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Pre-storage melatonin treatment maintains cell membrane integrity, reduces fruit browning and decay incidence in guava (*Psidium guajava*)

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ABSTRACT

An experiments were conducted during 2021–22 at ICAR-Indian Agricultural Research Institute, New Delhi to study the impact of melatonin (MT) on key characteristics of guava (*Psidium guajava* L. cv. Barafkhana). Guava fruits were treated with different MT concentrations (200, 400, 600 and 800 µM) and stored at 10±1°C with 85–90% RH. The research revealed that treating with 600 µM MT notably decreased polyphenoloxidase activity by 57%, leading to delayed peel browning and also suppressed polygalacturonase (PG) activity by approximately 45% and pectine methylesterase activity by about 73%, resulting in higher firmness (5.33 N). Following a 20-days period of cold storage, significant reductions in electrolyte leakage, hydrogen peroxide accumulation and lipoxygenase activity were observed, thereby preserving cell membrane integrity. Additionally, MT stimulated the antioxidant defense system, boosted proline content accumulation, and enhanced phenylalanine ammonia-lyase activity. Together, these effects contributed to enhanced resistance against postharvest fungal decay (8.75%). In conclusion, MT at a concentration of 600 µM proves to be an effective postharvest treatment for maintaining texture, reducing fruit decay and extending the shelf-life of guava during cold storage.

Keywords: Decay incidence, Firmness, Lipoxygenase, Peel discoloration, Phenylalanine ammonia-lyase

Guava (*Psidium guajava* L.), native to tropical America and belonging to the Myrtaceae family, is a prominent fruit grown in tropical and subtropical regions worldwide. Its fruits are high in dietary fibre, pectin, antioxidants, vitamins (A and C), and essential minerals (Ca, Fe, P) (Anjum *et al.* 2020). Membrane integrity is crucial in the ripening of fruits; however, the rise in free radicals during ripening causes lipid peroxidation, escalating membrane permeability and disrupting metabolic processes (Mirshekari *et al.* 2020). Despite being advantageous, storing guava below 10°C causes chilling injury, and characterized by peel and pulp browning, abnormal ripening, and increased susceptibility to diseases (Fan *et al.* 2022). The best alternative to overcome these problems could be the use of physical treatments (ozonation, irradiation, edible coating etc.), chemical substances (nitric oxide, ascorbic acid, 1-methylcyclopropene etc.), plant-based compounds (brassinosteroids, methyl jasmonate, salicylic acid, plant extracts, essential oil etc.) and bioagents (Anjum *et al.* 2020). Exogenous melatonin treatment can induce fruit disease resistance and chilling tolerance, delay fruit ripening and senescence, minimize browning and membrane deterioration (Gao *et al.* 2018, Luo *et al.* 2021).

Melatonin (MT), improves fruit quality which is linked to its ability to boost the accumulation of antioxidants, such as total phenols, flavonoids, ascorbic acid, and anthocyanins (Rastegar *et al.* 2020). MT's antioxidant properties enhance enzyme activity and reduced reactive oxygen species, and extending shelf life (Ma *et al.* 2021). Further, MT sustains membrane integrity, retards ripening, and enhances disease resistance (Dong *et al.* 2021). However, the impact of MT dipping on guava's membrane integrity, browning and decay remains unexplored. Hence, the current investigation was framed to understand the influence of MT application on the physical and biochemical attributes of guava.

MATERIALS AND METHODS

Fruit collection and melatonin treatment: The experiments were conducted during 2021–22 at ICAR-Indian Agricultural Research Institute, New Delhi. Guava (cv. Barafkhana) was harvested at the physiologically mature green stage (TSS 9.20) and brought to the postharvest handling laboratory for the treatment application. A total

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of 125 fruits with uniform colour, size, and shape without any defects, diseases, pests, or bruises were chosen and divided into five groups. These fruits were then immersed in varying concentrations of MT solution (200, 400, 600, and 800 µM), along with Tween-80 (0.1%) as a surfactant. Control fruits were dipped in distilled water. Following treatment, the fruits were air-dried, packed in corrugated fiberboard boxes, and stored at a temperature of 10±1°C and 85–90% relative humidity. Throughout the storage period of 20 days, the fruits were assessed at intervals of 0, 5, 10, 15 and 20 days for physico-chemical parameters with three replications for each treatment.

