Journal of Food Science and Technology (Iran)

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Scientific Research

Effect of melatonin treatment on enzymatic browning, nutritional quality and shelf life of fresh-cut apple fruit

Boukaga Farmani*[1] and Morteza Soleimani Aghdam[2]

[1]Associate Professor Department of Food Science and Technology, Ahar Faculty of Agriculture and Natural Resources, University of Tabriz, Tabriz, Iran.
[2]Associate Professor Department of Horticultural Science, Imam Khomeini International University, Qazvin, Iran

ARTICLE INFO

Article History:

Received: 2025/04/13

Review: 2025/10/28

Accepted: 2025/11/10

Keywords:

Melatonin treatment,
browning index,
antioxidant capacity,
fresh-cut apple fruit.

DOI: **10.48311/fsct.2026.84022.0**

*Corresponding Author E-

bfarmani@tabrizu.ac.ir

ABSTRACT

Effect of melatonin treatment on nutritional quality and enzymatic browning of fresh-cut apple fruit was investigated. In the first stage, apple slices were treated with melatonin at concentrations of 0, 1, 10, 100 and 1000 µM for 5 min. By investigating the browning index in treated samples and stored at refrigerated temperature for 7 days, concentration of 10 µM melatonin with the lowest browning index (50.77) was selected for further experiments. In the second stage, fresh-cut apples were treated with 10 µM melatonin to determine the quality characteristics of treated samples compared to the control during 7 days of storage at 4 °C ($P < 0.05$). Results showed that treated samples significantly reduced the content of malondialdehyde from day 0 (1.4 µmol kg^{-1} FW) to day 7 (1.3 µmol kg^{-1} FW) and hydrogen peroxide (H_2O_2) from day 0 (37 mmol kg^{-1} FW) to day 7 (31 mmol kg^{-1} FW). Treatment with melatonin during 7-day storage caused an increasing trend in total phenol content from day 0 (475 mg GAE kg^{-1} FW) to day 7 (523 mg GAE kg^{-1} FW), antioxidant capacity from day 0 (47%) to day 7 (64%) and inhibition of ascorbic acid reduction in samples. In addition, melatonin treatment increased activity of phenylalanine ammonia lyase from day 0 (23 U g^{-1} FW) to day 7 (31 U g^{-1} FW) and decreased the activity of polyphenol oxidase from day 0 (21 U g^{-1} FW) to day 7 (3 U g^{-1} FW) during storage ($P < 0.05$).

1- Introduction

Granny Smith apple (*Malus domestica* L.) is a popular fruit among consumers due to its nutritional value and sensory properties. Recently, fresh-cut apple has been rapidly growing in the food category due to its convenience, nutritional value, and freshness [1]. Mechanical damage during fresh-cut fruit processing causes a series of biological and chemical changes such as browning, off-flavor, senescence, softening, and spoilage, ultimately leading to shorter shelf life. Therefore, improving the quality and extending storage life are key technologies for fresh-cut fruit processing [2]. Surface browning is the main cause of fresh-cut fruit spoilage during post-harvest handling, especially in apples, which are rich in polyphenolic compounds and susceptible to enzymatic browning. Many experiments have been conducted to preserve apple fruit against enzymatic browning, such as semi-thermal pretreatment, controlled atmosphere, modified atmosphere inside the package, coating with xanthan gum-cinnamic acid and chitosan-rosemary extract [3]. However, some of these treatments are not commercially applicable and the development of an effective technique to control browning and improve the nutritional quality of fresh-cut fruit is essential. Melatonin (N-acetyl-5-methoxytryptamine) is a hormone discovered in various types of fruits and vegetables such as apples, cherries, grapes, pears, tomatoes, etc. [4]. Melatonin can protect cell structure, prevent DNA damage, reduce peroxide levels by eliminating free radicals, and thus increase resistance to oxidation and reduce lipid peroxidation [5]. Melatonin has also been reported to inhibit plant tissue browning and has been suggested as an effective antioxidant in preventing the oxidation of phenolic compounds [6]. Studies in recent years have revealed that melatonin treatment can promote tomato fruit ripening and improve its storage quality [7]. Melatonin reduces chilling injury in peach fruit by reducing fatty acid content and phenolic compound metabolism and prevents physiological deterioration of the product [8]. Many studies have been conducted on various fresh-cut fruits to increase shelf life, but the investigation on the effects of exogenous melatonin on enzymatic browning and nutritional quality of fresh-cut apple fruit is limited. Therefore, the current research is focused on selecting the appropriate concentration of melatonin to investigate its effects on enzymatic browning, phenolic compounds, ascorbic acid content, antioxidant capacity, and PAL and PPO enzyme activities in fresh-cut apple fruit during storage at refrigerated temperature.

