Journal of Agriculture and Food Research 19 (2025) 101704

Contents lists available at ScienceDirect

Journal of Agriculture and Food Research

journal homepage: www.sciencedirect.com/journal/journal-of-agriculture-and-food-research

The influence of exogenous melatonin treatment on quality, nutritional profile, and antioxidant system of fresh-cut carambola during cold storage

Farah Anum Mohd Yusof [a], Ezzat Mohamad Azman [a], Noranizan Mohd Adzahan [a], Noor Liyana Yusof [a,b,*]

[a] Food Technology Department, Faculty of Food Science and Technology, Universiti Putra Malaysia, Serdang, 43400, Selangor Darul Ehsan, Malaysia
[b] Halal Products Research Institute, Universiti Putra Malaysia, Serdang, 43400, Selangor Darul Ehsan, Malaysia

ARTICLE INFO

Keywords:
Fresh-cut fruits
Antioxidants
Melatonin
Vacuum impregnation

ABSTRACT

Carambola, also known as starfruit, is a tropical fruit that is rich in vitamins, minerals, antioxidants, and is attractive in shape. The consumption of fresh-cut fruits has become increasingly popular owing to its convenience. However, it has limited marketability because of quality loss. This study aimed to investigate the effect of exogenous melatonin on the quality of fresh-cut carambolas and its relation to the antioxidant system. Vacuum impregnation with melatonin (MT) at 100 μM maintained the nutritional value and quality of fresh-cut carambola after 16 days of storage at 4 °C. These findings indicate that MT reduced the titratable acidity (TA) and total soluble solids (TSS) of the fruit, resulting in a higher pH value than untreated carambolas. A high initial hydrogen peroxidase, malondialdehyde, and polyphenol oxidase activity was observed in MT-treated fresh cut carambolas. Upon four days of storage, malondialdehyde content and hydrogen peroxide content decreased compared to untreated carambolas, resulting from the upregulated antioxidant system. Higher total phenolic, total flavonoid, catalase, and peroxidase activities recorded upon MT treatment were reflected by greater DPPH and ABTS scavenging activities. High hydrogen peroxide content during the initial storage may serve as a signalling molecule that upregulates the antioxidant system, resulting in fresh-cut carambola with better retention in quality and nutritional value. This study suggests that MT treatment can be used to maintain the quality and extend the shelf life of fresh-cut fruits.

1. Introduction

Carambola is a tropical fruit rich in vitamins, minerals, dietary fibre, and antioxidants, that are beneficial for human health [1]. Its oblong shape with three to six longitudinal ribs, with five being the most common, results in a star-shaped cross section upon cutting. Its succulent pulp, distinct flavour, health benefits, and aesthetic appeal draw the attention of consumers. Fresh cut starfruit, can be consumed directly or used in salads, juices, and garnishes [2]. Over the years, the trend and consumption of minimally processed fruits have become increasingly popular, especially among consumers who prefer healthy, convenient, and fresh produce [3]. However, fresh cut fruits have limited marketability owing to quality loss. With time, the nutritional value, weight, flavour, appearance, and texture of fresh cut fruit deteriorate.

Reactive oxygen species (ROS) is often associated with oxidative stress in fresh produce. ROS are reactive molecules produced from oxygen metabolism, including hydrogen peroxide (H_2O_2), superoxide anion (O_2-), and hydroxy radical ($^{\bullet}OH$) [4]. ROS concentrations are determined by the availability and composition of enzymatic and non-enzymatic antioxidant systems. ROS can be either beneficial or harmful, depending on its concentration. At moderate levels, it can act as a second messenger in the intracellular signalling cascade that mediates various plant responses [5]. At high levels, ROS may damage various biomolecules such as lipids, proteins, DNA and RNA, which then leads to a series of events, including lipid peroxidation, modification of proteins, and DNA damage [6]. This ultimately shortens the shelf life of fresh-cut fruits.

Recently, quality maintenance of fruits and vegetables using melatonin has gained interest. Melatonin, N-acetyl-5-methoxytryptamine, is an indolamine signalling molecule that mediates the stress response, increases the activities of antioxidant system, and extends the post-harvest storage of horticultural produce [7]. Previous study showed that

* Corresponding author. Food Technology Department, Faculty of Food Science and Technology, Universiti Putra Malaysia, Serdang, 43400, Selangor Darul Ehsan, Malaysia.
E-mail addresses: farahanumyusof@gmail.com (F.A. Mohd Yusof), noorliyana@upm.edu.my (N.L. Yusof).

https://doi.org/10.1016/j.jafr.2025.101704
Received 8 November 2024; Received in revised form 30 January 2025; Accepted 31 January 2025
Available online 2 February 2025

the application of melatonin to fresh cut pear fruit alleviates browning and improves the nutritional quality of the fruit [8]. Melatonin treatment also capable enhancing antioxidant capacity, slowing transpiration and respiration, and chilling injury providing a multifaceted approach to preserve produce quality [9,10]. In fresh cut brocollini, melatonin extends the shelf life by reducing weight loss, preserving colour quality, and enhancing antioxidant, phenolic, and flavonoid content [10]. Despite these advancements, the precise mechanisms by which melatonin interacts with the antioxidant system and influences oxidative stress in fresh-cut fruits remain poorly understood. This lack of clarity limits the optimization and broader application of melatonin-based treatments in postharvest management.

To address this knowledge gap, the objective of this study was to investigate the effects of melatonin on the nutritional and quality attributes of fresh-cut carambola during low-temperature storage. This study specifically aimed to elucidate the relationship between melatonin application and the antioxidant system, including enzymatic and non-enzymatic antioxidants, and its role in modulating oxidative stress and maintaining fresh cut carambola quality.

2. Materials and methods

2.1. Plant materials

According to the specifications outlined in the 2017 Malaysian Standard for Fresh Carambola (MS 1127:2017), carambola of the B10 cultivar at maturity stage 5 were selected for this study. These fruits were acquired from the commercial farm H&M Senaling Enterprise in Kuala Pilah, Negeri Sembilan, Malaysia, and subsequently transported to Universiti Putra Malaysia in a corrugated box. Within 24 h of purchase, the fruits were washed with 0.05 % sodium hypochlorite solution and allowed to air dry at room temperature for 60 min. Prior to vacuum impregnation process, the carambola was sliced into 10 mm thickness.

2.2. Vacuum impregnation

Vacuum impregnation (VI) was performed in a desiccator attached to a vacuum controller (VACUUBRAND GMBH + CO KG, Wertheim, Germany) and a vacuum pump, as explained by Panarese et al. [11]. The carambola slices were immersed in a container with 100 μM melatonin solution, and the VI procedure was conducted at 25 °C ± 2 °C. To determine the minimal absolute pressure required to prevent tissue damage and achieving maximum weight increase, a preliminary experiment was carried out. This led to the selection of a 350 mbar pressure protocol. The pressure was dropped from atmospheric pressure to 350 mbar in 6 min during the first stage of the VI process, and it remained there for 4 min. In the subsequent phase of the process, the atmospheric pressure was returned within 6 min and kept for another 6 min at atmospheric pressure. A total of 22 min was taken for one treatment cycle. Excess solution was blotted with tissue paper and a weight gain of 11.1 ± 0.5 % was recorded.

