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Effect of melatonin application on biochemical changes and maintaining the quality of persimmon

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ABSTRACT

The purpose of this study is to determine the effects of melatonin dips on cold storage of the non-astringent 'Fuyu' persimmon cultivar. Persimmon fruits were subjected to different concentrations of melatonin (0 (control), 0.01, 0.1, and 1 mmol l^{-1} melatonin) and then stored at 0-1°C and 90-95 % relative humidity (RH) for 80 days. Weight loss, respiration rate, fruit firmness, total soluble solids (TSS), titratable acidity (TA), ascorbic acid, total phenolic, calyx appearance and chilling injury (CI) were examined at 20-day intervals. In the study, increasing doses of melatonin were found to be more effective in extending postharvest life. Among concentrations of melatonin application, 1 mmol l^{-1} melatonin-treated persimmon showed the preferable quality properties and lowest CI index. Moreover, this melatonin concentration caused a significant decrease in respiration rate and weight loss rate and maintained the fruit firmness, TSS and TA. While total phenolic content and ascorbic acid content were maintained by melatonin, it was determined that melatonin application had a non-significant effect on calyx appearance. Results obtained suggest that treating persimmon fruit with 1 mmol l^{-1} concentration of melatonin is effective in improving the postharvest quality and opens the possibility that higher concentrations of melatonin may be also effective.

Keywords: *Diospyros kaki* L., postharvest, melatonin, quality, chilling injury**.**

INTRODUCTION

Persimmon fruits are climacteric fruits, and inhibition of ethylene biosynthesis or action can play an important role in slowing the ripening process and increasing the storage life (Oz, 2011; Luo, 2007). Moreover, they are extremely susceptible to physiological defects, particularly skin and flesh discoloration during cold storage and shelf life, which may be associated with preharvest factors and storage conditions (Lee *et al*., 1993). Therefore, there is a need to provide postharvest technologies for postharvest losses in persimmons.
Plant melatonin is a versatile biological signal and its most prominent roles in plants include delay senescence, seed germination, plant development, flowering, photosynthesis and plant defense (Wang *et al*., 2020). Arnao and Hernandez-Ruiz (2018) reported that its role in fruit maturation and postharvest processes as a gene regulator of ethylene-related factors is relevant. The use of melatonin for the preservation of various postharvest fruits and vegetables is among the new research topics. It has been determined that exogenous melatonin applications after harvest in fruits and vegetables remove excess reactive

oxygen species (ROS) by increasing antioxidants, enzymes and enzymes associated with the repair of oxidized proteins (Kasım and Kasım, 2021). Previous reports have indicated that exogenous melatonin applications improve the quality of fruits and vegetables (Liu *et al*. 2018; Xu *et al*., 2019). Melatonin acts as an antioxidant by activating the antioxidant system and increasing the efficiency of other enzymatic and non-enzymatic antioxidants within the cell (Hu *et al*., 2016). The application of litchi and strawberry with melatonin enhanced the concentration of anthocyanin and phenolics content, maintaining the nutritional quality of the fruits (Aghdam and Fard, 2017; Zhang *et al*. 2018). Gao *et al*. (2016) and Zhai *et al*. (2018) reported that the melatonin application to peach pear fruits significantly reduces respiratory rate and inhibits respiratory climacteric under cold storage conditions. In addition, exogenous melatonin application alleviated CI and reduced the decay rate in plum, peach guava, sweet cherry and nectarine fruits (Bal, 2019; Cao *et al*., 2018; Wang *et al*., 2019; Bal, 2021). However, there is no information about the effects of melatonin as a postharvest application on postharvest life and persimmon fruit quality. The purpose of this study was to examine the potential use of melatonin as a postharvest tool to preserve the quality and extend postharvest life of persimmon cv. Fuyu.