2-Materials and Methods

Treatment of apple slices: Granny Smith apples with almost uniform size, color, shape and no damage were purchased from the local market and immediately transported to the laboratory. The apples were disinfected with 200 µL L^{-1} sodium hypochlorite (NaClO) solution for 2 min, then rinsed with distilled water 3 times and dried at room temperature. Also, all tools used were disinfected with NaClO solution to prevent further contamination. The apples were peeled and after removing the central part, each apple was cut into 12 equal slices and categorized into 15 groups of 12. Fresh slices were immediately treated with different concentrations of melatonin solution (0, 1, 10, 100 and 1000 µM) for 5 min (in 3 replicates). The treated fresh-cut apples were packed in polyethylene plastic bags and stored at 4°C with 95% relative humidity until the necessary tests

were performed on days 0, 2, 3, 4, 5, 6 and 7. For other tests, the samples were frozen with liquid nitrogen and stored at -80°C.

Measurement of browning index: Color changes of surface of fresh-cut apples were investigated using image analysis [9]. For this purpose, apple slices were photographed with a digital camera using a simulated Hunterlab device and the color index values (L*, a*, b*) were extracted in Photoshop software and then the browning index and percentage were calculated using the following equations. The standard index in color tests was barium sulfate powder.

$$1) \quad BI = \frac{(X-0.31)}{0.172} \times 100$$

$$X = \frac{a* +1.75 \times L*}{5.645 \times L* +a* -3.012 \times b*}$$

$$2) \quad BI\ increase\% = \frac{(BIx-BI0)}{BI0} \times 100$$

Measurement of Malondialdehyde and Hydrogen Peroxide

1) Malondialdehyde (MDA) content was measured by method of Sun et al. (2011) [10]. To begin 0.1 g of fresh-cut apple was homogenized in 1 mL of cold trichloroacetic acid (1 gL^{-1}) and centrifuged for 10 min at 4°C (12,000 rpm). Next, 0.6 mL of the extract was mixed with 1 mL of thiobarbituric acid (6.7 g L^{-1}) and reacted for 20 min at 95°C and quickly cooled on ice, then centrifuged for 10 min at 4°C (10,000 rpm). The absorbance was read at 450, 532 and 600 nm, respectively, and the following formula was used to calculate MDA and the result was expressed as µmol kg^{-1} FW.

$$3) \quad MDA\ (\mu mol\ g^{-1}) = 6.45 \times (A_{532} - A_{600}) - 0.56 \times A_{450}$$

2) Hydrogen peroxide (H_2O_2) content was measured according to method of Liu et al. (2018) [11]. To begin 0.9 mL of cold 1% trichloroacetic acid was added to 0.1 g of fresh-frozen sliced apples and centrifuged (12,000 rpm) for 10 min after mixing for 15 min at 4°C. Then, 1 mL of potassium iodide (1 mol L^{-1}) and 1 mL of potassium phosphate buffer (10 mM, pH 7) were added into 0.6 mL of the upper phase and homogenized. The absorbance at 390 nm was measured using a spectrophotometer. The H_2O_2 content was calculated based on the H_2O_2 standard curve and the result was expressed in mmol kg^{-1} FW.