Estimation of peel browning, decay incidence and firmness: Peel browning was assessed based on a modified scale as reported by Anjum *et al.* (2020). The scale consisted of 0 = no browning (0%), 1 = 1–20% browning, 2 = 21–40% browning, 3 = 41–60% browning, 4 = 61–80% browning and 5 = 81–100% browning. Decay incidence was determined by counting fruits with dark brown or blackish spots and expressing it as a percentage (%) of the initial fruit count (Dhami *et al*. 2023). Guava firmness was measured using a texture analyzer (Model: TA+Di, Stable Micro Systems, UK) as described by Bhan *et al.* (2023). Measurements were taken at three random points (top, middle and bottom) on each fruit using a 2 mm diameter cylindrical probe with specific compression speed settings (Pre-test, 3 mm/s; Test speed, 1 mm/s; Post-test speed, 5 mm/s; Distance, 10 mm). The peak force value, reported in newton (N), represented fruit firmness.

Estimation of cell wall modifying and defence related enzymes: Various cell wall modifying enzymes [Polygalacturonase (PG), Pectin methylesterase (PME) and Lipoxygenase (LOX)] and defence related enzymes [(Polyphenoloxidase (PPO), Phenylalanine Ammonia-Lyase (PAL) and Peroxidase (POD)] were analyzed after MT treatment in guava fruits using standard methods with slight modifications, except in few cases. To obtain the crude enzyme, 1 g of guava sample was homogenized in a solution of 0.2 M sodium phosphate buffer (*p*H 6.5) with the addition of 1% polyvinylpolypyrrolidone. Following centrifugation (10,000 × g, 20 min, 4°C), the resulting supernatant was employed for subsequent enzymatic analysis. The activity of PG (Tang *et al*. 2020), PME (Bhan *et al.* 2022), PPO (Njie *et al.* 2022), PAL (Njie *et al.* 2022), POD (Rastegar *et al.* 2020) and LOX (Bhan *et al.* 2022) were estimated in both MT-treated and control fruits.

Estimation of proline content, electrolyte leakage (EL), hydrogen peroxide (H_2O_2) and total antioxidant activity: The proline levels, H_2O_2 content and total antioxidant activity were estimated after MT treatment as per the procedures outlined by Kebbeh *et al.* (2023), Dong *et al*. (2021) and Liu *et al.* (2018), respectively with some modifications, and expressed as µM/g FW (Fresh Weight) for proline and H_2O_2 content, and µM Trolox Equivalent (TE) per gram FW for total antioxidant activity. However, the EL was estimated using a conductivity meter (Cole-Parmer Instrument, EC METER 1481-6) as per the method given by Ma *et al*. (2021) and the results were expressed as per cent (%).

Statistical analysis: The data on various physico-chemical attributes of MT-treated guava fruit were subjected to one-way analysis of variance (ANOVA). The significance of differences in the treatments across different days of storage was tested by F-test, and the treatment means were compared by least significant differences at P=0.05 using the statistical software SPSS version 16.0.

RESULTS AND DISCUSSION

Peel browning, decay incidence and firmness: There were significant variations in the browning, decay incidence and firmness of guava fruits across the treatments over the storage periods (Table 1). The browning and decay incidence were significantly decreased, while firmness of fruits increased in MT-treated fruits as compared to control treatment. However, at the end of storage, MT-600 µM treated fruits exhibited significantly slower browning process (1.4) and decay incidence (8.75%), and highest firmness of 5.33 N as compared to control (Table 1). The suppression of peel browning with MT treatment could be associated with reduction in electrolyte leakage and hindered activity of PPO and POD enzymes. Likewise, Luo *et al.* (2021) reported reduced browning of longan pericarp due to MT treatment. Further, the minimum decaying could be due to upregulation of the antioxidant defense system including higher total antioxidants, proline content and POD activity by MT. Similarly, Promyou *et al.* (2023) found reduced postharvest fungal decay of strawberries due to MT treatment during storage. The enhanced firmness in MT-treated fruits might be attributed to the reduction in the activity of softening enzymes such as PG and PME. Dong *et al.* (2021) also reported increase in the firmness of stored mangoes due to MT treatment.

