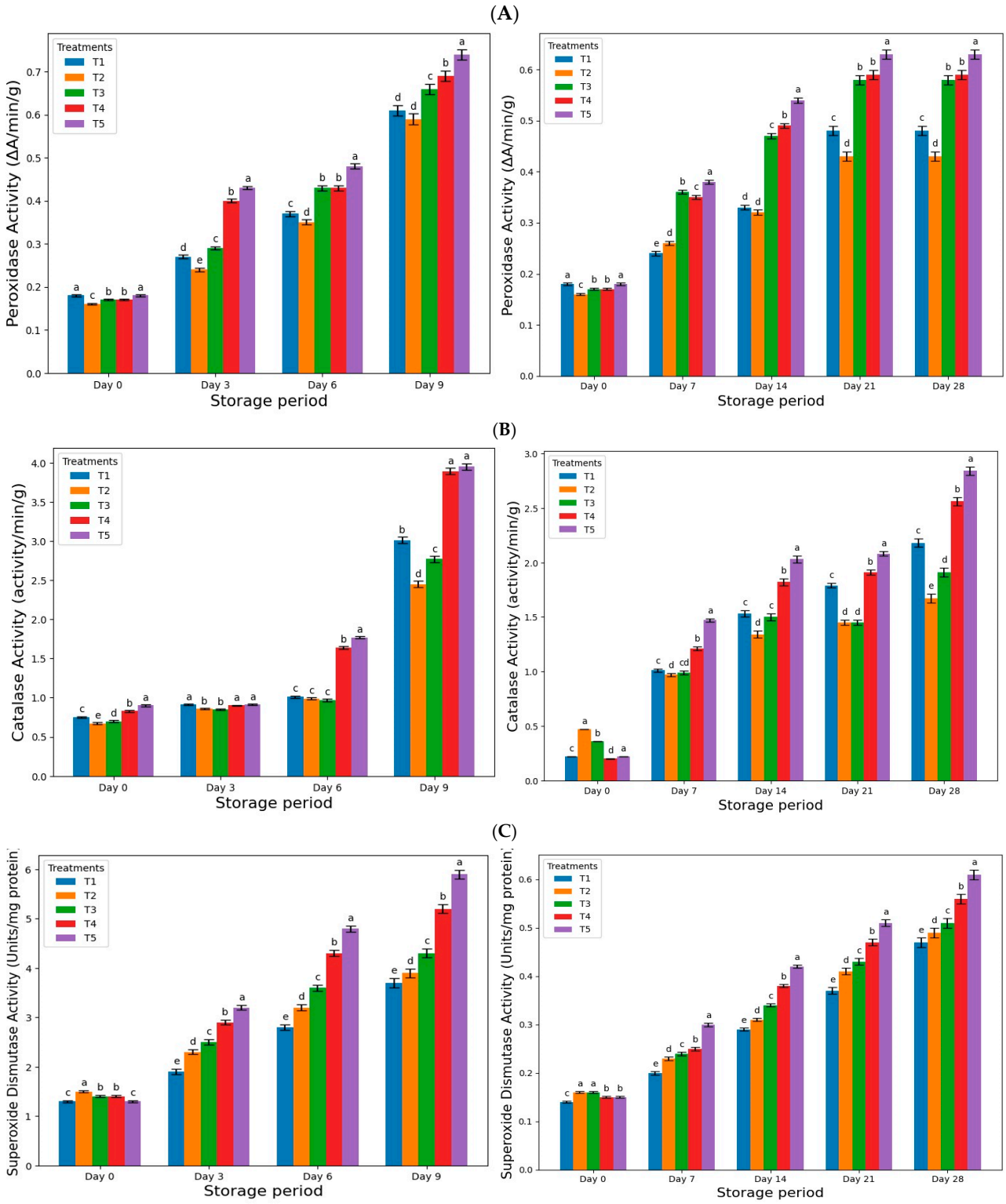

Figure 3. The influence of EMT treatment on peroxidase (**A**), catalase (**B**), superoxide dismutase (**C**) of papaya fruits during ambient and cold storage. Lines representing the standard error of the means ($p \leq 0.05$) are drawn from data derived from the mean of four replicates. Treatment details: T_1—Control; T_2—Pre-harvest spray of 1.0 mM Melatonin + Postharvest dip of 1.0 mM Melatonin; T_3—Pre-harvest spray of 1.0 mM Melatonin + Postharvest dip of 1.5 mM Melatonin; T_4—Pre-harvest spray of 1.5 mM Melatonin + Postharvest dip of 1.0 mM Melatonin; T_5—Pre-harvest spray of 1.5 mM Melatonin + Postharvest dip of 1.5 mM Melatonin. Different letters indicate significance of statistical difference among the treatments.