Measurement of phenylalanine ammonia lyase and polyphenol oxidase enzyme activities

1) The activities of phenylalanine ammonia lyase (PAL) and polyphenol oxidase (PPO) enzymes were evaluated according to method of Gao et al. (2017) [12]. For PAL enzyme activity, 0.1 g of fresh-cut apples were mixed with 0.9 mL of 0.2 M borate buffer (pH 8.8) for 10 min at 4°C and centrifuged (10,000 rpm). Subsequently, 0.2 mL of the upper phase was mixed with 1.28 mL of borate buffer and 0.4 mL of 50 mM L-phenylalanine and incubated for 30 min at 30°C. Then, 80 µL of 6 M hydrochloric acid was added to stop the reaction, the absorbance was measured at 290 nm, and the result was expressed in U g^{-1} FW (U = 0.1 Δ290 nm min^{-1}).

2) For PPO enzyme activity, 0.2 g of fresh-cut apple was mixed with 1 mL of 0.1 M phosphate buffer (pH 6.5) for 10 min at 4°C and centrifuged (8000 rpm). Next, 0.2 mL of the upper phase was mixed with 0.6 mL of phosphate buffer and 0.2 mL of 0.2 M catechol and incubated first for 10 min at 37°C and then for 5 min at 95°C. Then 80 µL of 6 M hydrochloric acid was added to stop the reaction, the absorbance was measured at 420 nm and the result was expressed in U g^{-1} FW (U = 0.1 Δ420 nm min^{-1}) [12].

Measurement of total phenol content: The total phenol content of apple slices was evaluated by the Folin-Ciocalteau method [12]. After extracting and preparing the extract from fresh-cut apples and adding desired chemical solutions, the absorbance of samples was measured at 750 nm with a spectrophotometer and the total phenol was expressed as mg GAE kg^{-1} FW.

Measurement of ascorbic acid content: Ascorbic acid content of extracts from fresh-cut apple samples was measured by oxidation-reduction titration method using iodine solution. Through the standard curve of potassium iodate-ascorbic acid, the ascorbic acid content was calculated as mg kg^{-1} FW [13].

Antioxidant capacity measurement: Antioxidant capacity was measured by DPPH free radical scavenging method [14]. The absorbance of samples (control and extract) at 517 nm was measured with a spectrophotometer and antioxidant capacity was calculated as a percentage of DPPH scavenging capacity from the following equation:

$$4) \quad DPPH \text{ inhibition}\% = \frac{Ac - As}{Ac} \times 100$$

Where: Ac is absorbance of the control (DPPH solution) and As absorbance of the sample extract.

Statistical analysis: This study was conducted in a completely randomized design using a factorial method, in which the effects of melatonin concentration and storage time on quality characteristics of fresh-cut apples were investigated. Comparison of means was performed using Duncan's method to select the optimal melatonin treatment and t-student test to investigate the effect of the optimal treatment on quality, nutritional and shelf life characteristics. SPSS software (Version 22 SPSS Inc. Chicago, USA) was used for statistical analysis at a probability level of 5% ($P < 0.05$).

3-Results and Discussion

Effect of melatonin treatment on BI of fresh-cut apples: To determine the appropriate concentration of exogenous melatonin on quality of fresh-cut apples, five concentrations of melatonin were tested along with a control. Rapid enzymatic browning is the main limiting factor in processing and sale of fresh-cut apples. In fresh-cut apples, BI is considered as an important quality indicator in browning process. The results in Figure 1 show that browning index decreased from 1 to 10 µM melatonin (the lowest value) and increased from 10 to 100 and 1000 µM. So that the control sample (without melatonin treatment) had the highest (77.55) and the sample treated with 10 µM melatonin had the lowest (50.77) browning index ($P < 0.05$).

