Response of melatonin on postharvest qualities and shelf life of pineapple cv. Kew at ambient storage

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Abstract
Melatonin is a pleiotropic molecule which plays a pivotal role in extending shelf life and maintaining the postharvest quality of fruit. Pineapple fruits harvested at different stages of maturity had marked variations in physico-biochemical qualities and shelf life during storage. The present study aimed to determine the potential effectiveness of different concentrations of postharvest melatonin applications on pineapple cv. Kew fruit in ambient storage. Different concentrations of melatonin (MT) solution viz. T1: MT at 0.25 mM, T2: MT at 0.5 mM, T3: MT at 1 mM, T4: MT at 2 mM, T5: MT at 4 mM and T6: MT at 8 mM were used for the study and compared with T7: untreated fruits (control). At 20 days after storage, fruits dipped in 8 mM melatonin resulted in lowest physiological weight loss (11.84%), fruit decay (13.33%), decrease in fruit length (3.29%) and diameter (3.42%), juice content (58.27%), titratable acidity (0.91%), flesh translucency (2.0) and crown condition (2.6) these resulted in the decrease with the increase in concentration of melatonin. Maximum shelf life (23.33 days), fruit firmness (39.24 Ncm²), TSS (10.3⁰ Brix), total sugar (10.10%), reducing sugar (8.85%), TSS: Acid ratio (11.30), ascorbic acid (19.3 mg 100g⁻¹), total Carbohydrate (10.08%), total phenol (35.64mg 100g⁻¹) and antioxidant activity (48.14%) increased depending on increment in melatonin concentration as compared to control. Results of the study showed that Melatonin @ 8mM has a marked influence in extending the shelf life while maintaining the physico-chemical quality of stored pineapple and thus can be a good option in using the technique for commercial shelf life extension in ambient conditions.

Keywords: Biochemicals, Melatonin, Physico-chemical changes, Pineapple, Quality, Shelf life

INTRODUCTION
Pineapple (Ananas comosus L.) is an important fruit in the Bromeliaceae and an excellent source of vitamins like A, B and C and minerals viz. Fe, P and Ca. Besides, it is a good source of health-promoting plant chemicals. Bromelain is a protein digestive enzyme found in the pineapple plant’s fruit and stem (Kamol et al., 2014). Currently (2020-2021) in India, pineapple is produced 1774 thousand MT from 107 thousand hectares (Anonymous 2021). India ranked 6th with a share of about 8% of world production. More than 40% of the total production of pineapple is contributed by Northeast regions of the country (Anonymous 2018), with 90-95% of its produce being organic (Sema et al., 2011). Pineapple fruit showed ample variation in fruit physico-
chemical qualities and storage when harvested at different maturity stages (Dhar et al., 2008). Harvesting pineapple fruits at correct maturity stage is crucial as it is non-climacteric in nature and after harvest, its eating quality does not enhance, nor does its sugar content increase. Therefore, to be acceptable to the intended market pineapple fruits should be picked at optimum maturity and ripeness stage (Joy and Rajuva, 2016). Pineapple fruit, being a perishable crop, needs proper postharvest handling to ensure the fruit’s quality. India alone is estimated to experience postharvest losses of about 32% from total production, where a major loss of 9.63% was due to over-ripening, followed by 8.96% of mechanical damages. The loss due to spoilage was 5.01% followed by 4.85% loss due to immature/unmarketable sizes, 1.55% loss contributed by storage conditions and 2.12% due to other factors (Ningombam et al., 2019). To reduce postharvest losses in pineapples, several postharvest handling practices can be followed (Hossain and Bepary, 2015). Different chemical reagents can be used to enhance the shelf life of fruits and vegetables (Kuang et al., 2008; Wang et al., 2012). Melatonin is a multifunctional signaling molecule chemically denoted as N-acetyl-5-methoxytryptamine that is distributed far and wide in various plant species (Tan et al., 2002) and an endogenous indoleamine, marked ability of scavenging free radicals and an antioxidant (Reiter et al., 1999). Different plant physiological activities are marked influenced by melatonin, starting from plant growth, root development, flowering, fruit development and ripening, plants response to biotic and abiotic stress and up to senescence, etc. Melatonin, a safe and non-toxic substance, is profusely under use in maintaining postharvest qualities and shelf life of fruits and vegetables apart from its uses in crop production (Fan et al., 2022). Recent reports suggested that melatonin regulates various physiological processes like fruit ripening and prevents postharvest senescence of fruits like bananas, peaches, grapes, strawberries and tomatoes (Mukherjee, 2019). Besides, melatonin improves postharvest ripening and quality of different climacteric as well as non-climacteric fruits (Sun et al., 2015). Fruits like apples, bananas, cherries, grapes, pineapples, tomatoes and strawberries are commonly used in the healthy human diet and proves to be a good source of natural melatonin (Reiter et al., 2005; Sae-Teaw et al., 2013). Melatonin, when used exogenously tested to be an effective postharvest tool which encourages ripening and improves postharvest qualities of tomatoes, delays senescence and increases chilling tolerance of stored peach (Cao et al., 2016; Gao et al., 2016), attenuating postharvest decay and maintaining nutritional properties of stored strawberry (Liu et al., 2018). It has been proven that melatonin applied exogenously delayed the postharvest ripening of fruits like apples (Onik et al., 2021), sweet cherries (Tijero et al., 2019), bananas (Li et al., 2019; Hu et al., 2017), pears (Liu et al., 2019), peaches (Gao et al., 2016) and mangoes (Liu et al., 2020). Studies have reported incidence of decay has been reduced in several fruits during postharvest storage, which occurs with exogenous melatonin treatment, including litchis (Zhang et al., 2021), strawberries (Aghdam and Fard, 2017), kiwifruits (Hu et al., 2018) and peaches (Gao et al., 2016). Currently the most common postharvest handling method is soaking or spraying melatonin solution (Ze et al., 2021). However, there is scanty information on postharvest use of melatonin in pineapple. Therefore, the present investigation was conducted to study the influence of melatonin on the postharvest qualities and shelf life of pineapple cv. Kew at ambient storage.

