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ORIGINAL PAPER

Melatonin treatment improves nutritional value and antioxidant enzyme activity of *Physalis peruviana* fruit during storage

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Abstract
Physalis peruviana is a perishable fruit with a restricted shelf-life that exhibits a climacteric performance. The aim of this study was to evaluate the effect of different concentrations of melatonin treatment (0, 100, 200 and 300 µmol L^{-1}) on extending shelf life and improving nutritional quality of *P. peruviana* fruit storing at 10 ± 1 °C and 90 ± 1% relative humidity for 21 days. The results showed that application of melatonin at 200 and 300 µmol L^{-1} was notably effective in reducing weight loss of fruit. Senescence of Physalis fruit was obviously retarded by melatonin treatment, as revealed by the firmness, total soluble solids (TSS) content, titratable acidity (TA), and TSS:TA ratio of the fruit. Melatonin treatment at 200 µmol L^{-1} melatonin significantly reduced the respiration rate. At 300 µmol L^{-1} melatonin increased the total phenolic and carotenoid, resulting in the higher antioxidant capacity and nutritional value. Melatonin significantly increased the activity of catalase, superoxide dismutase and ascorbate peroxidase enzymes during storage. The optimum concentration of melatonin for extending the postharvest life and improving the quality of Physalis fruit was 200 or 300 µmol L^{-1}. These findings recommended that melatonin treatment may be a convenient method to extend the postharvest life and to improve nutritional quality in Physalis fruit.

Keywords Enzyme activity · Melatonin · *Physalis peruviana* · Postharvest life · Quality · Respiration rate

Introduction

Physalis (*Physalis peruviana* L.) belonging to the Solanaceae family, has been categorised into the small-fruit group and is originated from tropical south America. Since this plant shows the rapid growth and high yield, it has been introduced as one of the promising tropical fruits [1, 2] that cultivated in tropical regions worldwide, in some part of Iran, and other countries in last two decades. Physalis fruit is small and sweet, contains high levels of A and C vitamins as well as carotenoids, flavonoids, and alkaloids [3]. Researchers have found that Physalis showed a significant medicinal property [1, 4–6]. Considering the involvement in the list of "superfruits", Physalis has stimulated the interest of many researchers. This important position is due to the significant economic value, ornamental, medicinal and nutritional attributes [5, 7].

Physalis is a perishable fruit with a restricted shelf-life that shows a climacteric pattern with an enhancement of ethylene biosynthesis at the climaterium phase [1]. During ripening, the green color of fruit changes into orange owing to chlorophyll degradation and carotenoid accumulation and gradual softening happens (Fig. 1) [1]. Thus, there is an urgent need for reducing decay and extending the postharvest life of Physalis fruit. To date, various postharvest treatments including refrigeration and 1-methylcyclopropene (1-MCP) [1], edible coating [2], chitosan coating [8], chitosan and Rue (*Ruta graveolens* L.) essential oil [9], and gelatin and calcium chloride [10] have been applied into postharvest preservation of the physalis fruit. Nevertheless, some of these methods are not commercially reasonable

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Fig. 1 Different stages of fruit maturation in *Physalis peruviana* L.

due to low customer preference or need for verifying the effectiveness. Accordingly, it is still required to find ways to slow down senescence to reduce the post-harvest loss and to improve the quality of physalis fruit.

Melatonin (N-acetyl-5-methoxytryptamine) is a naturally occurring low molecular weight indole-based metabolite was identified in plants in 1995 [11]. The biosynthesis of melatonin is accomplished via the shikimate pathway and from its amino acid precursor tryptophan [12]. Melatonin, as an evolutionary biological signal regulating various plant biological processes including seed germination, vegetative and reproductive growth, maturation, senescence, and responses to biotic and abiotic stresses [13–19]. Also, decisive aspect is its role in fruit ripening, preservation of various fruits, and improving fruit quality [19, 20]. Melatonin also shows high capabilities in scavenging toxic reactive oxygen species (ROS) and antioxidant capacity in some fruits and vegetables, such as bananas, cucumber, cassava, peaches, mango, and litchi [14, 18, 21]. Recently, exogenous melatonin treatment has been applied to retard changes in color, firmness, total soluble solids content and titratable acidity of strawberry fruit [22], enhance the accumulation of total phenols and anthocyanins of litchi [16], retard the decrease of kiwifruit hardness [23], decline surface browning of fresh-cut pear fruit [24], and inhibit the respiration rate of climacteric fruit [25].

However, information regarding the effect of melatonin on postharvest quality and nutritional values of Physalis is limited. Therefore, the present study aimed to evaluate the effects of postharvest application of exogenous melatonin on reducing respiration rate, improving Physalis fruit quality and nutritional value during storage. The findings may obviously help in the development of new postharvest technique to extend the shelf life of Physalis.