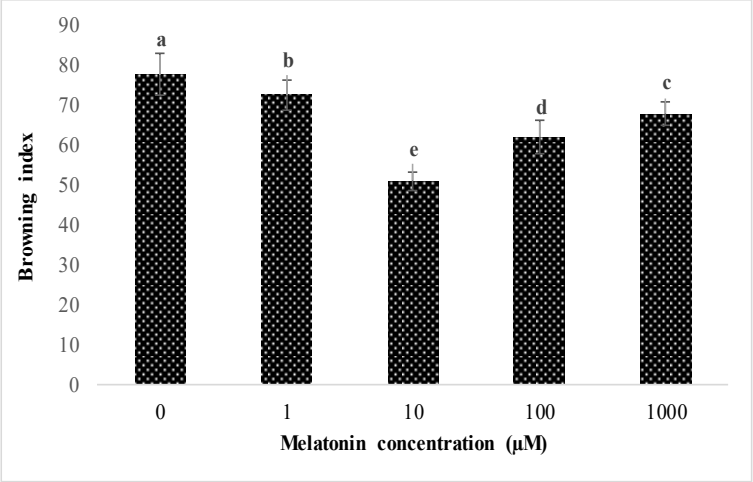

Fig. 1. Effects of melatonin concentration on browning index of fresh-cut apple fruit.
Values with different small letters are different at $P < 0.05$.

After determining the appropriate concentration of exogenous melatonin treatment, the treatment and storage of fresh-cut apples for 7 days with 10 µM melatonin and its comparison with control sample were evaluated. According to Figure 2, the browning index gradually increased in treated and control samples, but compared to the control, melatonin treatment inhibited the browning index during storage. So that after 7 days, the BI of fresh-cut apples treated with melatonin was significantly lower (41.86) than that of fresh-cut apples in the control (61.53) ($P < 0.05$). Similar results were also observed by Zhang et al. (2018) and Zhang et al. (2019) for litchi and pear fruits treated with melatonin, respectively [15, 16].

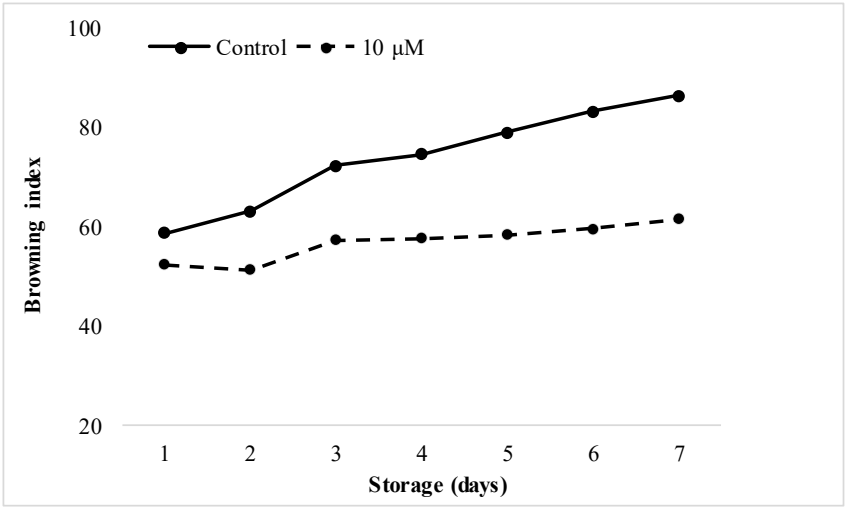

Fig. 2. Effects of storage time on browning index of fresh-cut apple fruit.

Effect of melatonin treatment on MDA and H_2O_2 content of fresh-cut apple: Lipid peroxidation is an important marker for membrane system damage and cellular metabolism degradation. To determine lipid

peroxidation in plant cell membranes, MAD and H_2O_2 are examined [8]. The accumulation of MAD and H_2O_2 can cause the destruction of cell membrane composition, development of brown polymer accumulation, and browning of the pericarp [17]. Figure 3a shows the changes in MDA content in fresh-cut apple fruit. The MDA content in control sample increased gradually from day 0 (1.5 µmol kg^{-1} FW) to day 7 (3.9 µmol kg^{-1} FW), but in fresh-cut apples treated with 10 µM melatonin, it increased from day 0 (1.4 µmol kg^{-1} FW) to day 5 (1.7 µmol kg^{-1} FW) and then decreased (1.3 µmol kg^{-1} FW) during storage. Therefore, fresh-cut apples treated with melatonin had lower MDA content during storage at refrigerated temperature compared to the control sample due to improved antioxidant capacity ($P < 0.05$).