MATERIALS AND METHODS

Experimental site
The experiment was conducted in Postharvest Laboratory, Department of Horticulture, Aromatic and Medicinal Plants, Mizoram University, situated at Tanhlil-Aizawl, Mizoram.

Fruit material and melatonin treatment
Fully matured green fruits grown in open fields at Aizawl, Reiek block of Mamit district of Mizoram were chosen for samples. Freshly harvested pineapple cv. Kew fruits were thoroughly washed under running water and air drying at room temperature. For each treatment, fruits were tagged and subjected to six melatonin (MT) treatments by dipping in viz., T1: MT at 0.25 mM, T2: MT at 0.5 mM, T3: MT at 1 mM, T4: MT at 2 mM, T5: MT at 4 mM, T6: MT at 8 mM and T7: Control for 60 minutes followed by air drying in ambient condition. Then all the melatonin-treated pineapples were kept on an open shelf and covered with a perforated net for the duration of storage at an ambient temperature range of 20 to 25 °C and relative humidity of 65 to 85%. The physical and biochemical parameters of the fruits were recorded each 5 days after storage (DAS).

Fruit peel/skin and flesh colour
Fruits for each treatment in pineapple for skin and flesh colour were measured with a digital held colour meter and expressed as L*, a*, b* (as per Commission International d’ Eclairage,1976) where L* value for bright dark scale, a* value is positive for red whereas negative for green and b* value is positive for yellow while negative for blue colour.

Physiological weight loss
At a 5-day interval, fruit weighed was measured using a digital electronic balance. The following equation was used for calculating percentage weight loss:-
Percentage weight loss at nth day = (Fruit weight at 0 day - fruit weight at nth day) / Fruit weight at 0 day × 100

\textbf{Percent decrease in fruit length and diameter}
The length and diameter of fruits for each treatment were measured using a digital slide caliper at 5 days intervals. The following equation was used to calculate per cent decrease in fruit length and diameter:

\[
\text{Percent decrease (nth day)} = \frac{\text{Initial length / diameter at 0 day} - \text{length/diameter at nth day}}{\text{Initial length / diameter at 0 day}} \times 100
\]

\textbf{Juice percentage (%)}
Juice percentage of fruit was calculated with the help of the given formula:

\[
\text{Percentage of juice in fruit pulp} = \frac{\text{Weight of fruit juice x 100}}{\text{Weight of fruit pulp}}
\]

\textbf{Fruit firmness, scoring for crown condition and flesh translucency}
The texture/firmness of pineapple fruit samples was recorded using a handheld digital penetrometer device. Crown condition, flesh translucency, etc. visual quality indices were recorded per the standard procedure (Mandal et al., 2015). Scores for crown condition and flesh translucency were as follows:

\textbf{Crown condition}
Good, fresh and green: Score 1; Good with slightly yellow at tips: Score 2; Moderate, dry tips and yellowing: Score 3; Bad, dry tips and more yellowing: Score 4; Severe yellowing: Score 5

\textbf{Flesh translucency}
100% opaque: Score 1; Opaque with slight translucency (less than 50%): Score 2; Opaque with moderate translucent (more than 50%): Score 3; 100% translucent: Score 4

\textbf{Biochemical parameters}
Fruit juice was extracted by cutting the pineapple flesh, crushing it in mortar and pestle, and then strained with the muslin cloth. Fresh extracted juice was used to estimate total soluble solids (TSS) and was expressed in degree brix after temperature correction at 20°C. Fresh juice’s total and reducing sugar content was estimated using Fehling’s reagents (A&B) through the copper reduction method and methylene blue as an indicator (AOAC, 1990). Titratable acidity of the freshly extracted pineapple juice was measured by titration against sodium hydroxide (N/10) and phenolphthalein indicator (AOAC, 1990). Fruit ascorbic acid content (Vitamin -C) was estimated by titration method using 2,6 dichlorophenol indophenol dye (AOAC, 1990; Ranganna, 1997), and Folin-Ciocalteau method was followed to determine total phenols by using 80% ethanol. Folin-Ciocalteau reagent and 20% sodium carbonate and a digital Spectrophotometer at 650nm were used for recording the absorbance (Sadasivam and Manickam, 1996). For estimation of total carbohydrates, the Anthrone method was followed using Anthrone reagent and HCl and absorbance at 630 nm was recorded with a digital Spectrophotometer (Sadasivam and Manickam, 1996). Antioxidant activity of the fruit juice was determined using methanol and DPPH following DPPH assay and absorbance was measured using a digital spectrophotometer at 516 nm (Pekkarinen et al., 1999). The percentage of antiradical activity was calculated by following the equation:

\[
\text{% Antiradical activity} = \frac{\text{Absorbance (Control) – Absorbance (Sample)}}{\text{Absorbance (Control)}} \times 100
\]

\textbf{Decay percentage and shelf life}
Visual observations were done to determine the decay percentage of stored pineapple. The decayed fruits were divided by the total number of fruits to calculate the decay percentage of stored pineapple fruits (Pila et al., 2010). Visual observation of spoilage, fruit decay and physico-chemical parameters were considered along with days count from harvest to days with the highest visual appearance and edible marketable quality for calculating the shelf life (days) of the stored pineapple fruits under different melatonin treatments along with control (Pila et al., 2010; Mandal and Vanlalawmpua, 2020).

\textbf{Statistical analysis}
Data analysis was done using SPSS software version 22 for statistical inference by following the method of Complete Randomized Design (CRD) (Gomez and Gomez, 1984).

\textbf{RESULTS}

\textbf{Physiological weight loss}
Physiological weight loss of pineapple samples varied significantly due to the different postharvest treatments at room temperature. Regardless of the treatments, weight loss in pineapple fruits increased for 20 days storage. At 20 days after storage (DAS), among all the treatments, the highest physiological weight loss was observed in fruits at control, having 28.71% and the fruits treated with 8.0 mM (T6) melatonin had the least physiological weight loss of 11.84%. Fruits treated with melatonin resulted in weight loss significantly lower than fruits in control (Table 1).

\textbf{Percent decrease in length and diameter}
The length and diameter of pineapple fruits gradually decreased during ambient storage. Fruit length and diameter decreased most in control fruits with 13.05%
and 11.86% decreases at 20 DAS, respectively (Table 1). At 20 DAS, fruits treated with 8.0 mM melatonin (T₀) had the least decline in length and diameter, with 3.29% and 3.42%, respectively.

**Fruit firmness**

Loss in firmness was significantly reduced by melatonin during ambient storage when compared to fruits at control. The fruits at control began to lose firmness and had the lowest firmness with a decreasing trend in values of 38.69 N, 31.32 N, 28.76 N, and 20.52 N for 5, 10, 15 and 20 DAS (Table 2). Meanwhile fruits treated with 8.0 mM melatonin (T₀) recorded highest firmness in decreasing order of 55.53 N, 45.73 N, 41.47 N, and 39.24 N at 5, 10, 15 and 20 DAS.