Cell wall modifying and defence related enzymes: There were significant differences in the activity of various enzymes, viz. PG, PME, PPO, PAL, POD and LOX in guava fruit after MT imposition (Fig. 1a-1f). The activity of PG, PME, LOX and PPO were significantly decreased, while the activity of PAL and POD was significantly increased over the period of 20-days storage in MT treated fruits as compared to control fruits (Fig. 1a-1f). However, at the end of storage, the activity of PG (11.61 µg min/g/FW), PME (284.41 µM min/g/FW), LOX (2.28 µM min/g/FW) and PPO (4.31 U min/g/FW) was significantly lowest in MT- 600 µM treated fruits (Fig. 1a-1f). Notably, the activity of PAL (4.46 U min/g/FW) and POD (23.14 U min/g/FW) was significantly highest at the end of storage in MT treated fruits as compared to control fruits, showcases MT's effectiveness in curbing biotic and abiotic stress, minimizing fruit decay, and enhancing antioxidant processes during guava's cold storage (Fig. 1a-1f). Similarly, the delay in PG activity and suppression of fruit softening was also observed in MT-treated mangoes (Liu *et al.* 2020) and pears (Zhai *et al*. 2018). Further, previous studies also confirmed that MT treatment reduce the loss of fruit firmness by hindering the activity of cell wall degrading enzymes such as PG

Table 1 Effect of melatonin treatment on physical characteristics of guava fruit during cold storage

Parameter	Treatment (MT)	Storage days				
		0	5	10	15	20
Browning index	Control	0 ± 0	0 ± 0	1.5 ± 0.06	3.61 ± 0.16	5.0 ± 0.05
	200 µM	0 ± 0	0 ± 0	0.6 ± 0.03	2.41 ± 0.04	4.2 ± 0.3
	400 µM	0 ± 0	0 ± 0	0.4 ± 0.03	1.51 ± 0.04	3.0 ± 0.07
	600 µM	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1.4 ± 0.1
	800 µM	0 ± 0	0 ± 0	0 ± 0	0.6 ± 0.03	2.5 ± 0.06
	F-value	NA	NA	435.12	374.36	96.85
	P-value	<0.001	<0.001	<0.001	<0.001	<0.001
	LSD (*P*=0.05)	NA	NA	0.09	0.24	0.46
Decay incidence (%)	Control	0 ± 0	4.15 ± 0.19	44.15 ± 2.7	77.85 ± 2.38	95.71 ± 5.73
	200 µM	0 ± 0	3.82 ± 0.27	23.46 ± 0.4	40.16 ± 0.88	63.45 ± 0.85
	400 µM	0 ± 0	0 ± 0	9 ± 0.36	21.88 ± 0.35	57.39 ± 3.07
	600 µM	0 ± 0	0 ± 0	0 ± 0	3.45 ± 0.11	8.75 ± 0.39
	800 µM	0 ± 0	0 ± 0	6.66 ± 0.45	9.56 ± 0.23	14.56 ± 0.17
	F-value	NA	225.84	199.48	679.64	152.80
	P-value	<0.001	<0.001	<0.001	<0.001	<0.001
	LSD (*P*=0.05)	NA	0.463	3.98	3.66	9.37
Firmness (N)	Control	24.91 ± 0.14	13.23 ± 0.17	8.19 ± 0.11	4.27 ± 0.13	1.16 ± 0.13
	200 µM	24.85 ± 0.13	13.76 ± 0.18	8.86 ± 0.17	4.61 ± 0.12	1.97 ± 0.16
	400 µM	24.7 ± 0.18	14.45 ± 0.11	9.37 ± 0.12	5.08 ± 0.12	3.22 ± 0.11
	600 µM	24.63 ± 0.18	16.48 ± 0.13	11.76 ± 0.1	7.16 ± 0.12	5.33 ± 0.14
	800 µM	24.61 ± 0.16	15.16 ± 0.14	9.86 ± 0.15	5.39 ± 0.11	3.92 ± 0.11
	F-value	0.75	80.09	112.15	95.04	172.06
	P-value	0.58	<0.001	<0.001	<0.001	<0.001
	LSD (*P*=0.05)	NA	0.45	0.41	0.37	0.40
EL (%)	Control	5.77 ± 0.18	12.8 ± 0.21	27.75 ± 0.28	44.74 ± 2.02	66.91 ± 1.27
	200 µM	5.54 ± 0.37	11.13 ± 0.78	23.3 ± 0.51	39.92 ± 2.04	61.84 ± 3.02
	400 µM	5.59 ± 0.25	7.77 ± 0.39	18.48 ± 1.19	35.15 ± 2.32	57 ± 0.84
	600 µM	5.82 ± 0.28	4.84 ± 0.13	11.12 ± 0.63	27.99 ± 0.89	50.35 ± 2.34
	800 µM	5.68 ± 0.33	6.08 ± 0.07	12.93 ± 0.75	30.3 ± 1.48	52.23 ± 2.08
	F-value	0.16	69.66	90.16	14.25	11.02
	P-value	0.95	<0.001	<0.001	<0.001	<0.001
	LSD (*P*=0.05)	NA	1.29	2.34	5.80	6.56