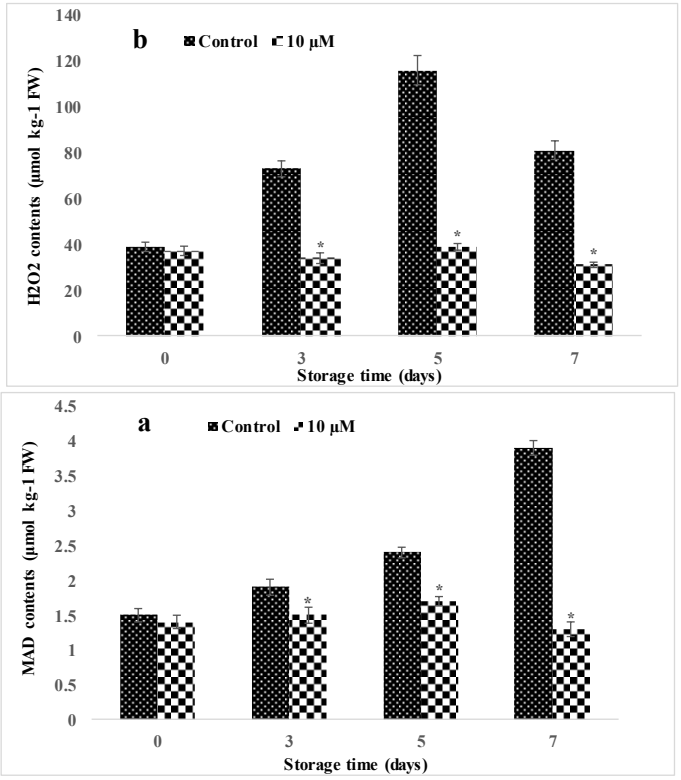

Fig. 3. Effects of melatonin treatment on content of MDA (a) and H_2O_2 (b) in fresh-cut apple fruit. Statistical significance was confirmed at *$P < 0.05$.

As shown in Figure 3b, the H_2O_2 content of control increased until day 5 (116 mmol kg^{-1} FW) and then decreased on day 7 (81 mmol kg^{-1} FW). Meanwhile, the H_2O_2 content of fresh-cut apples treated with melatonin (µM 10) remained lower than that of control during the storage period from day 0 (37 mmol kg^{-1} FW) to day 7 (31 mmol kg^{-1} FW) ($P < 0.05$). Similar results were observed with the MDA and H_2O_2 contents of melatonin-treated strawberries, which were significantly lower than those of the control [11]. Previous studies have shown that melatonin treatment increases the endogenous melatonin content of tomatoes and strawberries [18]. In addition, melatonin treatment was reported to increase the activity of reactive oxygen species (ROS) scavenging enzymes such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD),

and ascorbate peroxidase (APX). Therefore, H_2O_2 levels were lower in melatonin-treated cucumber and peach [6]. Our results showed that melatonin treatment at concentration of 10 µM decreased H_2O_2 accumulation in fresh-cut apples either directly or indirectly through the enhancement of endogenous melatonin accumulation and the release of ROS scavenging enzyme activity.

Effect of melatonin treatment on total phenolic content, ascorbic acid and antioxidant capacity of fresh-cut apples: Some bioactive compounds with antioxidant activity, such as phenolic compounds, have beneficial effects on human health. Phenolic content has been reported to be related to enzymatic browning in apple fruit [3]. According to Figure 4a, phenolic compounds in fresh-cut apples without melatonin treatment (control) gradually decreased from mg 460 GAE kg^{-1} FW on day 0 to 390 mg GAE kg^{-1} FW on day 7 during 7 days of storage. In contrast, in samples treated with melatonin, the amount of phenolic compounds gradually increased from 475 mg GAE kg^{-1} FW on day 0 to 525 mg GAE kg^{-1} FW on day 7 during storage ($P < 0.05$). Therefore, it is concluded that melatonin treatment (10 µM) significantly affected the phenolic compounds content of fresh-cut apples during storage at refrigerated temperature, which is consistent with the results of research of Xu et al. (2018) [19].