2.3. Storage condition and sample preparation

Carambola slices, subjected to either vacuum impregnation or left untreated (acting as the control), were individually enclosed in polypropylene containers, each weighing approximately 200 g. These containers were subsequently stored at a temperature of 4 °C for a period of 16 days. Sampling of the carambola slices took place at 4 days intervals. The experimental setup involved three repetitions for each treatment.

2.4. Total soluble solid, titratable acidity and pH

Fruit samples were homogenized using distilled water, where then the pH, total soluble solids (TSS) and titratable acidity were measured as detailed by Mohd Suhaimi et al. [12]. The pH was measured with digital pH metre (Delta 320 A, Metler Toledo). A digital refractometer (PR-201α, ATAGO, Tokyo, Japan) was used to measure TSS, which was then expressed as %. Titratable acidity was calculated based on the amount of sodium hydroxide titrated into the homogenate with phenolphthalein as indicator and expressed in % of malic acid equivalent.

2.5. H_2O_2 content

Hydrogen peroxide (H_2O_2) content was determined according to Liu et al. [13] with minor modification. Of the fruit sample, 1 g were homogenized with 5 mM of 0.1 % (w/v) trichloroacetic acid, TCA followed by centrifugation at 12 000 *g* for 15 min. 0.5 mL of the supernatant was then added to 0.5 mL of 10 mM potassium phosphate buffer, KH_2PO_4 of pH 7.0 along with 1 mL of 1 M potassium iodide, KI. The absorbance was read at 390 nm with at spectrophotometer and the content of H_2O_2 was measured using a standard curve. The content of H_2O_2 was reported as μmol g^{-1} of fresh weight.

2.6. Malondialdehyde content

Content of malondialdehyde (MDA) was measured based on method described by Sun et al. [14]. Of 1 g of pericarp tissue were homogenized with 5 mL of 100 g L^{-1} trichloroacetic acid before centrifuged (KUBOTA 3740, Kubota Corporation, Tokyo, Japan) at 10 000×*g* for 20 min at 4 °C. 2 mL of the supernatant were then added to 2 mL of 0.67 % of thiobarbituric acid (TBA) before being boiled for 20 min. The mixture was centrifuged under the same conditions as previous. The absorbance was taken at 450, 532 and 600 nm using spectrophotometer (GENESYS 30, Thermo Scientific, Waltham, MA, USA). MDA content was calculated using the following equation, MDA content (μmol kg^{-1}) = [6.45 × (A532 – A600) – (0.56 × A450)] × 5.

2.7. Antioxidant extraction

Extraction was carried out according to Lim et al. [15] with minor modification. Fruit sample, 1 g was homogenized with 10 mL of 60 % ethanol followed by stirring for 10 min. The homogenate was then centrifuged (KUBOTA 3740, Kubota Corporation, Tokyo, Japan) at 12 000 *g* for 10 min at 4 °C.

2.8. DPPH free radical-scavenging assay

The determination of antioxidant capacity of the fruit extract using free radical scavenging effect was carried out according to Alothman et al. [16] with minor modification. Ethanolic extract of 0.1 mL was added to 3.9 mL of 0.0250 g L-1 DPPH methanolic solution. The mixture was then incubated in the dark for 30 min. Spectrophotometer (GENESYS 30, Thermo Scientific, Waltham, MA, USA) was used to record the absorbance value at 515 nm. The result was reported as the percentage of DPPH radical inhibition, calculated based on Equation (1):

$$\%\ \text{DPPH inhibition} = \left(\frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}}\right) \text{x } 100 \qquad \text{Equation 1}$$

Where Abs control is the absorbance of DPPH solution without extract and abs sample is absorbance of the sample.

2.9. ABTS inhibition

The antioxidant capacity in the reaction with ABTS radical cation was determined according to Liu et al. [13]. The assay was performed by preparing 7 mM ABTS solution and 2.45 mM potassium persulfate where the mixture was left in the dark for 16 h prior to use at room temperature. The solution was diluted to attain the absorbance of 0.7 ± 0.1 at 734 nm using spectrophotometer (GENESYS 30, Thermo Scientific,

Waltham, MA, USA) before used as test reagent. The ethanolic extract of the sample was then added to the ABTS solution. The absorbance at 734 nm was recorded after 6 min of incubation, where the percentage decrease of the absorbance was also calculated using Equation (1).

2.10. Total phenolic content

Total phenolic content was measured using Folin-Ciocalteau colorimetric method as detailed by Rastegar et al. [17]. About 1.5 mL of 10 % Folin-Ciocalteau reagent was mixed with 0.3 mL of ethanolic extract and 1.2 mL of 7 % sodium carbonate solution. The absorbance was measured at 750 nm using a spectrophotometer (GENESYS 30, Thermo Scientific, Waltham, MA, USA) following a 90 mim incubation period. The findings were presented as mg of gallic acid equivalent per 100 g of fresh weight (mg GAE per 100 g FW).

2.11. Total flavonoid content

Total flavonoid content was assayed according to methodology described by Zainudin et al. [18]. 4 mL of distilled water were mixed with 1 mL of ethanolic extract and 0.3 mL of 5 % sodium nitrite followed by 5 min incubation period. 0.3 mL of 10 % aluminium chloride and 2 mL of 1 M sodium hydroxide were added to the reactant mixture. With distilled water, the total volume was marked up until 10 mL. The absorbance was measured at 510 nm using a spectrophotometer (GENESYS 30, Thermo Scientific, Waltham, MA, USA). To express the data in mg of quercetin equivalent per 100 of fresh weight (mg QE per 100g FW), a reference curve was built using quercetin.

2.12. PPO and POD activities

2.12.1. Enzyme extraction

The extraction of enzymes was conducted with a modified method based on the procedure outlined by Moosa et al. [19]. A 5 g portion of the fruit sample was ground in 10 mL of 0.2 M potassium phosphate buffer (KH_2PO_4) with a pH of 6.8. The homogenate was centrifuged (KUBOTA 3740, Kubota Corporation, Tokyo, Japan) at 12 000 g at 4 °C for 15 min, and the resulting supernatant was collected for the assessment of PPO and POD activities.

2.12.2. POD and PPO activities assay

Peroxidase (POD) activity assay was established using a reaction mixture containing 0.1 mL of 50 mM guaiacol and 0.1 mL of 1 % hydrogen peroxide in 2.6 mL of 0.2 M KH_2PO_4 buffer of pH 6.8. 0.2 mL of enzyme extract was added to the reaction mixture where the absorbance was recorded at 470 nm using spectrophotometer (GENESYS 30, Thermo Scientific, Waltham, MA, USA). The activity of POD was calculated based on the extinction coefficient of 26.6 mM^{-1} cm^{-1} for guaiacol and expressed as μmol min^{-1} g^{-1} of fresh weight (U g^{-1}) [20]. The activity of the enzyme was calculated based on the following Equation (2):

$$\textit{Enzyme activity}\ (\mathrm{U/L}) = \frac{\Delta \mathrm{Abs} \times \text{Total volume}}{\Delta t \times \varepsilon \times l \times \text{Volume enzyme}} \qquad \text{Equation 2}$$

Where, Δ abs is the change in absorbance, Δ time taken for change in absorbance (min), ε is the extinction coefficient (M^{-1} cm^{-1}), and l is the cuvette width (cm).

