



# Melatonin and Chitosan Coating Effects on Banana Postharvest Life and Physiological Traits

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## ABSTRACT

Fruit ripening involves marked physiological and biochemical changes that affect fruit color, taste, aroma, texture, and nutritional value. A significant amount of research has shown that chitosan interacts with other postharvest treatments, which can enhance the impact of the chitosan coating. This study considered the effects of chitosan coating and melatonin on banana shelf-life and fruit quality at the green mature stage. The studied variables had three levels of melatonin 0, 75, and 150 mg L<sup>-1</sup>, two levels of chitosan coating 0, 0.25, and 0.5%, and three storage durations 3, 6, and 9 days. Fruits were harvested at the green mature stage and stored at 25 °C and 80% RH for 9 days. The results showed that melatonin and chitosan coating interacted and significantly affected green mature banana quality and shelf-life during the storage time. Chitosan and melatonin interaction delayed the decrease in chlorophyll and the increase in carotenoids, fruit maturity, and color change. Our results showed that on the ninth day of storage under chitosan (0.5%) treatment along with melatonin at a concentration of 150 mg L<sup>-1</sup>, the total chlorophyll content became 3.89% lower than that under control conditions. The level of antioxidant enzyme activity in the treated samples after 3, 6, and 9 days of storage was more than in the control sample. The results showed that chitosan (0.5%) and melatonin (150 mg L<sup>-1</sup>) successfully increased the shelf life of banana fruits.

## Introduction

Fruits harvested at the immature stage are more likely to suffer physical damage and transpiration losses during postharvest and have lower qualities than fruits harvested at the proper maturity stage (Avila et al., 2006; Rodrigues et al., 2012). Enhancing our available knowledge of polyamine metabolism during postharvest would

be relevant to understanding the ripening behavior of fruits (Singh et al., 2019). Conversely, fruits harvested at full maturity better maintain their quality due to high acidity and lower weight loss (Rincón et al., 2016). Delay in fruit harvest leads to over-ripening in very soft fruits with floury texture and causes insipid flavor soon after harvest (Singh et al., 2019; Rincón et al., 2016).

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One of the most critical waste factors after harvesting banana fruit is the undesirable effects of low temperatures in storage conditions (Siyari et al., 2011). Since banana is a tropical product, due to the presence of saturated fatty acids in its membrane, it freezes at low temperatures, and due to the reduction of oxygen, fermentation occurs. In addition to the apparent damage and softening of the tissues, the chemical composition of the fruit also changes (Sayari et al., 2011).

In recent years, consumer preferences have shifted to the demand for a lower consumption of chemical preservatives (or antimicrobials) in food while preferring more natural and fresh food characteristics (Liato et al., 2017). Thus, researchers have been investigating safer alternative substances to reduce the use of synthetic fungicides in fruits (Oliveira et al., 2014). Edible coatings (primarily comprising proteins, lipids, or polysaccharides) are considered an environmentally-friendly technology that can help to extend the shelf-life of fruits through the reduction of moisture loss and respiration rate, thereby preventing physical damage and enhancing product appearance (Fagundes et al., 2014).

There has been a significant increase in the number of studies on melatonin in plants recently. This substance has multiple functions in organisms, including their potential in plant physiology. Its role as an anti-stress agent against stress-causing factors, such as drought, salinity, low and high temperature, ultraviolet rays, and toxic chemicals, has been the subject of frequent research. Among the many functions of melatonin in plants, the role of antioxidants and growth initiation is remarkable (Paredes et al., 2009; Parck, 2011). Melatonin is a polar molecule with physiological and cellular functions in various fields (Giglou et al., 2023). This substance was introduced in 1958 as a neurohormone that acts by being secreted into the bloodstream in biological systems (Carrillo-Vico et al., 2013). It regulates many physiological relationships, such as sleep rhythms, feelings, body temperature, appetite, sexual behaviors, and the immune system in bodies (Giglou et al., 2023; Vico et al., 2013).

Plants treated with melatonin under water-deficit stress increased significantly in seed germination and root growth intensity and showed antioxidant defense. This occurrence was due to melatonin consumption, which reduces the oxidative stress from water-deficit stress to its lowest value (Fagundes et al., 2014). The effect of stress became alleviated in apples treated with melatonin in most of the morphophysiological traits compared to the control. Moreover,

melatonin reduced the levels of hydrogen peroxide due to the increased stimulation of antioxidant enzymes such as catalase, ascorbate peroxidase, and peroxidase, and the transfer of potassium and sodium ions, based on which melatonin significantly helped to reduce the effects of stress (Paredes et al., 2009; Fagundes et al., 2014).

Edible coatings are an environmentally friendly technology that helps to minimize moisture loss, oxidation, or gas exchange processes to improve the quality and shelf life of fruits (Basit et al., 2019). Chitosan is a biodegradable and biocompatible polymer derived from natural renewable resources. It has numerous applications in various fields, one of which is in edible films and coatings. Furthermore, chitosan cannot penetrate tissues within muscle organs or even fruit cover due to its insolubility in water; therefore, it appears as a safe physical modulator of the fruit biological system (Romanazzi et al., 2017). It has antibacterial and antifungal properties, which qualify it for food protection; however, its weak mechanical properties and gas and water-vapor permeability limit its uses. These complexities offer an exciting potential for fresh food consumption. Chitosan derivatives have broad applications for their advantages due to their prominently active amino groups (Morgado et al., 2013; Tang et al., 2015).