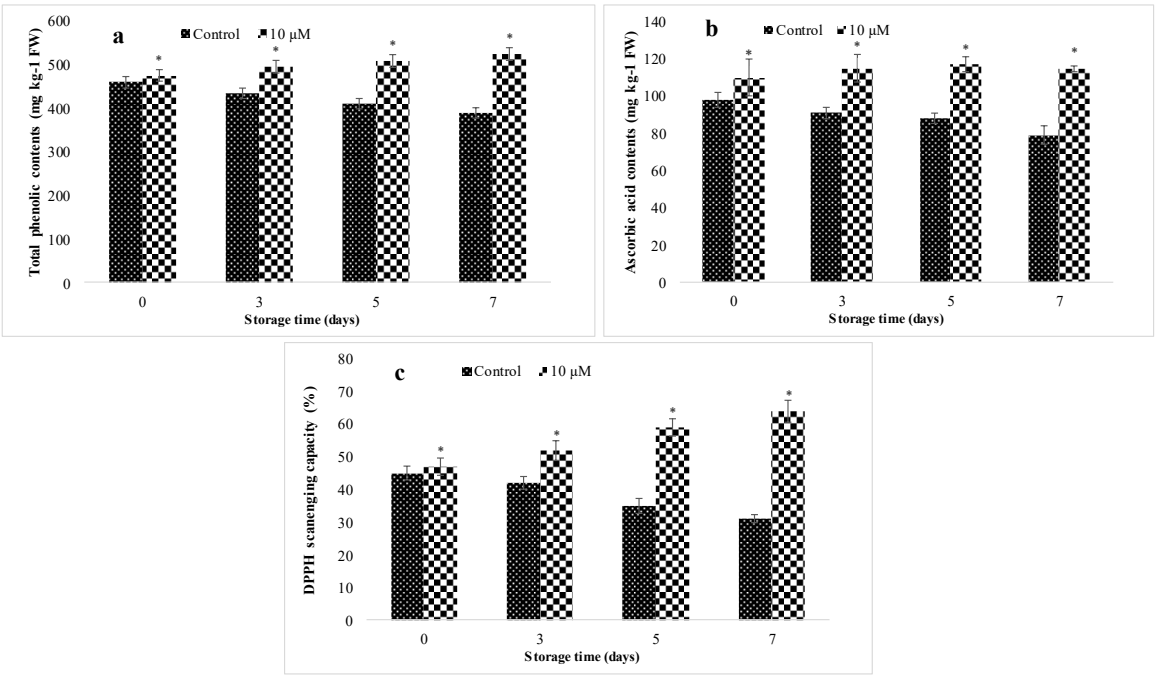

Fig. 4. Effects of melatonin treatment on total phenolic content (a), ascorbic acid content (b) and DPPH scavenging capacity (c) in fresh-cut apple fruit.

Statistical significance was confirmed at *$P < 0.05$.

As shown in Figure 4b, ascorbic acid content in control samples gradually decreased during the refrigerated storage period, from 98 mg kg^{-1} FW on day 0 to 79 mg kg^{-1} FW on day 7, which could be due to the degradation of cell membrane structure in fresh-cut apple fruit. Phenolic compounds are rapidly oxidized, and ascorbic acid, as a strong antioxidant, has the ability to reduce the browning of the samples. Therefore, the ascorbic acid content in control samples decreased rapidly during storage, but

in fresh-cut apples treated with melatonin, the ascorbic acid content remained stable from 110 mg kg^{-1} FW on day 0 to 115 mg kg^{-1} FW on day 7, so the ascorbic acid content was significantly higher than that in the control ($P < 0.05$).

