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Research article

Melatonin immersion improves postharvest quality and antioxidant capacity of 'Nam Dok Mai' mangoes during storage at ambient temperature

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Abstract

Importance of the work: Quality loss and delays in ripening in mangoes were explored based on melatonin (MT) immersion as a natural, effective postharvest treatment under ambient conditions.

Objectives: To evaluate MT efficacy in preserving the quality, delaying ripening and enhancing oxidative stress resistance in mangoes under ambient storage.

Materials and Methods: The mangoes were immersed in water (control) or in a solution of 0.5 mM MT for 1 hr and thereafter stored at room temperature for 10 days.

Results: Mangoes treated with 0.5 mM MT had nearly 17% and 60% lower respiration rates and ethylene production, respectively, than untreated ones at the climacteric peak during storage, contributing to a slower ripening process and delayed progression of both peel and pulp coloration. In terms of internal quality, MT application effectively slowed the decline in firmness, suppressed the rise in the total soluble solids-to-titratable acidity ratio. In addition, the MT-treated fruits had enhanced antioxidant defense, as shown by elevated activities of superoxide dismutase, catalase and ascorbate peroxidase. These responses were accompanied by significantly lower levels of hydrogen peroxide, malondialdehyde and lipoxygenase activity, suggesting reduced oxidative membrane damage. Collectively, these findings demonstrated that MT immersion contributed to better quality preservation and improved resistance to postharvest deterioration and disease through modulation of oxidative stress and antioxidant metabolism.

Main finding: Subjecting mangoes to postharvest MT treatment was a feasible approach to preserve their postharvest quality and to stimulate antioxidant capability.

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Introduction

Mango (*Mangifera indica* L.) cultivar 'Nam Dok Mai No. 4' is one of the most well-liked commercial mango cultivars in both the domestic and export markets, as, when ripe, its fruit has a lovely exterior with a golden-yellow skin, a desirable flavor and a sweet aroma (Tangpao et al., 2022). These mangoes are an excellent source of dietary fiber, pro-vitamin A, vitamin C, carotenoids and phenolics, which are bioactive compounds (Guiamba, 2016). Rapid ripening and senescence are the primary obstacles to quality maintenance and also limit the shelf life of postharvest mangoes (Zhang et al., 2022). Mangoes are classified as climacteric fruits and many researchers have demonstrated that ethylene (C_2H_4) is a crucial factor in accelerating the ripening processes in mango fruit, leading to a shorter shelf-life and limiting export opportunities (Zaharah and Singh, 2010). Various technologies have been used to maintain quality, control the ripening process and delay the senescence of mango fruit, such as nitric oxide (Zaharah and Singh, 2010), titanium dioxide (Chaishome et al., 2019), 1-methylcyclopropene incorporated with packaging (Kwanhong et al., 2018) and 6-benzylaminopurine (Zhang et al., 2022). Normally, the 'Nam Dok Mai No.4' mango cultivar starts to ripen after harvest within 4–8 d under room temperature, and by day 4 they exhibit clear ripening progress, including distinct changes in peel coloration. (Lin Aung et al., 2021). This rapid ripening limits storage life and presentation for sale. This ripening is also a favorable condition for anthracnose in mangoes (Fitzell, 1979). Consequently, technological or chemical approaches are needed to delay ripening and control anthracnose. Specifically, the industry requires postharvest technology that would delay the ripening process and extend the shelf-life of mangoes without any negative effects on fruit quality.

Melatonin (MT; N-acetyl-5-methoxytryptamine), is a mammalian pineal gland-secreted brain hormone that has been linked to the sleep-wake cycles of mammals (Dubocovich et al., 2010). MT is a tryptophan derivative found in all living creatures (Tang et al., 2020; Wu et al., 2021) and has been detected in several plant parts such as leaves, flowers, fruits and seeds (Jemima et al., 2011; Reiter et al., 2015). MT acts as a plant growth regulator in several physiological processes, including plant development, flowering, fruit development, fruit ripening, senescence, controlling disease and promoting the postharvest quality of perishable crops (Hernández-Ruiz and Arnao, 2018; Xu et al., 2019; Arnao and Hernández-Ruiz, 2019). In addition, MT enhances plant tolerance to environmental factors by functioning as an antioxidant to neutralize reactive oxygen species (ROS), to stimulate the antioxidant mechanism and to boost the effectiveness of other antioxidants in plants exposed to biotic and abiotic stresses (Debnath et al., 2019). Other studies on this topic have suggested that MT could reduce fungal decay, delay ripening and senescence, attenuate postharvest physiological deterioration and prolong the durability of various fresh produce such as peaches (Gao et al., 2016), pears (Zhai et al., 2018), tomatoes (Liu et al., 2019), bananas (Hu et al., 2017) and strawberries (Liu et al., 2018). MT decreased C_2H_4 production by inhibiting the activity of 1-aminocyclopropane-1-carboxylic acid synthase (ACS) and 1-aminocyclopropane-1-carboxylic acid oxidase (ACO), which are C_2H_4 biosynthesis genes (Hu et al., 2017; Liu et al., 2019; Liu et al., 2020). Conversely, there have been reports indicating that postharvest treatment with exogenous MT accelerated the ripening process and improved the tomato quality (Sun et al., 2015). Furthermore, MT treatment decreased the rate of respiration, C_2H_4 production and oxidative damage to cells, as well as preserving cell integrity in cucumbers (Xin et al., 2017). In litchi fruit, MT treatment delayed pericarp browning and senescence because it maintained redox homeostasis by improving the antioxidant capacity and repairing damaged protein oxidation (Zhang et al., 2018). These studies have provided evidence to supports the idea that MT exhibits high potential in postponing ripening and senescence and in retaining the quality of fruits and vegetables. MT is generally safe to use and is not harmful for consumption when used under appropriate limits (Givler & Givler, 2023)). Furthermore, it is of interest because there is no published report of its effects on the 'Nam Dok Mai' mango cultivar. Hence, the aim of the current research was to examine the effects of postharvest MT treatment on delaying ripening, maintaining postharvest quality and enhancing the antioxidant capacity in 'Nam Dok Mai' mangoes under ambient temperature.

