Philippine Journal of Crop Science (PJCS) April 2025, 50 (1): 19-28

https://doi.org/10.63568/pjcs.v50i1.1295

FULL PAPER

Physico-chemical Responses of Mango (*Mangifera indica* L. cv. Carabao) Fruit to Melatonin Under Chilling Stress

Abigail P. Daria[1*], Elda B. Esguerra[2,3], Josephine U. Agravante[2,3], and Rona Kay G. Franco[3]

[1]Department of Crop Science, College of Agriculture, Food, Environment, and Natural Resources, Cavite State University-Main Campus, Indang, Cavite, Philippines; [2]Institute of Crop Science, College of Agriculture and Food Science (CAFS), University of the Philippines Los Baños, College (UPLB), Laguna 4031 Philippines; [3]Postharvest Horticulture Training and Research Center, CAFS, UPLB, College, Laguna 4031 Philippines.
*Corresponding author, apdaria@cvsu.edu.ph

'Carabao' mango fruit is highly susceptible to chilling injury (CI) when stored at sub-optimal temperature for extended period. Melatonin (MLT) was reported to ameliorate chilling injury (CI), hence this study aimed to enhance the cold tolerance of 'Carabao' mango fruits through MLT treatment. Mature green mango fruits were dipped for 10 min in varying concentrations (0.1, 0.5 and 1.0 mM) of MLT then stored at 7°C for 6 wk followed by poststorage at 24°C. Significant amelioration of CI with MLT was achieved during the 3-week storage at 7°C. Melatonin at 1.0 mM delayed the onset of CI and reduced the incidence of CI symptoms like lenticel spotting, peel discoloration, vascular streaking, pulp cavitation, and flesh browning. MLT however, did not significantly retard the rate of ripening of mangoes during storage at 7°C. Since ripening was not completely retarded during cold storage, total soluble solids (TSS) content increased although TSS of MLT–treated fruit was lower than the control. On the other hand, titratable acidity upon withdrawal from cold storage and at the ripe stage was not affected by MLT treatment. The high visual quality rating was maintained for t3 wk at 7°C in both treated and control fruit.

Keywords: chilling injury, *Mangifera indica*, melatonin

INTRODUCTION

Mango (*Mangifera indica* L.) is considered as one of the most important tropical fruits worldwide in terms of production, consumption, and nutritional value (Tharanathan et al. 2006; Singh et al. 2013). Mango ranks third as the most important fruit crop in the Philippines next to banana and pineapple, and one of the country's top agricultural exports (Philippine Mango Industry Roadmap 2017-2022). In 2020, the Philippines ranked 14th with an export share of 1.75% and export value of USD 65.54M, where the top 5 export destinations were United States, Hong Kong, Japan, Canada, and South Korea (Jang et al. 2020).

Mango being a tropical fruit is highly susceptible to chilling injury (CI) when stored at temperatures below 10°C for extended periods. CI is a physiological disorder as a consequence of exposure to low but non-freezing temperature for extended period (Raison and Lyons, 1986). In 'Carabao' mangoes kept at 5°C, CI first appeared as light brown irregular specks on the peel which later expanded and coalesced leading to peel discoloration (Agillon and Lizada, 2010). In the study of Cantre et al. (2017), the first symptoms of CI in 'Carabao' mangoes were brown spots and peel discoloration with high incidence of lenticel spotting after 7 d of storage at 4°. CI results in loss of sensory quality and consumer acceptability due to poor ripening, pitting, off-flavor development, and decay (Jackman et al. 1988).

Various methods have been employed to alleviate CI such as manipulation and/or modification of the storage environment exemplified by temperature conditioning, atmosphere modification, and increased relative humidity. The other techniques are directed to the commodity like waxing/edible coating, modified atmosphere packaging, breeding for resistant lines, and chemical treatments (Peng et al. 2013). Among the chemical treatments, polyamines (Koushesh Saba et al. 2012), methyl jasmonate (González-Aguilar et al. 2001), salicylic acid (Aghdam et al. 2012), and melatonin (Aghdam et al. 2019; Madebo et al. 2021) have been reported to induce cold tolerance in several fruits. Melatonin (MLT; N-acetyl-5-methoxytryptamine) is an indole compound that shares the same initial biosynthetic pathway with indole−3−acetic acid (IAA) (Fan et al. 2018) with tryptophan as the precursor. It is produced in the mitochondria and chloroplasts of leaves and/or roots, then translocated to the other plant organs (Arnao and Hernández-Ruiz, 2013; Tan et al. 2013). MLT is reported to have numerous beneficial effects on fruits and vegetables which include stimulating ethylene production, delaying senescence, alleviating CI, controlling disease and physiological disorders, and coping to environmental stresses (Arnao and Hernández-Ruiz 2015). In mango cultivar Sensation, a 10-min dip in 0.2 mM MLT retarded ripening measured as higher green peel color (lower a* values), higher firmness and titratable acidity (TA), and lower total soluble solids (TSS) and TSS/TA ratio (Awad and Adel 2021). Dong et al. (2021) similarly reported inhibition of ripening of 'Keitt' mangoes when treated with 100 to 200 µM MLT manifested as inhibition of softening and retardation of chlorophyll degradation. MLT treatment of 'Dashehari' mangoes resulted in lower weight loss, higher ascorbic acid content and TA, lower pH and TSS content than the control fruit during the 28-day storage at 5°C

(Bhardwal et al. 2022). In the study of Kebbeh et al. (2023) in 'Keitt' mangoes treated with 0.1 to 0.2 mM MLT, they reported that high firmness was maintained only during cold storage but decreased when transferred to room temperature. The reduced activity of cell wall-degrading enzymes resulted in the maintenance of fruit firmness of 'Guiqi' mangoes treated with 1.0 mM MLT (Njie et al. 2023). Recent study of Charoenphun et al. (2025) in 'Nam Dok Mai' mangoes, MLT at 1.0 to 2.0 mM effectively maintained the color characteristics and preserved fruit firmness.

With the improved cold tolerance associated with MLT treatment, it will then be possible to use refrigerated sea shipment of mangoes at sub-optimal temperatures such as 10°C and below to reach distant markets of Europe and North America. Thus, this study on 'Carabao' mango aimed to enhance the fruit's cold tolerance through establishing the optimum concentration of MLT that will ameliorate CI symptoms during storage at 7°C for 6 wk and poststorage at 24°C, and to determine the physicochemical changes associated with MLT treatment.

MATERIALS AND METHODS

Preparation of Experimental Materials and Treatment Concentration

Fully mature 'Carabao' mango fruits were harvested at 115 d from flower induction from a farm in Barangay Siranglupa, Calamba City, Laguna, Philippines. Mangoes were sorted at the farm as to freedom from defects and those that exhibit tinge of yellow peel color were culled. Mangoes were then transported on the same day to the Postharvest Horticulture Training and Research Center (PHTRC) Laboratory at the University of the Philippines Los Baños (UPLB) for experimental set-up. In the laboratory, fruit maturity was determined by floatation in 1% salt solution. Since the storage duration at 7°C was 6 wk and to control decay which might interfere with the observation, mangoes were then dipped in fungicide (125 ppm azoxystrobin) for 5 min.