DPPH assay was used to evaluate the antioxidant capacity of fresh-cut apples in this study. The changes in DPPH radical scavenging capacity of samples during refrigerator storage are shown in Figure 4e. DPPH scavenging activity significantly decreased in control fresh-cut apples from day 0 (45%) to day 7 (31%) during storage, which is consistent with previous studies on strawberries [20]. However, in melatonin-treated samples, it increased from day 0 (47%) to day 7 (64%) during storage ($P < 0.05$). The results also showed a clear trend between the increase in phenolic compounds and antioxidant capacity in melatonin-treated samples. The mechanism of melatonin treatment to reduce browning is through the elimination of free radical content in fresh-cut apples by inhibiting the conversion of o-diphenolic compounds to quinones [15].

Effect of melatonin treatment on PAL and PPO activity of fresh-cut apples: PAL enzyme plays a key role in the metabolism of phenolic compounds [21]. Figure 5a shows the comparison of PAL enzyme activity in control and melatonin (10 µM) treated fresh-cut apples. In treated fresh-cut apples, PAL enzyme activity increased significantly from day 0 (23 U g^{-1} FW) to day 7 (31 U g^{-1} FW), but in the control sample, it showed a decreasing trend from day 0 (19 U g^{-1} FW) to day 7 (8 U g^{-1} FW) during storage at refrigerated temperature ($P < 0.05$).

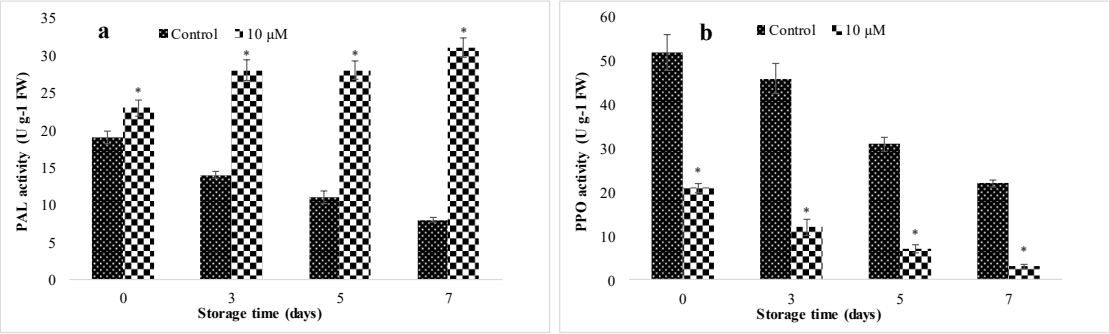

Fig. 5. Effects of melatonin treatment on the enzyme activity of PAL (a) and PPO (b) in fresh-cut apple fruit.

Statistical significance was confirmed at *$P < 0.05$.

PPO enzyme plays a key role in enzymatic browning [22]. The results of Figure 5b showed that PPO enzyme activity in control and melatonin-treated samples decreased during the storage period at refrigerated temperature. However, melatonin treatment significantly decreased PPO enzyme activity in treated fresh-cut apples compared to the control sample during storage from day 0 (21 U g^{-1} FW) to day 7 (3 U g^{-1} FW) ($P < 0.05$).

4-Conclusion

It can be concluded that melatonin treatment at a concentration of 10 µM was the most effective dose for controlling enzymatic browning and maintaining the quality of fresh-cut apples. In this treatment, MDA and H_2O_2 content significantly decreased during storage

at refrigerated temperature compared to the control. Total phenolic content, ascorbic acid, and antioxidant capacity increased significantly at this melatonin concentration. The results also showed that during storage of fresh-cut apples, compared to the control sample, the activity of PAL enzyme (phenolic compounds biocenter pathway) increased and, on the contrary, the activity of PPO enzyme (enzymatic browning factor) decreased. Therefore, melatonin treatment can be a useful strategy for maintaining quality and inhibiting enzymatic browning in fresh-cut apples.

Acknowledgements

This article is an extract from the research project No. 931, Section 6, dated 03/07/1400 and funded by the grant of University of Tabriz.