Materials and Methods

Mango preparation

Mango cv. 'Nam Dok Mai No. 4' fruits were obtained from a plantation in Prachinburi province, Thailand. The mangoes were harvested at commercial maturity (95–100 d after blooming). After harvest, the fruits were immediately delivered to the Division of Postharvest Technology at King Mongkut's University of Technology Thonburi, Thailand within 4 hr. Following that, the fruits underwent a screening process in which their size was

assessed for uniformity, with each fruit weighing 350–400 g. Additionally, the fruits were carefully examined to ensure they were devoid of any faults or signs of disease. Then, each fruit was rinsed with tap water and soaked in a 200 parts per million (ppm) solution of sodium hypochlorite for 5 min and finally treated with a 250 ppm solution of prochloraz for another 5 min. Following the cleaning process, the fruits were subjected to a drying period for 30 min at room temperature (RT) prior to further treatments.

Treatments

The mangoes were divided into four groups (30 fruits per group) and immersed in distilled water (control) or in MT solutions at concentrations of 0.1 mM, 0.5 mM or 1 mM for 1 hr at RT. To prevent the decomposition of MT, these treatments were regulated during low-light conditions (Zhang et al., 2018; Liu et al., 2020). Following the completion of each treatment, the mangoes were stored at RT (25°C, 85 ± 1% relative humidity) for 10 d. Then, every second day, the mangoes were examined for physical and chemical parameters: visual appearance, respiration rate, C_2H_4 production, color (peel and pulp), texture, titratable acidity (TA), the total soluble solids (TSS) content, the TSS-to-TA ratio, ferric reducing antioxidant power (FRAP) and the free radical scavenging ability of DPPH (2,2-diphenyl-1-picrylhydrazyl), total phenolic, ascorbic acid, hydrogen peroxide (H_2O_2), malondialdehyde (MDA) contents and the function of antioxidant enzymes comprising superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX).

Color measurement

The color attributes of mango skin and pulp samples were assessed using a CR 400 Minolta colorimeter (Minolta, Japan). Three specific areas (upper, middle, bottom) of the fruit were chosen for color assessment. The values of lightness (L*), chroma, hue angle and color difference (rE*) were recorded.

Disease incidence and disease severity

Disease incidence was assessed by recording the number of infected fruits in each treatment group during the storage period. The results were expressed as a percentage of diseased fruits relative to the total number of fruits examined using Equation 1 (Safari et al., 2020; Brito et al., 2021):

$$\text{Disease incidence} = (\text{number of infected fruits} / \text{Total number of fruits}) \times 100 \quad (1)$$

Disease severity was evaluated using a visual scale (0–4), where" 0 = healthy fruit with no visible disease symptoms; 1 = symptoms covering up to 25% of the surface; 2 = 26–50%; 3 = 51–75%; and 4 = severe infection covering more than 75% of the fruit surface. The severity score for each treatment was calculated as the average of all observed fruits in the treatment.

Respiration rate and ethylene production

Each mango was weighed and placed in a 1,600 mL airtight plastic container. Then, the fruit was incubated for 2 hr at RT. Subsequently, 1 mL of gas sample in the headspace was removed using a plastic syringe and introduced into a gas chromatograph (GC-2014, Shimadzu, Japan) to determine CO_2 production. The respiration rate of each sample was expressed in milliliters of CO_2 per kilogram of sample per hour (mL CO_2/kghr). For C_2H_4 production determination, a gas sample of 3 mL was removed using a plastic syringe and injected into a gas chromatograph (GC-8A, Shimadzu, Japan). The production of C_2H_4 was expressed in milliliters of C_2H_4 per kilogram per hour (mL C_2H_4/kg/hr).

Firmness

The flesh firmness of each mango pulp sample was assessed using a texture analyzer (TA-XT2; Stable Micro Systems; UK) fitted with a cylindrical probe (5.0 mm diameter). Following the removal of the peel, the determination was carried out with the probe moving at a rate of 1.0 mm/s, reaching a depth of 5.0 mm. The hardness measurement was expressed in newtons (N).

Total soluble solids-to titratable acidity ratio

The TSS of each mango was measured using a digital refractometer (PAL-1; ATAGO Co., Ltd.; Japan). The mango juice was extracted and the TSS was quantified and expressed in °Brix units. The TA of mangoes was determined based on AOAC (2000). Each 20 g sample was homogenized and then passed through a filter cloth. Then 2 mL of mango juice were combined with a drop or two of 1% phenolphthalein. The resulting blend underwent titration with 0.1 N NaOH until it turned a pale pink color. The volume of 0.1 N NaOH utilized was then noted. The TA was quantified in grams of malic acid per gram of fresh weight (g malic acid /g FW). The TSS-to-TA ratio was computed by dividing the TSS content by the TA value.

Ferric reducing antioxidant power, 2,2-diphenyl-1-picrylhydrazyl free radical scavenging ability and total phenolic contents

Each sample (2 g) of mango pulp was blended with 10 mL of 60% ethanol. The resulting homogenate was passed through Whatman No. 1 filter paper. The antioxidant capacity was assessed using the FRAP method in accordance with Benzie and Strain (1996). The FRAP reagent was prepared using a 10:1:1 mixture of acetate buffer (pH 3), 10 mM of 2,4,6-tripyridyl-s-triazine and 20 mM ferric chloride hexahydrate, respectively. The reaction was initiated by combining 0.1 mL of the extract with 2 mL of the FRAP reagent, then incubating the mixture for at least 30 min at 25°C. The absorbance was recorded at a wavelength of 630 nm. The data were reported as µmole equivalent Trolox units per kilogram (µmol TE/kg). The extract was collected and used to assay free radical scavenging activity, with the DPPH test performed following the procedure described by Ramadan et al. (2003). A sample (0.1 mL) of the extract was mixed with 2.85 mL of 0.2 mM DPPH in methanol and kept in darkness for 30 min at RT. The DPPH concentration was assessed using a spectrophotometer (model UV 1800; Shimadzu; Japan), with the absorbance measured at 515 nm. The ability to scavenge DPPH radicals was expressed as the percentage inhibition. The total phenolic content was assessed based on the procedure established by Slinkard and Singleton (1977). For the reaction mixture, an aliquot of 0.1 mL of the solution was combined with 1.0 mL of Folin-Ciocalteu reagent at a concentration of 25% (weight per volume). Afterward, the mixture was supplemented with 2.0 mL of saturated Na_2CO_3 solution and then incubated at RT for 30 min. The optical density (OD) was measured at 750 nm using a UV-1800 spectrophotometer (Shimadzu; Japan). A reference blank composed of distilled water and reagents was utilized and the findings were expressed as micrograms of gallic acid equivalent per kilogram (µg GA/kg).