Analytical grade MLT was purchased from Sigma Aldrich Co. based in Singapore through a local chemical supplier and was kept in the biofreezer (-60°C) until use. Since MLT is insoluble in water, pre-weighed amount of MLT was dissolved in 1 mL absolute ethanol then diluted with distilled water to obtain the desired concentrations of 0.1, 0.5, and 1.0 mM following the procedures of Ba et al. (2021). To avoid light-induced degradation of MLT, the prepared solutions were kept in dark bottles and the experiment was carried out under dark condition. The concentrations tested were based from the previous studies on MLT treatment of various cultivars of mango such as Sensation (0.2 mM; Awad and Adel, 2021), Keitt (0.1 - 0.2 mM; Dong et al. 2021), and unnamed cultivar (0.1-1.0 mM; Rastegar et al. 2020).

Mango fruits were dipped for 10 min in freshly prepared MLT of varying concentrations with Tween 20 as wetting agent. After MLT treatment at 24°C, mangoes were air-dried for about 2 h (also under dark condition) and then packed in corrugated retail fruit box that contained 5 mangoes and stored at 7°C for 6 wk. Withdrawal from 7°C storage was done every 2 wk.

Table 1. Rating scale for the different symptoms of chilling in mango fruit.

CI Symptom	Rating/Description	Reference
External		
Pitting	1: None 2: 1-25% of surface area affected 3: 26-50% of surface area affected 4: More than 50% of surface area affected	Zhao et al. 2006
Peel discoloration	1: None 2: 1-25% of surface area affected 3: 26-50% of surface area affected 4: >50% of surface area affected	Rodeo and Esguerra 2013
Lenticel spotting	1: None 2: Light spots not more than 25% of the surface 3: Light spots not more than 50% of the surface 4: Scattered spots more than 50% of the surface	Hofman et al. 2010 with modifications
Internal		
Vascular browning or streaking and pulp discoloration	1: No discoloration 2: 1-25% brown flesh/ vascular area affected 3: 26-50% brown flesh/ vascular area affected 4: 50-75% brown flesh/ vascular area affected 5: 75-100% brown flesh/ vascular area affected	Westra et al. 2016 with modifications
Cavitation	1: None 2: 1-25% of pulp affected 3: 25-50% of pulp affected 4: > 50% of pulp affected	Jacobi et al. 1996 with modifications

Data Gathering

During wkly monitoring of physical changes at 7˚C, 12 labeled fruit per treatment were used. The physical changes that were monitored every wk for 6 wk focused on external CI symptoms incidence and severity such as pitting, lenticel spotting, and peel discoloration using the ratings indicated in Table 1. The CI severity was determined in each treatment by multiplying the number of fruit in each injury level by their score, and then dividing this sum by the total number of fruits assessed per treatment.

Other physical changes that were monitored included changes in peel color and visual quality. The rate of ripening based on peel color change was determined using the following peel color index (PCI) established at PHTRC: 1- green, 2 - green with tinge of yellow, 3 - more green than yellow, 4 - more yellow than green, 5 - yellow with green tip, and 6 - full yellow. Change in visual quality was assessed following the visual quality rating (VQR) scale used in PHTRC as follows: 9,8 - excellent, field fresh, 7,6 - good, minor defects, 5-4 - fair, moderate defects, limit of marketability; 3- poor, serious defect, 2- limit of edibility, and 1-non-edible.

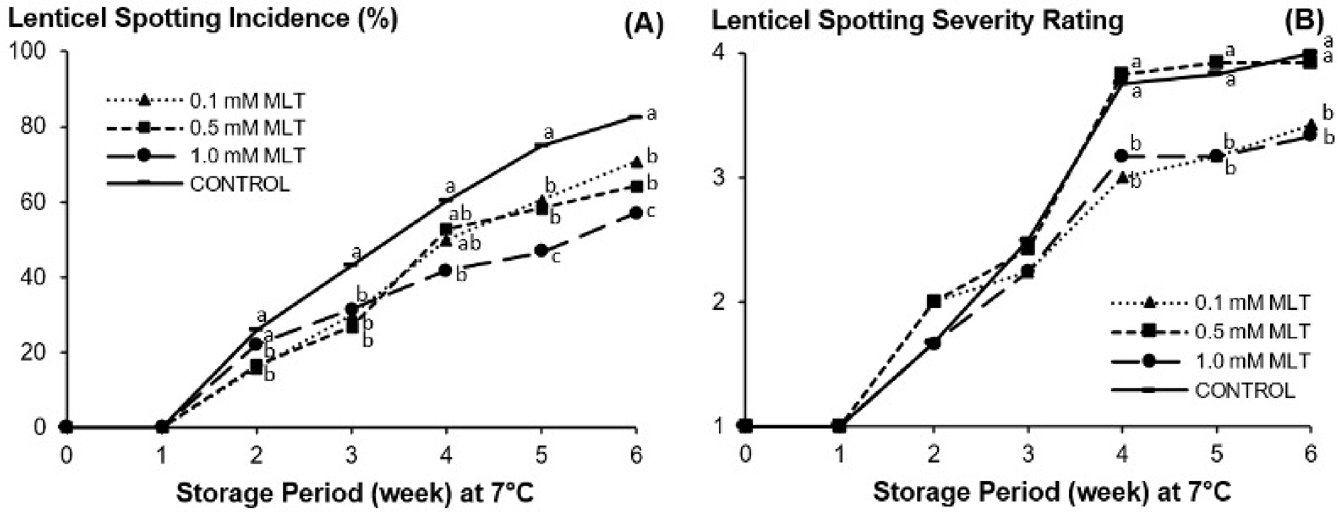

Figure 1. Lenticel spotting incidence (A) and severity (B) of 'Carabao' mango fruits treated with varying concentrations of melatonin (MLT) and stored at 7°C for six weeks. Means at each storage period with the same letter are not significantly different at 5% level, LSD (n=12).

Data gathered during destructive sampling were firmness, total soluble solids (TSS) content, titratable acidity (TA), and internal CI symptoms like cavitation (spongy air pockets in the pulp), flesh browning, and vascular discoloration. The remaining 9 fruit from each treatment were transferred to 24°C for ripening. Observation on peel color change, disease development, visual quality change and further development of external CI symptoms were done every other day. At the ripe stage after withdrawal from cold storage, the 9 mango fruit used in continuous monitoring were evaluated for internal CI symptoms, while 6 fruits per treatment were used for destructive sampling of firmness, TSS, and TA determination.

Fruit firmness was measured at the middle portion (opposite sides of the fruit with peel) using fruit pressure tester (AIKOH Digital Force Gauge SX Series Model-2256) with pointed plunger. Firmness was expressed as kg-force.For TSS content, 20 g of mango pulp taken at the mid-portion of the fruit was added with 100 mL of distilled water for homogenization using osteorizer blender. Homogenized mixture was filtered in a wad of cotton. TSS of the extract was determined using pocket refractometer (Atago Pocket Refractometer PAL-1). TA was determined by titration using 5 mL of aliquot and 10 mL of distilled water with 0.095 N NaOH and 2-3 drops of phenolphthalein indicator.

Experimental Design and Statistical Analysis

The experiment was laid out in a Completely Randomized Design having 3 replications per treatment. Mangoes were packed in corrugated retail box with each box representing a replicate consisting of 5 fruits. There were separate boxes of fruit for continuous monitoring during cold storage and during withdrawal every 2 wk. Three replicates or boxes were withdrawn at each period. Parametric variables were subjected to Analysis of Variance (ANOVA) using the SAS software version 9.1. Non-parametric variables were subjected to Kruskal-Wallis test using SPSS version 27. LSD was used in determining the significant differences among treatment means at $P \leq 0.05$ and $P \leq 0.01$ probability levels.