To our knowledge, little information is available regarding the effect of chitosan coating and melatonin on the postharvest quality of banana fruits. Thus, this study evaluated how green-mature bananas can change from multiple perspectives in response to chitosan and melatonin treatments before and during storage. The current research considered fruit color changes and the conversion of chlorophyll pigments into carotenoids in bananas stored at 25 °C for nine days. It also included an assessment of the nutrient profile of the bananas after the storage period, hypothesizing that the nutritional value of the bananas can become less disrupted in storage because of the treatments.

## Materials and Methods

### *Plant materials and experimental design*

Banana fruits were harvested in their green mature stage. The fruits were selected for uniformity, shape, medium shape, color, and size. The banana fruits were first weighed, and then medium-sized fruits were used. Any blemished or diseased fruit was discarded. Chitosan (C<sub>12</sub>H<sub>24</sub>N<sub>2</sub>O<sub>9</sub>) (Sigma Aldrich Corporation-India) had a medium molecular weight (CAS number: 9012-76-4) and standard melatonin was used

(Sigma Aldrich Corporation-India) (CAS number: 73-31-4). A concentration of 0.5% chitosan was prepared by dissolving 5 g of chitosan in 1,000 mL of distilled water and gradually adding 10 mL acetic acid. The final pH became 5.6. The solution was heated and constantly agitated for 24 h. The final solution was adjusted to 1,000 mL of distilled water. Chitosan was prepared at two different concentrations, i.e., 0.25 and 0.5% (w/v), in an aqueous acetic acid solution (0.5% v/v). For melatonin, 150 mg was prepared by dissolving 150 mg of melatonin in 1,000 mL of distilled water. Melatonin was prepared at two different concentrations, 75 and 150 mg L<sup>-1</sup>.

After cooling at 20 °C, the banana fruits (10 fruits per sample) were dipped separately in the chitosan and melatonin solution for 60 seconds to allow for chitosan and melatonin adhesion to the whole fruit surface and create a uniform film. The samples were dried at room temperature. The same number of fruits (10 fruits per sample) in the control group were dipped in distilled water. Then, the fruits were stored at 20 °C and 85% RH for nine days. At 3-day intervals, one group was taken at random and transferred to 20 °C (shelf-life) for one day and subjected to physicochemical analysis.

### Color measurements

Banana fruit color was non-destructively determined using a colorimeter (CR-10 plus, Konica Minolta Inc., Tokyo, Japan) according to a method by Giglou et al. (2023). The color difference indices ( $\Delta E$ ), C (chroma), and H (hue) were calculated based on 'L' values (lightness), 'a' (green to red), and 'b' (blue to yellow).

### Chlorophyll a and b, total chlorophyll, and carotenoids

For this purpose, 100 mg of fresh leaf tissue was ground entirely and homogenized with 10 mL of 80% acetone in a Chinese mortar. The resulting solution was centrifuged at 4 °C for 10 min at 6000 rpm, and then the supernatant was separated. Light absorption values of each solution were read at wavelengths of 470, 645, and 663 nm by a spectrophotometer (HACH Company, Loveland, USA). Then, the chlorophyll a, b, and carotenoid contents were calculated as mg g<sup>-1</sup> fresh weight of fruit tissue using the following equation (Arnon, 1949):

$$\begin{aligned} \text{Chlorophyll } a &= 12.25 A_{663} - 2.79 A_{645} \\ \text{Chlorophyll } b &= 21.5 A_{645} - 5.1 A_{663} \\ \text{Total chlorophyll} &= \text{Chl } a + \text{Chl } b \end{aligned}$$

### Carotenoids

$$= \frac{(1000 A_{470} - 1.82 \text{ chl } a - 85.25 \text{ chl } b)}{198}$$

### Total phenol content

To measure the total phenol content, 2 mL of sodium carbonate (2%), 2.8 mL of distilled water, and 100  $\mu$ L of Folin-Ciocalteu phenol reagent (50%) were added to 100  $\mu$ L of plant extract. Half of their absorbance time was recorded at 720 nm compared to the control. Gallic acid was used as a standard for illustrating a standard curve. The total phenol content of the extracts per mg was the equivalent of glycogen acid per gram of plant dry weight (Meda et al., 2005).

### Measurement of catalase

A method by Boominathan and Doran (2002) was used for measuring catalase enzyme in the leaves. For this purpose, 900  $\mu$ L of the reaction solution comprised 10 mM solution of hydrogen peroxide in phosphate that buffered saline without PVP and 100  $\mu$ L of the enzyme extract in a glass cell. After adding H<sub>2</sub>O<sub>2</sub> in the reaction solution, it decreased immediately due to the decomposition of H<sub>2</sub>O<sub>2</sub> and catalase action, measured at a wavelength of 240 nm and within 1 min by a spectrophotometer (Uvi Light XS 5 SECOMAM). CAT activity was measured and calculated accordingly.

### Measurement of peroxidase enzyme activity (POD)

Peroxidase enzyme activity was measured using a method by Upadhyaya et al. (1985). The reaction mixture consisted of 2.5 mL of 50 mM phosphate buffer (pH = 7) containing 1 mL of 1% guaiacol, 1 mL of 1% hydrogen peroxide and 0.1 mL of extract. Peroxidase enzyme activity was calculated by measuring the increase in absorbance in 1 min at a wavelength of 420 nm by a spectrophotometer. The following equation was used for measuring the activity level of the extinction coefficient (26.6 mM<sup>-1</sup>cm<sup>-1</sup>).

$$\begin{aligned} \text{Units (Mm min}^{-1}\text{)} &= \\ &= \frac{\frac{\text{doD}}{\text{min (slop)}} \times \text{Vol. of assay (0.0003)}}{\text{Extinction coefficient (0.0436)}} \end{aligned}$$

### Measurement of superoxide dismutase enzyme activity SOD

The measurement of superoxide dismutase enzyme activity was performed by the method of Giannopolitis and Ries (1997) and by measuring the light inhibition of nitro blue tetrazolium chloride at a wavelength of 560 nm (Giannopolitis and Ries, 1997).