Ascorbic acid content

The ascorbic acid level was measured following protocol outlined by Hashimoto and Yamafuji (2001). Each sample (3 g) of mango pulp was blended with 12 mL of 5% metaphosphoric acid and the resulting mixture was passed through Whatman No.1 filter paper. The test was initiated by combining 0.2 mL of the preparing filtrate with 0.2 mL of 0.02% 2,6-dichlorophenol indophenol, 0.4 mL of 2% thiourea and 0.2 mL of 2% dinitrophenol, respectively, then incubating the mixture at 37°C for 3 hr. Subsequently, 1 mL of 85% sulfuric acid was added and mixed well, followed by incubation at RT for 30 min. The ascorbic acid level was assessed by recording the absorbance at 540 nm using an ultraviolet (UV)-1800 spectrophotometer (Shimadzu Corporation; Japan). The results were reported as micrograms of ascorbic acid per kilogram (µg ascorbic acid/kg).

Antioxidant enzyme activity assays: superoxide dismutase, catalase and ascorbate peroxidase

Each sample (5 g) was blended with 10 mL of 50 mM sodium phosphate buffer (pH 7) and 0.2 g of polyvinyl pyrrolidine to obtain crude enzyme. After the extraction process, each extract was stored in an ice bath until the SOD, CAT and APX activity assays. The SOD activity was measured according to the method proposed by Ukeda et al. (1997). Each 0.3 mL aliquot of the enzyme extract was combined with a solution containing 0.5 mL of 50 mM sodium phosphate buffer (pH 7), 0.1 mL of 3.0 mM xanthine, 0.1 mL of 3.0 mM ethylenediaminetetraacetic acid (EDTA), 0.05 mL of 0.75 mM 3'-{1-[(phenylamino)-carbonyl]-3,4-tetrazolium}bis (4-methoxy-6-nitro) benzenesulfonic acid hydrate and 0.1 mL of 0.14 U xanthine oxidase. The SOD activity was quantified by the decrease in the OD at 470 nm after a 20 min incubation period, with activity expressed as Units per gram (Units/g). The CAT activity was assessed using method described by Aebi (1984). A 0.3 mL aliquot of the enzyme extract was combined with 2.0 mL of 0.036% H_2O_2 and the activity was monitored at 240 nm OD for 90 sec. The CAT activity was reported in Units per gram. The APX activity was determined following the method of Cuvi et al. (2011). A 0.3 mL aliquot of the enzyme extract was combined with a solution consisting of 0.5 mL of 50 mM sodium phosphate buffer (pH 7), 2 mL of 0.1 mM EDTA, 0.15 mL of 5.0 mM ascorbic acid and 0.3 mL of 20 mM H_2O_2. The APX activity was monitored at a 290 nm OD for 90 sec and reported in Units per gram.

Hydrogen peroxide

The H_2O_2 level was measured following the procedure outlined by Sagisaka (1976). Each sample (2 g) was combined with 10 mL of 5% trichloroacetic acid (TCA), homogenized and centrifuged at 12,000×*g* at 4°C for 10 min. A sample (0.2 mL) of the supernatant was mixed with the prepared solution including 1 mL of 50% TCA, 1 mL of 10 mM ferrous ammonium sulfate and 0.5 mL of 2.5 M potassium thiocyanate. The OD of 480 nm was recorded. The data were recorded as micromoles of H_2O_2 per kilogram (µmol H_2O_2/kg).

Lipoxygenase activity

Each sample (5 g) of mango pulp was blended in 15 mL of cold 0.2 M Tris-HCl buffer (pH 8.0). After homogenization, the crude was centrifuged at 15,000×*g* for 20 min at 4°C. The lipoxygenase (LOX) activity was assessed according to the protocol outlined by González-Aguilar et al. (2004). Each sample (150 µL) of the crude enzyme was added with 2.85 mL of substrate containing linoleic acid (157.2 µL), Tween 20 (157.2 µL) and distilled water (10 mL). The obtained mixture was clarified by treating it with 50 mL of 20 mM NaOH for 10 min at 25°C. Afterward, the volume was adjusted to 200 mL by adding 0.2 M phosphate buffer (pH 7.0) to the mixture. The LOX activity was quantified by monitoring the increase in the 234 nm OD wavelength after a 2 min incubation period. The results were reported as Units per gram.

Malondialdehyde content

The MDA level was measured following the procedure of Wang et al., (2004). Each sample (2 g) of flesh was blended well in 10 mL of 0.1% TCA. Afterward, the homogenate was centrifuged at 12,000×*g* for 10 min at 4 °*C*. *After* centrifugation, 1.0 mL of the supernatant was combined with 1 mL of 1.5% TCA containing 0.5% thiobarbituric acid. Then, the resulting solution was heated at 95°C for 20 min and cooled in the ice bath. Absorbance readings were recorded at wavelengths of 532 nm and 600 nm. Subsequently, the results were reported as nanomoles of MDA per kilogram (nmol MDA/kg).

Statistical analysis

The experiment was performed using a completely randomized design. Data were subjected to analysis of variance using SPSS software (version 18; SPSS Inc.; USA). Mean differences were analyzed using Duncan's multiple range test ($n = 5$). Differences were considered significant at $p < 0.05$ and highly significant at $p < 0.01$.