3.4. Effect of EMT Treatment on Ethylene, CO_2 Evolution Rate and Textural Characteristics

Throughout the storage period, the respiration rate decreased, with melatonin-treated fruits showing a lower respiration rate than the control fruits. Especially, CO_2 evolution (Figure 4) was higher in control fruit (5.1%, 8.2%, and 9.3% on the 3rd, 6th, and 9th day, respectively) compared to the combined application of the pre-harvest spray and the postharvest dip of 1.5 mM melatonin, which had respiration rates of 4.9%, 6.2% and 7.3% on the same days. Similarly, the ethylene evolution rate was higher in control fruits (0.2 ppm, 0.3 ppm, and 0.8 ppm on the 3rd, 6th, and 9th day, respectively). In contrast, EMT fruits had ethylene evolution rates of 0.1 ppm, 0.2 ppm, and 0.5 ppm, respectively (Figure 4).

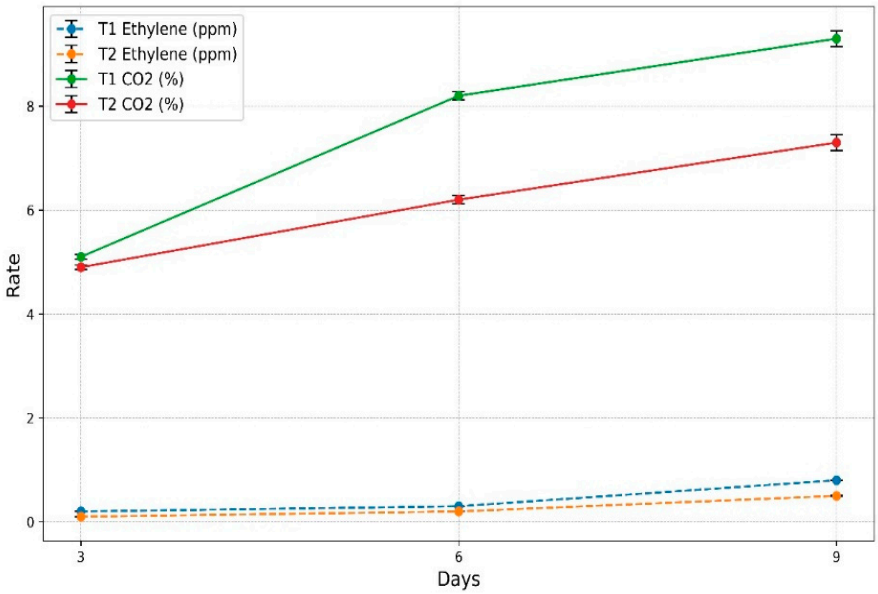

Figure 4. The effect of EMT treatment on ethylene evolution rate and respiration rate (CO_2) of papaya fruits during ambient storage. The lines show the standard error of the means ($p \leq 0.05$) based on data gathered from the mean of four replicates. Treatment details: T_1—Control; T_2—Pre-harvest spray of 1.5 mM Melatonin + Postharvest dip of 1.5 mM Melatonin.

Textural attributes decreased as the storage duration was prolonged, while melatonin-treated fruits showed less ripening enzyme activity than control fruits, as in Table 3. The hardness (2768.78 g, 1523.07 g, 362.11 g), adhesiveness (210.95 g/s, 149.35 g/s, 73.29 g/s), resilience (3.08%, 1.84%, 0.66%) cohesion (0.27, 0.25, 0.15), springiness (90.37%, 84.20%, 83.70%), gumminess (680.14, 346.01, 134.80), and chewiness (614.65, 306.09, 127.14) of control fruit was lower compared to melatonin-treated fruit, which had a hardness of 2608.05 g, 2008.04 g, 1906.22 g, an adhesiveness of 438.14, 167.62, 201.25 g/s, a resilience of 3.25%, 2.51%, 1.98%, a cohesion of 0.43, 0.37, 0.29, a springiness of 94.32%, 92.84%, 90.37%, a gumminess of 1113.78, 579.77, 279.37, and a chewiness of 1034.02, 523.94, 233.84 on 3rd, 6th, and 9th day of ambient storage.

Table 3. Effect of combination of pre-harvest and postharvest application of melatonin on texture in papaya cv. CO 8.