MATERIALS AND METHODS

Non-astringent 'Fuyu' persimmon fruits (*Diospyros kaki* L.) were picked from an orchard in Tekirdag, Turkey. Fruits were harvested at their commercial maturity stage (yellowish-green), and transferred to the laboratory immediately. Persimmon fruits were selected for uniform size, firmness, color, and no disease symptoms, then randomly divided into four groups with three replicates each (9 kg per replicate). For applications, four solutions were prepared (control (distilled water), 0.01 mmol l^{-1}, 0.1 mmol l^{-1} and 1 mmol l^{-1} melatonin containing 0.05% Tween 80) and immersed in the solution for 30 minutes. Then, the fruits were dried in air at room temperature for approximately 1 hour and were packed in a plastic basket with polypropylene trays. Fruits were stored at 0-1^{o}C and 90–95% humidity for up to 80 days. Persimmons were analyzed for the physico-chemical and biochemical attributes at 0, 20, 40, 60, and 80 days.

Weight loss was determined by weighing the persimmon fruits before and after storage. Results are expressed as the percentage of weight loss concerning the first weights. The handheld penetrometer equipped with an 8-mm diameter plunger probe was used for the measurement of the firmness of persimmon fruit. It was expressed in Newtons (N).

For the analysis of TSS content and TA of each application, a homogenous juice sample was prepared. In the juice, TSS was determined with a hand refractometer (%). Acidity was evaluated by titration method and calculating the result as g 100 g^{-1} of malic acid.

For measurement of the respiration rate of persimmon fruit was used the static method was in a closed system with a gas analyzer (Systech Gaspace Advance, UK) at 20°C. (CO_2 kg $^{-1}$h $^{-1}$). Ascorbic acid content was measured in persimmon fruits by the titration method according to the method defined previously by AOAC (1990) and expressed as mg 100 g^{-1}.

To measure the total phenolic content, the Folin Ciocalteu reagent method was used (Slinkard and Singleton 1977) and expressed as mg 100g^{-}1 gallic acid equivalent fw.

The appearance of the calyx was determined with a 0–4 scale (Golding *et al*., 2020), where 0= fully green, fresh; 1= slight browning; 2= moderate browning/drying, 3= severe browning/drying and 4= completely brown/dried. CI degree (jelling and browning) shown in a 0 to 4 stage rating: 0 as (no symptoms), 1 (<25% symptoms), 2 (25%–50% symptoms), 3 (50%–75% symptoms), and 4 (>75% symptoms) (Nasr *et al*. 2021). CI index (%) = [∑ (CI levels) x (number of fruits at this level)/ (total number of fruits in the application) x 4] x 100. The experiment of the storage quality data was subjected to analysis of variance using SPSS software (Ver. 15). Means of different applications were compared by the LSD test ($p < 0.05$). The values are reflected as the means with their standard error.

RESULTS AND DISCUSSIONS

According to Figure 1(a), the weight loss of persimmon fruits showed an increasing trend with the advancement of maturity stages and storage time regardless of the applications. During the investigation period, no significant difference was noticed between the weight loss results by control and by melatonin applications except to 1 mmol l^{-1} melatonin. At the end of the experiment, the highest weight loss (7.4%) was found in treated fruits with 0.01 mmol l^{-1} melatonin, while the lowest weight loss (6.3%) was found in treated fruits with 1 mmol l^{-1} melatonin application. Less weight loss was recorded in 1 mmol l^{-1} melatonin treated fruit as compared to the control fruit, which might be associated with the ability of melatonin to maintain cellular stability (Madebo *et al*., 2021) and also the suppression of respiration (Wang *et al*., 2020). This indicates was consistent with Liu *et al*. (2018) and Bal (2019) who found that melatonin application significantly delayed the increase in weight loss in strawberry and plum fruit during storage.