Results and Discussion

Respiration rate and ethylene production

Fig. 1 illustrates the impacts of MT on the respiration rate and C_2H_4 production of the mangoes during storage. In both the control and MT treatments, there was a steady increase in the respiration rate over the initial 4 d of storage, reaching its peak on day 4, followed by a subsequent decline (Fig. 1A). The respiration peak of the MT treated mangoes was highly significantly lower than that of the mangoes in the control group. After reaching the peak, the respiration rate of both the non-treated and MT-treated mangoes decreased to a similar extent during storage. The C_2H_4 production of both the control and MT treated mangoes increased throughout storage (Fig. 1B), with that of the control fruits increasing highly significantly compared to the mangoes treated with MT. These findings reveal that immersing mangoes in 0.5 mM MT for 1 hr lowered the climacteric respiration peak and retarded the increase in C_2H_4 production during storage. According to Liu et al., (2020), postharvest MT application delayed the respiration peak and C_2H_4 production in 'Guifei' mangoes during storage by inhibiting ACC synthase (ACS) and ACC oxidase (ACO) activities. Furthermore, earlier research has revealed that exogenous MT application suppressed expression of the *ACS* and *ACO* genes in bananas (Hu et al., 2017), pears (Zhai et al., 2018) and tomatoes (Liu et al., 2019). In addition, Onik et al., (2021) reported that MT treatment led to the down-regulation of the expressions of genes,

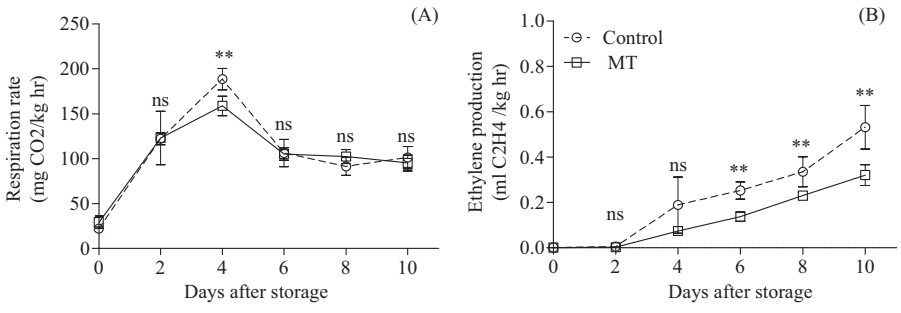

Fig. 1 Effects of 0.5 mM melatonin treatment on 'Nam Dok Mai No. 4' mangoes compared with untreated controls during storage at 25°C for 10 d: (A) respiration rate; (B) ethylene (C_2H_4) production. Value represent the mean ± SD ($n = 5$). Asterisks indicate significant differences between treatments at each storage period [* $p < 0.05$; ** $p < 0.01$; ns = non-significant difference ($p \geq 0.05$)].

such as *MdACO1*, *MdACS1*, *MdAP2.4* and *MdERF109*, in apples during postharvest storage, resulting in the suppression of increased C_2H_4 production. Based on the current results, the MT treatment delayed the progress of ripening in the 'Nam Dok Mai No.4' mangoes by decelerating the peak of respiratory climacteric activity and decreasing the generation of C_2H_4 during storage. Furthermore, this occurred simultaneously with the alterations in visual appearance and pulp quality.

Peel color development

The effects of 0.5 mM MT immersion on peel color progression in 'Nam Dok Mai No. 4' mangoes are presented in Fig. 2. There was a notable delay in peel yellowing in the MT-treated fruit compared to the control, as visually observed throughout storage. The alterations in color characteristics of the mango peel (L*, chroma, hue angle), are displayed in Figs. 2A–C, indicating a gradual decrease in the L* and hue angle values of the mango skin across all treatment groups during storage. There were no significant changes in the L* values across all treatments during storage. The chroma value of the mangoes increased throughout the entire storage duration. There was a highly significant greater chroma value in the non-MT-treated fruit compared to the MT-treated groups during storage for 8 d. However, on day 10 of storage, the chroma values of all groups were similar. During storage, the hue value decreased in all treatments. The observed increase in the yellowness of the mango fruit epidermis during storage was associated with a simultaneous reduction in the hue value, reflecting the visual color change over time. No noticeable differences in the hue values were observed among the MT-treated groups throughout the storage period. Furthermore, based on the results, there was a greater reduction in the hue value in the control set than in the mangoes treated with MT. The ΔE* values of mango peel increased progressively during storage in the both the control and MT-treated groups. However, the MT-treated mangoes showed a markedly more gradual increase in ΔE* values than the control, indicating that MT application effectively delayed the peel color changes associated with ripening (Fig. 2D). The visual color development (increase in yellowness) was an indication of the maturation process in the 'Nam Dok Mai No. 4' mangoes. The effectiveness of MT in retarding the ripening process of mangoes was clearly demonstrated by the delayed development of peel yellowness, as reflected in the decrease in hue value observed during storage. Similarly, Liu et al. (2020) reported that applying exogenous MT delayed the ripening process and color development in mangoes, which was attributed to retardation in the climacteric peak rate and C_2H_4 production during the ripening process. Onik et al. (2021) suggested that MT suppressed the C_2H_4 biosynthesis system in apples during storage. Overall, based on these results, postharvest MT treatment slowed down the ripening process in mangoes. Furthermore, in the current study, applying MT suppressed the progression of disease incidence in the mangoes during storage. Other studies have shown that MT treatment effectively decreased anthracnose occurrence in bananas (Li et al., 2019a), papayas (Fan et al., 2022a) and guavas (Fan et al., 2022b) during storage. In addition, in grape berries (Gao et al., 2020) and tomatoes (Liu et al., 2019),

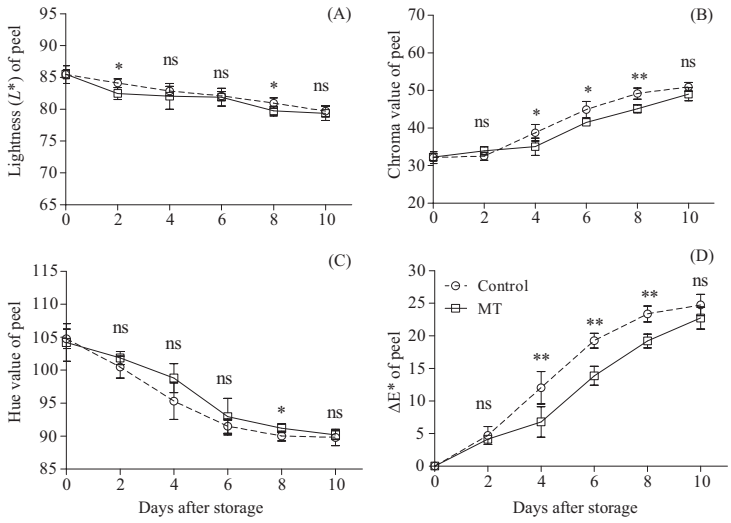

Fig. 2 Effects of 0.5 mM melatonin treatment on peel color attributes of 'Nam Dok Mai No. 4' mangoes compared with untreated controls during storage at 25°C for 10 d: (A) lightness (*L**); (B) chroma; (C) hue angle; (D) color difference (ΔE*). Values are mean ± SD (n = 5). Asterisks indicate significant differences between treatments at each storage period [* $p < 0.05$; ** $p < 0.01$; ns = non-significant difference ($p \geq 0.05$)].

the ability of MT treatment to augment disease resistance has been attributed to the stimulation of several defense-related genes, including pathogen recognition genes (Moustafa-Farag et al., 2020). Based on these findings, fruit immersion in MT at 0.5 mM concentration was effective in postponing the onset of disease and the development of peel yellowing in the mangoes stored at RT.