Parameters	Storage Period Treatment	Day 3	Day 6	Day 9
Hardness	T_1	2768.78 ^{a}	1523.07 ^{b}	362.11 ^{b}
	T_2	2608.05 ^{b}	2008.04 ^{a}	1906.22 ^{a}
	SE (d)	33.83	31.10	9.47
	CD	82.79	76.12	23.18

Table 3. *Cont.*

Parameters	Storage Period Treatment	Day 3	Day 6	Day 9
Adhesiveness	T_1	−73.29 [a]	−149.35 [a]	−210.95 [a]
	T_2	−167.62 [b]	−201.25 [b]	−438.14 [b]
	SE (d)	1.52	3.36	4.34
	CD	3.73	8.23	10.64
Resilience	T_1	3.08 [b]	1.84 [b]	0.66 [b]
	T_2	3.25 [a]	2.51 [a]	1.98 [a]
	SE (d)	0.06	0.03	0.02
	CD	0.16	0.07	0.06
Cohesion	T_1	0.27 [b]	0.25 [b]	0.15 [b]
	T_2	0.43 [a]	0.37 [a]	0.29 [a]
	SE (d)	0.003	0.006	0.001
	CD	0.008	0.016	0.002
Springiness	T_1	90.37 [b]	84.20 [b]	83.70 [b]
	T_2	94.32 [a]	92.84 [a]	90.37 [a]
	SE (d)	1.27	0.95	0.54
	CD	3.12	2.34	1.33
Gumminess	T_1	680.14 [b]	346.01 [b]	134.80 [b]
	T_2	1113.78 [a]	579.77 [a]	279.37 [a]
	SE (d)	10.98	7.12	2.06
	CD	26.86	17.43	5.05
Chewiness	T_1	614.65 [b]	306.09 [b]	127.14 [b]
	T_2	1034.02 [a]	523.94 [a]	233.84 [a]
	SE (d)	10.43	5.02	1.99
	CD	25.53	12.30	4.87

Four replicates are averaged to obtain the data. Significant differences are shown at $p \leq 0.05$ for mean values with distinct letters. Treatment details: T_1—Control; T_2—Pre-harvest spray of 1.5 mM Melatonin + Postharvest dip of 1.5 mM Melatonin under ambient storage.

3.5. Correlation Matrix

The analysis performed by correlating several parameters (Figure 5) showed that PLW was correlated positively with respiration gases and ripening enzymes, and negatively correlated with firmness, antioxidant enzymes, and textural attributes. Firmness was positively related to antioxidant enzymes and textural qualities but negatively correlated with ripening enzymes. In contrast, textural attributes were negatively correlated with ripening enzymes and respiration rates but positively correlated with antioxidant enzymes.

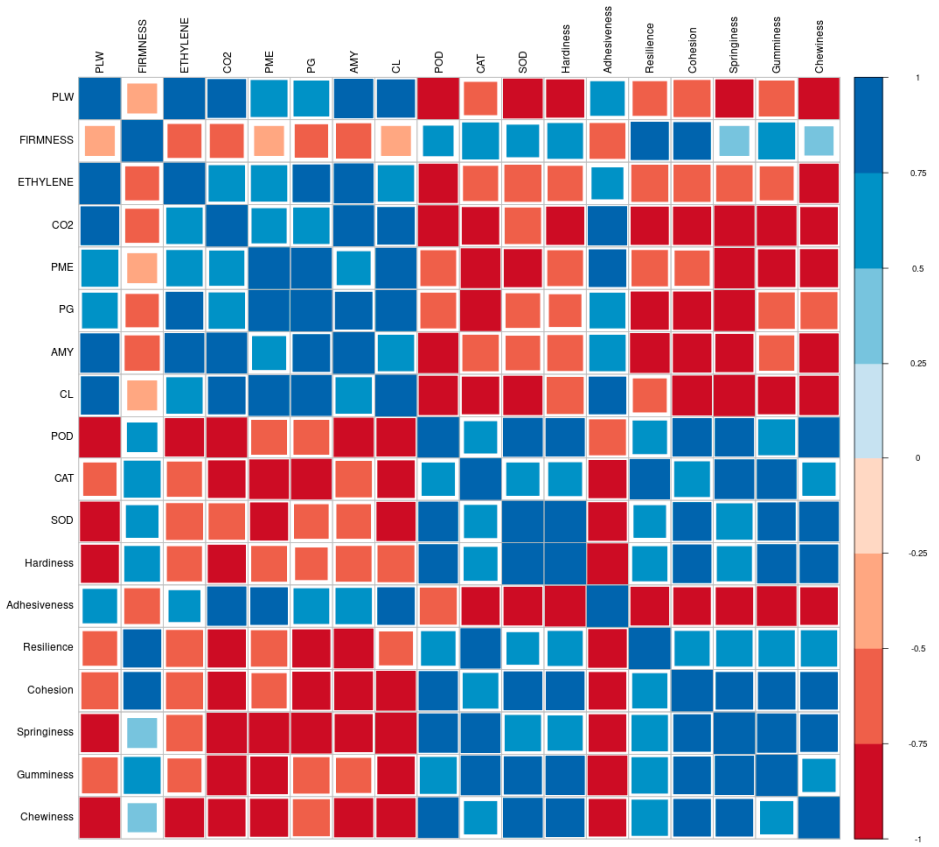

Figure 5. Correlation correlogram of each variable of EMT-treated papaya fruits during ambient storage. The strength of the correlation is shown by the color: a positive correlation is shown by the color blue, and a negative correlation is shown by the color red. (PLW—Physiological loss in weight, PME—Pectin methylesterase, PG—Polygalacturonase, AMY—Amylase, CL—Cellulase, POD—Peroxidase, CAT—Catalase, SOD—Superoxide dismutase).