RESULTS

Incidence and Severity of Chilling Injury

External Chilling Injury Symptoms

Lenticel Spotting (LS) Incidence and Severity. LS first appeared as green spots which later turned into dark brown to black spots as storage period was extended to 6 wk, LS became noticeable on the 2nd wk and progressed as the duration of storage at 7°C increased (Figure 1A and B). On the 2nd and 3rd wk, significant differences were observed among treatments where MLT-treated fruit had lower incidence than control fruit. On the 3rd wk, LS incidence was reduced by almost 30% with 1.0 mM MLT. From the 4th until the 6th wk of storage, incidence of LS in all treatments further increased with more than 50% of fruit exhibiting LS. Mangoes treated with 1.0 mM MLT had lower incidence than the rest of the treatments.

As to the severity of LS, during the first 3 wk, severity was rated as slight (less than 10% of the peel with LS) and differences among treatments were not significant. On the 4th until the 6th wk of storage at 7°C, all MLT-treated and control fruit had more than 50% of the surface covered with LS. However, the 0.1 and 1.0 mM MLT-treated mangoes had significantly lower severity rating than the control and 0.5 mM MLT treatment.

In most cases, CI symptoms developed progressively when fruits are brought to higher temperatures. During poststorage at 24°C, the initially low LS incidence on the day of withdrawal from wk 2 storage at 7°C rapidly increased in all treatments. The control and 0.1 mM MLT-treated mangoes had significantly higher incidence than the rest of the MLT treatments, the pattern of which persisted until day 5 at 24°C. The green spots during storage at 7°C turned into brown to black spots as ripening progressed. The 1.0 mM MLT-treated fruit had the lowest incidence from day 1 until day 9 of ripening, while the control fruit got the highest. On the 4th wk of withdrawal when the incidence was already high, the proportion of mangoes with LS

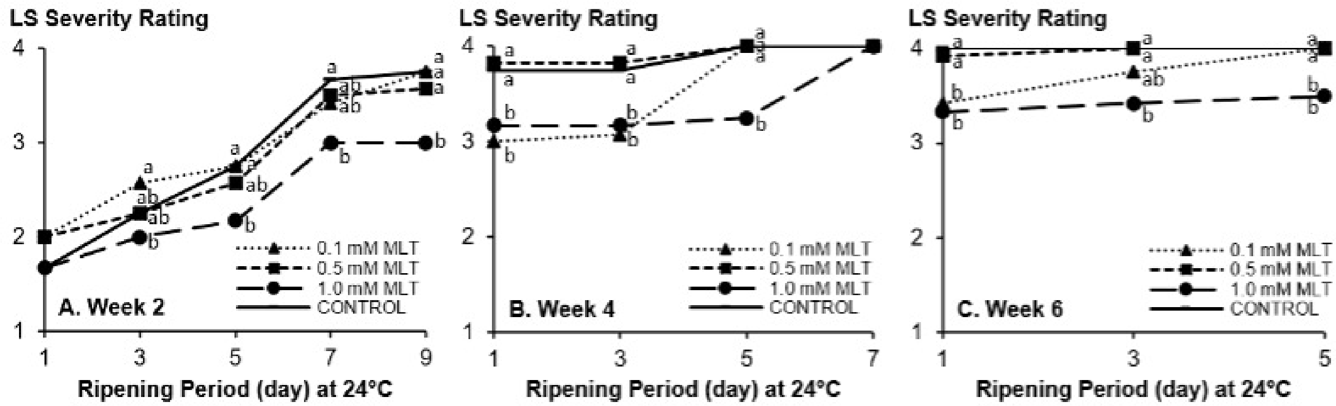

Figure 2. Severity of lenticel spotting of 'Carabao' mango fruits during poststorage ripening at 24°C on Week 2 (A), 4 (B), and 6 (C). Means at each ripening period with the same letter are not significantly different at 5% level, LSD (n=12).

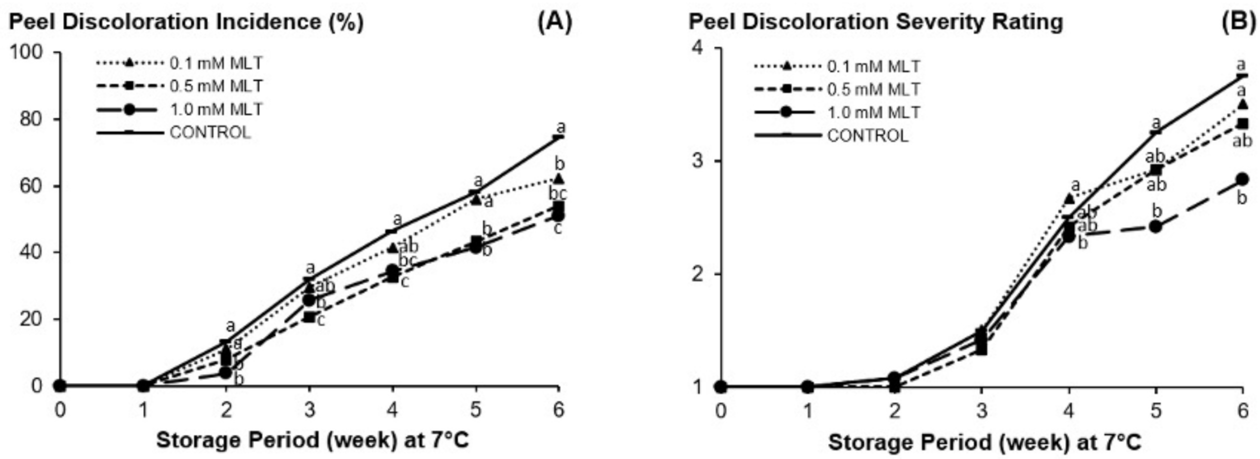

Figure 3. Peel discoloration incidence (A) and severity (B) of 'Carabao' mango fruits treated with varying concentrations of melatonin (MLT) and stored at 7°C for six weeks. Means at each storage period with the same letter are not significantly different at 5% level, LSD (n=12).

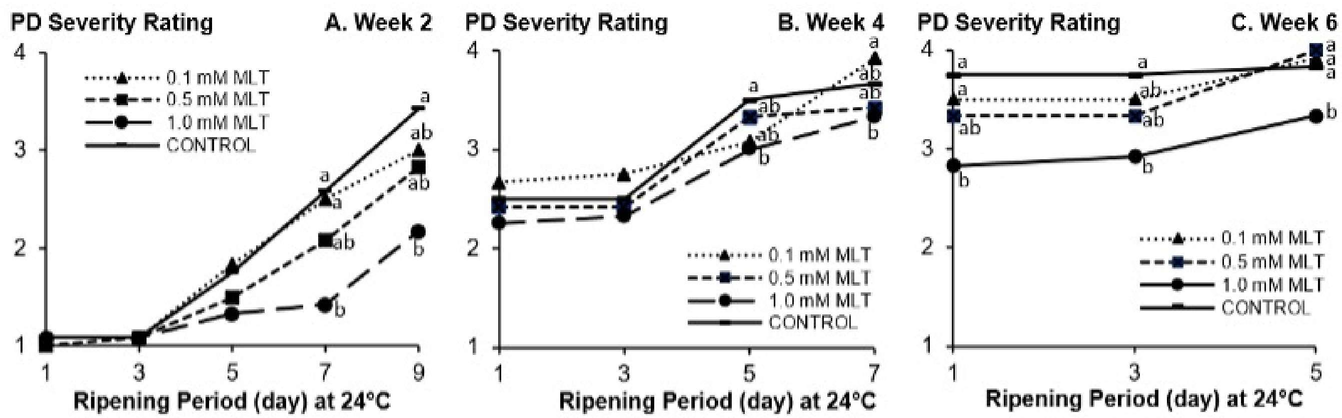

Figure 4. Severity of peel discoloration of 'Carabao' mango fruits during posstorage ripening at 24°C on Week 2 (A), 4 (B), and 6 (C). Means at each ripening period with the same letter are not significantly different at 5% level, LSD (n=12).