## Ethylene

To measure ethylene production, banana fruits at different ripening stages were harvested and measured according to Jian et al. (2019).

## Statistical analysis

This research was carried out as a factorial experiment in a completely randomized design (CRD). Chitosan (0, 0.25, and 0.5%), melatonin (0, 75, and 150 mg L<sup>-1</sup>), and storage time (3, 6, and 9 days) were the independent variables. Empirical data were analyzed in a factorial experiment based on a completely randomized design using ANOVA analysis and Duncan's multiple range tests ( $p \leq 0.05$ ) by SAS 9.1 for Windows (SAS Institute Inc. Carolina, USA) and SPSS21 for Windows (IBM SPSS Statistics Statistical Procedures Companion, New York, USA).

## Results

### Chlorophyll and carotenoids

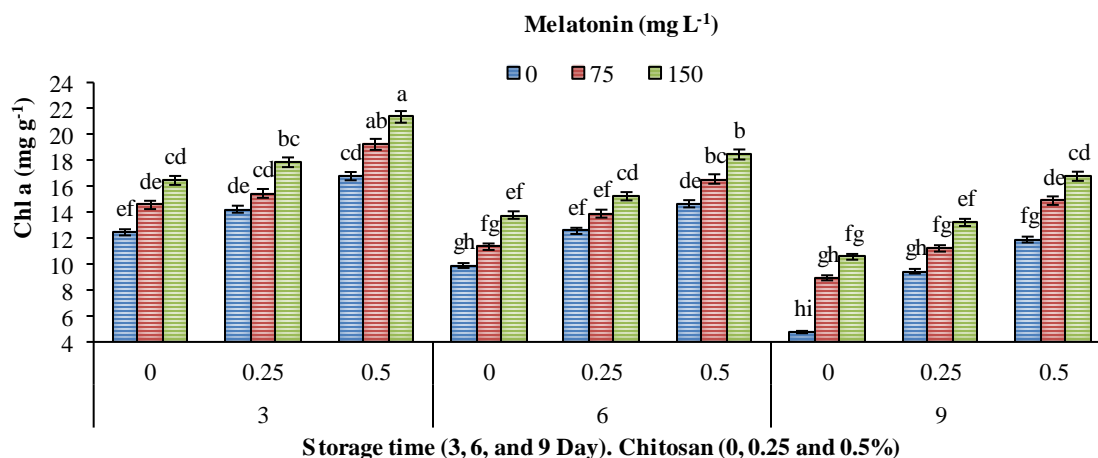
The amount of chlorophyll in banana fruits harvested at the mature green decreased during storage. In contrast, the carotenoid content increased during storage and with fruit maturity (Figs. 1-4). However, this reduction of chlorophyll in the control fruits (without melatonin and chitosan treatment) was more than in the treated fruits. Also, the increase in carotenoid content during storage in control fruits was more than in the samples treated with chlorophyll and carotenoids, thus reaching the highest chlorophyll a (21.35 mg g<sup>-1</sup>) (Fig. 1), b (5.21 mg g<sup>-1</sup>) (Fig. 2), and total chlorophyll (Fig. 3). After three days of storage, fruits harvested at the mature green stage were treated with chitosan

(0.5%) and melatonin (150 mg L<sup>-1</sup>). Also, it led to the highest carotenoid content after nine days of storage. Banana fruits without melatonin and chitosan treatments were in the control group and showed the least values (Fig. 4a and b).

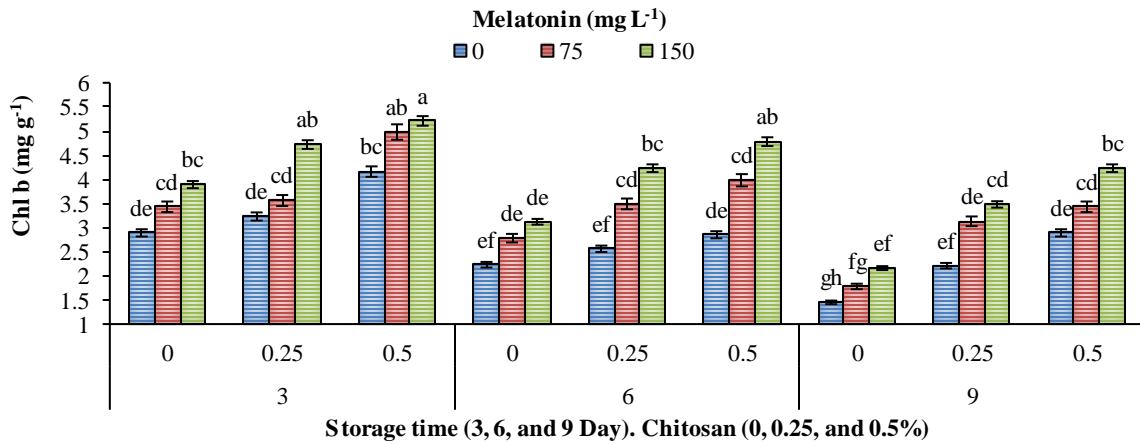
### Color changes

As mentioned in the chlorophyll and carotenoid section, banana skin color changed in response to the treatments, and the fruit color changed from green to yellow.