MT, Melatonin; EL, Electrolyte leakage.

and PME in jujube fruits (Tang *et al.* 2020). However, the increased activity of PPO enzyme is linked to peel browning by oxidizing phenolic compounds into quinones. In current study, reduced PPO activity in MT-treated guava fruits led to less skin browning. Similarly, Gao *et al*. (2018) also reported less skin browning in peaches due to MT treatment. Further, MT-induced PAL activity led to increased total phenols, inhibiting fungal decay and extending shelf life in mangoes (Nije *et al.* 2022). Furthermore, the increase in POD activity during storage was also noticed in other fruit crops such as mango (Rastegar *et al.* 2020) and strawberries (Promyou *et al.* 2023). Current findings showed that MT treatment reduced the LOX activity, thereby maintaining membrane integrity of guava fruits during cold storage. Likewise, the suppression of LOX activity was also observed in sapota fruits treated with MT (Mirshekari *et al.* 2020).

Proline, electrolyte leakage, hydrogen peroxide and total antioxidant activity: There were significant differences in the content of proline, EL, H_2O_2 (Table 1) and total antioxidant activity in MT-treated guava fruits (Table 2). The proline content and EL were significantly increased, total antioxidant activity decreased significantly, while H_2O_2 increased and then declined over the period of 20-days storage. However, at 20 days of storage, the proline content (26.96 µM/g/FW) and total antioxidant activity (3.67 µM TE/g/FW) were significantly higher, while H_2O_2 (45.63 µM/g/FW) and EL