further increased with almost 90% of the fruit exhibiting LS when the fruit ripened. The 1.0 mM MLT treatment had lower incidence than the rest of the treatments. The same pattern was observed in mangoes kept at 7°C for 6 wk then transferred to 24°C except that the 1.0 mM MLT treatment had a significantly lower incidence than the rest of the treatments.

Regarding LS severity, it became more apparent as ripening progressed (Figures 2A to C). On the 2nd wk of withdrawal, severity of LS was significantly higher in the control fruit compared to 1.0 mM MLT-treated fruit. On the 4th and 6th wk of withdrawal, 1.0 mM MLT-treated fruit got the significantly lowest severity rating.

Peel Discoloration Incidence and Severity. Peel discoloration or peel browning is another major CI symptom exhibited by mangoes during extended storage at sub-optimal temperature. Peel discoloration was not yet apparent in all fruit during the 1st wk of storage at 7°C. From the 2nd wk onwards, significant differences were observed, wherein the 0.5 mM and

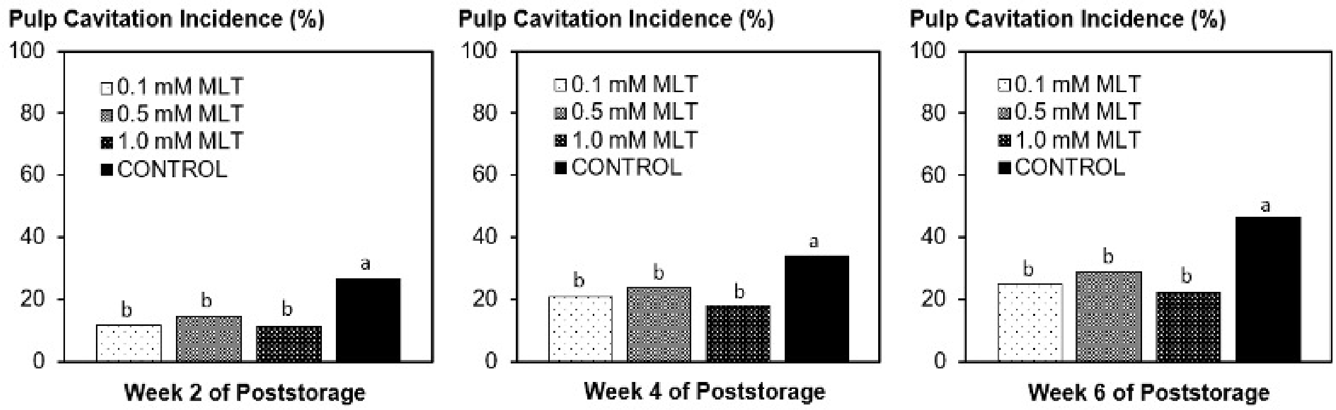

Figure 5. Incidence of pulp cavitation of 'Carabao' mango fruits at the ripe stage. Mangoes were dipped for 10 min in varying concentrations of melatonin (MLT) and stored at 7°C then withdrawn every two weeks for ripening at 24°C. Means at each poststorage period with the same letter are not significantly different at 5% level, LSD (n=9).

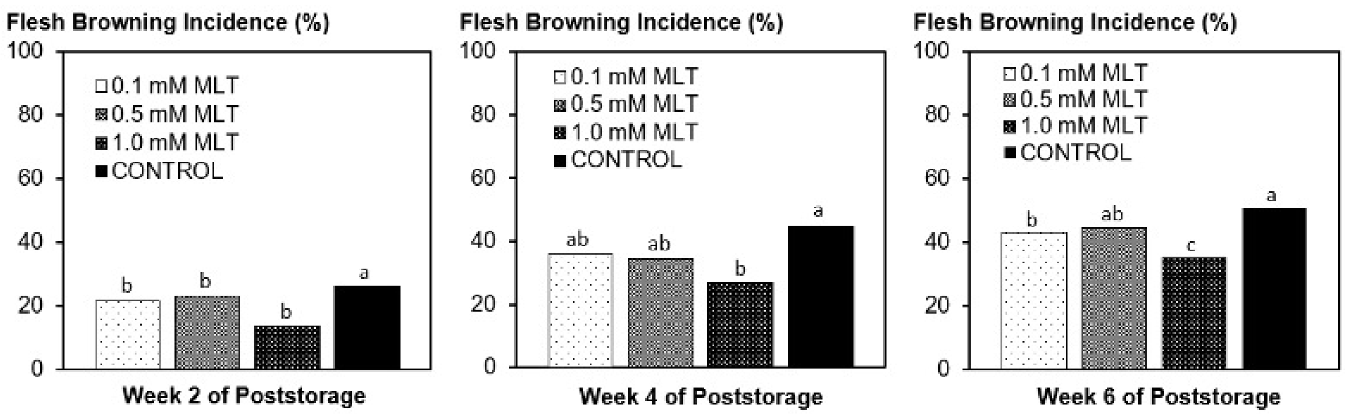

Figure 6. Incidence of flesh browning of 'Carabao' mango fruits at the ripe stage. Means at each poststorage period with the same letter are not significantly different at 5% level, LSD (n=9).

1.0 mM MLT-treated fruit got lower incidence than the control fruit. The severity of peel discoloration was lowest in 1.0 mM MLT-treated fruit although no significant differences were obtained (Figure 3A & B).

During poststorage at 24°C, the incidence of peel discoloration progressed rapidly as the fruit started to ripen. The dark gray patches on the peel became severely manifested as the fruit reached the ripe stage. On wk 2, the 0.5 mM and 1.0 mM MLT-treated fruit got lower incidence compared to the other treatments. On wk 4 and 6 of withdrawal, 1.0 mM MLT-treated fruit had the lowest incidence, while control fruit got the highest. The highest severity of brown to grayish peel discoloration was exhibited by control fruit in all wk of withdrawal and poststorage (Figure 4).

IInternal Chilling Injury Symptoms

Pulp Cavitation Incidence and Severity. Internal CI symptoms became apparent only when cold-stored mangoes are ripened at 24°C. Pulp cavitation appeared as small, white, and starchy spots in the inner mesocarp. During poststorage at 24°C and when the fruit reached the ripe stage, the incidence of pulp cavitation was highest in the control fruit regardless of the previous duration of storage at 7°C (Figure 5). When the duration of cold storage was extended to four (4) and 6 wk, the incidence increased with the control fruit exhibiting the highest (34-46%) while the 1.0 mM MLT-treated mangoes got the lowest (17-22%). While the incidence of pulp cavitation increased as the duration of storage at 7°C increased, the severity of cavitation on the other hand, was very slight regardless of the storage period, and whether the mangoes were treated with MLT or not (data not shown). The severity of pulp cavitation was highest in control fruit, while the 1.0 mM MLT-treated mangoes got the lowest, but no significant difference was observed.