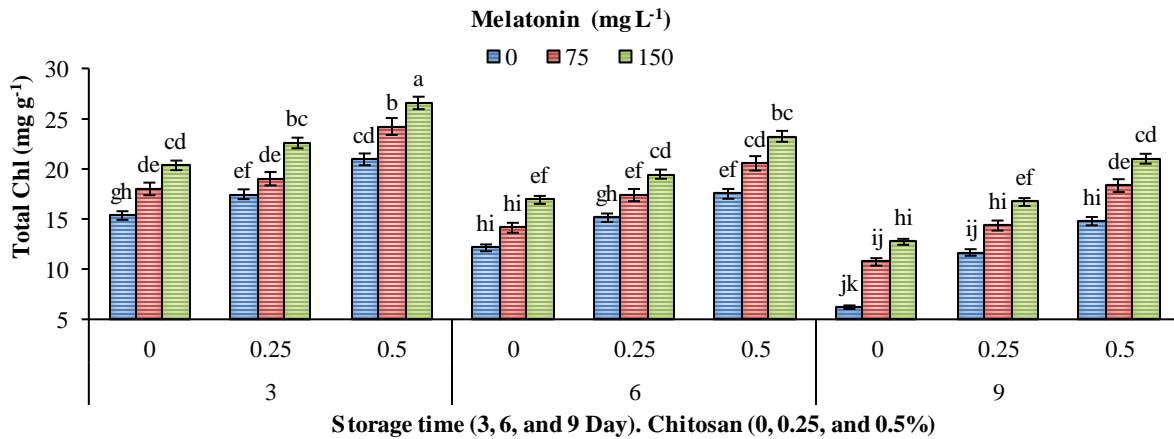
During storage, the color of the fruit skin changed from green to yellow from 3 to 9 days after storage due to decreased chlorophyll, which increased in the control samples more than in treated samples with chitosan and melatonin. Among the treatments, the changes in the investigated color indices in samples treated with chitosan were less than in those treated with melatonin. The changes were most significant in the a\* index in control fruits after nine days of storage. The lowest changes were in samples treated with 0.5% chitosan (13.56) and in samples treated with melatonin (-11.23) (Table 1). Moreover, in the examination of color indices, the highest increases in b\*, L\*, C, and H occurred in the control samples after nine days of storage. Also, regarding the effects of chitosan on color indices, the lowest b\* (20.88), L\* (19.08), C (12.78), and H (10.34) indices occurred in samples treated with chitosan (0.5%) after three days of storage (Table 1). In examining the effects of melatonin, the lowest of these indicators occurred in samples treated with melatonin (150 mg L<sup>-1</sup>) after three days of storage (Table 1).



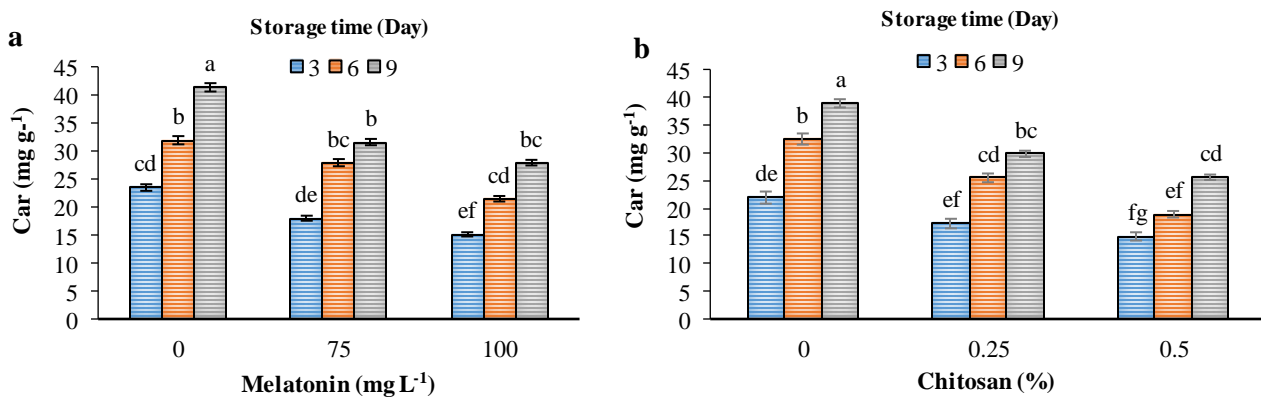
**Fig. 1.** Changes in the amount of chlorophyll an influenced by melatonin and chitosan in storage time. Whiskers indicate standard deviation. The values marked with the same letter do not differ significantly according to the Duncan's multiple range tests ( $P < 0.05$ ).



**Fig. 2.** Changes in the amount of chlorophyll b, influenced by melatonin and chitosan in storage time. Whiskers indicate standard deviation. Values marked with the same letter do not differ significantly according to Duncan’s multiple range test ( $p \leq 0.05$ ).



**Fig. 3.** Changes in total chlorophyll content, influenced by melatonin and chitosan in storage time. Whiskers indicate standard deviation. Values marked with the same letter do not differ significantly according to Duncan’s multiple range tests ( $p \leq 0.05$ ).



**Fig. 4.** Melatonin (a) and chitosan (b) and their effects on the carotenoid content of banana fruits during storage. Whiskers indicate standard deviation. Values marked with the same letter do not differ significantly according to Duncan’s multiple range tests ( $p \leq 0.05$ ).