Polyphenol oxidase (PPO) activity was determined using a reaction mixture consisting of 0.4 mL of 100 mM catechol, 2.2 mL of 0.2 KH_2PO_4 buffer of pH 6.8, 0.2 mL of chilled acetone and 0.2 mL of enzyme extract. Absorbance was measured at 420 nm using a spectrophotometer (GENESYS 30, Thermo Scientific, Waltham, MA, USA). The activity of PPO was calculated based on Equation (2) with extinction coefficient of 3400 mM^{-1} cm^{-1} for catechol and expressed as μmol min^{-1} g^{-1} of fresh weight (U g^{-1}) [19].

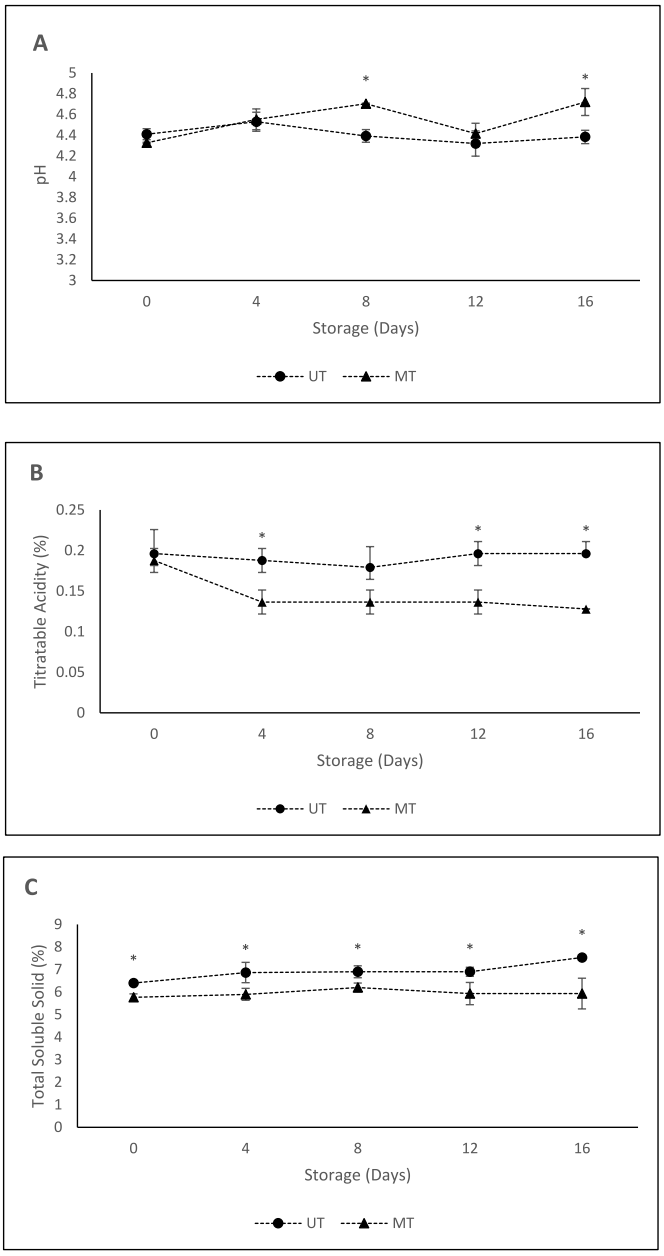


Fig. 1. pH **(A)**, titratable acidity **(B)** and total soluble solid **(C)** of carambola during 16 days of cold storage. Values were stated as mean ± standard deviation (SD). T-test was used to compare differences between means from three replicates of two treatments *$p < 0.05$ denoted the significance.

2.13. CAT and APX activities

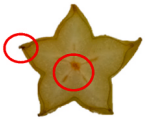
2.13.1. Enzyme extraction

Sliced carambola, 2 g was homogenized in 5 mL of 50 mM phosphate buffer (pH 7.0). The homogenate was then centrifuged (KUBOTA 3740, Kubota Corporation, Tokyo, Japan) at 12 000×*g* for 15 min at 4 °C.

2.13.2. CAT and APX activities

Catalase (CAT) and ascorbate peroxide (APX) was determined using the method described by Yao et al. [21] with minor modification. CAT reaction solution consists of 2.6 mL of 50 mM phosphate buffer (pH 7.0) and 0.2 mL of 0.75 % H_2O_2 was added with 0.2 mL of enzyme extract. The reduction in absorbance was measured at 240 nm using a spectrophotometer. For APX activity, the reaction mixture consists of 2.6 mL of 50 mM phosphate buffer (pH 7.0), 0.2 mL enzyme extract, 0.1 mL of 10

Table 1
Cross-sectional images of carambola slices treated with melatonin and untreated carambola over a 16-day cold storage period.

	Day 0	Day 4	Day 8	Day 12	Day 16
UT					
MT					

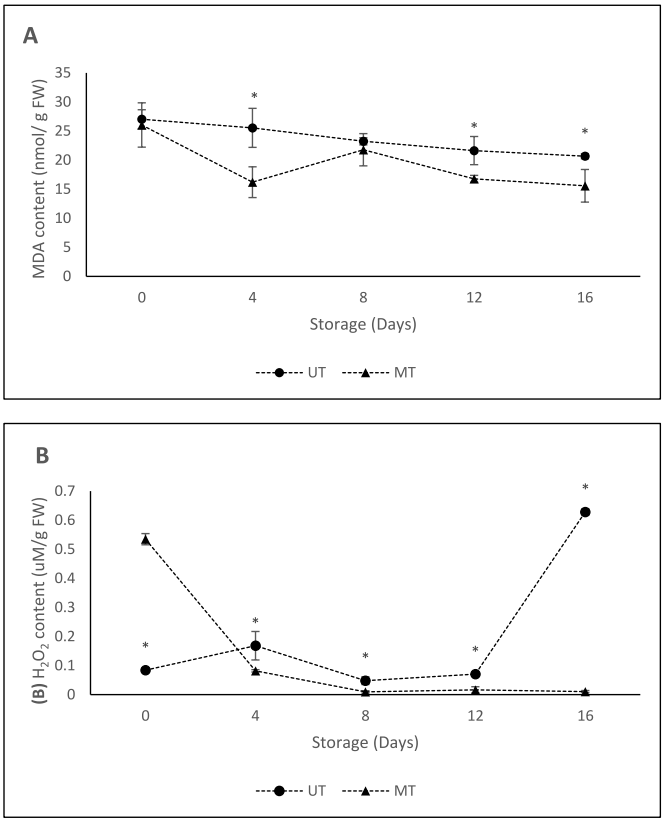


Fig. 2. MDA content **(A)** and H_2O_2 content **(B)** of carambola during 16 days of cold storage. Values were stated as mean ± standard deviation (SD). T-test was used to compare differences between means from three replicates of two treatments *p < 0.05 denoted the significance.

mM ascorbic acid, and 0.1 mL of 3 % H_2O_2. The reaction started with the addition of H_2O_2 and the absorbance decrease at 290 nm was recorded. The activity of CAT and APX was measured based on Equation (1) with extinction coefficient 43.6 M^{-1} cm^{-1} for CAT and 2.8 mM^{-1} cm^{-1} for APX where it was then expressed as μmol min^{-1} g^{-1} of fresh weight (U g^{-1}).