Flesh Browning Incidence and Severity. Flesh browning during poststorage and ripening at 24°C was *observed*in all treatments from the 2nd wk of withdrawal until the 6th wk. The 1.0 mM MLT-treated fruit got the lowest incidence of 13-35% from the 2nd until the 6th wk of poststorage at 24°C and was significantly different with the rest of the treatments (Figure 6). The severity of flesh browning however, was very slight in all treatments (data not shown).

Vascular Streaking Incidence and Severity. In 'Carabao' mangoes, vascular browning appeared as string-like discolored vascular tissues vertically aligned in the pulp of chill-injured ripe fruit. When stored for 4 and 6 wk, vascular streaking was evident in all treatments with the MLT-treated mangoes having significantly lower incidence than the control (Table 2). The severity, however, was rated as slight and the 1.0 mM MLT treatment had the lowest rating.

Rate of Ripening

Low temperature storage effectively retarded the rate of ripening with the mangoes remaining green (PCI 1) for 3 wk at 7°C particularly in MLT-treated mangoes.

Table 2. Incidence of vascular streaking of 'Carabao' mango fruit at the ripe stage.[1]

Treatment	Incidence (%) / Poststorage Period (week)		
	2	4	6
0.1 mM MLT	0.00 b	13.33 b	25.93 b
0.5 mM MLT	11.11 a	23.70 ab	25.56 b
1.0 mM MLT	0.00 b	15.93 ab	21.48 b
CONTROL	0.00 b	25.93 a	36.30 a

[1]Mangoes were dipped for 10 min in varying concentrations of melatonin (MLT), stored at 7°C for six weeks then transferred to 24°C for ripening after 2, 4 and 6 weeks of cold storage. Means within columns with the same letter are not significantly different at 5% level, LSD (n=9).

Table 3. Change in firmness of 'Carabao' mango fruit treated with varying concentrations of melatonin (MLT) and stored at 7°C for six weeks[1].

Treatment	Firmness (kg-force) / Storage Period (week)[1]		
	2	4	6
0.1 mM MLT	6.01 a	5.21 a	2.16 b
0.5 mM MLT	5.81 a	4.79 a	2.18 b
1.0 mM MLT	5.42 a	4.36 a	2.23 b
CONTROL	6.12 a	5.24 a	2.65 a

[1]Means within columns followed by the same letter are not significantly different at 5% level LSD (n=6).

Some fruit however, were at the color break stage (PCI 2) especially in the control. On the 4th wk, all MLT-treated mangoes reached PCI 2, while the control fruit were at the turning stage (PCI 3 - more green than yellow) and was significantly different with the MLT treatment (Figure 7). The effect of MLT on ripening was further apparent as the storage period was extended to 6 wk. On wk 6, the 0.1 and 1.0 mM MLT-treated mangoes were still at PCI 4 (more yellow than green) whilethe other 2 treatments had mangoes that were almost near the yellow stage with tinge of green (PCI 5).

When cold-stored mangoes were transferred to higher temperatures, peel color change progressed rapidly but the brightyellow peel color was not achieved. The MLT-treated fruit exhibited slower rate of ripening compared with the control fruit particularly during the early days of poststorage at 24°C.Mangoes cold-stored for 2 to 6 wk then ripened at 24°C exhibited pale yellow peel at the ripe stage regardless of the treatment.

Visual Quality Rating

Consumer's acceptability of fresh produce depends on its overall visual quality and postharvest treatments are generally aimed at maintaining the visual quality of the produce. The visual quality rating of 9 (excellent, field fresh) of mangoes in all treatments was maintained during the 2-wk storage at 7°C (Figure 8). On the 3rd wk onwards, VQR decreased when lenticel spotting and disease like anthracnose became noticeable particularly in the control fruit. On the 6th wk, mangoes in all treatments attained the VQR of 5 (fair, moderate defects, limit of marketability). The rate of decrease in VQR of the MLT-treated and the control fruit was almost similar.

Firmness

During storage at 7°C for 2 wk, a decrease in firmness from 9.56 kg-force to about 5.4 to 6.1 kg-force was noted in all treatments. When storage at 7°C was 4

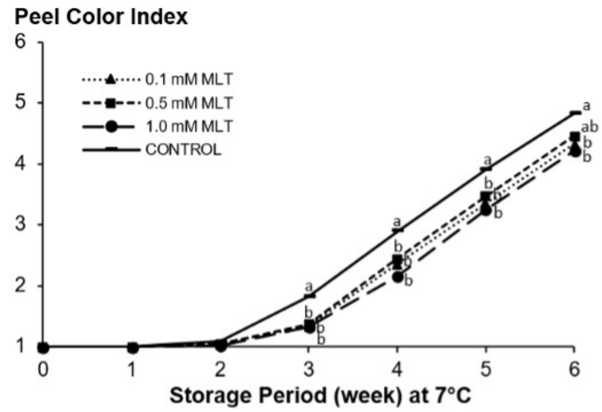

Figure 7. Rate of ripening based on peel color change of 'Carabao' mango fruits treated with varying concentrations of melatonin (MLT) and stored at 7°C for six weeks. Means with the same letter at each storage period are not significantly different at 5% level, LSD (n=12).

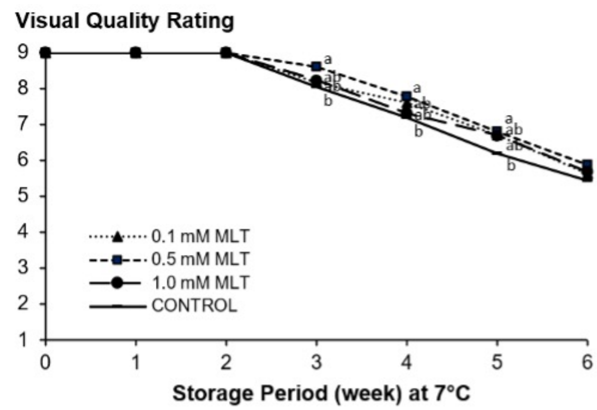

Figure 8. Change in the visual quality rating of 'Carabao' mango fruits treated with varying concentrations of melatonin (MLT) and stored at 7°C for six weeks. Means at each storage period with the same letter are not significantly different at 5% level, LSD (n=12).

wk, firmness decreased to about 4.3 to 5.2 kg-force when mangoes were at the turning stage (PCI 3, more green than yellow), and differences among treatments were not significant (Table 3). On the 6th wk of withdrawal, dramatic decrease in firmness was obtained in all fruit regardless of the treatment. The control fruit had significantly higher firmness value than the MLT-treated fruit. During poststorage at 24°C, a drastic decrease in firmness was obtained in all fruit regardless of the treatment (data not shown).