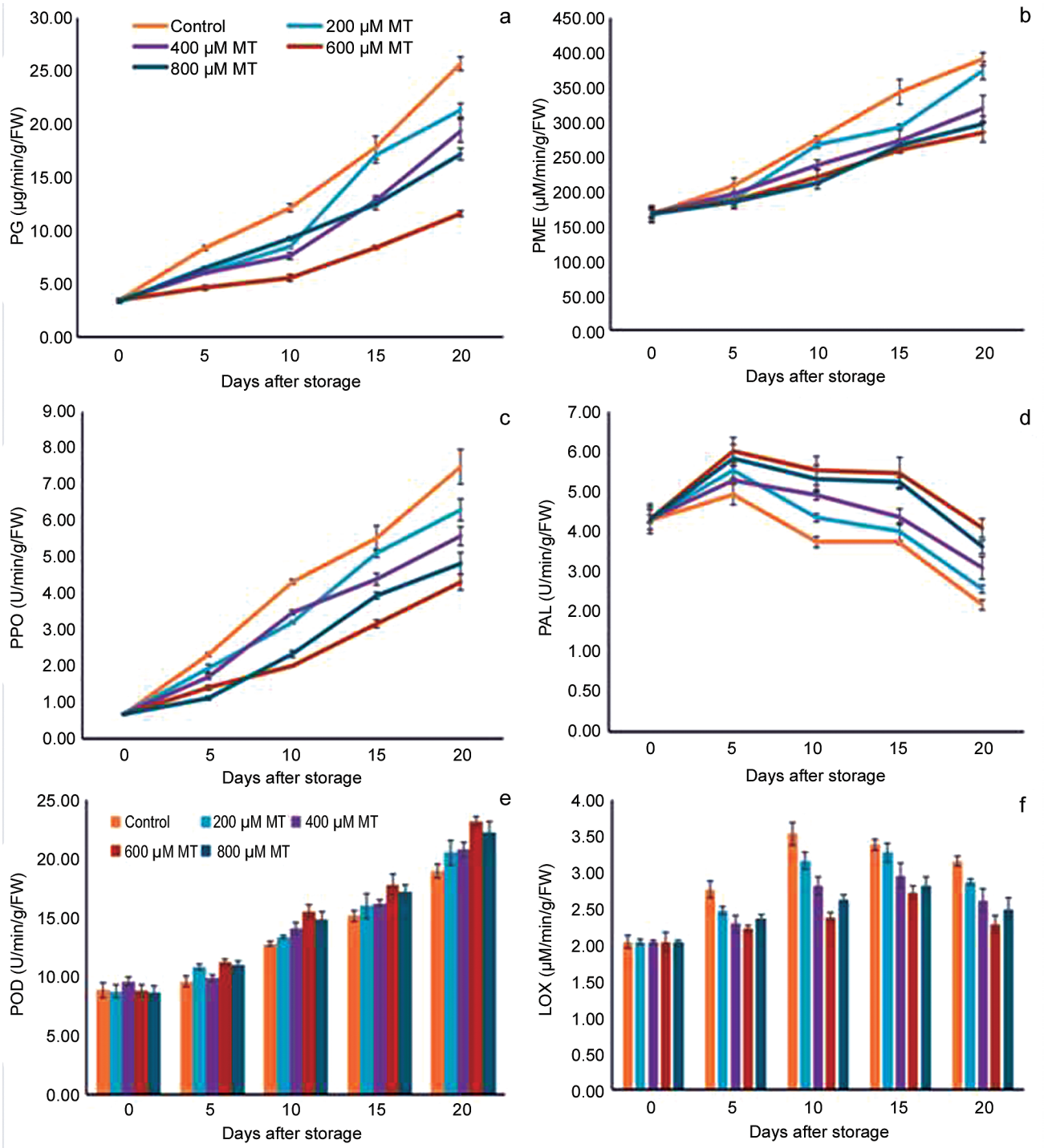

Fig. 1 Effect of melatonin on the activity of (A) Polygalacturonase (PG); (B) Pectinmethylesterase (PME); (C) Polyphenoloxidase (PPO); (D) Phenylalanine ammonia-lyase (PAL); (E) Peroxidase (POD); and (F) Lipoxygenase (LOX) enzymes during cold storage (10±1; 85–90% RH) of guava fruit.

(50.35%) were significantly lowest in MT-600 µM treated guava fruits as compared to rest of the treatments (Table 2). The results of current investigation showed that exogenous MT treatment increased the proline content in guava fruits providing resistance against oxidative stress and fruit decay. Kebbeh *et al.* (2023) also reported that MT-induced proline accumulation in mangoes. Further, antioxidant activity plays a critical role in scavenging the free radicals and boosting the immune system response in fruits. Current study reported the higher antioxidant activity in MT-treated guavas. Previous study also showed that MT treatment significantly increased the DPPH scavenging capacity in strawberry fruit during storage (Liu *et al.* 2018). The minimum damage to cell membrane with MT treatment may be due to the alleviation in H_2O_2 and malondialdehyde accumulation. Similarly, reduction of membrane deterioration has been

Table 2 Effect of melatonin treatment on proline, H_2O_2 content and total antioxidant activity (DPPH) of guava fruit during cold storage