Pulp color attributes

The impact of MT immersions on the pulp color attributes of 'Nam Dok Mai No. 4' mangoes was depicted in Fig. 3A–D. It shows the alteration of pulp color attributes, including L*, chroma, hue angle, chroma values and rE* respectively, during storage. The findings indicated that L* and hue angle values exhibited a downward trend, while the chroma value showed an upward trend during the storage time. This was consistent with the change in visual appearance and the development of peel yellowness. The L* value of the untreated mangoes presented a more pronounced downward trend than MT-treated fruits. On storage days 6 and 8, the L* value of the control group showed significant reduction compared to MT treatment ($p < 0.05$ and $p < 0.01$, respectively). During the entire storage period, the pulp L* value of MT-treated mangoes was comparable. Over a period of 4 days in storage, the chroma value of both treatments exhibited a marked increase. The chroma value of control mangoes had a greater increase compared to mangoes treated with MT. A significant difference in the increased chroma value of the control mangoes compared with MT-treated fruits was observed on day 2 ($p < 0.01$). After being stored for 4 days, the chroma value of both treatments was comparable and showed a gradual increase. The data demonstrate a decline in the hue value of mango pulp in all treatments throughout storage, which suggests an increase in the yellowness of the pulp. During storage for 2 days, the hue value of mangoes treated with 0.5 mM MT seemed consistent and the hue value was notably higher than that of the control fruits ($p < 0.05$). Subsequently, both treatments presented the reduction of hue value and they became similar after 8 days of storage. By the end of the 10-day storage period, we found that the hue value of 0.5 mM MT-treated mangoes was markedly greater than that of the control fruits ($p < 0.01$).

Firmness and TSS/TA ratio

The impact of MT immersions on the flesh firmness, TSS/TA ratio and pulp color attributes of 'Nam Dok Mai No. 4' mangoes was depicted in Fig. 4A–B. The flesh firmness exhibited a consistent downward trend, indicating that the fruits progressively softened as the storage duration extended. A sharp decrease in pulp firmness was noted in all treatments during the first 4 days of storage. Subsequently, the reduced firmness of the mango flesh underwent only modest alterations throughout the remaining storage period. Notably, the fruits subjected to the 0.5 mM MT treatment showed significantly greater firmness than non-treated fruit for the whole storage period ($p < 0.01$). Prior studies have shown that MT treatment suppressed the breakdown of the cell wall and the enzymatic activities of cell wall hydrolases, including polygalacturonase,

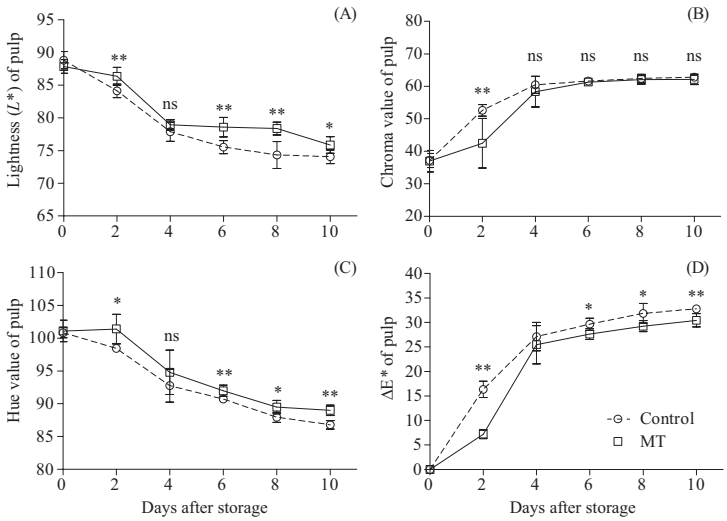

Fig. 3 Effects of 0.5 mM melatonin treatment on pulp color attributes of 'Nam Dok Mai No. 4' mangoes compared with untreated controls during storage at 25°C for 10 d: (A) *L**; (B) chroma; (C) hue angle; (D) color difference (ΔE*). Values is mean ± SD (n = 5). Asterisks indicate significant differences between treatments at each storage period [* $p < 0.05$; ** $p < 0.01$; ns = non-significant difference ($p \geq 0.05$)].

pectin methylesterase and β-galactosidase, play a role in maintaining the firmness of mangoes during storage by delaying cell wall degradation (Liu et al., 2020; Njie et al., 2023). The increased TSS/TA ratio is an indicator of the ripening progression in mangoes during storage, as shown in Fig. 4B. The increased TSS/TA ratio in the untreated mango was considerably higher than that of all mangoes treated with MT during the entire storage period ($p < 0.01$). During storage for 6 days, the mangoes subjected to 0.5 mM MT immersion exhibited a lower TSS/TA ratio increase compared to non-treated mangoes. This demonstrated that MT treatments at the concentration of 0.5 mM, could postpone 'Nam Dok Mai No. 4' mango ripening. These findings imply that MT immersions could delay the pulp softening retardation, the increased TSS/TA ratio and the pulp color development of mangoes during storage. According to the results, immersing mangoes with MT may effectively slow down the ripening process during storage. This effect is particularly noticeable when using a concentration of 0.5 mM and immersing for 1 hr. In a similar vein, prior research has shown that postharvest MT treatment can successfully delay the ripening process and maintain the postharvest quality of several fruits, including peaches (Gao et al., 2016), tomatoes (Liu et al., 2019), bananas (Hu et al., 2017; Wang et al., 2021), strawberries (Liu et al., 2018) and mangoes (Liu et al., 2020; Njie et al., 2023). The ripening and maturation process of mangoes is notably influenced by respiration rate, C_2H_4 production and biological oxidation reactions, which have a substantial impact on various aspects such as color, sugar content, acidity, texture and the release of aromatic compounds (Singh et al., 2013). The application of 0.5 mM MT reduced respiration and ethylene production and enhanced antioxidant activity in mangoes, which in turn delayed softening and moderated the development of TSS/TA, resulting in a slower progression of ripening under ambient conditions than in untreated fruit.