Total Soluble Solids (TSS) Content and Titratable Acidity (TA)

During storage at 7°C, an increase in TSS content was observed in all fruit regardless of the treatment although the mangoes were still green and firm on the 2nd wk. Mangoes from the control had significantly higher TSS than the MLT-treated fruit. Withdrawal on wk 4 when mangoes were starting to ripen, further resulted in an increase in TSS in all treatments with values of 16.1 to 19.8°Brix, with the control having the highest TSS value (Figure 9). At a more advanced stage of ripeness on wk 6, only a slight increase in TSS was obtained. During poststorage and ripening at

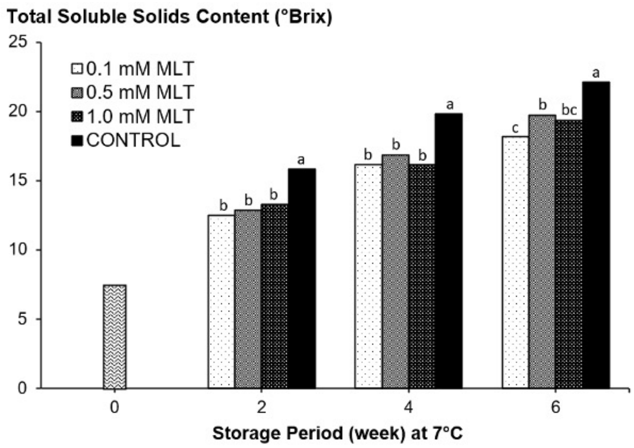

Figure 9. Total soluble solids content of ‘Carabao’ mango fruits treated with varying concentrations of melatonin (MLT) and stored at 7°C for six weeks. Means at each storage period with the same letter are not significantly different at 5% level, LSD (n=6).

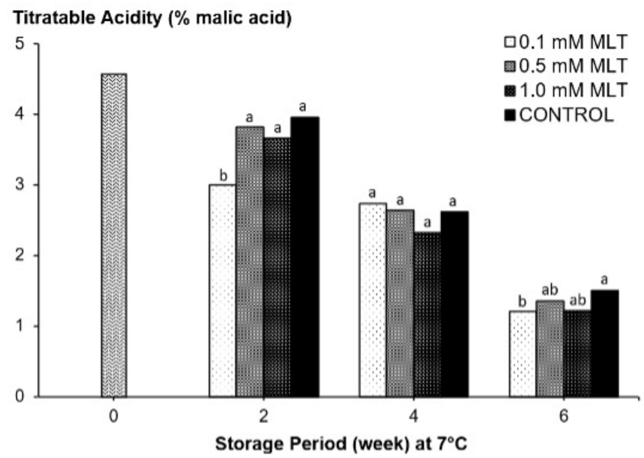

Figure 10. Titratable acidity of ‘Carabao’ mango fruits treated with varying concentrations of melatonin (MLT) and stored at 7°C for six weeks. Means at each storage period with the same letter are not significantly different at 5% level, LSD (n=6).

24°C, TSS content increased to 21-23°Brix with the control fruit having the higher TSS than the treated fruit.

In general, most fruits are acidic at the unripe stage due to the high content of organic acids. ‘Carabao’ mango fruits at the green mature stage have a TA of 5.11%. During storage at 7°C, a slight decrease was noted on all fruits and the 0.1 mM MLT-treated mangoes got the lowest TA value (Figure 10). As storage at 7°C was extended to four (4) wk, TA considerably decreased, and the 1.0 mM MLT-treated fruit had the lowest TA value indicating that it is more acidic, while 0.1 mM MLT-treated fruit had the highest value. On wk 6, significant differences were noted as TA values of fruit continued to decrease wherein 0.1 mM MLT-treated fruit got the lowest, while control fruit got the highest value. During poststorage at 24°C and when mangoes were already ripe, TA values decreased to 0.4% regardless of the treatment and duration of cold storage.

DISCUSSION

Carabao’ mango fruits are susceptible to CI when stored below 10°C for extended period (Agillon and Lizada, 2010; Rodeo, and Esguerra, 2013). In mango, the most common symptoms of CI are pitting, peel and pulp discoloration, lenticel spotting (LS), cavitation, and vascular browning or streaking (Brecht et al. 2012; Agillon and Lizada, 2010; Gonzales-Aguilar et al. 2000). In ‘Carabao’ mango, lenticel spotting and discoloration was attributed to intracellular water leakage in the damaged cells of the outer mesocarp which contributed to the decrease in porosity and pore connectivity during CI development (Cantre et al. 2014). In ‘Tommy Atkins’ and ‘Keitt’ mango, the tissues around lenticels became darkened due to the collapse of endomembrane resulting in the release of polyphenol oxidase (PPO) which interacts with the phenolics (Du Plooy et al. 2004).

Peel browning has been linked to enzymatic conversion of phenolics to o-quinones which then polymerize to brown pigments (Chidtragool et al. 2011). Chilling-sensitive crops lose their cells’ turgor and cytoplasm compartmentalization causing swelling of organelles which leads to substrate leakage required by PPO for the synthesis of the brown substances (Kratsch and Wise, 2000) hence the observed flesh browning of ‘Carabao’ mangoes in the present study.

Internal CI symptoms like pulp cavitation, flesh browning and vascular streaking or discoloration were not apparent during storage at 7°C. In some chilling-sensitive crops, CI symptoms are rarely observed during storage at sub-optimal temperatures, but the symptoms become apparent upon transfer to high temperatures (Wang, 1993). This was attributed to slow metabolism of the fruit during cold storage that affect the development of internal CI symptoms (Chongchatuporn et al. 2013). The aforementioned findings were also observed in the present study. Upon transfer to 24°C for ripening, external CI symptoms developed progressively concomitant with the manifestation of internal CI symptoms. Pulp cavitation appeared as small, white, and starchy spots in the inner mesocarp which Cantre et al. (2017) attributed to intracellular water leakage that diffuse and evaporate on the fruit’s surface leading to cavity formation in the pulp. Flesh browning and vascular streaking on the other hand, resulted from decompartmentalization thus the intermixing of PPO and phenolics leading to brown pigment formation.

One of the strategies in controlling or ameliorating CI that is directed to the commodity is through chemical treatments such as MLT. It is a powerful free-radical scavenger and has a direct antioxidant activity (Arnao and Hernández-Ruiz, 2015). MLT was reported to ameliorate CI in mango cv. Sensation (Awad and Adel, 2021), Langra and GulabJamun (Bhardwaj et al. 2022), Keitt (Ping et al. 2023), and Guifei (Xu et al. 2023). In ’Guiqi’ and ‘Tainong 1’ mangoes, MLT treatment significantly reduced the PPO activity by upregulating phenylammonialyase (PAL) genes and downregulating the PPO genes during storage (Njie et al. 2022). Daria (2023) similarly demonstrated that 1.0

mM MLT treatment of 'Carabao mangoes reduced PPO activity and consequently flesh browning. As an antioxidant, Njie et al. (2022) reported that ascorbate peroxidase (APX) activity was significantly higher in MLT-treated 'Guiqi' mangoes thus inducing the cold tolerance of mango fruit to oxidative stress resulting in lower incidence and severity of CI symptoms than untreated fruit. In this study on 'Carabao' mango, the amelioration of both external and internal CI symptoms particularly with 1.0 mM MLT can be attributed to the increased activity of antioxidant enzymes such as catalase (CAT) and APX (Daria, 2023).

The reported beneficial effect of MLT was its effectiveness in retarding the ripening of mango cultivars such as Keitt (Dong et al. 2021), Sensation (Awad and Adel, 2021) and 'Nam Dok Mai' Charoenphun et al. (2025). However, in 'Carabao' mango, the desired dramatic effect of controlling ripening was not achieved. MLT treatment of 'Carabao' mangoes did not prevent ripening during storage at 7°C and normal ripening ensued during poststorage at 24°C. Mangoes subjected to MLT treatment were at PCI 3 (more green than yellow) and at PCI 4 (more yellow than green) in the control fruit after 6 wk at 7°C. One possible reason is the observation that 'Carabao' mango fruit starts producing ethylene 10 d before harvest maturity (Lizada 1991) hence ripening has been initiated even before harvest. Moreover, ripening is a programmed change hence low temperature and MLT treatment will only slow down the process.