4. Discussion

Papayas' shelf life has been extended and postharvest loss has decreased due to the investigation and development of numerous pre- and postharvest methods over the last few decades. Nitric oxide, salicylic acid, and edible coatings [13], hot water [31], 1-MCP [32], and chitosan and hot water dip combination treatments [11] have all been linked to extending fruit ripening and maintaining quality throughout the period following harvest. Nevertheless, the majority of these methods encounter certain issues when they are being used. For instance, determining the proper concentration of 1-MCP therapy might be challenging because an improper treatment would result in a ripening issue in the fruit, known as "rubbery texture," and although papaya fruits treated with hot water had a lower incidence of anthracnose, the fruit ripened more quickly [31]. Melatonin is a substance that is harmless and good for the environment, and postharvest preservation has drawn more attention in recent days. Hence, numerous studies have demonstrated that applying melatonin to postharvest fruit can delay its ripening and senescence while extending its shelf life duration.

Melatonin's effects as a pre-harvest treatment on fruit growth and ripening have yet to be well studied. However, reports of differing outcomes, depending on the type of fruit, amount applied, and timing of application, exist. The ideal time to apply melatonin as a pre-harvest treatment in controlling ethylene evolution and extending shelf life is when the papaya reaches the mature green stage or just before the start of ripening (also known as the early breaker stage or pre-climateric phase) [33]. At the respective stage of application of melatonin, it functions as an ethylene synthesis regulator by suppressing the expression of genes (such as the ACS and ACO genes) involved in ethylene synthesis. This can slow down

the ripening process, which is especially helpful when ethylene production starts to spike during the breaker stage [34]. Moreover, while applying the pre-harvest spray, melatonin enhances the fruit's endogenous antioxidant system, which becomes increasingly important during the pre-ripening phase. Fruits go through major physiological and biochemical changes at the breaker stage that cause them to ripen. When melatonin is administered at this point, it helps to postpone these changes, especially by reducing the rate of cell wall deterioration and softening, preserving the firmness of the fruit, and increasing its shelf life [35]. In grapevines at the pre-veraison stage, melatonin administration once or twice at 0.43 mM considerably enhanced (6.6%) the size of the grape berry, which was associated with an increase in endogenous melatonin concentration [36]. In the "Prime Giant" sweet cherry, melatonin, when applied at pit hardening at concentrations of 0.1 and 0.01 mM, inhibited fruit ripening [37]. Apricot yield and fruit weight were boosted by a foliar spray of melatonin. However, on-tree ripening remained unaffected [38]. Therefore, preliminary studies with pre-harvest spray of melatonin in papaya were conducted, and based on the results, a combination experiment with pre-harvest spray and the postharvest dip was carried out in this study.

According to this study's findings, which align with most previous studies on other fruit crops, the papaya fruit's softening, ethylene content peak, and respiration rate can all be delayed with the proper melatonin treatment. Every melatonin treatment may successfully lower the respiration rate and ethylene during the subsequent storage, demonstrating a greater impact than the climacteric fruit-ripening phase.

The main causes of weight loss in fruits during both ambient and cold storage (Table 1) are quickly increased by elevated respiration and ethylene production. Similarly, a noteworthy rise in weight loss was noted by Ong et al. [9] during the papayas' postharvest ripening period. Over the course of the storage periods, the EMT therapy dramatically reduced the rate of weight loss and senescence compared to the control. Similar results have been documented, showing that during cold storage, strawberries treated with EMT showed significantly reduced rates of senescence and weight loss [34]. Weight loss rises during storage in papaya fruit [8] and cherries [39] because of increased respiration rate, rate of evaporation, as well as quick use of metabolites that are kept for metabolic processes within cells. Similarly, EMT therapy significantly decreased peach quality degradation and weight loss during storage, according to Gao et al. [40]. Recent research has shown that EMT therapy greatly enhanced resistance, postponed senescence, and decreased weight loss in sweet cherry fruits against abiotic stress [19].