**Table 1.** Changes in color indices under the influence of chitosan and melatonin during storage.

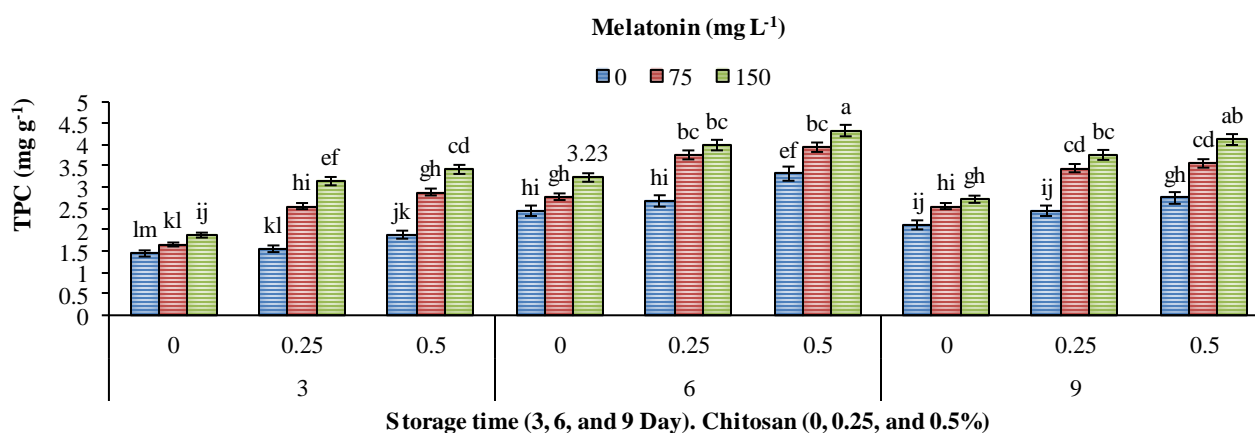
ST (Day)	Chitosan (%)	Melatonin (mg)	a*	b*	L*	C	H
3	0	-	-18.23 <sup>bc</sup>	25.78 <sup>bc</sup>	23.87 <sup>de</sup>	18.67 <sup>cd</sup>	17.88 <sup>bc</sup>
	0.25	-	-21.32 <sup>b</sup>	22.34 <sup>cd</sup>	21.45 <sup>ef</sup>	14.56 <sup>ef</sup>	12.88 <sup>ef</sup>
	0.5	-	-24.67 <sup>a</sup>	20.88 <sup>de</sup>	19.08 <sup>fg</sup>	12.78 <sup>fg</sup>	10.34 <sup>gh</sup>
6	0	-	-12.45 <sup>ef</sup>	27.45 <sup>b</sup>	28.34 <sup>b</sup>	21.67 <sup>b</sup>	19.88 <sup>b</sup>
	0.25	-	-16.27 <sup>cd</sup>	24.33 <sup>cd</sup>	25.78 <sup>cd</sup>	17.33 <sup>cd</sup>	15.23 <sup>de</sup>
	0.5	-	-19.87 <sup>bc</sup>	22.90 <sup>cd</sup>	24.33 <sup>cd</sup>	15.89 <sup>ef</sup>	13.34 <sup>ef</sup>
9	0	-	-8.34 <sup>fg</sup>	31.88 <sup>a</sup>	33.67 <sup>a</sup>	25.88 <sup>a</sup>	26.98 <sup>a</sup>
	0.25	-	-11.23 <sup>fg</sup>	26.88 <sup>b</sup>	29.88 <sup>b</sup>	21.34 <sup>b</sup>	18.98 <sup>bc</sup>
	0.5	-	-13.56 <sup>ef</sup>	25.67 <sup>bc</sup>	26.88 <sup>cd</sup>	20.89 <sup>b</sup>	17.89 <sup>bc</sup>
3	-	0	-17.34 <sup>b</sup>	26.88 <sup>cd</sup>	28.56 <sup>bc</sup>	19.88 <sup>ef</sup>	18.23 <sup>de</sup>
	-	75	-20.78 <sup>ab</sup>	23.5 <sup>de</sup>	26.67 <sup>de</sup>	16.88 <sup>gh</sup>	14.88 <sup>gh</sup>
	-	150	-22.55 <sup>a</sup>	22.88 <sup>ef</sup>	24.89 <sup>ef</sup>	15.54 <sup>hi</sup>	13.23 <sup>hi</sup>
6	-	0	-13.89 <sup>cd</sup>	29.33 <sup>b</sup>	32.89 <sup>b</sup>	24.56 <sup>bc</sup>	21.34 <sup>cd</sup>
	-	75	-14.89 <sup>cd</sup>	26.56 <sup>cd</sup>	29.88 <sup>bc</sup>	21.33 <sup>cd</sup>	17.88 <sup>ef</sup>
	-	150	-17.34 <sup>b</sup>	24.88 <sup>de</sup>	27.89 <sup>de</sup>	19.88 <sup>ef</sup>	15.68 <sup>gh</sup>
9	-	0	-6.89 <sup>gh</sup>	33.88 <sup>a</sup>	37.67 <sup>a</sup>	28.98 <sup>a</sup>	28.67 <sup>a</sup>
	-	75	-10.45 <sup>ef</sup>	28.89 <sup>bc</sup>	31.89 <sup>b</sup>	26.33 <sup>ab</sup>	23.38 <sup>bc</sup>
	-	150	-11.23 <sup>ef</sup>	29.88 <sup>b</sup>	30.11 <sup>bc</sup>	21.45 <sup>cd</sup>	19.34 <sup>de</sup>

C (chroma) and H (hue) were calculated based on the values of 'L\*' (lightness), 'a\*' (green to red), and 'b\*' (blue to yellow). The values marked with the same letter do not differ significantly according to Duncan's multiple range test ( $p \leq 0.05$ ).