The observation that ripening was not completely retarded with MLT treatment was supported by the results on fruit firmness. The firmness of treated fruit continuously decreased during storage concomitant with the progression of ripening. The control fruit had higher firmness value than the MLT-treated fruit which is contrary to the results of Rastegar et al. (2020) and Kebbeh et al. (2023). The decrease in firmness particularly during the later period of storage at 7°C is attributed to the depolymerization of the cell wall pectin matrix and hemicelluloses by hydrolytic enzyme action under the control of ethylene (Cao et al. 2018.).

As the fruits ripen, TSS content increased which is due to the conversion of starch into simple sugars mainly by the action of hydrolytic enzymes (Kittur et al. 2001). The TSS content of treated fruit was lower than the control fruits. This showed that MLT regulates α-amylase activity (Han et al. 2017) during cold storage and even at high temperatures as reported in other mango cultivars (Kebbeh et al. 2023; Ping et al. 2023).

During low temperature storage and when ripening is initiated, the amount of acids decreased since these are utilized as substrates during respiration (Hatami et al. 2013). MLT-treated 'Carabao' mangoes had lower TA than the control fruit contrary to the response of mango cultivars Sensation (Awad and Adel, 2021), and Dasherari (Bhardwal et al. 2022).

CONCLUSION AND RECOMMENDATION

From the results of the study, 'Carabao' mangoes treated with 1.0 mM MLT for 10 min can be stored for 3 wk at 7°C wherein significant amelioration of both external and internal CI symptoms was obtained. Extending the storage at 7°C for 6 wk likewise ameliorated CI but the incidence increased although the severity of symptoms was rated as slight. The reported inhibition of ripening with MLT however, was not observed in the case of 'Carabao' mango although the rate of peel color change was lower in MLT-treated fruit than the control. Moreover, the fruit had high marketability in terms of visual quality and treated mangoes ripened normally and lasted in the shelf for 3 d. Being a naturally-occurring plant hormone, the use of MLT as a postharvest treatment to ameliorate CI induced by extended exposure to sub-optimal temperature can be recommended.

ACKNOWLEDGEMENT

Support from the Department of Science and Technology (DOST)-ASTHRDP for funding this dissertation of the senior author is gratefully acknowledged. Support from PHTRC-UPLB-CAFS for the use of the facilities and analytical-grade reagents for various physicochemical analyses through its Core Project titled "Physiological and Biochemical Responses of Mango (*Mangifera indica* L. cv. Carabao) Fruit to Salicylic Acid and Melatonin" is also acknowledged.

REFERENCES

Aghdam MS, Luo Z, Jannatizadeh A, Sheikh-Assadi M, Sharafi Y, Farmani B, Fard JR, Razavi F. 2019. Employing exogenous melatonin applying confers chilling tolerance in tomato fruits by upregulating ZAT2/6/12 giving rise to promoting endogenous polyamines, proline, and nitric oxide accumulation by triggering arginine pathway activity. Food Chem. 275:549-556. https://doi:10.1016/j.foodchem. 2018.09.157.

Aghdam MS, Asghari M, Moradbeygi H, Mohammadkhani N, Mohayeji M, Rezapour-Fard J. 2012. Effect of postharvest salicylic acid treatment on reducing chilling injury in tomato fruit. Rom. Biotechnol. Lett. 17:7466-7473.

Agillon AB, Lizada MCC. 2010. Responses of 'Carabao' mango (*Mangifera indica*) fruit to chilling stress. Acta Hort. 877:467-474. https://doi.org/10.17660/ActaHortic.2010.877.60.

Arnao MB, Hernández-Ruiz J. 2013. Growth conditions determine different melatonin levels in Lupinusalbus L. J Pineal Res. 55:149–155.

Awad M, Adel AL. 2021. Postharvest salicylic acid and melatonin dipping delay ripening and improve quality of 'Sensation' Mangoes. Philipp. Agric. Scientist. 104:34-44.

Ba L, Cao S, Ji N, Ma C, Wang R, Luo D. 2021. Exogenous melatonin treatment in the postharvest storage of pitaya fruits delays senescence and regulates reactive oxygen species metabolism. Food Sci. Technol. 42: e15221. https://doi: 10.1590/fst.15221.

Bhardwaj R, Pareek S, Mani S, Dominguez-Avila J, Gonzales-Aguilar G. 2022. A melatonin treatment delays postharvest senescence, maintains quality, reduces chilling injury, and regulates antioxidant metabolism in mango fruit. J. Food Quality.1, 18. https://doi.org/10.1155/2022/2379556

Brummell DA. 2006. Cell wall disassembly in ripening fruit. Funct. Plant Biol. 33:103-119.

Cantre D, Herremans E, Verboven P, Ampofo-Asiama J, Hertog MLATM, Nicolaï BM. 2017. Tissue breakdown of mango (*Mangifera indica* L. cv. Carabao) due to chilling injury. Postharvest Biol. Technol. 125:99-111. https://doi.org/10.1016/j.postharvbio.2016.11.009.

Cantre D, Herremans, E., Verboven, P., Ampofo-Asiama, J. And Nicolaï, B., 2014. Characterization of the 3-D microstructure of mango (*Mangifera indica* L. cv. Carabao) during ripening using X-ray computed microtomography. Innov. Food Sci. Emerg. Technol. 24:28–39. https://doi.org/10.1016/j.ifset.2013.12.008.

Cao S, Shao J, Shi L, Xu L, Shen Z, Chen W, Yang Z. 2018. Melatonin increases chilling tolerance in postharvest peach fruit by alleviating oxidative damage. Scientific Reports. 8 (1). https://doi.org/10.1038/s41598-018-19363-5.

Charoenphun N, Lekjing S, Venkatachalam K. 2025. Effect of exogenous melatonin application on maintaining physicochemical properties, phytochemicals, and enzymatic activities of mango fruits during cold storage. *Horticulturae* 2025, *11*(2), 222; https://doi.org/10.3390/horticulturae11020222.

Chidttragool S, Ketsa S, Bowen J, Ferguson IB, VN Doorn WG. 2011. Chilling injury in mango fruit peel: cultivar differences are related to the activity of phenylalanine ammonia lyase. Postharvest Biol. Technol. 62: 59-63. https://doi.org/10.1016/j.postharvbio.2011.04.011.

Chongchatuporn U, Ketsa S, Van Doorn WG. 2013. Chilling injury in mango (*Mangifera indica*) fruit peel: Relationship with ascorbic acid concentrations and antioxidant enzyme activities. Postharvest Biol. Technol., 86: 409-417. https://doi.org/10.1016/j.postharvbio.2013.07.023.

Daria AP. 2023. Physiological and Biochemical Responses of Mango (*Mangifera indica* L. cv. Carabao) Fruit to Melatonin Under Chilling Stress. PhD Dissertation. University of the Philippines Los Baños, College, Laguna. (Available at UPLB Library)

Dong J, Kebbeh M, Yan R, Huan C., Jiang T, Zheng X. 2021. Melatonin treatment delays ripening in mangoes associated with maintaining the membrane integrity of fruit exocarp during postharvest. Plant PhysiolBiochem.169:22–28.https://doi.org/10.1016/j.plaphy.2021.10.038.