Antioxidant capacity, total phenolic content and ascorbic acid

The impacts of MT immersion on antioxidant activities (FRAP and DPPH radical scavenging activity), along with the concentrations of total phenolic and ascorbic acid in the mangoes, are depicted in Fig. 5. Both phenolic compounds and ascorbic acid are recognized for their antioxidant capacity. The MT treatment demonstrated preservation and enhancement of both compounds in the mangoes during storage (Fig. 5A–B). Throughout the storage period, the levels of both total phenolic compounds and ascorbic acid in the MT-treated mangoes were significantly greater compared to the control mangoes ($p < 0.05$ and $p < 0.01$). Furthermore, we observed an elevation in the levels of these compounds in both mango groups after storage for 6 days. Both mango groups showed a decrease in DPPH radical scavenging activity throughout the keeping period. The DPPH radical scavenging activity in mangoes treated with MT was potentially greater than that of the control mangoes. At storage days 8 and 10, mangoes treated with MT demonstrated significantly higher DPPH radical scavenging activity compared to the control mangoes ($p < 0.05$) (Fig. 5C). The findings also show that the MT treatment significantly increased FRAP in the mangoes compared to the control mangoes within the initial 2 days of storage ($p < 0.01$) (Fig. 5D). Subsequently, the FRAP levels in both groups of mangoes declined, with the FRAP levels in MT-treated mangoes consistently remaining higher than those in the control group over the entire storage duration ($p < 0.05$ and $p < 0.01$). These findings demonstrate that MT immersion effectively preserved antioxidant activity and certain biologically active compounds in the mangoes during storage. Likewise, earlier studies have found that MT can significantly impact the levels of various metabolites in fruits, including ascorbic acid, phenols and antioxidants. This effect has been reported in guavas (Fan et al., 2022b), peaches (Cao et al., 2018), strawberries (Aghdam and Fard, 2017),

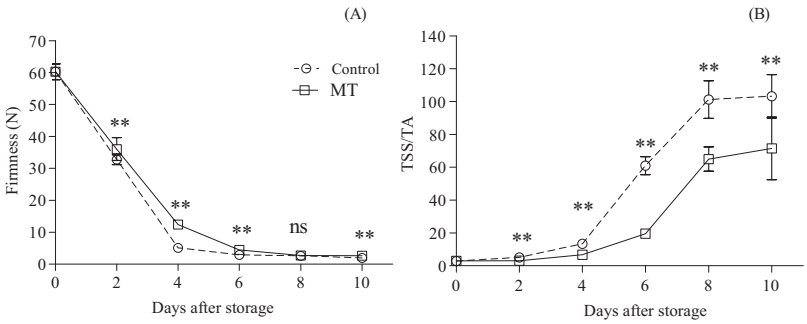

Fig. 4 Effects of 0.5 mM melatonin treatment on firmness and TSS/TA ration of 'Nam Dok Mai No. 4' mangoes compared with untreated controls during storage at 25°C for 10 d: (A) firmness (B) TSS/TA. Values is mean ± SD (n = 5). Asterisks indicate significant differences between treatments at each storage period [* $p < 0.05$; ** $p < 0.01$; ns = non-significant difference ($p \geq 0.05$)].

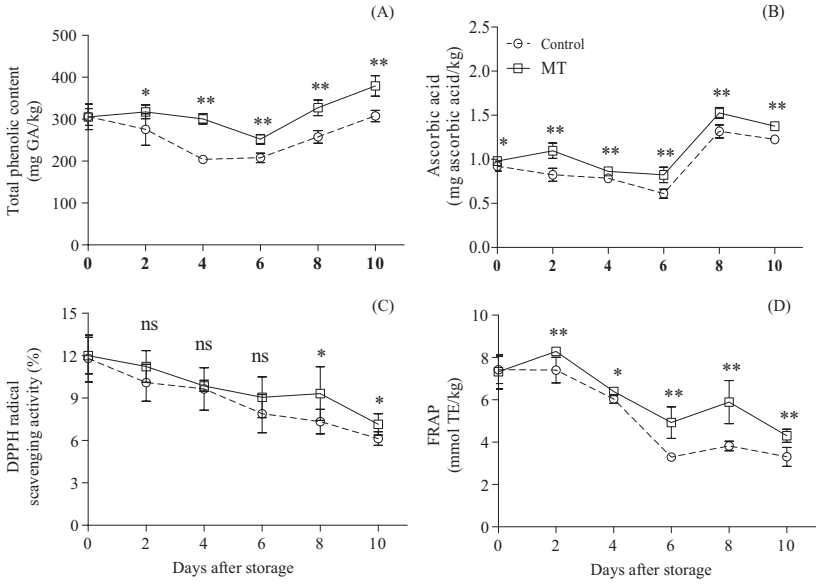

Fig. 5 Effects of 0.5 mM melatonin treatment on 'Nam Dok Mai No. 4' mangoes compared with untreated controls during storage at 25°C for 10 d: (A) total phenolic content; (B) ascorbic acid content; (C) 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity; (D) ferric reducing antioxidant power (FRAP). Value is mean ± SD (n = 5). Asterisks indicate significant differences between treatments at each storage period [*$p < 0.05$; ** $p < 0.01$; ns = non-significant difference ($p \geq 0.05$)]; GA = gallic acid.

litchi (Zhang et al., 2018) and blueberries (Shang et al., 2021). Qu et al. (2022) suggested that MT can stimulate the production of phenylpropanoid metabolites in blueberries by influencing the jasmonic acid signaling system during storage. Additionally, Wang et al. (2023) stated that MT considerably improved the important enzymes' activity involved in phenylpropanoid metabolism in apples. These enzymes such as 4-coumarate CoA ligase, cinnamic acid 4-hydroxylase, phenylalanine ammonia lyase, polyphenol oxidase, laccase, glucose-6-phosphate dehydrogenase and glucose-6-phosphate isomerase. Additionally, MT-induced ascorbic biosynthesis in kiwifruit has been reported by Luo et al. (2022). According to their findings, MT enhanced the gene expression associated with ascorbic acid biosynthesis, including *AcGME2* and *AcGalDH*, as well as *AcGalLDH*.