### Total phenol content (TPC)

Total phenol content increased from the beginning of storage to the sixth day. Meanwhile, total phenol content decreased on the ninth day as the storage continued (Fig. 5). Using chitosan and melatonin improved the total phenol content. During the decreasing trend in total phenol content from the sixth day onwards, samples

treated with chitosan and melatonin showed a slower decrease compared to the control samples (Fig. 5). The highest total phenol content (4.33 mg g<sup>-1</sup> DW) appeared in banana fruits in response to chitosan (0.5%) and melatonin (150 mg L<sup>-1</sup>) after six days of storage. Its lowest amount (1.45 mg g<sup>-1</sup> DW) occurred in the control sample on the third day of storage (Fig. 5).

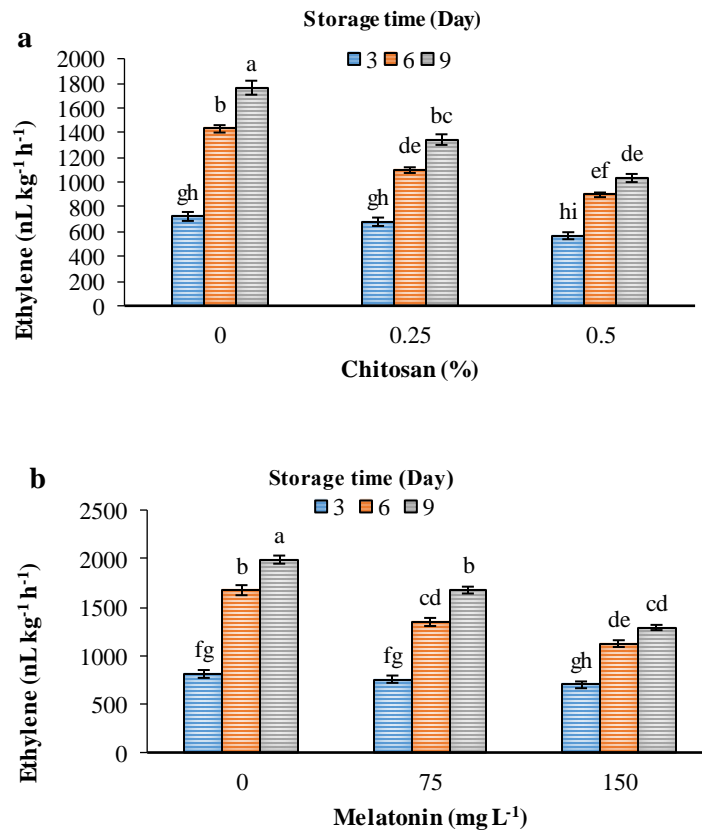


**Fig. 5.** Changes in total phenol content influenced by melatonin and chitosan in storage time. Whiskers indicate standard deviation. Values marked with the same letter do not differ significantly according to Duncan's multiple range test ( $p \leq 0.05$ ).

## Ethylene

In studying the effects of chitosan (Fig. 6a) and melatonin (Fig. 6b) on ethylene release in fruits harvested at the mature green stage during storage, the amount of ethylene increased during storage, but using these two substances reduced the release of ethylene in the fruit during nine

days of storage compared to the control samples. Therefore, the maximum amount of ethylene released from banana fruits after nine days of storage occurred in the control samples. Also, the results showed that different concentrations of chitosan had a more influential role than melatonin in reducing ethylene release from the fruits (Fig. 4)



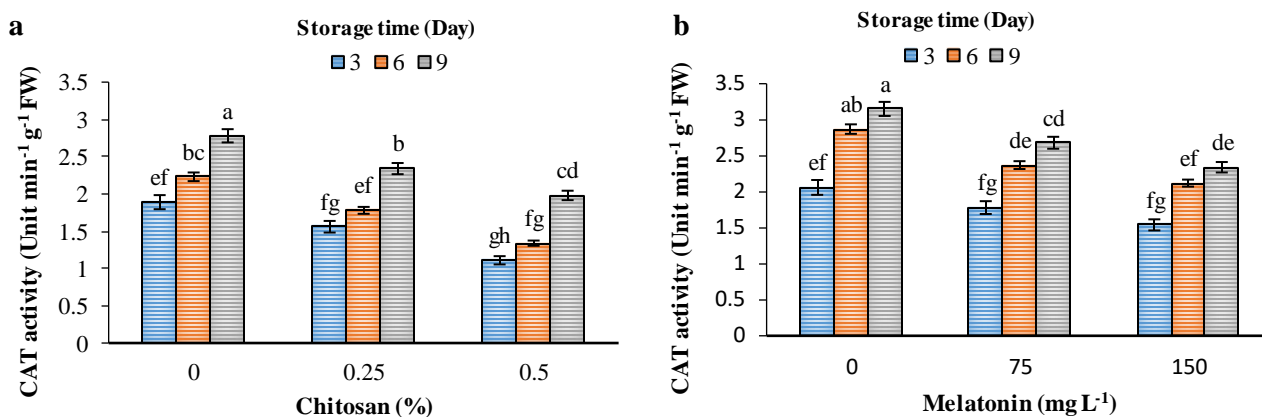
**Fig. 6.** Ethylene release rate from fruits after using chitosan (a) and melatonin (b) during storage. Whiskers indicate standard deviation. Values marked with the same letter do not differ significantly according to Duncan's multiple range test ( $p \leq 0.05$ ).