Materials and methods

Fruit treatment

Green-Yellow Physalis fruits were collected from a commercial greenhouse of Shiraz in Fars Province (Lat. 29° 36′ 37.12″ N and Long. 52° 31′ 52.07″ E), which is located in the southwest of Iran. Physalis fruit of uniform size, bright coloration, without mechanical damage and symptoms of pest infestation were selected. Then they were transported to the laboratory in less than an hour using cardboard boxes. Thirty fruits per treatment were immersed into solutions of 100, 200 and 300 μmol L^{-1} melatonin for 5 min. (melatonin was first dissolved in ethanol and then diluted in water to the desired concentrations). Treatment using distilled water was served as the control. Treated fruit were air-dried at room temperature for 30 min. and then packaged in 0.03 mm polyethylene fresh-keeping bags ($10 \times 5 \times 20$ cm^3) with a perforation ratio at 3% and then stored at 10 ± 1 °C and 90 ± 1% relative humidity (RH) for 21 days. The fruit were removed from storage at intervals of one week for evaluation during twenty-one-day period.

Weight loss

Fruit weight loss was measured by a digital balance. Fruit was individually weighed at the beginning of storage and during each storage time. Weight loss was calculated using the following equation [26]:

$$weightloss(\%) = \frac{\text{Initialweightofeachsample(g)} - \text{Finalweightofeachsample(g)}}{\text{Initialweightofeachsample(g)}} \times 100$$

Evaluation of the fruit respiration rate

The content of CO_2 produced by the fruit respiration was determined by a CO_2 sensor (Altro, Taiwan). A specified amount of fresh Physalis was placed in an airtight glass chamber, incubated for 30 min at 25 °C and finally the results were expressed as mg of CO_2 per kg of fresh fruit in an hour [27].

Total soluble solid content (TSS), Titratable acidity (TA) and TSS:TA ratio

TSS was measured with a hand-held refractometer (ATAGO-B933475) and expressed as percent [28]. TA was determined by titration method using 0.1 N NaOH up to pH 8.2, according to Hosseinifarahi et al. [29]. The results were expressed in citric acid percentage. The TSS:TA ratio was calculated by dividing TSS by TA.

Firmness of the fruits

The firmness of fruits (10 fruits for each replication) was measured by a hand-held penetrometer (I-OSK- 10576). The firmness of each fruit was measured at two opposite points of the equatorial area by using a 3 mm probe. The results were expressed as kilo Pascal [30].

Antioxidant activity

Three milliliters of 80% acetone was added to 1.0 g of fruit samples and the suspension was kept overnight at 4 °C. The extract was centrifuged for 2 min (1000 g) and the supernatant was used for the evaluation of total phenolic content and antioxidant activity.

Antioxidant capacity was determined using the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) free radical scavenging assay described by Martins et al. [31] with some modifications. Briefly, 950 µL of 0.1 mM methanolic DPPH solution was mixed with 50 µL of methanolic extract. The mixture was placed at room temperature for 30 min and UV absorbance at 517 nm was recorded. DPPH scavenging activity was measured using the following equation:

DPPH free radical scavenging capacity (% inhibition) = 100 $(Abs_{control} - Abs_{sample})/Abs_{control}$

Antioxidant enzymes extraction and activity assay

The method described by Taghipour et al. [32] was performed used for the preparation of tissue extracts for antioxidant enzyme activity including catalase (CAT), ascorbate peroxidase (APX) and superoxide dismutase (SOD). 1.0 g of frozen fruit sample was homogenized in 4 mL of potassium phosphate buffer and the homogenate was centrifuged for 10 min (10,000 × g and 4 °C), and the supernatants was used for studying the following enzymes activity.

Catalase activity was measured by adding 950 µL of reaction solution (50 mM phosphate buffer, pH 7.0, 15 mM H_2O_2) to 50 µL of enzyme extract. The activity of CAT was reported in decreasing in absorbance at 240 nm and outlined in terms of unit per g of fresh weight [32].

The activity of the SOD enzyme was calculated according to the method of Taghipour et al. [32] and the absorbance of the reaction mixture (50 mM phosphate buffer (pH 7.0), 50 µL of enzyme extract, 13 mM *L*-methionine, 0.1 mM EDTA, 75 µM nitro-blue tetrazolium and 2 µM riboflavin) was taken at 560 nm. The reaction was started by using the light source of 15 W fluorescent lamps for 15 min. The result was expressed as unit per g of fresh weight.

APX activity was measured as the decrease in absorbance at 290 nm for 1 min in a 1 mL reaction mixture containing 950 µL of 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbate, 0.15 Mm H_2O_2, 0.1 mM EDTA and 50 µL of enzyme extract. The activity of APX was reported in unit per g of fresh weight [32].