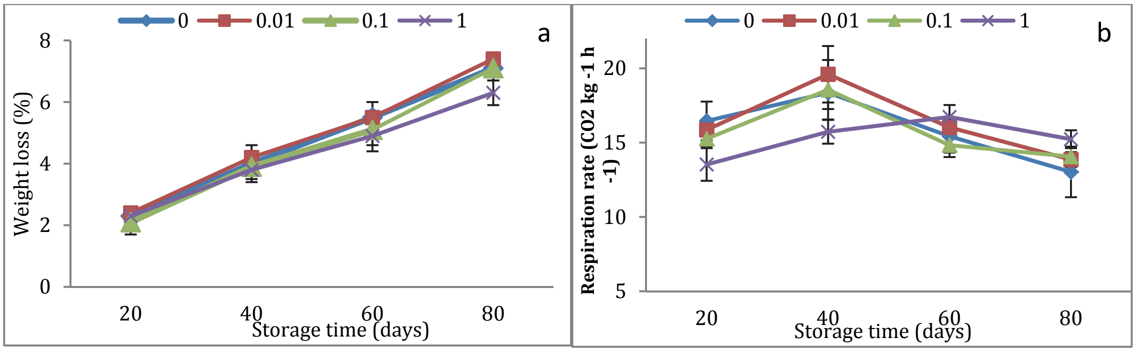

Figure 1 (a, b). The effect of different levels of postharvest application of melatonin on weight loss (a) and respiration rate (b) of persimmon cv. "Fuyu"

In persimmon fruit, the fruit depends on the calyx for gas exchange, as there are no lenticels or stomata on the fruit surface, which is covered with a waxy cuticle (Perez-Munuera *et al*., 2009). As shown in Figure 1(b), persimmon fruits showed an increase in the respiratory rate, then a decline in the later stages of storage. All the applications reached maximum climacteric on the 40th and 60th day of storage. Control, 0.01 mmol l^{-1} and 0.1 mmol l^{-1} melatonin application groups produced the highest amount of CO_2 (18.36 CO_2 kg^{-1} h^{-1}, 19.6 CO_2 kg^{-1} h^{-1} and 18.56 CO_2 kg^{-1} h^{-1} respectively) on 40th day while 1 mmol l^{-1} melatonin dose had the highest CO_2 production (16.73 CO_2 kg^{-1} h^{-1}) on 60th day. Among all melatonin applications, 1 mmol l^{-1} melatonin application had the smallest climacteric peak value and its peak respiratory rate was delayed by 20 days. The present study showed that postharvest 1 mmol l^{-1} melatonin application significantly inhibited the respiratory intensity of persimmon fruit, and also delayed the climacteric peak time. The current result was also supported by the beneficial effects of melatonin on delaying the ripening and inhibiting respiration rate of peach (Gao *et al*., 2016; Zhai *et al*., 2018), and sweet cherry (Bal *et al*., 2022). In the study, in parallel to flesh gelling development, a decrease in fruit firmness was observed in treated and non-treated fruits during storage, without differences among the applications. In addition, a negative relationship between weight loss and softening of persimmon was observed (Koyuncu *et al*., 2005). Figure 2 confirms that the fruit firmness (initial value 73.3 N) decreased with the increase in the storage period. Fruits treated with 1 mmol l-1 melatonin had the highest firmness value at

80 d, although they lost about 54% of the firmness value. The lowest flesh firmness was in fruit from the 0.01 mmol l^{-1} melatonin application (23.3 N) at the end of storage, and the results of the control fruit did not differ significantly from the 0.01 mmol l^{-1} melatonin application. The decelerated firmness loss with the postharvest melatonin application may be due to the effect of melatonin on decreasing activities of fruit softening enzymes which are responsible for hydrolyzing glycosidic linkage in the cell wall integrity, which is directly related to ripening and senescence. Similarly, decreased fruit softening by melatonin applications were reported in sweet cherry (Wang *et al*., 2019), mango (Liu *et al*., 2020) and nectarine (Bal, 2021).