## Enzyme activity

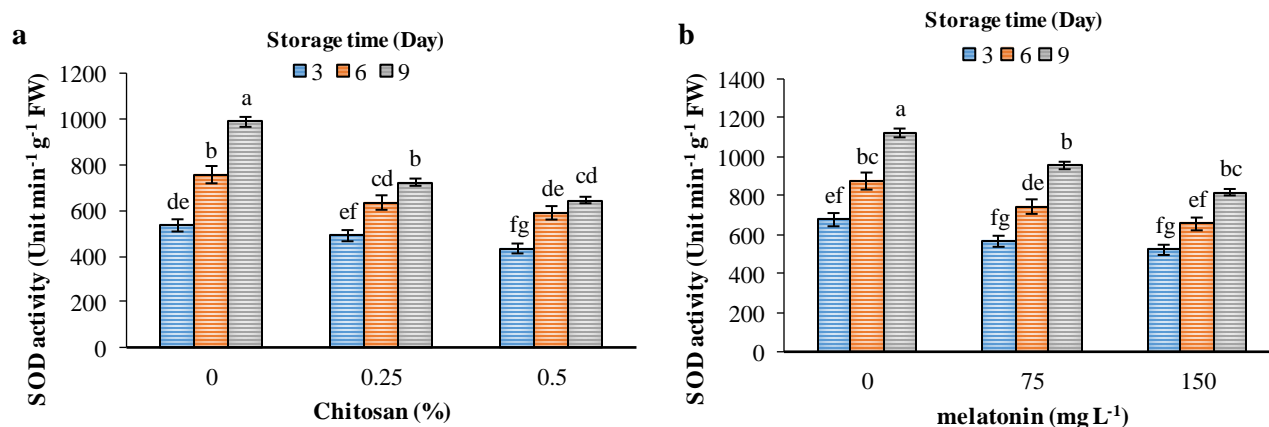
Antioxidant enzyme activity in green-mature banana fruits increased through storage. This increase reached its maximum after nine days of storage. On the other hand, through time and upon reaching full maturity, control samples showed more antioxidant enzyme activity in all three stages of postharvest (3, 6, and 9 days after storage) compared to the treated samples (Figs. 7-9). Our results showed that CAT and SOD activity levels became the lowest due to 0.5% chitosan application compared to the untreated samples on the ninth day. This value for CAT and SOD enzymes was equal to 1.98 (Fig. 7a) and 645 (Fig. 8a). Investigating the effects of melatonin during storage showed that CAT and SOD activity

levels were 2.34 (Fig. 7b) and 819 (Fig. 8b), respectively. Thus, the present results showed that chitosan was more effective in reducing CAT and SOD activities (Figs. 7 and 8) compared to the effect of melatonin.

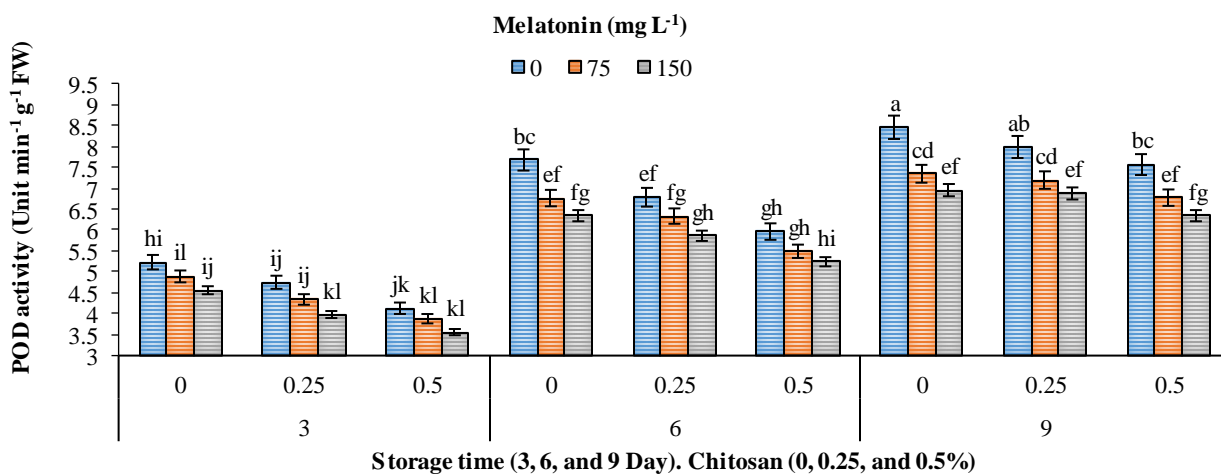
In evaluating POD activity, similar to the results on CAT and SOD enzyme activity, this enzyme increased its activity during storage and fruit maturity. However, using melatonin and chitosan delayed the fruit ripening process and reduced its activity compared to untreated samples. The highest POD enzyme activity in the control samples occurred after nine days of storage, while the lowest level occurred on the ninth day in samples treated with chitosan (0.5%) and melatonin (150 mg L<sup>-1</sup>) (Fig. 9).



**Fig. 7.** CAT activity in fruits under the effect of chitosan (a) and melatonin (b) during storage. Whiskers indicate standard deviation. Values marked with the same letter do not differ significantly according to Duncan's multiple range test ( $p \leq 0.05$ ).



**Fig. 8.** SOD activity in fruits under the effect of chitosan (a) and melatonin (b) during storage. Whiskers indicate standard deviation. Values marked with the same letter do not differ significantly according to Duncan's multiple range test ( $p \leq 0.05$ ).



**Fig. 9.** Changes in POD activity influenced by melatonin and chitosan in storage time. Whiskers indicate standard deviation. Values marked with the same letter do not differ significantly according to Duncan's multiple range test ( $p \leq 0.05$ ).