Total phenolic content

Total phenolic content was determined using the Folin–Ciocalteu colorimetric method outlined by Pennycooke et al., [33] with some modifications. For this purpose, 135 µL of the fruit extracts was added to 750 µL of Folin–Ciocalteu reagent (10%) and 600 µL of 7.5% sodium carbonate. After incubating at room temperature for 60 min, the absorbance was read spectrophotometrically at 765 nm. The results were expressed as mg gallic acid per g of fruit fresh weight. Gallic acid was used as the standard for plotting the calibration curve.

Carotenoid content

To measure the carotenoid content of the sample, 0.1 g of the fruit peel was extracted in an extraction solvent containing 7 mL of dimethyl sulfoxide. After incubation at 65 °C for 30 min, the extract was adjusted to 10 mL and the absorbance was measured at 645, 663, and 470 nm. The content of carotenoid was reported in terms of micrograms per gram of fresh weight [34].

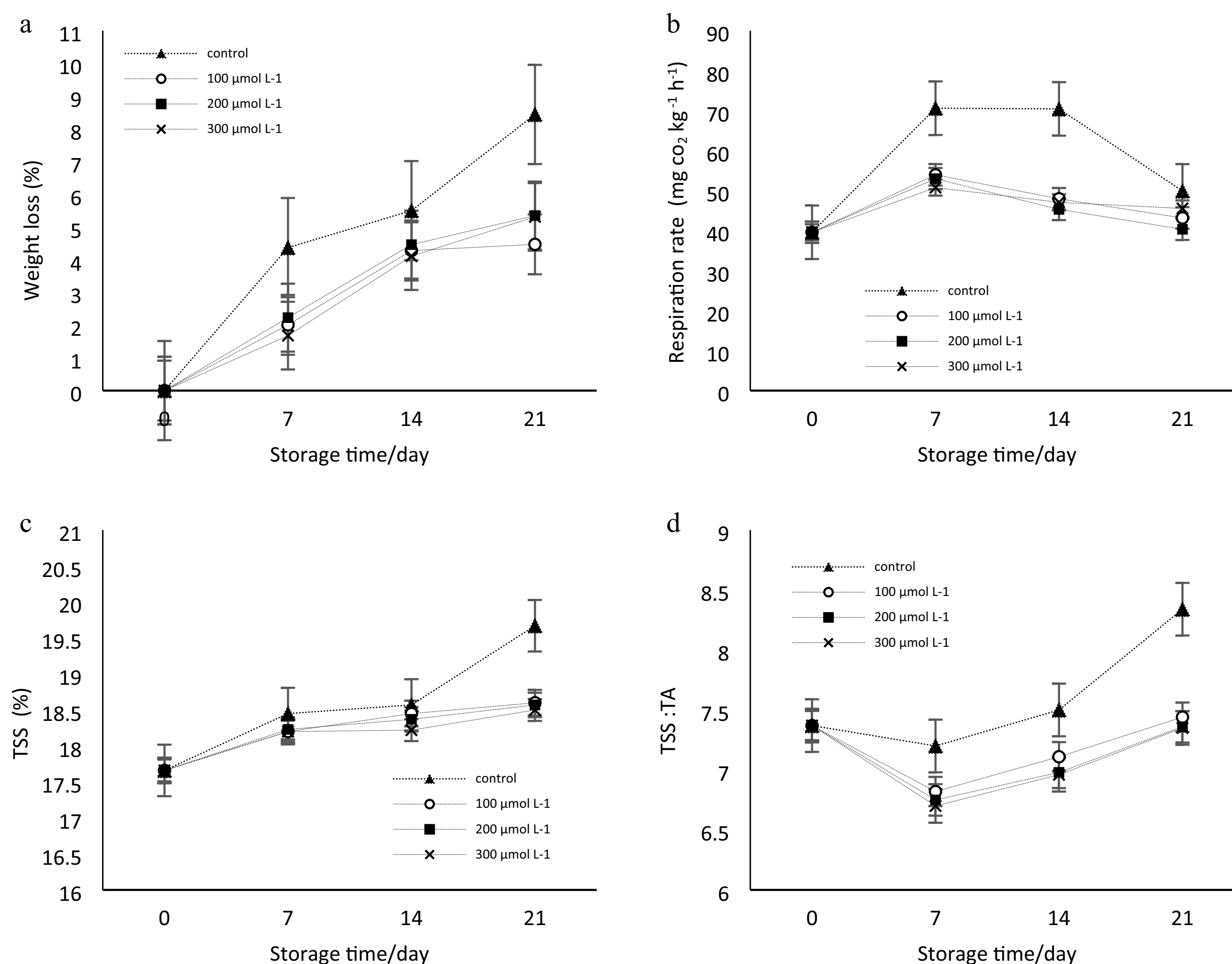

Fig. 2 The interaction between melatonin treatment and storage periods on weight loss (**a**), respiration(**b**), TSS (**c**) and TSS:TA ratio (D) of Physalis fruit during 21 days' storage at 10 ± 1 °C and 90% RH 85± 1%. Values are means ± SD from three replicates (n=3). Statistical analysis was performed using LSDtest