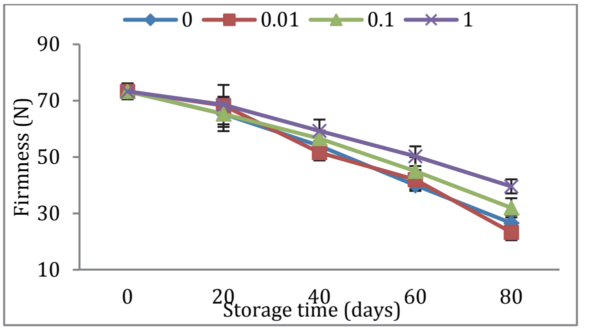

Figure 2. The effect of different levels of postharvest application of melatonin on the firmness of persimmon cv. "Fuyu"

In the present study, irrespective of melatonin applications, the TSS of fruits increased during storage (Figure 3a). The increase in TSS contents of the fruits during the storage period might be due to the continuous respiration and water loss of fruits. This result is parallel to that of Ozdemir *et al*. (2009) and Khan *et al*. (2017), who found that TSS in persimmon fruits increases by extending the storage duration. At the end of the storage, maximum TSS (17.8%) was noticed in 0.01 mmol l^{-1} melatonin followed by control fruit (17.4%) and minimum (15.9%) was present in fruit of 1 mmol l^{-1} melatonin application. Overall results revealed the ability of 1 mmol l^{-1} melatonin application for suppressing the accumulation of TSS in stored persimmon fruits as compared to low melatonin doses and control fruit. Previous studies have reported preventing an increase in fruit TSS with melatonin for plum (Bal, 2019), strawberry (Liu *et al*., 2018) and mango (Bhardwaj *et al*., 2022). Fruit acidity expressed a behavior opposite to the TSS accumulation and decreased with fruit ripening during the storage period.

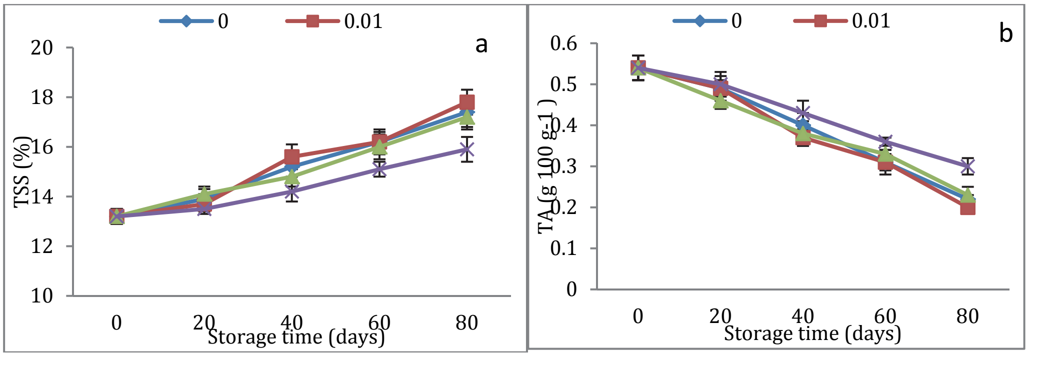

Figure 3 (a, b). The effect of different levels of postharvest application of melatonin on TSS (a) and TA (b)of persimmon cv. "Fuyu"

In the study, TA content, which was 0.54 g 100 g^{-1} at the harvest, gradually decreased in the control and the treated fruit, as seen in Figure 3b. The decline in the titratable acidity is often used as an indicator of maturity as organic acids decrease during the ripening process. However, no significant difference in TA content was observed between untreated and melatonin treated persimmon fruits within the first 60 d of storage. However, there are no significant differences between applications within the first 60 days of storage in terms of acidity. At 80 d of storage, 1 mmol l^{-1} melatonin application significantly delayed the decrease in TA content compared to untreated and other melatonin applications. At the end of the experiment, the highest TA was recorded in 1 mmol l^{-1} melatonin application (0.30 g 100 g^{-1}), while the lowest TA was recorded in 0.01 mmol l^{-1} melatonin application (0.20 g 100 g^{-1}) followed by control fruit (0.22 g 100 g^{-1}). This study demonstrates that with proper dosage of melatonin could retain the level of TA content in persimmon fruit, which is consistent with most of the studies so far such as strawberry (Liu *et al*., 2018), sweet cherry (Wang *et al*., 2019) and guava (Mirshekari and Madani, 2022).