**Juice percentage**

Pineapple juice content also decreased along with physiological changes such as fruit weight, length and firmness (Table 2). The juice content of the fruit kept increasing during the entire storage period. Control (T7) fruits recorded the highest juice content with 69.13%, 55.53%, 45.73% and 39.24% decreases at 20 DAS, respectively (Table 3). In case of flesh colour, fruits at control (T₇) had maximum flesh colour accumulation (deep golden yellow, L: 38.56, a: 3.38, b: 35.87) whereas, fruits treated with Melatonin at 8.0 mM (T₀) had white to creamy yellow colour (L:62.41, a:-1.91, b:28.29).

**Fruit skin and flesh colour**

The skin and flesh colour of pineapple fruits were measured at 20 DAS. The final result was illustrated using a colorimeter and it manifested that stored pineapple fruits when treated with Melatonin at 0.25 mM (T₁) have the maximum peel colour (dark orange; L:42.55, a: 48.88, b: 36.71) while full orange colour can be found in fruits at control (T₇; L: 44.43, a: 43.55, b: 43.77). Fruits at T₀ (i.e. treated with melatonin at 8.0 mM) remained green to turning orange colour (L:47.95, a:5.17, b:26.37) after 20 days of storage (Table 3). In case of flesh colour, fruits at control (T₇) had maximum flesh colour accumulation (deep golden yellow, L:38.56, a: 3.38, b: 35.87) whereas, fruits treated with Melatonin at 8.0 mM (T₀) had white to creamy yellow colour (L:62.41, a:-1.91, b:28.29).

**Flesh translucency and crown condition**

At 20 DAS, fruits at control had maximum flesh translucency with increasing scores of 4.0 fruits treated with 8.0 mM (T₀) melatonin had the lowest flesh translucency with 2.0 at 20 DAS (Fig 1). Untreated fruits, i.e. control (T₇) showed the highest crown condition score at 20 DAS with an average score of 5.0 and 8.0 mM melatonin-treated (T₀) fruits had the least change in pineapple crown condition with a score of 2.6 respectively (Fig 2).

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### Table 1. Effect of treatment of melatonin on weight loss, decrease in fruit length and fruit diameter of pineapple

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Physiological Weight Loss (%)</th>
<th>Decrease in fruit length (%)</th>
<th>Decrease in fruit breadth (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DAS</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>T₁: MT0.25mM</td>
<td></td>
<td>5.43</td>
<td>12.63</td>
</tr>
<tr>
<td>T₂: MT0.50mM</td>
<td></td>
<td>5.26</td>
<td>11.36</td>
</tr>
<tr>
<td>T₃: MT1.0mM</td>
<td></td>
<td>4.42</td>
<td>10.47</td>
</tr>
<tr>
<td>T₄: MT2.0mM</td>
<td></td>
<td>3.17</td>
<td>9.45</td>
</tr>
<tr>
<td>T₅: MT4.0mM</td>
<td></td>
<td>2.78</td>
<td>9.38</td>
</tr>
<tr>
<td>T₆: MT8.0mM</td>
<td></td>
<td>2.25</td>
<td>8.24</td>
</tr>
<tr>
<td>T₇: Control</td>
<td></td>
<td>8.13</td>
<td>13.42</td>
</tr>
<tr>
<td>SEm ±</td>
<td></td>
<td>0.69</td>
<td>0.75</td>
</tr>
<tr>
<td>CD at 5%</td>
<td></td>
<td>1.21</td>
<td>1.31</td>
</tr>
</tbody>
</table>

*Melatonin=MT, Days after storage=DAS*

### Table 2. Effect of treatment of melatonin on fruit firmness and juice content of stored pineapple

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Firmness (N cm⁻²)</th>
<th>Juice Content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DAS</td>
<td>5</td>
</tr>
<tr>
<td>T₁: MT0.25mM</td>
<td>44.43</td>
<td>36.64</td>
</tr>
<tr>
<td>T₂: MT0.50mM</td>
<td>48.77</td>
<td>37.38</td>
</tr>
<tr>
<td>T₃: MT1.0mM</td>
<td>48.97</td>
<td>38.81</td>
</tr>
<tr>
<td>T₄: MT2.0mM</td>
<td>52.27</td>
<td>39.64</td>
</tr>
<tr>
<td>T₅: MT4.0mM</td>
<td>53.68</td>
<td>41.86</td>
</tr>
<tr>
<td>T₆: MT8.0mM</td>
<td>55.53</td>
<td>45.73</td>
</tr>
<tr>
<td>T₇: Control</td>
<td>38.67</td>
<td>31.32</td>
</tr>
<tr>
<td>SEm ±</td>
<td>1.05</td>
<td>1.18</td>
</tr>
<tr>
<td>CD at 5%</td>
<td>1.84</td>
<td>2.06</td>
</tr>
</tbody>
</table>

*Melatonin=MT, Days after storage=DAS*
The highest TSS content at 20 DAS was recorded in fruit treated with 8.0 mM melatonin (T₆) having 10.34° Brix. The least TSS content recorded was in control (T₇) with 5.75° Brix at 20 DAS respectively (Table 4).