2.14. Statistical analysis

The data was analysed using the Minitab Statistical Software (Minitab 21, LLC, Dayton, OH, USA) and is presented as mean ± standard deviation (SD). A *t*-test was used to compare the differences between means from three biological replicates of both samples on the same day, with a significance level of $P < 0.05$ (*). To compare the differences between means from three biological replicates of the same treatment on different days, a one-way analysis of variance (ANOVA) was used with a significance level of $P < 0.05$. The Tukey-Kramer multiple comparison test was then used to determine the true differences.

3. Result

3.1. pH, titratable acidity, and total soluble solid

As depicted in Fig. 1 (A), the pH of melatonin (MT)-treated carambolas exhibited a gradual increase over the storage period. Notably, on days 8 and 16, the MT-treated carambolas displayed a significantly higher pH compared to the untreated (UT) carambolas. The pH of untreated carambolas remained consistently stable throughout the storage duration. In Fig. 1 (B), it is evident that the titratable acidity (TA) of MT-treated carambolas decreased after 4 days of storage, while no significant changes in TA were observed in UT carambolas. On days 4, 12, and 16, the TA of MT-treated carambolas was significantly higher than that of untreated carambolas. Total soluble solid (TSS) of untreated carambola were significantly higher than MT-treated throughout the storage, with increasing trend, as shown in Fig. 1 (C). The TSS of MT-treated carambola remains unchanged during the storage.

3.2. Physical appearance of fresh-cut carambola

The physical appearance of the fresh cut carambolas is shown in Table 1. Severe browning developed as early as 4 days of storage and continued to worsen with time at the centre of the untreated carambola slices. On day 12, the untreated carambola slices exhibited shrivelling at the corners, accompanied by browning at the centre. In contrast, the melatonin-treated (MT) carambola slices showed minimal browning, and no shrivelling was observed at the ribs.

3.3. Malondialdehyde and hydrogen peroxide content

In Fig. 2 (A), MT-treated carambola recorded a lower MDA content than untreated carambola on days 4, 12 and 16. On day 4, the MDA content of MT-treated carambola dropped significantly. For the rest of the storage period, the MDA levels remained the same, with insignificant variations. For untreated carambola, MDA content decrease steadily with significant decrease only recorded on day 16 of storage.

As illustrated in Fig. 2 (B), the melatonin-treated carambola consistently exhibited a significantly lower concentration of H_2O_2 compared to the untreated carambola, except for the initial storage period. The H_2O_2 content in the melatonin-treated carambola significantly decreased on day 4, and this pattern persisted throughout the storage duration. In contrast, the untreated carambola displayed a progressive increase in H_2O_2 content from day 4 of storage, with a significant spike observed on day 16.

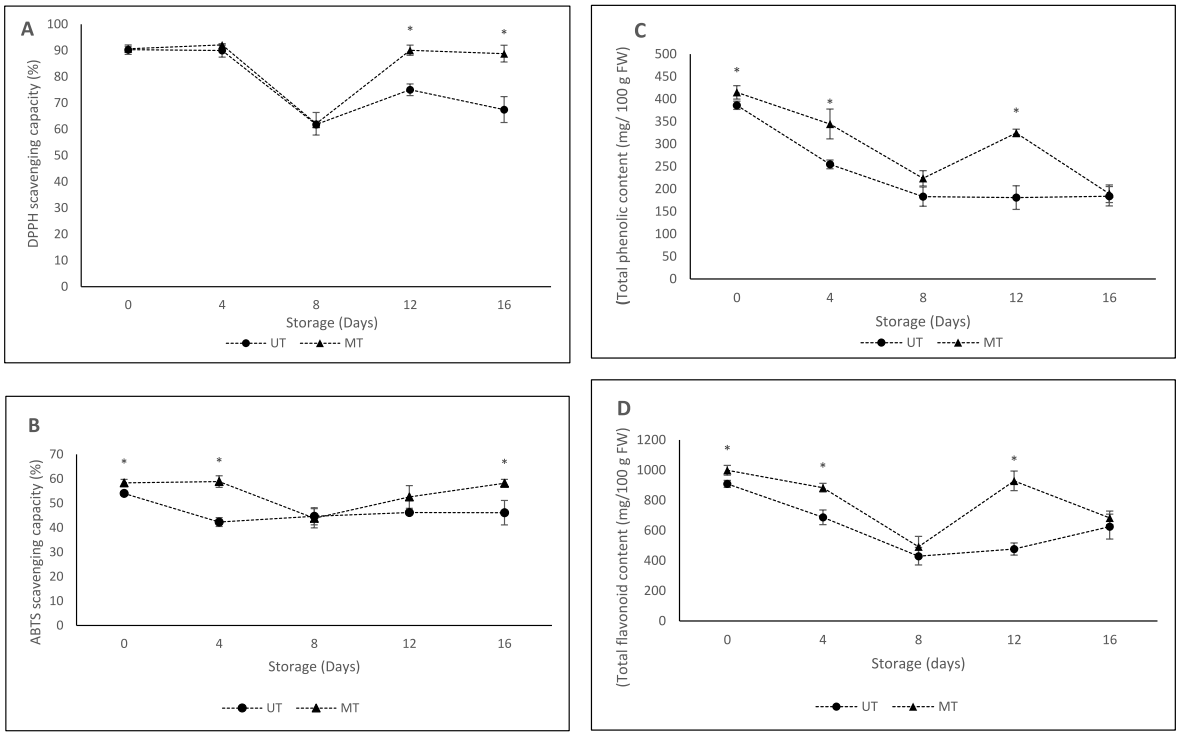


Fig. 3. DPPH scavenging activity **(A)**, ABTS scavenging activity (B), total phenolic content **(C)**, and total flavonoids content **(D)** of carambola during 16 days of cold storage. Values were stated as mean ± standard deviation (SD). T-test was used to compare differences between means from three replicates of two treatments *p < 0.05 denoted the significance.