Parameter	Treatment (MT)	Storage days				
		0	5	10	15	20
Proline (µM/g FW)	Control	7.08 ± 0.3	9.9 ± 0.34	11.73 ± 0.52	17.23 ± 0.62	20.66 ± 1.02
	200 µM	7.48 ± 0.41	11.49 ± 0.56	14.72 ± 0.38	19.09 ± 1.31	23.97 ± 1.12
	400 µM	7.38 ± 0.29	9.64 ± 0.51	14.07 ± 0.9	19.76 ± 0.3	23.18 ± 0.53
	600 µM	7.36 ± 0.39	11.87 ± 0.26	15.7 ± 0.46	20.64 ± 0.99	26.96 ± 0.87
	800 µM	7.34 ± 0.36	12.53 ± 0.43	15.16 ± 0.43	20.46 ± 0.21	25.03 ± 0.42
	F-value	0.18	8.66	7.44	2.98	7.81
	P-value	0.943	0.003	0.005	0.073	0.004
	LSD (*P*=0.05)	NA	1.37	1.81	NA	2.66
DPPH (µMTE/g FW)	Control	6.27 ± 0.36	5.29 ± 0.12	4.58 ± 0.27	3.54 ± 0.13	1.98 ± 0.07
	200 µM	6.3 ± 0.23	5.55 ± 0.06	4.71 ± 0.27	3.87 ± 0.18	2.54 ± 0.07
	400 µM	6.25 ± 0.21	5.73 ± 0.11	4.9 ± 0.15	4.07 ± 0.05	2.97 ± 0.14
	600 µM	6.25 ± 0.26	6.01 ± 0.13	5.43 ± 0.26	4.69 ± 0.22	3.67 ± 0.09
	800 µM	6.29 ± 0.19	5.85 ± 0.18	5.18 ± 0.16	4.26 ± 0.05	3.3 ± 0.12
	F-value	0.01	5.35	2.37	9.54	44.66
	P-value	1.000	0.014	0.123	0.002	<0.001
	LSD (*P*=0.05)	NA	0.39	NA	0.45	0.32
H_2O_2 (µM/g FW)	Control	8.35 ± 0.06	35.43 ± 0.31	68.42 ± 0.62	96.53 ± 0.79	82.76 ± 0.63
	200 µM	8.72 ± 0.1	30.45 ± 0.63	60.45 ± 0.38	84.34 ± 0.6	68.95 ± 0.82
	400 µM	8.54 ± 0.17	27.81 ± 0.54	54.81 ± 0.41	73.79 ± 0.35	62.69 ± 0.39
	600 µM	8.27 ± 0.03	18.45 ± 0.14	45.44 ± 0.22	62.73 ± 0.6	45.63 ± 0.64
	800 µM	8.49 ± 0.19	21.93 ± 0.29	49.93 ± 0.57	67.87 ± 0.57	54.57 ± 0.56
	F-value	2.00	262.64	388.09	520.00	517.66
	P-value	0.17	<0.001	<0.001	<0.001	<0.001
	LSD (*P*=0.05)	NA	1.33	1.46	1.90	1.98

MT, Melatonin; FW, Fresh weight; DPPH-2, 2-Diphenyl-1-picrylhydrazyl.

reported in MT-treated navel oranges (Ma *et al*. 2021) and rambutan (Wei *et al.* 2022). Our results suggest that MT treatment effectively reduced H_2O_2 content in guava (Table 1), indicating a decrease in membrane lipid peroxidation and thus preservation of membrane integrity. Dong *et al.* (2021) also reported reduction in H_2O_2 content in mangoes due to exogenous MT treatment.

The current investigation found that treatment with 600 µM of MT best-preserved fruit firmness over a 20-days storage period by inhibiting cell wall hydrolyzing enzyme activities. MT treatment also triggered defence-related compounds and enzymes, leading to reduced decay. Moreover, immersing the fruit in MT solution prevented lipid peroxidation and oxidative stress burst, thus maintained membrane stability. Hence, exogenous MT treatment is an effective strategy to retain membrane integrity and enhance defence against postharvest decay in guava fruit during cold storage.

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