## Discussion

In this study, the changes in color indices during the storage period indicated the conversion of chlorophyll to carotenoids (Figs. 3 and 4). Gradually, the amount of chlorophyll decreased and caused the appearance of carotenoid pigments in the skin of banana fruits. The downward trend of changes in the  $a^*$  index and the increase in the  $b^*$  index (Table 1) indicated this issue in line with previous results by Heydaranazhad et al. (2019).

Our results indicated that during fruit maturity and changes in fruit color, the most significant change in the hue angle and C occurred in the control sample (Table 1), which indicates that the fruit color shifted from green to yellow during the postharvest period of examination. Color is a significant quality aspect of unprocessed and processed foods, and their changes reflect chemical changes due to storage processes such as browning, frying, and drying (Sahin et al., 2006). During fruit maturity, phytochemical changes affect the antioxidant activity and nutrition quality in fruits and vegetables at specific times (Conforti et al., 2007). On the other hand, our results showed that using chitosan and melatonin decreased the rate of change in the  $a^*$  and  $b^*$  index, followed by the deterioration of chlorophyll and the appearance of carotenoid pigments. Chitosan treatment can restrict water transfer or dehydration, gas exchange, and nutrient loss by acting as a protective fruit, thus reducing weight loss in fruits at postharvest (Yuzhao et al., 2020).

Changes in the total phenol content of fruit in the postharvest period depend on various factors such as genotype, stage of harvesting, variety, growth season conditions, storage life, and storage time (Valero, 2013; Heydarnajad Giglou and Torabi Giglou, 2023). Phenolic compounds are potent inhibitors of oxidative stress and participate in the collection or removal of hydrogen peroxide in collaboration with peroxides. Here, chitosan and melatonin were also affected by the increase in phenol compounds compared to the control (Fig. 5). The effect of chitosan on total phenol content and fruit coloring appeared in various experiments (Heydarnazhad et al., 2019; Rajestary et al., 2021; Wu et al., 2021). In line with the results of our research, Liu et al. (2018) investigated the effect of melatonin on the quality and lifespan of strawberries at postharvest. They found that postharvest treatments with melatonin reduced the loss of fruit juice, delayed fruit aging, and increased the amount of total phenol. Total flavonoids and antioxidants correlated with

values of fruit color, TA, firmness, and soluble solids during cold storage.

Antioxidant activity in chitosan has been the subject of considerable research focus (Park et al., 2004). Chitin and chitosan increase the amount of phenolic compounds that play a role in plant defense mechanisms (Pu et al., 2009). Emami Bastegani et al. (2016) showed that with the increase in chitosan concentration, the phenol content increases, which is consistent with the results of the present study. Taheri (2015) and Coqueiro et al. (2011) stated in their results that chitosan acts as a defense barrier against environmental stresses by increasing the production of phenolic compounds. Malekpoor et al. (2015) reported that stimulants such as chitosan may cause the formation of secondary metabolites such as phenolic and flavonoid compounds by activating genes and various biosynthetic pathways and enzymes, which is consistent with the results of the present study regarding the increase in POD, SOD, and CAT activity.

Mechanical damages may happen during transportation and storage. The activation of senescence processes during postharvest can increase the accumulation of reactive oxygen species (ROS), such as superoxide radical and hydrogen peroxide, which are primary factors in destroying biological membranes, thus affecting quality and marketability (Beltagi-El et al., 2022; Ma et al., 2022). Antioxidant defense systems in plants have evolved to limit ROS accumulation and inhibit oxidative damage; catalase and superoxide dismutase are two antioxidant enzymes (Chen et al., 2021; Sun et al., 2020).

Under abiotic stress, oxygen molecules receive an electron and become a negative radical – superoxide ( $O_2^-$ ) cannot transfer through biological membranes, and the accumulation of this compound inside the cell has a high affinity that damages internal organs and compounds. A cell becomes like biological membranes and DNA (Joshi et al., 2001). Upon receiving another electron, the superoxide radical becomes hydrogen peroxide ( $H_2O_2$ ), and superoxide dismutase carries out the reaction (Malekzadeh et al., 2014; Wang et al., 2022). This reaction has a product ( $H_2O_2$ ) that can easily pass through biological membranes, and the remaining  $H_2O_2$  inside the cell can convert into water and oxygen with the help of the catalase enzyme (CAT). Thus, CAT and SOD enzymes clean the excess reactive oxygen species that accumulate as a result of the stress response (Mukhtar et al., 2022). Previous reports showed that melatonin may act as an antioxidant compound, eliminate free radicals, and protect biomolecules from free radical

damage (Li et al., 2022; Zhao et al., 2017; Normohammadi et al., 2021).

## Conclusion

Through the storage period, the chlorophyll content decreased, correlating with changes in carotenoids, ethylene release, and antioxidant enzyme activities. Exogenous melatonin and chitosan increased the superoxide dismutase, catalase, and peroxidase. The effect of chitosan compared to melatonin during the storage period was more significant on SOD and POD activities. Regarding changes in color indices  $a^*$ ,  $L^*$ , and  $b^*$ , chitosan delayed banana color change more than melatonin during storage; therefore, chitosan can increase the shelf life of banana fruits. Also, the results showed that chitosan (0.5%) and melatonin (150 mg L<sup>-1</sup>) had an influential role in increasing the shelf life of banana fruits. Melatonin and chitosan can simultaneously increase the storage period and maintain banana fruit quality.

## Conflict of Interest

The authors indicate no conflict of interest in this work.

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