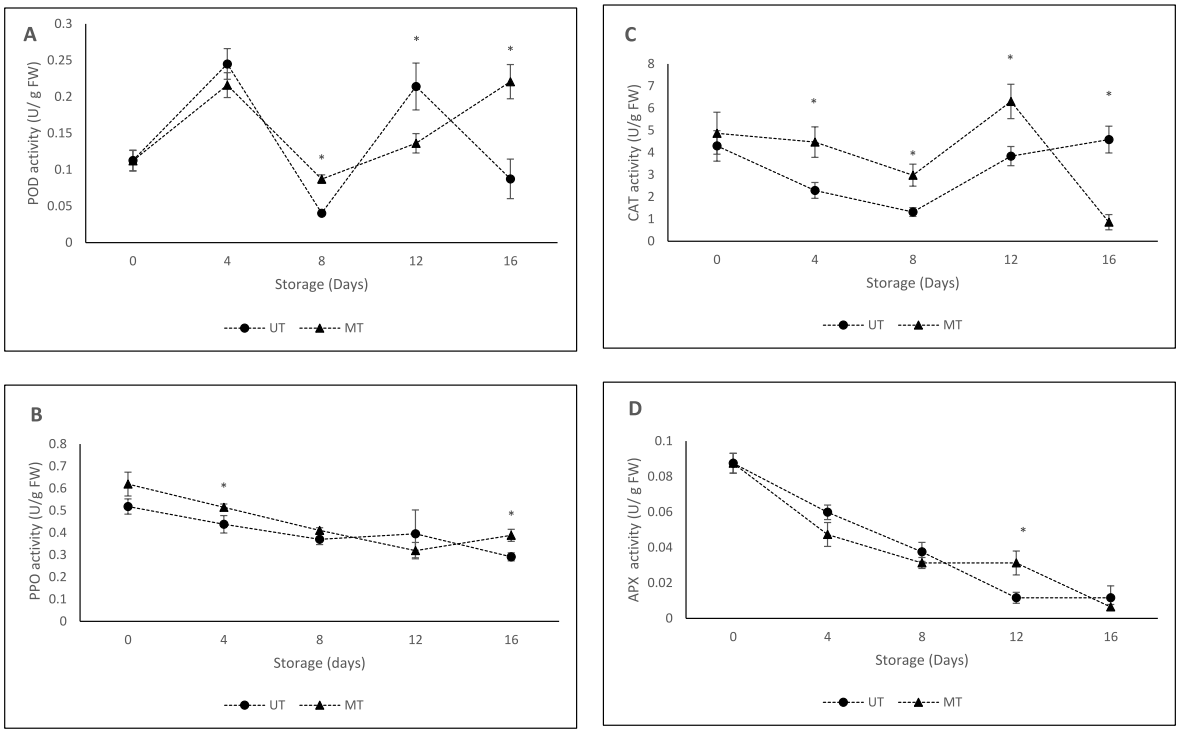


Fig. 4. Peroxidase activity **(A)**, Polyphenol oxidase activity **(B)**, catalase activity **(C)**, and ascorbate peroxidase **(D)** of carambola during 16 days of cold storage. Values were stated as mean ± standard deviation (SD). T-test was used to compare differences between means from three replicates of two treatments *p < 0.05 denoted the significance.

3.4. Antioxidant capacity

From the data in Fig. 3 (A), MT-treated carambola had significantly higher DPPH scavenging activity than untreated carambola on days 12 and 16. In MT-treated carambola, the scavenging capacity decreased significantly on day 8 before rebound back on day 12 to its original

capacity. In untreated carambola, the DPPH scavenging capacity also dropped on day 8, before increase on day 12 although not as high as its original capacity. DPPH inhibition then decreased on day 16.

As depicted in Fig. 3 (B), the melatonin-treated carambola exhibited significantly higher ABTS scavenging activity on days 0, 4, and 16. The ABTS scavenging capacity decreased on day 4 of storage but remained consistent for the remaining storage period. On day 8, the melatonin-treated carambola showed a reduction in ABTS scavenging capacity. Fortunately, there was an increase in ABTS inhibition on day 12, returning to its initial inhibition capacity. As shown in Fig. 3 (C), the total phenolic content (TPC) of MT – treated carambola was significantly greater on days 0, 4, and 12 compared to the untreated carambola. The TPC decreased until day 8 in MT – treated carambola, followed by a surge on day 12.

Based on Fig. 3 (D), day 0, 4, and 12 also recorded a higher total flavonoids content (TFC) for MT-treated carambola. The same pattern as TPC emerged, with sudden decrease on day 8, followed by dramatic rise on day 12 and degradation on day 16 for the MT-treated carambola. The untreated carambola exhibited a comparable pattern with a substantial decrease on day 8. The Total Flavonoid Content (TFC) exhibited a gradual increase for the remaining storage period, albeit at a slower rate when compared to those treated with melatonin.

3.5. Antioxidant enzymes activities

From Fig. 4 (A), MT – treated carambola on days 0, 8 and 16 has higher peroxidase (POD) activity than untreated carambola. Only on day 12, untreated carambola recorded a higher enzymatic activity compared to the MT – treated carambola. Similar as the non-enzymatic antioxidant analysis, a drop on the enzymatic activity of both samples also recorded on day 8 of storage. The POD activity of the MT – treated carambola rise on day 12 and recovered back its initial level by day 16.

As depicted in Fig. 4 (B), the melatonin-treated carambola exhibited higher polyphenol oxidase (PPO) activity on days 4 and 16 compared to the untreated carambola. A declining trend was observed in the melatonin-treated sample, with the lowest activity recorded on day 12. In contrast, the untreated carambolas only displayed a significant reduction in PPO activity on day 16. MT – treated carambola presented an elevated catalase (CAT) activity on day 4, 8 and 12, based on Fig. 4 (C). On day 16, however, untreated carambola had higher activity than MT – treated carambolas. Significant reduction on day 8 recorded in MT – treated carambola before increased on day 12, followed by a dramatic drop. In contrast, the untreated carambola displayed a significant reduction as early as day 4 of storage before experiencing an increase on day 12. Based on Fig. 4 (D), MT – treated carambola had a higher ascorbate peroxidase (APX) activity on day 12. Both samples showing a declining trend, with a significant reduction as early as day 4 of storage.

4. Discussion

The quality of fruit is commonly assessed through parameters such as pH, titratable acidity (TA), and total soluble solids (TSS) Botondi et al. [22]. This study reveals that treating fresh-cut carambola with MT resulted in a reduction of TSS and TA, leading to a higher pH value compared to the untreated carambola. Cutting and other processing activities, in this case vacuum impregnation, may increase the respiration rate of the fruit. During respiration, organic acids such as malic acid may serve as an alternative respiratory substrates during storage, leading to a reduction in the TA levels [23]. As TA decreased, the pH value increased depending on the presence of acidic compounds. It is noteworthy that although MT-treated carambola exhibited lower TSS and TA than untreated carambola, its TSS and TA levels remained relatively stable for the most part during storage. This is in line with previous study conducted by Wang et al. [24] where the author stipulated that the MT treatment alleviated the degradation of nutrients, resulting in a maintained level of TA.

Maintaining the appearance of fresh cut fruits poses several challenges, including cut-surface browning, decay, shrinkage, and water-soaked appearance. These challenges limit the quality, marketability, and shelf-life of fresh cut fruit. The findings of this study suggest that MT treatment can effectively mitigate severe browning and shrinkage associated with these challenges. Cut-surface browning in the MT – treated carambola was minimal in comparison to untreated carambola. Xiao et al. [25] reported that the application of MT to pears, apples, potatoes, and taros reduces surface browning in fresh cut produce. Melatonin acts as a potent antioxidant and induces antioxidant system of the fruit. The increased level of non-enzymatic antioxidant and enzymatic antioxidant helps maintain cellular integrity and reduces browning which ultimately prevents decay [26]. By improving cell membrane stability and reducing electrolyte leakage, melatonin also helps maintaining turgor pressure within fruit cells [25]. This stabilization is crucial for preventing shrinkage and maintaining firmness, which helps preserve their visual quality. By addressing these critical issues, melatonin treatment effectively prolongs the shelf life of fresh-cut fruit, ensuring better quality and marketability for consumers.

The generation of malondialdehyde (MDA) is linked to the process of lipid peroxidation occurring in the plant cell membrane, and it serves as a biomarker for this oxidative reaction. Membrane lipid peroxidation is a consequence of the oxygenation of polyunsaturated fatty acids, leading to the formation of conjugated dienes and MDA, catalyzed by lipoxygenase (LOX) [8]. In this study, the higher level of MDA in the initial storage period can be attributed to the cutting action which caused tissue wounding. This is supported by a study conducted by Hu et al. [27], who discovered that membrane lipid peroxidation increased with wounding intensity. After a few days of storage, the MDA level decreased, with prominent decrease in MT-treated carambolas. Similar results were reported by Gao et al. [28], where MT treatment lowered the lipoxygenase and MDA content in peach fruits, suggesting that MT could reduce membrane lipid peroxidation.