Persimmon fruit is a good source of ascorbic acid and polyphenols that have antioxidant properties. In the study, initial ascorbic acid content of persimmon fruits (44.5 mg 100 g^{-1}) decreased with the progress in the storage period (Figure 4, a). This result agrees with Khan *et al*. (2017) and Direito *et al*. (2021) who found that ascorbic acid of persimmon fruits decreased during storage. However, this decrease was less pronounced in fruits treated with melatonin. At the end of the experiment, ascorbic acid was minimum (27.4 mg 100 g^{-1}) in the control application while maximum (31.3 mg 100 g^{-1}) in 1 mmol l^{-1} melatonin application. Melatonin treated persimmon fruits showed less reduction in ascorbic acid, probably due to the fact that melatonin delays the oxidation of ascorbic acid by inhibiting the enzyme responsible for this oxidation. Similar results were also reported by Gao *et al*. (2016) and Bal (2021) who documented that melatonin prevents the reduction of ascorbic acid and maintains the quality of peach and nectarine fruits during the storage period. Ma *et al*. (2021) also reported that ascorbic acid content in oranges showed a downward fluctuating trend in the control group and melatonin application inhibited vitamin C degradation, producing higher levels by 17.45% compared to the control fruits during storage.

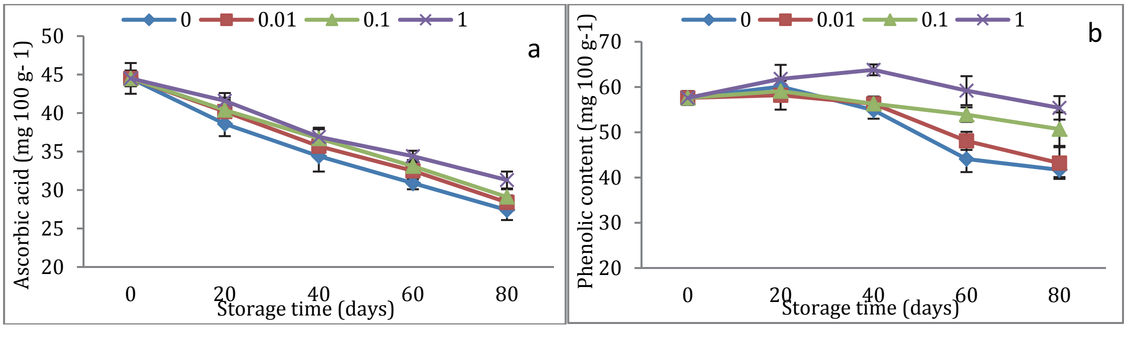

Figure 4 (a, b). The effect of different levels of postharvest application of melatonin on ascorbic acid (a) and phenolic content (b) of persimmon cv. "Fuyu"

Persimmon fruit is a good source of nutritional antioxidants, carotenoids and polyphenols. In the study, total phenolic content was 57.6 mg 100 g^{-1} at the harvest. Regardless of the applications, total phenolic content increased during the first period of storage, then decreased (Figure 4, b). There was no significant difference between the total phenolic content of the control group and that of the treated groups at 20 d. However, in remaining analysis periods, 1 mmol l^{-1} melatonin application effectively suppressed the phenolics degradation compared with control. At the end of the storage, persimmon subjected to 1 mmol l⁻¹ melatonin application had the greatest value of the phenolic content (55.4 mg 100 g⁻¹) followed by 0.1 mmol l⁻¹ melatonin application, while the control fruit had the lowest phenolic content (41.7 mg 100 g⁻¹) with no significant difference with the phenolic content of the fruit treated by 0.01 mmol l⁻¹ melatonin application. This protective effect of melatonin on phenolic content is in agreement with previous results obtained by Aghdam and Fard (2017) in strawberry fruit, Bal *et al*. (2022) in sweet cherry fruit and Bhardwaj *et al*. (2022) in mango fruit. Michailidis *et al*. (2021) also noted that melatonin application induced phenolic compound accumulation, through the up-regulation of various related genes at harvest and specifically at cold storage for 12 days. Persimmon fruit possesses a large green four-lobed calyx which is considered a gas exchange organ. The calyx darkens during fruit ripening, which affects external fruit quality (Vilhena *et al*., 2022). In the present study, the calyx looked green and fresh at harvest, and then in parallel with the storage period, an increase in desiccation symptoms was determined. Drying and browning symptoms started from the apical part of the lobes toward the basal part. This browning reaction is believed to be induced by water loss and chlorophyll degradation in calyx (Golding *et al*., 2020). All applications displayed a similar pattern for the calyx appearance during storage and did not show significant differences (Figure 5, a). At the end of the experiment, the lowest calyx browning/drying was exhibited in 1 mmol l⁻¹ melatonin treated fruits (2.7), the highest calyx browning/drying was recorded in control and 0.01 mmol l⁻¹ melatonin application fruit (3) followed by 0.1 mmol l⁻¹ melatonin application (2.9).