Data Availability

The data used to support the finding of this study are available from the corresponding author upon request.

Conflict Of Interest

The authors have no conflicts interest to report.

Funding Statemant

The researchers did not receive any specific grant from funding agencies the public, commercial or not-for-profit sectors.

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سایت مجله: www.fsct.modares.ac.ir

مقاله علمی-پژوهشی

اثر تیمار ملاتونین بر قهوه‌ای شدن آنزیمی، کیفیت تغذیه‌ای و ماندگاری میوه سیب تازه-برش

بیوک‌آقا فرمانی*[1] و مرتضی سلیمانی اقدم[2]

[1] دانشیار گروه علوم و صنایع غذایی، دانشکده کشاورزی و منابع طبیعی اهر، دانشگاه تبریز، تبریز، ایران.

[2] دانشیار گروه مهندسی علوم باغبانی، دانشکده کشاورزی و منابع طبیعی، دانشگاه بین‌المللی امام خمینی (ره)، قزوین، ایران.

اطلاعات مقاله

تاریخ های مقاله :

تاریخ دریافت: ۱۴۰۴/۰۱/۲۴

تاریخ داوری: ۱۴۰۴/۰۸/۰۶

تاریخ پذیرش: ۱۴۰۴/۰۸/۱۹

کلمات کلیدی:

تیمار ملاتونین،

شاخص قهوه‌ای شدن،

ظرفیت آنتی‌اکسیدانی،

میوه سیب تازه-برش

DOI: 10.48311/fsct.2026.84022.0

* مسئول مکاتبات:

bfarmani@tabrizu.ac.ir

چکیده

تاثیر تیمار ملاتونین بر کیفیت تغذیه‌ای و قهوه‌ای‌شدن آنزیمی میوه سیب تاره-برش بررسی شد. در مرحله اول، برش‌های سیب تازه-برش در ملاتونین با غلظت‌های ۰، ۱، ۱۰، ۱۰۰ و ۱۰۰۰ µM برای ۵ min تیمار شدند. با بررسی شاخص قهوه‌ای‌شدن در نمونه‌های نگهداری در یخچال (۴ °C) طی ۷ روز، غلظت ۱۰ µM ملاتونین با کمترین شاخص قهوه‌ای‌شدن (۵۰/۷۷) برای ادامه آزمایشات انتخاب شد. در مرحله دوم، سیب‌های تازه-برش با ۱۰ µM ملاتونین تیمار شدند تا طی ۷ روز نگهداری در ۴ °C، خصوصیات کیفی نمونه‌های تیماری در مقایسه با شاهد ($P < ۰/۰۵$) نشان داد تیمار نمونه‌ها به‌طور معنی‌دار باعث کاهش محتوای مالون‌دی‌آلدئید از روز صفر (۱/۴ µmol kg^{-1} FW) به روز ۷ام (۱/۳ µmol kg^{-1} FW) و پراکسید هیدروژن (H_2O_2) از روز صفر (۳۷ mmol kg^{-1} FW) به روز ۷ام (۳۱ mmol kg^{-1} FW) کاهش یافت. این تیمار طی دوره نگهداری، روند افزایشی در محتوای فنول کل از روز صفر (۴۷۵ mg GAE kg^{-1} FW) به روز ۷ام (۵۲۵ mg GAE kg^{-1} FW)، ظرفیت آنتی‌اکسیدانی از روز صفر (۴۷٪) تا روز ۷ام (۶۴٪) و مهار کاهش آسکوربیک اسید در نمونه‌ها را باعث شده بود. بعلاوه، تیمار ملاتونین باعث افزایش فعالیت آنزیم فنیل‌آلانین آمونیالیاز از روز صفر (۲۳ U g^{-1} FW) تا روز ۷ام (۳۱ U g^{-1} FW) و کاهش فعالیت آنزیم پلی‌فنول اکسیداز از روز صفر (۲۱ U g^{-1} FW) تا روز ۷ام (۳ U g^{-1} FW) طی نگهداری داشت ($P < ۰/۰۵$).