Polyphenol oxidase (PPO) is an oxidizing enzyme that is not only responsible for the browning of fresh cut fruit through the oxidation of o-diphenols to o-quinones [29]. PPO is additionally accountable for causing chilling injury in fruits by disrupting membrane integrity. The reaction includes the upregulation of H_2O_2 by PPO, which later promotes hydrogenation of membranous unsaturated fatty acids to form saturated fatty acids by phospholipase D, which is further oxidized to MDA by LOX [30,31]. Despite the higher PPO activity observed in MT-treated carambola compared to untreated carambola in this study, a noteworthy reduction in enzymatic activity was recorded on each day of observation for MT-treated carambola. Higher levels of H_2O_2 were initially recorded in MT-treated carambolas before decreasing throughout the storage period. Hence, it can be inferred that the upregulation of PPO activity subsequently triggers the H_2O_2 burst.

Based on a study by Sharafi et al. [32], MT treatment created a high concentration of H_2O_2, on the first 7 days of storage, served as signalling molecules in response for chilling stress. Aghdam et al. [33] found that in cut anthurium flowers, higher ROS scavenging system activities, phenylpropanoid pathway activity, and proline synthesis in response to melatonin application may arise from signalling H_2O_2.

Exogenous MT engaged with its primary cellular receptor, CAND/PMTR1. This interaction triggered the activation of response mechanisms through ROS signalling [34]. ROS are considered as the signalling molecules that contribute to the plant response to stimuli through stress-response network activation contributing to plant defense and stress tolerance [35]. In a study conducted by Gong et al. [36] using tomato plant, the application of melatonin increases the activity of Respiratory Burst Oxidase Homolog (RBOH), a protein responsible for apoplastic ROS generation, resulting increased level of H_2O_2. In the same study, when melatonin was administered to silenced RBOH lines in stressful conditions, they demonstrated heightened sensitivity. This indicates that disruption of the H_2O_2 and RBOH signalling pathway impaired the ability of MT to enhance plant stress tolerance effectively.

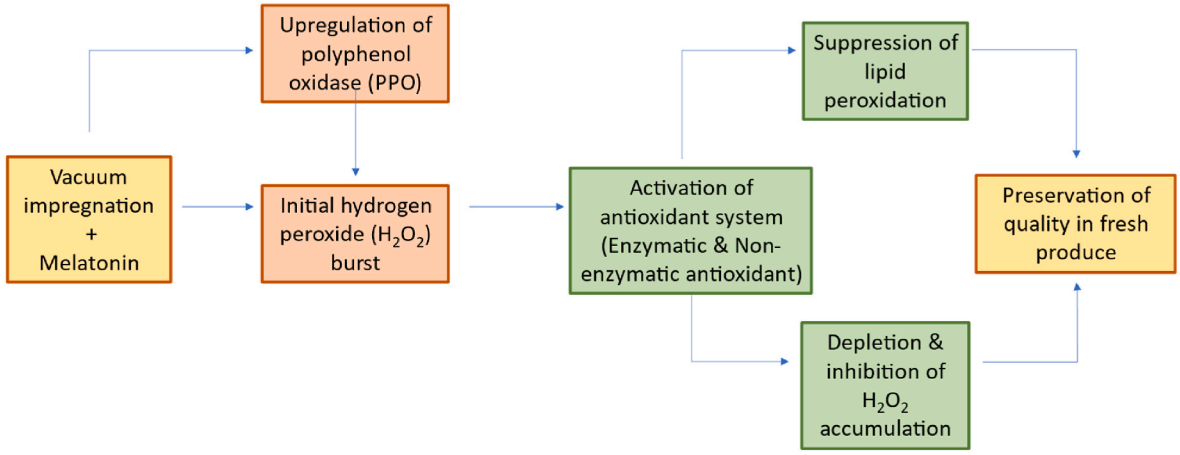


Fig. 5. Proposed model outlining the mechanism of antioxidant upregulation by hydrogen peroxide burst.

Under cold stress in plant, MT facilitate the formation of H_2O_2 by upregulating the expression of RBOHD and promoting the accumulation of cytosolic Ca^{2+}. The activation of CBF pathway-related genes by melatonin and the subsequent establishment of cold tolerance depend on the formation of a positive regulatory loop between H_2O_2 and Ca^{2+} [37]. ROS produced possess the ability to promote the expression of melatonin biosynthetic genes, leading to increase in the plant endogenous melatonin levels that then contribute to the enhanced plant antioxidant response [38]. This includes the increase in the formation of non-enzymatic antioxidant compounds such as phenolic compounds and enzymatic antioxidant such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and ascorbate peroxidase (APX) [5].

By utilizing this mechanism, fresh-cut fruits and vegetables can rapidly respond to bursts of reactive oxygen species (ROS), enhance their antioxidant defences, and mitigate oxidative damage to the produce tissue. This ultimately prolongs their shelf life and preserves their nutritional value. In present study, the application of exogenous MT significantly increased the accumulation of phenolics and flavonoids in fresh cut carambola. Simultaneously, the DPPH and ABTS radical scavenging capacities indicated a high antioxidant capacity. Previous study has shown that MT treatment maintained a higher concentration of total phenolics and flavonoids contributing to the overall antioxidant capacity, thus delaying senescence in mango fruit [39]. A study by Zheng et al. [8] demonstrated an enhanced total phenolic and antioxidant capacity due to MT treatment, which effectively preserved the nutritional quality and prevented browning in fresh cut pears.

Higher activity of POD, APX, and CAT in MT – treated carambola was also recorded in this study. In this experiment, melatonin enhanced the ability of the antioxidant system to recover after a significant decrease on day 8. When fresh-cut fruits are processed, it induced physical stress that may trigger various biochemical process. This initial stress often led to increase cellular respiration and production of reactive oxygen species which can damage cellular structures and deplete antioxidant compounds such as ascorbic acid and phenolic compounds [40]. Research indicates that during the first few days of storage, fresh-cut fruits may show a marked decline in their antioxidant capacity due to these stress-induced factors, including membrane breakdown and increased enzyme activity that promotes oxidation [41].

The subsequent increase on day 12 demonstrates the long-term benefits of melatonin in modulating stress responses and promoting secondary metabolite biosynthesis. Melatonin enhances the antioxidant capacity of fresh-cut fruits through a multifaceted approach that includes reducing ROS levels, activating antioxidant enzymes, stimulating phenolic compound synthesis, inhibiting senescence, and maintaining membrane integrity [8,10,42]. These mechanisms collectively contribute to recovery during storage, thereby improving the overall quality and shelf life of fresh-cut products.

In untreated carambola, after a significant decrease on day 8, the antioxidant system also recovered, but at a slower rate or stagnant, as reflected in the DPPH and ABTS scavenging capacity. Previous studies have shown that MT upregulates the activities of antioxidant enzymes to counter the negative of biotic and abiotic stresses. Rastegar et al. [17] reported that the application MT to mango, delays the fruit softening and maintained the nutritional quality as the activity of POD and CAT increased during the storage. In another study conducted by Bhardwaj, employing four distinct cultivars of mangoes, the treatment with melatonin (MT) upregulated the activities of six antioxidant enzymes. These enzymes included superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX), glutathione reductase (GR), and dehydroascorbate reductase (DHAR).