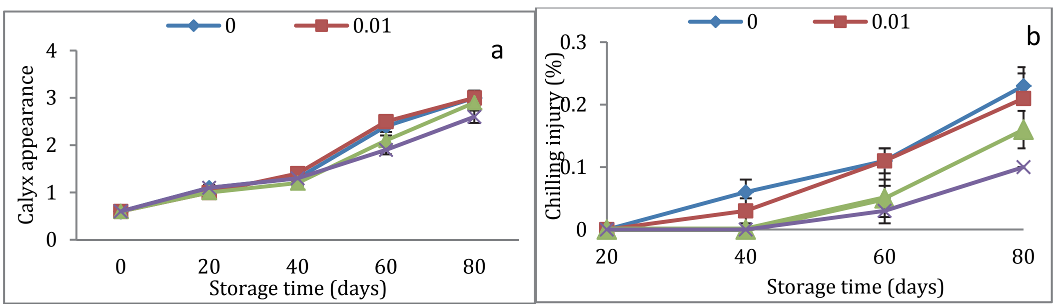

Figure 5 (a,b). The effect of different levels of postharvest application of melatonin on calyx appearance (a) and CI (b) of persimmon cv. "Fuyu"

'Fuyu' persimmons are very sensitive to CI, which is expressed by rapid fruit softening, flesh browning, translucency during and after storage (Crisosto, 2013). Figure 5(b) confirms that CI increased in control and treated fruits as the day of storage progresses. While no CI symptoms were observed during the first 20 days, CI symptoms were detected at a low level only in control and 0.01 mmol l⁻¹ melatonin treated fruits at 40 d. The findings showed that with increasing concentration of melatonin application, CI symptoms were observed less frequently during storage. The reduction in CI symptoms by melatonin may be related to increase the antioxidants in favor of membrane integrity and thus reduced cellular and tissue damage (Ze *et al*. 2021). The highest flesh jelling and browning symptoms were observed in control fruits and the lowest level of CI in 1 mmol l⁻¹ melatonin application. A low level of CI in the fruit treated by melatonin during cold storage was also reported for peach (Gao *et al*., 2016; Cao *et al*., 2018), pomegranate (Jannatizadeh, 2019), mango (Bhardwaj *et al*., 2022) and guava (Mirshekari and Madani, 2022). In agreement with those references, the current study confirms the exogenous melatonin application has been shown to improve chilling tolerance and reduce CI of postharvest fruit during cold storage.

CONCLUSIONS

The present results showed that the anti-ripening efficacy of melatonin applications could be significantly affected by the concentration applied, among other possible factors. Postharvest melatonin application, specifically using 1 mmol l^{-1} dose, protected the quality of persimmon fruit during storage. This application could prevent the reduction of physicochemical properties of the persimmon fruit, such as firmness, TA, ascorbic acid and phenolic compounds. Moreover, melatonin applications could successfully improve chilling tolerance and reduce CI symptoms. In conclusion, the prestorage dipping of 'Fuyu' persimmons in 1 mmol l^{-1} melatonin performed best to preserve their quality and can be effectively used for long-term storage.

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