Antioxidant enzymes

Figs. 6A–C show that there were increases during storage in antioxidant enzyme activity levels of SOD, CAT and APX, in the MT-treated mangoes and the control mangoes. However, immersing the mangoes in 0.5 mM MT for 1 hr enhanced the SOD, CAT and APX activities in the mangoes compared to the control group throughout storage. There were highly significant elevations in the SOD and CAT activity levels in the MT-treated mangoes, compared to the control mangoes, beginning on day 2 and persisting until the last day of the storage period. There was a sharp increase in the APX activity of both groups of mangoes following 4 d of storage. Additionally, the APX activity in the MT-treated mangoes remained higher ($p < 0.05$ and $p < 0.01$) than that in the control mangoes throughout storage. Other studies have indicated that MT enhanced the efficiency of the ROS-scavenging system, which includes antioxidant enzymes such as SOD, CAT, Peroxidase and APX (Gao et al., 2016; Tousi et al., 2020). According to Shang et al. (2021), MT treatment suppressed oxidative stress and membrane lipid peroxidation in blueberries by controlling ROS accumulation. These effects were linked to the enhanced expression of genes responsible for encoding antioxidant enzymes and the augmentation of antioxidant enzyme activities. The increase in antioxidant capacity could delay the effect of oxidation reactions by ROS (Paull and Chen, 2003). Comparable effects of MT have been observed in peaches (Gao et al., 2016), strawberries (Liu et al., 2018), cassava (Ma et al., 2016), fresh-cut sweet potatoes (Li et al., 2022) and mangoes (Bhardwaj et al., 2022). These findings implied that the MT treatment may be advantageous for preserving the metabolic equilibrium of ROS via the increment of antioxidant enzymes, hence reducing lipid peroxidation and maintaining quality in mango fruits.

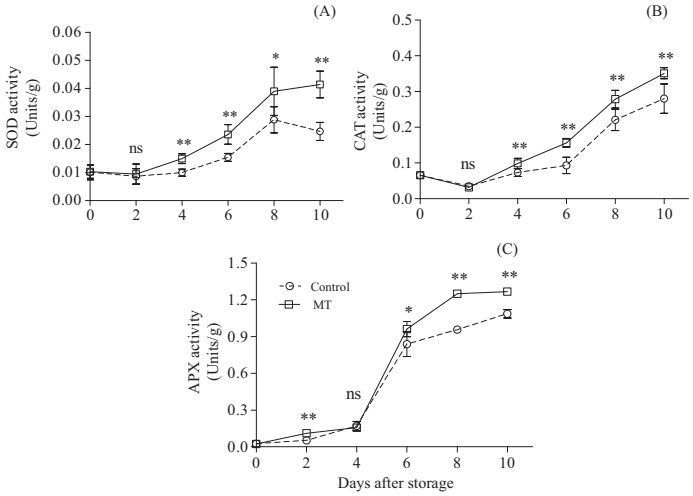

Fig. 6 Effects on antioxidant enzyme activity levels of 'Nam Dok Mai No. 4' mangoes treated with 0.5 mM melatonin and control fruits during storage at 25°C for 10 d: (A) superoxide dismutase (SOD); (B) catalase (CAT); ascorbate peroxidase (APX), where each value is mean ± SD (n = 5) and asterisks indicate differences between treatments for each storage period: * = significantly different at $p < 0.05$ and ** = highly significantly different at $p < 0.01$

Reactive oxygen species, lipoxygenase activity and malondialdehyde content

Notably, oxidative stress in plants is accompanied by the accumulation of ROS and a rise in LOX activity and MDA levels (Corpas et al., 2018). Increased oxidative stress accelerates the senescence process and reduces the storage longevity of fruits (Matamoros et al., 2010; Gao et al., 2016). The results in Fig. 7 depict the efficacy of MT in inhibiting increases in the H_2O_2, LOX activity and MDA levels in the mangoes during storage. The H_2O_2 content and LOX activity in the non-treated and MT-treated fruits gradually increased, then peaked on day 6 and day 8 of storage, respectively. Subsequently, they declined (Figs. 7A–B). The MDA content of both mango groups increased throughout the storage period (Fig. 7C). These findings reveal that the MT treatment markedly highly significantly decreased the levels of H_2O_2, LOX activity and MDA in the mangoes throughout storage, suggesting that the MT treatment was effective at mitigating increased oxidative stress and membrane lipid peroxidation in the mangoes during storage. The results of the current study were consistent with other studies reporting that MT functioned effectively as an ROS scavenger, safeguarding fruit from oxidative damage and maintaining cell membrane integrity in peaches (Gao et al., 2016), pears (Zhai et al., 2018), tomatoes (Liu et al., 2019), litchis (Zhang et al., 2018)

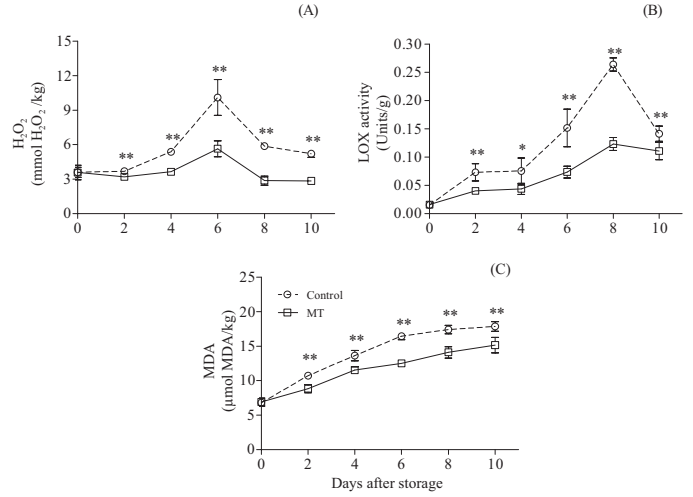

Fig. 7 Effects of 'Nam Dok Mai No. 4' mangoes treated with 0.5 mM melatonin and control fruits during storage at 25°C for 10 d on: (A) H_2O_2 level; (B), LOX activity; (C) MDA content, where each value is mean ± SD (n = 5) and asterisks indicate differences between treatments for each storage period: * = significantly different at $p < 0.05$ and ** = highly significantly different at $p < 0.01$

and mangoes (Bhardwaj et al., 2022; Njie et al., 2023). In addition, MT contributes to the preservation of redox homeostasis by modulating the repair of oxidatively damaged proteins and enhancing antioxidant capacity (Xin et al., 2017; Zhang et al., 2018). All of these mechanisms contribute to preserving the integrity of the membrane, which, in turn, helps to maintain fruit quality and prolong the postharvest life.