5. Conclusion

This study demonstrates that vacuum impregnation with exogenous melatonin (MT) significantly enhances the antioxidant capacity of fresh-cut carambola during cold storage. The treatment induces an initial burst of hydrogen peroxide (H_2O_2), likely functioning as a signalling molecule that activates oxidative stress responses. Elevated polyphenol oxidase (PPO) activity during the early storage phase is followed by an upregulation of key antioxidant enzymes, including peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX). Concurrently, the accumulation of phenolic and flavonoid compounds contributes to enhanced antioxidant defense system, effectively scavenging reactive oxygen species (ROS) and reducing H_2O_2 levels as storage progresses, as outlined in Fig. 5. This synergistic action between enzymatic and non-enzymatic antioxidants helps preserve the quality, nutritional value, and shelf life of fresh-cut carambola.

The practical implications of this work lie in its potential application within the fresh produce industry. Melatonin treatment offers a non-thermal, environmentally friendly approach to prolong the shelf life of minimally processed fruits and vegetables, aligning with consumer demand for natural and sustainable preservation methods. The findings suggest that vacuum impregnation with MT could be integrated into existing postharvest handling protocols to reduce quality loss, prevent browning, and extend the marketability of fresh-cut products. Such applications could be particularly valuable for export-oriented industries, where maintaining quality during long-distance transport is critical.

However, this study has several limitations. While the results confirm the efficacy of MT in enhancing antioxidant activity and preserving quality, the underlying biochemical mechanisms, particularly the interplay between H_2O_2 signalling and secondary metabolite

synthesis, remain incompletely understood. Additionally, the effectiveness of MT treatment may vary depending on fruit type, cultivar, and genotype. Future studies should explore the scalability of this technology, its compatibility with other preservation methods, and its impact on consumer safety and sensory attributes.

In conclusion, the findings underscore the potential of melatonin as a promising tool for advancing postharvest management strategies in the fresh produce industry. Further research should focus on unraveling the precise molecular pathways influenced by MT, assessing its applicability to other fruit and vegetable commodities, and investigating its long-term effects under commercial storage and distribution conditions.

CRediT authorship contribution statement

Farah Anum Mohd Yusof: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Ezzat Mohamad Azman:** Validation, Supervision, Resources, Funding acquisition. **Noranizan Mohd Adzahan:** Validation, Resources, Funding acquisition, Conceptualization. **Noor Liyana Yusof:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This study was funded by grants from Universiti Putra Malaysia UPM/800/3/3/13 GP-IPM/2018/9657000.

Data availability

Data will be made available on request.

References

[1] F. Ngaffo Mekontso, et al., Alleviation of postharvest chilling injury of carambola fruit by γ-aminobutyric acid: physiological, biochemical, and structural characterization, Front. Nutr. 8 (2021) 1–16, https://doi.org/10.3389/fnut.2021.752583. November.

[2] G.H.A. Teixeira, J.F. Durigan, A.S. Ferraudo, R.E. Alves, T.J. O'Hare, Multivariate analysis of fresh-cut carambola slices stored under different temperatures, Postharvest Biol. Technol. 63 (1) (2012) 91–97, https://doi.org/10.1016/j.postharvbio.2011.09.005.

[3] B. Yousuf, O.S. Qadri, A.K. Srivastava, Recent developments in shelf-life extension of fresh-cut fruits and vegetables by application of different edible coatings: a review, LWT–Food Sci. Technol. 89 (June 2017) 198–209, https://doi.org/10.1016/j.lwt.2017.10.051, 2018.

[4] B.C. Tripathy, R. Oelmüller, Reactive oxygen species generation and signaling in plants, Plant Signal. Behav. 7 (12) (Dec. 2012) 1621–1633, https://doi.org/10.4161/psb.22455.

[5] P. Sharma, A.B. Jha, R.S. Dubey, M. Pessarakli, Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions, J. Bot., Le 2012 (2012) 217037, https://doi.org/10.1155/2012/217037.

[6] S. Sachdev, S.A. Ansari, M.I. Ansari, M. Fujita, M. Hasanuzzaman, Abiotic stress and reactive oxygen species: generation, signaling, and defense mechanisms, Antioxidants 10 (2) (2021) 1–37, https://doi.org/10.3390/antiox10020277.

[7] H.M.S. Shah, Z. Singh, E. Afrifa-Yamoah, M.U. Hasan, J. Kaur, A. Woodward, Insight into the role of melatonin in mitigating chilling injury and maintaining the quality of cold-stored fruits and vegetables, Food Rev. Int. 40 (5) (2023) 1238–1264, https://doi.org/10.1080/87559129.2023.2212042.

[8] H. Zheng, W. Liu, S. Liu, C. Liu, L. Zheng, Effects of melatonin treatment on the enzymatic browning and nutritional quality of fresh-cut pear fruit, Food Chem. 299 (2019) 125116, https://doi.org/10.1016/j.foodchem.2019.125116. July.

[9] M.P. Madebo, S. Hu, Y. Zheng, P. Jin, Mechanisms of chilling tolerance in melatonin treated postharvest fruits and vegetables: a review, J. Funct.Foods 1 (2) (2021) 156–167, https://doi.org/10.1016/j.jfutfo.2022.01.005.

[10] M. Giraldo-Acosta, D. Ruiz-Cano, A. Cano, J. Hernández-Ruiz, M.B. Arnao, Extended post-harvest effect of melatonin in fresh-cut broccolini plants (Bimi®), Agronomy 13 (10) (2023), https://doi.org/10.3390/agronomy13102459.

[11] V. Panarese, P. Dejmek, P. Rocculi, F. Gómez Galindo, Microscopic studies providing insight into the mechanisms of mass transfer in vacuum impregnation, Innov. Food Sci. Emerg. Technol. 18 (2013) 169–176, https://doi.org/10.1016/j.ifset.2013.01.008.

[12] N.I. Mohd Suhaimi, A.A. Mat Ropi, S. Shaharuddin, Safety and quality preservation of starfruit (Averrhoa carambola) at ambient shelf life using synergistic pectin-maltodextrin-sodium chloride edible coating, Heliyon 7 (2) (2021) e06279, https://doi.org/10.1016/j.heliyon.2021.e06279.

[13] C. Liu, H. Zheng, K. Sheng, W. Liu, L. Zheng, Effects of melatonin treatment on the postharvest quality of strawberry fruit, Postharvest Biol. Technol. 139 (2018) 47–55, https://doi.org/10.1016/j.postharvbio.2018.01.016. January.

[14] H. jun Sun, M. li Luo, X. Zhou, Q. Zhou, S. juan Ji, Influence of melatonin treatment on peel browning of cold-stored 'nanguo' pears, Food Bioprocess Technol. 13 (8) (2020) 1478–1490, https://doi.org/10.1007/s11947-020-02489-8.

[15] Y.Y. Lim, T.T. Lim, J.J. Tee, Antioxidant properties of several tropical fruits: a comparative study, Food Chem. 103 (3) (2007) 1003–1008, https://doi.org/10.1016/j.foodchem.2006.08.038.

[16] M. Alothman, B. Kaur, A. Fazilah, R. Bhat, A.A. Karim, Ozone-induced changes of antioxidant capacity of fresh-cut tropical fruits, Innov. Food Sci. Emerg. Technol. 11 (4) (2010) 666–671, https://doi.org/10.1016/j.ifset.2010.08.008.