Fruits that have been treated with melatonin may still be firm in ambient and cold storage conditions, respectively, which could be a sign of reduced enzyme activity. The findings showed that melatonin treatments postponed the decline in firmness (Table 2), which was in line with previous work by Zhai et al. [41], who found that treatment with melatonin delayed cell wall breakdown and showed reduced tissue deformability. Fruit flesh firmness is a crucial characteristic that declines with fruit age due to the breakdown of cell wall components like cellulose, hemicellulose, and pectin compounds, as well as a drop in cell turgor pressure, a characteristic that establishes the fruit's capability for storage [42]. Additionally, Gao et al. [40] revealed that melatonin-treated peach fruits had increased firmness by up-regulating many efficient genes in the breakdown of cell walls during storage. Previous studies reported softening in fruit firmness due to water loss after picking due to intense respiratory transpiration in various other fruits such as banana [43], orange [44], and guava [45].

Papaya fruit is climacteric; hence, differences in its responsiveness to ethylene and consequent autocatalytic production can initiate and regulate the biochemical and molecular changes that occur throughout ripening. The plant hormone ethylene, produced by carbon dioxide, is crucial for ripening and senescence. In higher plants, ACS and ACO work in unison to produce ethylene. ACS and ACO activity in papaya fruits correlate with the transcription of their corresponding genes, which are minimal in mature, green pre-climacteric fruit and gradually rise during ripening [46]. According to the cur-

rent investigation, MT administration inhibited and delayed the generation of climacteric ethylene and the buildup of ACC, which is consistent with an MT-mediated decrease in ACS and ACO activity (Figure 4). Exogenous melatonin treatment has been demonstrated to decrease ethylene production and suppress the ACS and ACO expression in bananas and pears that have ripened in ambient circumstances, which is consistent with our findings [18,41]. Nevertheless, the production of ethylene in papaya fruits was slowed down by high CO_2 levels, which slowed down the maturation of fruit by lowering the expression of genes linked to ethylene production during fruit maturity (Figure 4). For 1-aminocyclopropane-1-carboxylic (ACC) oxidase, carbon dioxide functions as a cofactor [47]. The primary enzyme that produces ethylene, 1-aminocyclopropane-1-carboxylic (ACC), is inhibited by cold temperatures under cold storage conditions. Reconditioning the fruit at room temperature causes a sharp rise in ethylene production due to ACC formation, which, in turn, makes chilling injury more likely to arise [48]; hence, fruits ripen faster in ambient storage than in cold storage. It is discovered that MT causes a delay in the fruit's climacteric ascent. However, the administration of exogenous MT could change the climacteric increase. Similar findings regarding the way CO_2 affects ethylene were reported in peaches [49], lemons [50], and figs [51].

Pectin methylesterase, a member of the carbohydrate esterases class, initiates particular demethylesterification of homogalacturonan (HGA), releasing methanol and protons and generating negatively charged carboxyl groups. Following demethylesterification, HGA is linked to Ca^{2+} ions to create an "egg-box" type structure that supports the integrity of fruit tissue and the stiffness of cell walls [52]. It serves as a substrate for the enzymes that break down pectin. Because polygalacturonases need a non-esterified substrate, they work on polygalacturonic acid (PGA) after pectin methylesterase, breaking down the α-1,4 glycosidic linkages in pectic acid [52]. Polygalacturonase activity has a positive association with the softening of the fruit. Furthermore, similar findings were reported in table grape berries [53] and passion fruit [54]. Amylase is an endo-hydrolase that breaks down polysaccharide chains into maltose and glucose by hydrolyzing the alpha link. Depending on where the hydrolyzing location occurs, different types of amylase are classified: α-amylase breaks down long-chain carbohydrates by hydrolyzing 1,4-glycosidic bonds; β-amylase breaks down 1, 4-glucan linkages in non-reducing ends of polysaccharide chains; and γ-amylase hydrolyses both internal 1,6-glycosidic and terminal 1,4-glycosyldic links [55]. Similar work was reported by Goyal et al. [56] in mangoes, and the application of the pre-harvest spray and the postharvest dip of 1.5 mM slows the breakdown of the polysaccharide chains. The term "cellulase" describes a combination of three enzymes that break the glycosidic bonds in cellulose: endoglucanase, exoglucanase, and β-glucosidase. Endoglucanase is an enzyme that randomly breaks down cellulose to produce oligosaccharides with different diameters, which are then transformed by exoglucanase into tiny cello-oligosaccharides. The last process in the hydrolysis of cellulose is catalyzed by the rate-limiting enzyme β-glucosidase. It quickly converts the cello-oligosaccharides into glucose, a simpler sugar [52]. Such works were previously reported in kiwis [57], mangoes [58], and passion fruit [54]. By removing methyl-ester or acetyl groups, polymeric or single-sugar side chains from homogalacturonic acids, cleaving polymeric backbones, and consequently loosening the hydrogen bonding between cellulose microfibrils and glycans [52], the application of melatonin inhibited cell wall-degrading enzyme activities (e.g., pectin, cellulose) more under cold storage [59] and provided chilling tolerance than ambient storage conditions. In a nutshell, the study shows that melatonin downregulates the activity of all the ripening enzymes.