The current study also revealed that titratable acidity increased during storage period in all the treatments. The highest titratable acidity at 5, 10, 15 and 20 DAS was recorded in control (T₇) having 1.02%, 1.15%, 1.23% and 1.41% respectively. The lowest titratable acidity at 5, 10, 15 and 20 DAS was recorded in samples treated with 8.0mM melatonin (T₆) having 0.77%, 0.83%, 0.86% and 0.91% respectively (Table 4). The highest TSS:Acid ratio was recorded in 8.0 mM melatonin (T₆) treated fruits 11.36 at 20 DAS and the lowest TSS: Acid ratio at 20 DAS was recorded in control (T₇) with 4.08 (Table 4).

Total and reducing sugar
Total sugar decreased with storage, except for fruits treated with 4.0 mM (T₅) and 8.0 mM (T₆) at 10 DAS, which showed a slight increase at 12.68% and 12.82%, respectively. The highest total sugar content at 20 DAS was recorded in 8.0 mM melatonin-treated (T₆) fruits, which had 10.18%, and the least sugar content was found in the control (T₇) group, which had 4.79%, respectively. At 20 DAS, the highest reducing sugar was recorded in 8.0 mM melatonin (T₆) treated fruits with 9.15% and the least reducing sugar at control (T₇) with 3.82%, respectively (Table 5).

Ascorbic acid content
The highest ascorbic acid at 5, 10, 15 and 20 DAS was observed in 8.0 mM melatonin treatment (T₆) having 28.36 mg/100g, 25.56 mg/100g, 23.45 mg/100g and 19.32 mg/100g, respectively, whereas the least ascorbic acid was recorded at 5, 10, 15 and 20 DAS in control (T₇) with 16.42 mg/100g,13.68 mg/100 g,10.78 mg/100 g and 6.78 mg/100 g, respectively (Table 5).

Total carbohydrate, phenol content and antioxidant activity
Total carbohydrate content decreased with storage, and the highest carbohydrate content at 20 DAS was observed in 8.0 mM melatonin-treated (T₆) fruits, which was 10.08%. The least carbohydrate content was recorded at control (T₇) having 3.12%. The phenol content in stored pineapple kept decreasing during the entire storage period. At 20 DAS, samples treated with 8.0 mM melatonin (T₆) showed the highest phenol content of 35.64 mg/100g, while fruits at control (T₇) had the least phenol content of 4.28 mg/100g. The highest antioxidant activity at 20 DAS was observed in samples treated with 8.0 mM melatonin (T₆) having 48.14% and the least antioxidant activity was recorded in fruits at control (T₇) having 11.28% (Table 6).

Fruit decay and shelf life
The decay percentage in the stored fruits decreased with increase in the concentration of melatonin treatment. At 20 DAS, fruits at control (T₇) deteriorated the
most, having 73.33%, and the lowest decay percentage was found in fruits treated with 8.0 mM melatonin (T₈), which had 13.33%. Based on the experiment results, samples treated with 8.0 mM melatonin (T₈) have the longest shelf life of 23.33 days followed by 4.0 mM melatonin treated (T₄) fruits having 21.13 days. Fruits at control (T₀) had the shortest shelf life of 12.87 days (Fig. 3).

**DISCUSSION**

The present study found that physiological weight loss of the pineapple gradually increased with the duration of storage. Changes in the physiological weight of fruits after harvest generally happen due to the loss of moisture through respiration and transpiration (Lufu et al., 2019). However, the present study observed that the pineapple fruits treated with melatonin had considerably lower physiological weight loss than the control. Fruits treated with melatonin 8.0 mM (T₈) had the lowest physiological weight loss (Table 1). Similar observation was reported in the case of storage of mango (Rastegar et al., 2020), peach (Gao et al., 2016) and strawberry (Liu et al., 2018) when treated with melatonin exogenously. Melatonin may have maintained higher turgor pressure in cells, which caused the lower weight loss (Liu et al., 2018). In the present study, melatonin-treated pineapple fruits had a low percentage decrease in fruit length and diameter compared to the control (Table 1),signifying less shrinkage during storage and thus having higher fruit firmness than the control (Table 2). Pineapple fruits treated with melatonin @ 8.0 mM (T₈) had maximum fruit firmness at 20 DAS. Li et al. (2018) opined that improvement in water-retaining properties by decreasing the permeability of the cellular membrane had become responsible for the maintenance of fruit firmness. Researchers found enhanced activity of pectinmethyltransferase, β-galactosidase and polygalacturonase in postharvest softening of pineapple fruits and it was thought to decrease the fruit quality (Remon et al., 2003). Earlier reports showed that melatonin (MT) treatment in peaches increased firmness by increasing the expression of various important genes involved in cell wall disassembly during storage (Gao et al., 2016).