Du Plooy W, Van Der Merwe CF, Korsten L. 2006. Lenticel discoloration in mango (*Mangifera indica* L.) fruit- a cytological study of mesophyll cells from affected tissue. J. Hort. Sci. Biotechnol. 81:869-873.

Fan J, Xie Y, Zhang Z, Chen L. 2018. Melatonin: a multifunctional factor in plants. Int. J. Mol. Sci. 19(5):1528.

González-Aguilar GA, Buta JG, Wang CY. 2001. Methyl jasmonate reduces chilling injury symptoms and enhances color development of 'Kent' mangoes. J. Sci. Food Agric. 81:1244-1249.

Gonzales-Aguilar GA, Fortiz J, Ceuz R. Wang CY. 2000. Methyl jasmonate reduces chilling injury and maintains postharvest quality of mango fruit. J. Agr. Food Chem. 48:515-519.

Hatami M, Kalantari S, Delshad M. 2013. Responses of different maturity stages of tomato fruit to different storage conditions. Acta Hort. 1012: 857-864. 10.17660/ActaHortic.2013.1012.116.

Hofman PJ, Whiley A, Marques JR, Stubbings BA, Taylor L, Stirling LJ. 2010. Development of Best Practice Pre- and Postharvest of B74 Mango: Phase II. Final report MG06005. Horticulture Australia Ltd., Sydney.

Jackman RL, Yada RY, Marangoni A, Parkin KL, Stanley DW. 1988. Chilling injury. A review of quality aspects. J. Food Qual. 11(4): 253–278. https://doi.org/10.1111/j.1745-4557. 1988.tb00887.

Jacobi KK, Wong LS, Giles JE. 1996. Effect of hot air disinfestation treatment in combination with simulated air freight conditions on quality of 'Kensington' mango (*Mangifera indica* Linn.). Aust J ExpAgr. 36. https://doi.org/10.1071/EA9960739.

Jang H, Yoon J, Chege F, Razad H, Ndubiwa M, Mukherjee C. 2020. Harvest and Demand Updates for Mangoes: The Philippines, South Africa, Mexico, India - Market Analysis. Available at https://www.tridge.com/stories/harvest-and-demand-updates-for-mangoes-the-philippines-south-africa-mexico-and-india.

Kebbeh M, Dong J, Huan C, Liu Y, Zheng X. 2023. Melatonin treatment alleviates chilling injury in mango fruit 'Keitt' by modulating proline metabolism under chilling stress. J. Integrative Agric. 22(3): 935-944. https://doi.org/10.1016/j.jia.2023.02.008.

Kittur FS, Saroja N, Tharanathan R. 2001. Polysaccharide-based composite coating formulations for shelf-life extension of fresh banana and mango. Eur. Food Res. Technol. 213(4-5):306-311.

Koushesh Saba M, Arzani K, Barzegar M. 2012. Postharvest polyamine application alleviates chilling injury and affects apricot storage ability. J. Agric. Food Chem. 60(36):8947-8953. https://doi.org/10.1021/jf302088e.

Kratsch HA, Wise RR. 2000. The ultrastructure of chilling stress. Plant, Cell & Environment. 23 (4):337-350.

Lizada MC. 1991. Postharvest physiology of the mango: a review. Acta Hort. 291:437-453.

Madebo M, Luo SM, Zheng Y, Jin P, Wang L. 2021. Melatonin treatment induces chilling tolerance by regulating the contents of polyamine, -aminobutyric acid, and proline in cucumber fruit. J. Integrative Agric. 20:3060-3074. https://doi.org/10.1016/S2095-3119(20)63485-2.

Njie A, Dong X, Liu Q Lu C, Pan X, Zhang W, 2023. Melatonin treatment inhibits mango fruit (Cv. ‘Guiqi’) softening by maintaining cell wall and reactive oxygen metabolisms during cold storage. Postharvest Biol. Technol. 205:112500

Njie A, Zhang W, Dong X, Lu C, Pan X, and Liu Q. 2022. Effect of melatonin on fruit quality via decay inhibition and enhancement of antioxidative enzyme activities and genes expression of two mango cultivars during cold storage. Foods. 11: 3209. https://doi.org/10.3390/foods11203209.

Peng J, Cao SF, Zheng YH. 2013. Managing chilling injury. Acta Hort. 1012:1087-1095.

Philippine Mango Industry Roadmap 2017-2022. Department of Agriculture-High Value Crops Development Program. Philippines.

Ping X, Huber DJ, Gong D, Yun Z, Pan Y, Jiang Y, Zhang Z. 2023. Amelioration of chilling injury in ‘Guifei’ mango fruit by melatonin is associated with regulation of lipid metabolic enzymes and remodeling of lipidome. Postharvest Biol. Technol. 198. ISSN 0925-5214.https://doi.org/10.1016/j.postharvbio.2022.112233.

Raison JK, Lyons JM. 1986. Chilling injury: a plea for uniform terminology. Plant Cell Environ. 9:685-686.

Rastegar S, Hassanzadeh Khankahdani H, Rahimzadeh M. 2020. Effects of melatonin treatment on the biochemical changes and antioxidant enzyme activity of mango fruit during storage. Sci. Hortiulturae. 259:108835. https://doi.org/10.1016/j.scienta.2019.108835.

Rodeo AJ, Esguerra EB. 2013. Low temperature conditioning alleviates chilling injury in mango *(Mangifera indic*a L. cv. Carabao) fruit. Philipp. J. Crop Sci. 38:24-32.

Singh Z, Singh RK, Sane VA, Nath P. 2013. Mango postharvest biology and biotechnology. Crit. Rev. Plant Sci. 32:217-236.

Tan DX, Manchester LG, Liu XY, Rosales-Corral SA, Acunacastroviejo D, Reiter RJ. 2013. Mitochondria and chloroplasts as the original sites of melatonin synthesis: a hypothesis related to melatonin’s primary function and evolution in eukaryotes. J. Pineal. Res. 54:127-138. https://doi.org/10.1111/jpi.12026.

Tharanathan RN, Yashoda HM, Prabha TN. 2006. Mango (*Mangifera indica* L.), the king of fruits—an overview. Food Rev. Int. 22:95-123. https://doi.org/10.1080/87559120600574493.

Wang CY. 1993. Approaches to reduce chilling injury of fruits and vegetables. Hort. Rev. 15:63-95.

Westra EH, Mensink MGH, Pereira Da Silva FIDG. 2016. Greenchainge Fruit and Vegetables. Wageningen UR Food and Biobased Research. https://doi.org/10.18174/503215.

Xu P, Huber DJ, Gong D, Yun Z, Pan Y, Jiang Y, Zhang Z. 2023. Amelioration of chilling injury in ‘Guifei’ mango fruit by melatonin is associated with regulation of lipid metabolic enzymes and remodeling of lipidome. Postharvest Biol. Technol. 198:112233. https://doi.org/10.1016/j.postharvbio.2022.112233.

Zhao Z, Jiang W, Cao J, Zhao Y, Gu Y. 2006. Effect of cold-shock treatment on chilling injury in mango (*Mangifera indica* L. cv. ‘Wacheng’) fruit. J. Sci. Food Agric. 86:2458-2462.