In this study (Figure 1A,B) and (Figure 2A,B), control fruits exhibited higher activity of all the aforementioned enzymes in papayas. The peak activity of these enzymes was attained earlier in control fruits than in fruits treated with the pre-harvest spray and the postharvest dip of 1.5 mM melatonin (Figures 6 and 7). It is apparent that the delayed softening and enhanced shelf life of fruits dipped in melatonin might be due to the lower enzyme activity and delayed peak enzyme activity.

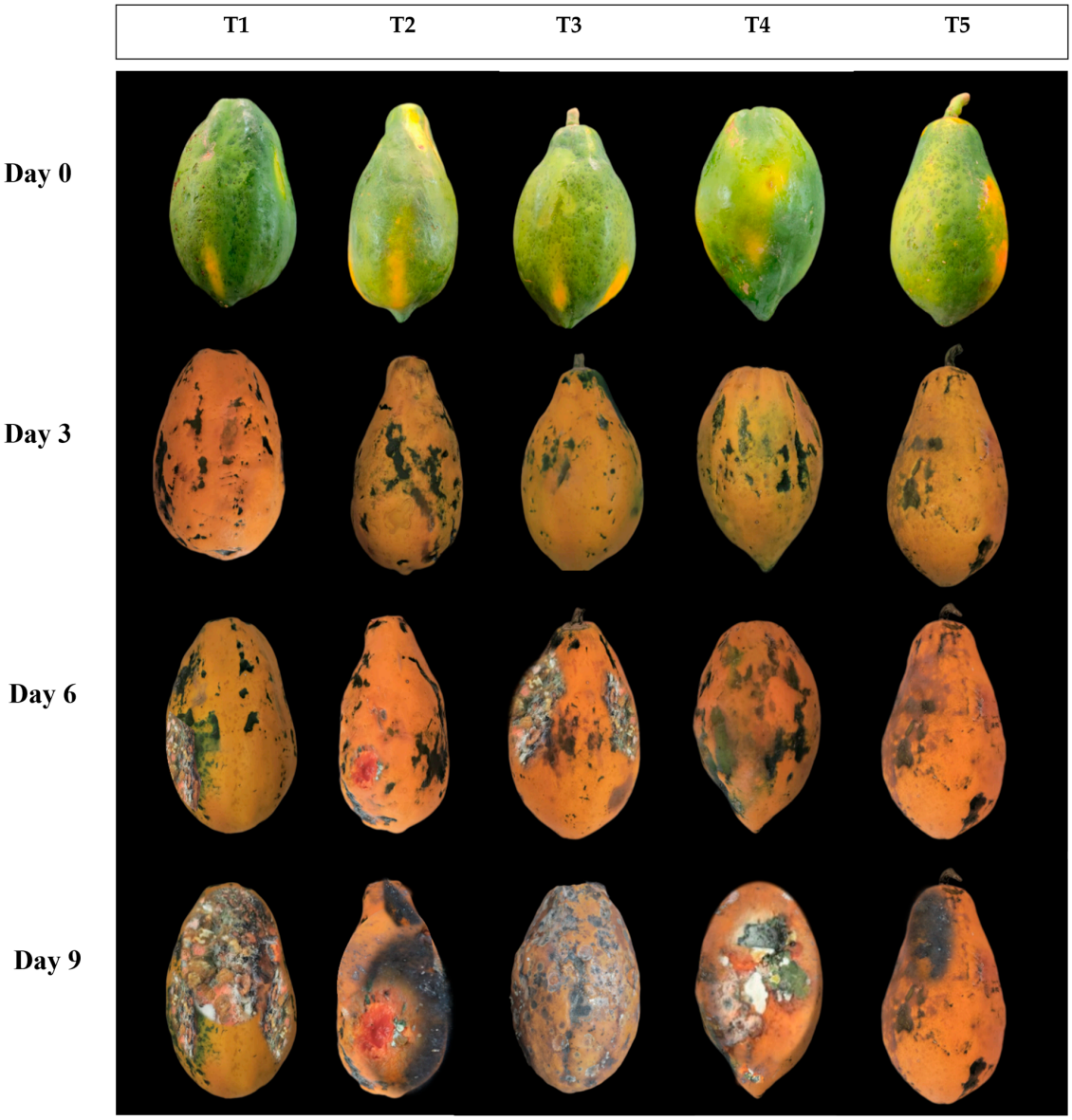

Figure 6. Effect of EMT treatment on visual quality under ambient storage conditions. Treatment details: T_1—Control; T_2—Pre-harvest spray of 1.0 mM Melatonin + Postharvest dip of 1.0 mM Melatonin; T_3—Pre-harvest spray of 1.0 mM Melatonin + Postharvest dip of 1.5 mM Melatonin; T_4—Pre-harvest spray of 1.5 mM Melatonin + Postharvest dip of 1.0 mM Melatonin; T_5—Pre-harvest spray of 1.5 mM Melatonin + Postharvest dip of 1.5 mM Melatonin.