The present study revealed that MT-treated pineapple fruits resulted in better skin and flesh colour retention than the control group (Table 3). Chlorophyll degradation of skin was delayed in MT treated pineapple fruits treated with melatonin @ 8.0 mM (T₈) treatment in peaches increased firmness by increasing the permeability of the cellular membrane had become responsible for the maintenance of fruit firmness. Researchers found enhanced activity of pectinmethyltransferase, β-galactosidase and polygalacturonase in postharvest softening of pineapple fruits and it was thought to decrease the fruit quality (Remon et al., 2003). Earlier reports showed that melatonin (MT) treatment in peaches increased firmness by increasing the expression of various important genes involved in cell wall disassembly during storage (Gao et al., 2016).

The present study revealed that MT-treated pineapple fruits resulted in better skin and flesh colour retention than the control group (Table 3). Chlorophyll degradation of skin was delayed in MT treated pineapple fruits

Table 4. Effect of treatment of melatonin on total soluble solids (TSS), acidity and TSS:Acid ratio of stored pineapple

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Total Soluble Solids (TSS) (°Brix)</th>
<th>Titratable acidity (%)</th>
<th>TSS: acid Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>T₁:MT0.25mM</td>
<td>15.84</td>
<td>12.62</td>
<td>8.75</td>
</tr>
<tr>
<td>T₂:MT0.50mM</td>
<td>15.32</td>
<td>13.46</td>
<td>9.28</td>
</tr>
<tr>
<td>T₃:MT1.0mM</td>
<td>14.84</td>
<td>13.54</td>
<td>9.56</td>
</tr>
<tr>
<td>T₄:MT2.0mM</td>
<td>12.35</td>
<td>14.08</td>
<td>10.52</td>
</tr>
<tr>
<td>T₅:MT4.0mM</td>
<td>13.26</td>
<td>14.38</td>
<td>11.34</td>
</tr>
<tr>
<td>T₆:MT8.0mM</td>
<td>12.56</td>
<td>14.62</td>
<td>11.75</td>
</tr>
<tr>
<td>T₇:Control</td>
<td>16.75</td>
<td>10.34</td>
<td>7.45</td>
</tr>
<tr>
<td>SEM±</td>
<td>0.66</td>
<td>0.65</td>
<td>0.57</td>
</tr>
<tr>
<td>CD at 5%</td>
<td>1.15</td>
<td>1.13</td>
<td>1.01</td>
</tr>
</tbody>
</table>

Melatonin=MT, Days after storage=DAS

Table 5. Effect of treatment of melatonin on total sugar, reducing sugar and ascorbic acid content of stored pineapple

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Total sugar (%)</th>
<th>Reducing sugar (%)</th>
<th>Ascorbic Acid (mg 100g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>T₁:MT0.25mM</td>
<td>13.96</td>
<td>10.78</td>
<td>8.13</td>
</tr>
<tr>
<td>T₂:MT0.50mM</td>
<td>13.85</td>
<td>11.35</td>
<td>8.67</td>
</tr>
<tr>
<td>T₃:MT1.0mM</td>
<td>13.72</td>
<td>11.26</td>
<td>8.97</td>
</tr>
<tr>
<td>T₄:MT2.0mM</td>
<td>12.58</td>
<td>11.92</td>
<td>9.54</td>
</tr>
<tr>
<td>T₅:MT4.0mM</td>
<td>12.32</td>
<td>12.58</td>
<td>10.28</td>
</tr>
<tr>
<td>T₆:MT8.0mM</td>
<td>11.32</td>
<td>12.82</td>
<td>10.79</td>
</tr>
<tr>
<td>SEM±</td>
<td>0.59</td>
<td>0.38</td>
<td>0.25</td>
</tr>
<tr>
<td>CD at 5%</td>
<td>1.05</td>
<td>0.67</td>
<td>0.44</td>
</tr>
</tbody>
</table>