[17] S. Rastegar, H. Hassanzadeh Khankahdani, M. Rahimzadeh, Effects of melatonin treatment on the biochemical changes and antioxidant enzyme activity of mango fruit during storage, Sci. Hortic. 259 (2020) 108835, https://doi.org/10.1016/j.scienta.2019.108835. April 2019.

[18] M.A.M. Zainudin, A.A. Hamid, F. Anwar, A. Osman, N. Saari, Variation of bioactive compounds and antioxidant activity of carambola (Averrhoa carambola L.) fruit at different ripening stages, Sci. Hortic. 172 (2014) 325–331, https://doi.org/10.1016/j.scienta.2014.04.007.

[19] A. Moosa, S.T. Sahi, S.A. Khan, A.U. Malik, Salicylic acid and jasmonic acid can suppress green and blue moulds of citrus fruit and induce the activity of polyphenol oxidase and peroxidase, Folia Hortic. 31 (1) (2019) 195–204, https://doi.org/10.2478/fhort-2019-0014.

[20] S.L. De Almeida, M.A.D.S. Morais, J.R.T. De Albuquerque, A.P. Barros Júnior, A.D. N. Simões, K.S. Fonseca, Polyphenol oxidase and peroxidase enzyme assays in sweet potato cultivars harvested at different times, Rev. Caatinga 32 (2) (2019) 537–542, https://doi.org/10.1590/1983-21252019v32n226rc.

[21] W. Yao, T. Xu, S.U. Farooq, P. Jin, Y. Zheng, Glycine betaine treatment alleviates chilling injury in zucchini fruit (Cucurbita pepo L.) by modulating antioxidant enzymes and membrane fatty acid metabolism, Postharvest Biol. Technol. 144 (2018) 20–28, https://doi.org/10.1016/j.postharvbio.2018.05.007.

[22] R. Botondi, M. Barone, C. Grasso, A review into the effectiveness of ozone technology for improving the safety and preserving the quality of fresh-cut fruits and vegetables, Foods 10 (4) (2021), https://doi.org/10.3390/foods10040748.

[23] M. Cofelice, F. Lopez, F. Cuomo, Quality control of fresh-cut apples after coating application, Foods 8 (6) (2019), https://doi.org/10.3390/foods8060189.

[24] D. Wang, et al., Melatonin treatment maintains quality and delays lignification in loquat fruit during cold storage, Sci. Hortic. 284 (2021), https://doi.org/10.1016/j.scienta.2021.110126. September 2020.

[25] Y. Xiao, J. Xie, C. Wu, J. He, B. Wang, Effects of melatonin treatment on browning alleviation of fresh-cut foods, J. Food Biochem. 45 (9) (Sep. 2021) e13798, https://doi.org/10.1111/jfbc.13798.

[26] Y. Zhang, et al., Delay of postharvest browning in litchi fruit by melatonin via the enhancing of antioxidative processes and oxidation repair, J. Agric. Food Chem. 66 (28) (Jul. 2018) 7475–7484, https://doi.org/10.1021/acs.jafc.8b01922.

[27] W. Hu, Y. Guan, Y. Ji, X. Yang, Effect of cutting styles on quality, antioxidant activity, membrane lipid peroxidation, and browning in fresh-cut potatoes, Food Biosci. 44 (2021) 101435, https://doi.org/10.1016/j.fbio.2021.101435.

[28] H. Gao, et al., Melatonin treatment delays postharvest senescence and regulates reactive oxygen species metabolism in peach fruit, Postharvest Biol. Technol. 118 (2016) 103–110, https://doi.org/10.1016/j.postharvbio.2016.03.006.

[29] S. Nogales-Delgado, Polyphenoloxidase (PPO): effect, current determination and inhibition treatments in fresh-cut produce, Appl. Sci. 11 (17) (2021), https://doi.org/10.3390/app11177813.

[30] A. Lukatkin, A. Brazaitytė, C. Bobinas, P. Duchovskis, Chilling injury in chilling-sensitive plants: a review, Zemdirbyste 99 (2012) 111–124.

[31] R. Shewfelt, B.A. Rosario, The role of lipid peroxidation in storage disorders of fresh fruits and vegetables, Hortscience 35 (Jul. 2000) 575–579, https://doi.org/10.21273/HORTSCI.35.4.575.

[32] Y. Sharafi, et al., Melatonin treatment promotes endogenous melatonin accumulation and triggers GABA shunt pathway activity in tomato fruits during cold storage, Sci. Hortic. 254 (2019) 222–227, https://doi.org/10.1016/j.scienta.2019.04.056. April.

[33] M.S. Aghdam, A. Jannatizadeh, M.S. Nojadeh, A. Ebrahimzadeh, Exogenous melatonin ameliorates chilling injury in cut anthurium flowers during low temperature storage, Postharvest Biol. Technol. 148 (2019) 184–191, https://doi.org/10.1016/j.postharvbio.2018.11.008. November 2018.

[34] J. Wei, et al., Phytomelatonin receptor PMTR1-mediated signaling regulates stomatal closure in Arabidopsis thaliana, J. Pineal Res. 65 (2) (Sep. 2018) e12500, https://doi.org/10.1111/jpi.12500.

[35] G.J. Ahammed, et al., Reactive oxygen species signaling in melatonin-mediated plant stress response, Plant Physiol. Biochem. 207 (2024) 108398, https://doi.org/10.1016/j.plaphy.2024.108398. January.

[36] B. Gong, Y. Yan, D. Wen, Q. Shi, Hydrogen peroxide produced by NADPH oxidase: a novel downstream signaling pathway in melatonin-induced stress tolerance in Solanum lycopersicum, Physiol. Plantarum 160 (4) (2017) 396–409, https://doi.org/10.1111/ppl.12581.

[37] J. Chang, et al., Positive interaction between h2o2 and ca2+ mediates melatonin-induced cbf pathway and cold tolerance in watermelon (Citrullus lanatus l.), Antioxidants 10 (9) (2021), https://doi.org/10.3390/antiox10091457.
[38] M.B. Arnao, J. Hernández-Ruiz, Melatonin: a new plant hormone and/or a plant master regulator? Trends Plant Sci. 24 (1) (2019) 38–48, https://doi.org/10.1016/j.tplants.2018.10.010.
[39] R. Bhardwaj, S. Pareek, S. Mani, J.A. Domínguez-Avila, G.A. González-Aguilar, "A melatonin treatment delays postharvest senescence, maintains quality, reduces chilling injury, and regulates antioxidant metabolism in mango fruit,", J. Food Qual. 2022 (2022) Ci https://doi.org/10.1155/2022/2379556.
[40] R.D. Iturralde-García, et al., Emerging technologies for prolonging fresh-cut fruits' quality and safety during storage, Horticulturae 8 (8) (2022), https://doi.org/10.3390/horticulturae8080731.
[41] D.M. Hodges, P.M.A. Toivonen, Quality of fresh-cut fruits and vegetables as affected by exposure to abiotic stress, Postharvest Biol. Technol. 48 (2) (2008) 155–162, https://doi.org/10.1016/j.postharvbio.2007.10.016.
[42] P. Borthakur, et al., Exogenous melatonin as pre- and postharvest application on quality attributes, antioxidant capacity, and extension of shelf life of papaya, Horticulturae 10 (10) (2024), https://doi.org/10.3390/horticulturae10101099.