Texture profile analysis (TPA) was used to determine the mechanical characteristics of the papaya fruit, including its hardness, adhesiveness, resilience, cohesiveness, springiness, gumminess, and chewiness. All the above parameters (Table 3) decreased as the days passed because of the demethylation of pectin methylesterase, which in turn increased the polygalacturonase activity, and we know from the above-mentioned biochemical process of PG that it has a positive relation with the softening of fruits. Cellulase is an enzyme that degrades the cell-wall cellulose, a major component of cell walls, which may work with pectic enzymes to soften the fruit, and ethylene may control cellulase activity [60]. Studies examining texture were previously reported in fruits like apricots [61] and kiwis [62]. However, when applied with 1.5 mM melatonin as a combination of the pre-harvest spray and the postharvest dip, it slowed the enzymatic reactions more than in control fruits, and hence, the shelf life extended. Furthermore, in the correlation matrix, it can be seen that the textural parameters were negatively related to ripening enzymes.

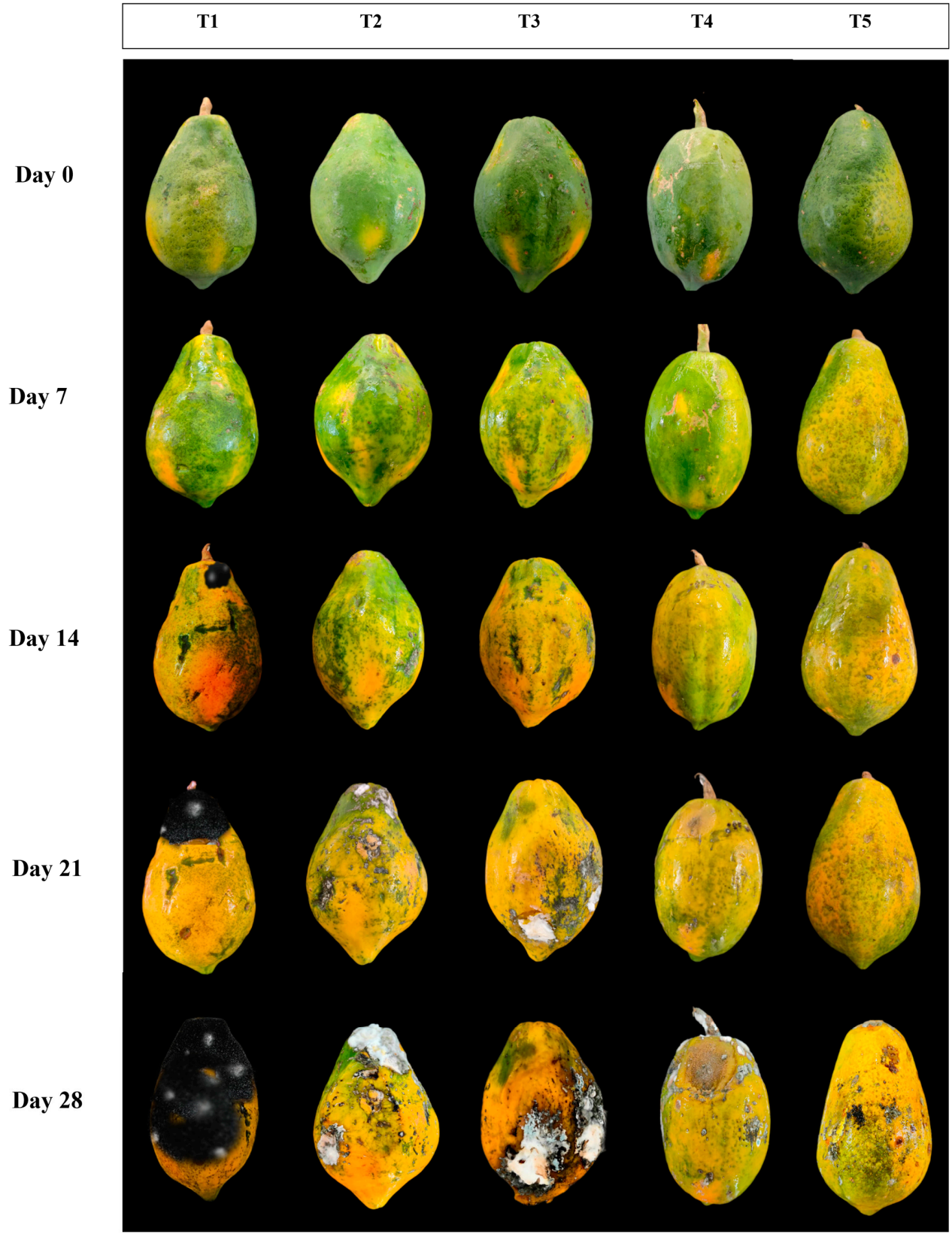

Figure 7. Effect of EMT treatment on visual quality under cold storage condition. Treatment details: T_1—Control; T_2—Pre-harvest spray of 1.0 mM Melatonin + Postharvest dip of 1.0 mM Melatonin; T_3—Pre-harvest spray of 1.0 mM Melatonin + Postharvest dip of 1.5 mM Melatonin; T_4—Pre-harvest spray of 1.5 mM Melatonin + Postharvest dip of 1.0 mM Melatonin; T_5—Pre-harvest spray of 1.5 mM Melatonin + Postharvest dip of 1.5 mM Melatonin.

The use of melatonin is crucial in augmenting the enzymatic activity that scavenges ROS and defends the integrity of the membrane inside fruit tissues [63,64]. According to Xu et al. [64], antioxidant enzymes such as SOD, POD, and CAT control the peroxidation of lipids and the buildup of ROS. Increased antioxidant enzyme activity may lessen lipid peroxidation, which helps delay senescence. Similar studies have been conducted with fruits like mangoes [65], avocados [66], and peaches [67]. Additionally, our results (Figure 3A–C) are consistent with earlier research showing that, in sweet cherries, SOD, POD and CAT activity increased after EMT according to Wang et al. [19]. Furthermore, EMT treatment increased the enzyme activity of antioxidants and decreased ROS biosynthesis, which in turn inhibited ethylene biosynthesis and stopped the ripening process of fruit, preventing chilling injury in cold storage [68]. Similar findings have been documented in fruits such as bananas [18], pears [41], and blueberries [69] at storage time. Therefore, oxidative stress decreased after EMT, which helped to down-regulate senescence and preserve papaya fruits' freshness for a longer period during storage in both ambient and cold.