Melatonin=MT, Days after storage=DAS
which caused delayed accumulation in the characteristic yellow colour development of the skin while maintained the white colour of flesh to be marked as delayed senescence. MT treatment affects chlorophyll content in rice and broccoli, delaying natural senescence (Liang et al., 2015; Ba et al., 2022). MT treatment may have retarded the colour change internally in the flesh of pineapple, which was associated with flesh yellowing and carotenoid synthesis and showed maintenance of L* and hue* values (Wu et al., 2020). MT treatment may have retarded the colour change internally in the flesh of pineapple, which was associated with flesh yellowing and carotenoid synthesis and showed maintenance of L* and hue* values (Wu et al., 2020). MT treatment may have retarded the colour change internally in the flesh of pineapple, which was associated with flesh yellowing and carotenoid synthesis and showed maintenance of L* and hue* values (Wu et al., 2020). MT treatment may have retarded the colour change internally in the flesh of pineapple, which was associated with flesh yellowing and carotenoid synthesis and showed maintenance of L* and hue* values (Wu et al., 2020). MT treatment may have retarded the colour change internally in the flesh of pineapple, which was associated with flesh yellowing and carotenoid synthesis and showed maintenance of L* and hue* values (Wu et al., 2020). MT treatment may have retarded the colour change internally in the flesh of pineapple, which was associated with flesh yellowing and carotenoid synthesis and showed maintenance of L* and hue* values (Wu et al., 2020). MT treatment may have retarded the colour change internally in the flesh of pineapple, which was associated with flesh yellowing and carotenoid synthesis and showed maintenance of L* and hue* values (Wu et al., 2020).

Application of melatonin also delays color evolution in litchi, strawberry and broccoli (Liu et al., 2018; Wu et al., 2020; Zhang et al., 2018). Cryptoxanthin, a significant carotenoid present in pineapple flesh, is responsible for the delay in the increase of carotenoids, which led to whiter and paler flesh showing reduction in carotenoid biosynthesis as a result of MT application (Paul, 1993). Pineapple fruits exhibited water soaking symptoms, which resulted in higher flesh translucency in the later part of the storage (Fig. 1). Electrolyte leakage is reported to be the reason for higher flesh translucency in pineapple when stored at ambient temperature (Chen and Paull, 2001). However, in the present study, it was found that fruits treated with MT 4 to 8 mM had lower score for flesh translucency (Fig. 1). Badiche-El Hilali et al. (2023) similarly observed that MT treatment caused reduction of electrolyte leakage dose dependent manner which may have caused lower flesh translucency. Juice content of the stored pineapple was increased with period of storage may be because of higher electrolyte leakage and senescence (Chen and Paull, 2001). At 20DAS, juice percentage was recorded highest in control (T7) compared with fruits treated with MT @ 8.0 mM (T6) (Table 2). MT was reported to retard senescence (Sharafi et al., 2021; Guillen et al., 2022) which may have caused lower juice percentage at MT treated fruits in the present study.

Present study manifested that MT (4-8 mM) treated pineapple fruits had higher TSS than control (Table 3). Treatment of peaches with a solution of 100µM melatonin for 2 h increased TSS value over control (Cao et al., 2016). Loss of soluble solids during storage in fresh cut pitaya fruits could be delayed by melatonin treatment (Ba et al., 2022). Besides, it was noted that level of titratable acidity in stored pineapple was efficiently maintained in MT @8.0mM (T6) treated fruits (Table 4). Wang et al. (2022) observed that MT treated papaya

Table 6. Effect of treatment of melatonin on total carbohydrates, total phenol and antioxidant activity of stored pineapple