Visual appearance, disease incidence and disease severity

The effects of 0.5 mM MT immersion are presented in Fig. 8A on the visual appearance, disease incidence and disease severity progression in 'Nam Dok Mai No. 4' mangoes. The disease symptoms, characterized by the emergence of black spots on the skin, appeared earlier in the control group (day 6) than in the MT-treated mangoes, with the latter also having lower disease incidence and severity. The application of MT immersions had a suppressive effect on the onset of diseases in mangoes during storage. On day 10 of storage, the control mangoes had 100% disease incidence, whereas the fruit treated with MT maintained 0% incidence up to day 8 and developed only 20% incidence thereafter. In terms of disease severity, the control group reached a severity score of 2, while the MT-treated mangoes recorded a markedly lower score of 0.4 (Fig. 8B).

In addition, disease symptoms, characterized by the emergence of black spots on the skin, appeared earlier in the control group (day 6) than in the MT-treated mangoes, with the latter also having lower disease incidence and severity. The application of MT immersions had a suppressive effect on the onset of diseases in mangoes during storage. On day 10 of storage, the control mangoes had 100% disease incidence, whereas the fruit treated with MT maintained 0% incidence up to day 8 and developed only 20% incidence thereafter. In terms of disease severity, the control group reached a severity score of 2, while the MT-treated mangoes recorded a markedly lower score of 0.4 (Fig. 8C). Li et al. (2019b) reported that application of MT postharvest significantly curbed fungal infection in cherry tomatoes, with notable efficacy against grey mold (*Botrytis cinerea*). In addition, they highlighted that this protective effect was associated with the upregulation of defense-related gene expression, which contributed to enhanced resistance mechanisms in the fruit. Additionally, Li et al (2019a) reported that melatonin treatment after harvest reduced anthracnose in bananas by enhancing gene expression related to stress response, signaling and secondary metabolite production, as well as modulating key hormone pathways, contributing to delayed ripening and improved resistance to *Colletotrichum musae*.

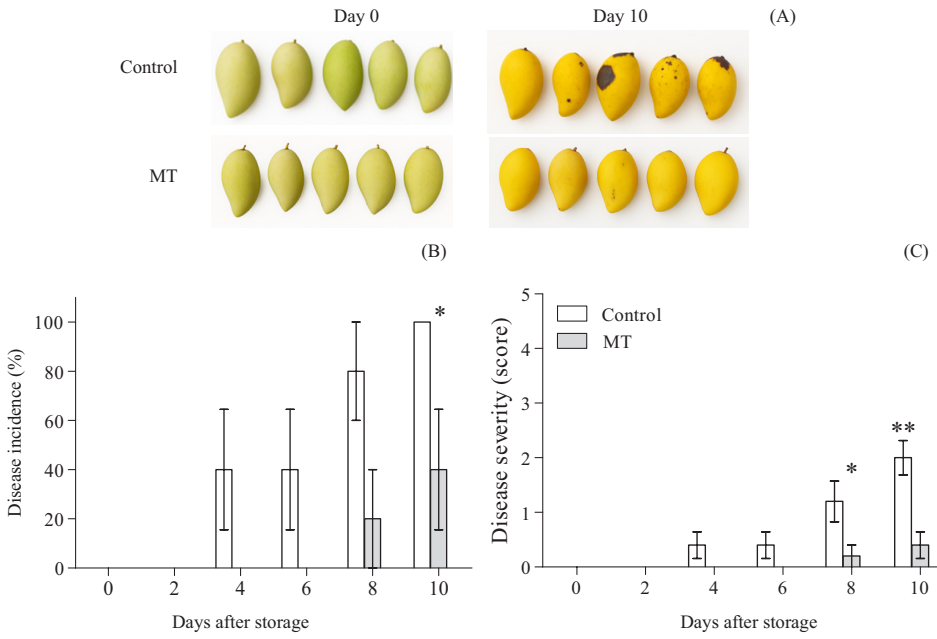

Fig. 8 Impacts on 'Nam Dok Mai No. 4' mangoes treated with 0.5 mM melatonin (MT) and control fruits during storage at 25°C for 10 d: (A) visual appearance; (B) disease incidence; (C) disease severity, where each value is mean ± SD (n = 5) and asterisks indicate differences between treatments for each storage period: * = significantly different at $p < 0.05$ and ** = highly significantly different at $p < 0.01$

Conclusion

Subjecting 'Nam Dok Mai No. 4' mangoes to MT immersion postponed the ripening process, including retardation of the peel and of pulp color development and pulp softening, as well as increasing the TSS-to-TA ratio. In addition, MT immersion retarded the onset of postharvest disease incidence in the mangoes. Compared to the other MT treatments, 0.5 mM MT was a suitable concentration to preserve mango postharvest quality. In addition, the mangoes subjected to 0.5 mM MT immersion had a lower respiratory climacteric peak and increased C_2H_4 production than the untreated mangoes. Furthermore, the MT treatment enhanced the antioxidant capacity, bioactive compounds and antioxidant enzyme activity levels, which were concomitant with the lower levels of parameters related to biological oxidation, such as H_2O_2, MDA content and LOX activity, in the MT-treated mangoes during storage. Conclusively, the postharvest application of MT was a feasible postharvest approach for preserving the postharvest quality, enhancing antioxidant capacity and decreasing biological oxidation in 'Nam Dok Mai No. 4' mangoes during storage at RT.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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