Hence, from our study, it can be conveyed that the combined application of a preharvest spray of 1.5 mM melatonin and a postharvest dip of 1.5 mM melatonin was better performing than the control. It also showed similar results for the above-discussed parameters under both ambient and cold storage conditions, except that weight loss, firmness, ripening enzymes (PME, polygalacturonase, amylase, and cellulase), and antioxidant enzyme (SOD, CAT, POD) activity under the cold storage condition (fruits extended until 28 days) followed a slower trend than in the ambient storage condition (fruits extended until 9 days) because fruits kept at temperatures below 13 °C suffer from chilling injury as a result of cellular energy being depleted by the oxidation of polyphenolic compounds rather than regular respiration, which raises the level of reactive oxygen species (ROS) [70]. By increasing antioxidant activity, melatonin shielded the fruits from chilling injury [71].

5. Conclusions

This study assessed the full range of favourable impacts of exogenous melatonin treatment on the quality of papaya fruits, including the evaluation of the primary physicochemical features, the activities of enzymes that cause ripening, the antioxidant activity, gases evolution rate (ethylene and CO_2), and textural characteristics. EMT treatment positively decreased weight loss and postponed postharvest senescence compared to the control. Additionally, the EMT treatment dramatically decreased the levels of ROS during ambient and cold storage by boosting the activities of antioxidant enzymes in papaya fruits. The differences in texture brought on by cell wall-degrading or ripening enzymes and direct or indirect impacts on the synthesis or action of ethylene produced by CO_2 may be responsible for the delay in softening and ripening of papayas in response to MT. The melatonin-treated fruits stayed healthy until the 8th day under ambient storage and for 28 days under cold storage. Consequently, applying EMT is a viable strategy for postharvest life extension, quality attribute preservation, and delayed ripening and senescence in papayas under both ambient and cold storage conditions. Future research could explore the use of melatonin in organic farming, its synergy with other biostimulants, nutrients, and hormones, breeding or genetically engineering crops for higher melatonin levels or responsiveness, and optimizing application methods like soil drenching and seed priming.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae10101099/s1. Table S1: Effect of EMT on pectin methylesterase, polygalacturonase, amylase and cellulase; Table S2: Effect of EMT on peroxidase, catalase and superoxide dismutase; Table S3: Effect of EMT on Ethylene and respiration evolution rate; Figure S1: Graphical representation of effect of EMT on physiological loss in weight and firmness; Figure S2: Graphical representation of effect of EMT on textural attributes.

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writing—review and editing. S.A.: resources, writing—review and editing. J.I.: writing—review and editing. E.T.: writing—review and editing. S.M.: conceptualization, methodology, writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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