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Total carbohydrates (%)</th>
<th>Total phenol (\text{mg} \text{100g}^{-1})</th>
<th>Antioxidant activity (% Inhibition DPPH)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>T1: MT0.25mM</td>
<td>13.08</td>
<td>9.67</td>
<td>7.66</td>
</tr>
<tr>
<td>T2: MT0.50mM</td>
<td>12.42</td>
<td>9.82</td>
<td>8.16</td>
</tr>
<tr>
<td>T3: MT1.0mM</td>
<td>11.85</td>
<td>10.79</td>
<td>8.71</td>
</tr>
<tr>
<td>T4: MT2.0mM</td>
<td>11.68</td>
<td>11.42</td>
<td>9.32</td>
</tr>
<tr>
<td>T5: MT4.0mM</td>
<td>11.72</td>
<td>11.82</td>
<td>10.54</td>
</tr>
<tr>
<td>T6: MT8.0mM</td>
<td>12.16</td>
<td>12.56</td>
<td>11.16</td>
</tr>
<tr>
<td>T7: Control</td>
<td>13.18</td>
<td>8.92</td>
<td>4.87</td>
</tr>
<tr>
<td>SEm±</td>
<td>0.21</td>
<td>0.27</td>
<td>0.25</td>
</tr>
<tr>
<td>CD at 5%</td>
<td>0.37</td>
<td>0.47</td>
<td>0.44</td>
</tr>
</tbody>
</table>

*Melatonin=MT, Days after storage=DAS

Fig. 3. Effect of treatment of melatonin on shelf life and fruit decay percentage of stored pineapples
fruits had better retention of titratable acidity than control. The present study revealed that total sugar and carbohydrate content drastically reduced during storage (Table 5,6). However, fruits treated with MT @ 8.0mM (T6) maintained high sugar content even after 20 DAS. Long-term storage of pineapple results in a drop in total sugar due to fruit senescence (Hong et al., 2013). Decrease in sugar content during longer storage periods may be due to sugar utilization by bacteria or fungus (Sayka et al., 2014). MT treated fruits was reported to have better retention of total sugar during storage (Wang et al., 2022).

Storage of pineapple in ambient conditions caused the loss in ascorbic acid content across the treatments (Table 5). Fruits treated with MT @ 8.0mM (T6) had the highest amount of ascorbic acid at 20 DAS compared with control. Due to conversion of L-ascorbic acid into dehydro ascorbic acid caused by ascorbic acid oxidase shows decline in ascorbic acid of the fruit at the time of storage (Choudhary et al., 2008). MT treatment reported to check oxidative stress, which mainly caused fruit ascorbic acid content loss during ripening (Galano et al., 2011; Gao et al., 2016). It was evident from the present study that total phenol content and antioxidant acidity of the stored pineapple were recorded as high in MT treated fruits (Table 6). Highest total phenol and antioxidant activity (% inhibition DPPH) were recorded in the case of the fruits treated with MT @ 8.0mM (T6) compared with the control (T7). Treatment of strawberries with melatonin increased DPPH scavenging capacity during storage (Liu et al., 2016; Aghdam and Fard, 2017). Researchers found that during storage the clearance of ABTS [2,2׳-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] and DPPH [2,2-Di(4-tert-octylphenyl)-1-picylhydrazyl] was significantly improved by melatonin treatment, correlated positively with antioxidant capacity and total phenol content (Liu et al., 2018; Puerta-Gomez and Cisneros-Zevallos, 2011).

The present investigation showed that pineapple fruits had maximum spoilage and consequently, the lowest shelf life at control compared with MT treated fruits (Fig.3). Pineapple treated with MT @ 8.0mM (T6) had minimum fruit decay and highest shelf life during ambient storage. Similarly, it was reported that exogenous application of melatonin had reduced fruit decay and extended shelf life in wax apples (Chen et al., 2020) and pear (Zhai et al., 2018). The scientists opined that the application of MT upregulates antioxidant enzymes like superoxide dismutase, ascorbate peroxidase and catalase (Gao et al., 2016; Aghdam and Fard, 2017; Liu et al., 2018) and ascorbate-glutathione cycle (Song et al., 2016; Cao et al., 2018) that causes a reduction in fruit decay and senescence. Moreover, MT increases the activity of glucose-6-phosphate dehydrogenase, phenylalanine ammonia-lyase and shikimate dehydro-
genase which promotes the accumulation of total phenols and thought to prolong the shelf life by inhibiting fungal decay of postharvest fruits (Gao et al., 2018).

Conclusion

The present study concluded that different concentrations of melatonin were applied to pineapple cv. Kew fruits dipped in 8.0 mM melatonin (T6) were the most effective for increasing shelf life, fruit firmness, TSS, total sugar, reducing sugar, ascorbic acid, total carbohydrate, phenol and antioxidant. Also, this treatment reduced postharvest weight loss, fruit decay, decrease in fruit length and diameter. These results suggest that using melatonin @8.0 mM is the most beneficial postharvest treatment for extending shelf life while maintaining the physicochemical qualities of pineapple stored at ambient temperature.

Conflict of interest

The authors declare that they have no conflicts of interest.

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