Phenylalanine ammonia-lyase (PAL) enzyme activity assay

The PAL activity was assayed based on the D'Cunha et al. [35] method and expressed as unit per g of fresh weight. In brief, 1 mL of 50 mM potassium phosphate buffer (pH 7), 0.5 mL of 10 mM phenylalanine, 0.4 mL of distilled water, and 0.1 mL of enzymatic extract were mixed for 1 h. at 37 °C. Following adding 0.5 mL of 6 M Hydrochloric acid, the reaction was ended and the absorbance was recorded in 290 nm. The content of PAL activity was expressed as unit per g of fresh weight.

Statistical analysis

A completely randomized design using factorial arrangement with 3 replications was used. The experimental data were subjected to analysis of variance (ANOVA) ($P < 0.05$), and the mean comparisons were performed using least significant differences. All statistical determinations were performed using SAS software package version 9.4.

Results and discussion

Weight loss

The postharvest weight loss in fresh fruits followed from the loss of water from the fruit surface by transpiration and respiration progressions. As shown in Fig. 2a, all fruits showed a progressive loss of weight with the advancement of storage time. The control Physalis fruits had higher loss of weight over all the storage period ($P < 0.05$). On the other hand, melatonin treatment significantly decreased the weight loss compared to the control

fruits during 21 d storage. At the end of the 21 days of storage, the weight losses of the treated *Physalis* fruits with 200 and 300 μmol L^{-1} melatonin were 5.35% and 5.31%, respectively; whereas the untreated fruit displayed about 8.44% weight loss. The reason for the preventing the weight loss in melatonin treated fruit was lower respiration rate along with better skin strength properties of the fruit, which confirmed slowing the process of fruit senescence and decay. Besides, many authors supported findings [14, 19, 22].

Respiration rate

Respiration rate reached a maximum value in control fruit, on day 7, however, the difference was not significant between day 7 and day 14 (Fig. 2b). Melatonin treatments declined respiration rate, and the difference between melatonin-treated and control was significant throughout the storage. Fruit treated with 200 μmol L^{-1} melatonin showed the lowest respiration rate at the end of the storage period.

Respiration rate is an outstanding indicative of fruit shelf life, which is corresponded to the metabolic activity of fruit. Physalis is a typical fruit that exhibits climacteric respiration [2]. The beneficial effect of exogenous melatonin treatments on lowering the respiratory consumption can also be attributed to improved preservation via a mechanism involving the prevention of respiration and delaying the Physalis ripening [19]. As the respiratory rate was declined, the weight loss of melatonin treated Physalis was decreased, and the TSS maintained at higher content.

The results of Liu et al. [36] showed that melatonin treatment in mango fruits led to the reduction of both the rate of respiration and a delay in presence of climacteric peak, which is consistent with the results of this research. In previous studies, beneficial effects of melatonin on preventing respiration rate and retarding the ripening of peach [18], pear [37] and broccoli [38] have been investigated.

TA, TSS and TSS:TA

Total acidity (TA) of Physalis fruit was significantly ($P < 0.05$) affected by the storage periods and showed a decreased trend with the progression of storage period. The lowest total acidity (2.47%) was registered in the end of storage (Fig. 3a, b). Nonetheless, the interaction between storage periods and melatonin treatment was found to be non-significant for total acidity in Physalis fruit (Data not shown).

Fruit TSS increased in both treated and untreated fruit during the 21 days of storage, but this increase was effectively delayed in response to melatonin treatments (Fig. 2c). At the end of storage, total soluble solids for control fruit was19.67% and for melatonin treated fruits was significantly lower than control fruits and maintained it better.

TSS:TA was increased during storage in all fruits. At the end of the 21 days of storage, fruits treated with melatonin showed significantly lower TSS:TA compared to the control (Fig. 2d).

TSS, TA concentrations and TSS:TA ratio are important factors to evaluate the fruit quality. Based on the available references, increasing the sugar content and decreasing the acidity of climacteric fruits with the advancement of storage time may be due to the development of the ripening process and this leads to change in hydrolytic processes and the transformation of polysaccharides into simple sugars. However, the melatonin treatment could retard the fruit senescence by inhibiting attributes of the flavour and taste of Physalis fruit. Previously, the positive effect of melatonin on maintaining the fruit quality has been reported [19, 36].

Firmness

Firmness is one of the important characteristics of fruits for evaluating its acceptability and increasing the potential for storage and mechanical damages [39, 40]. The firmness content was significantly ($P < 0.05$) affected by the melatonin treatment and storage periods (Data not shown). However, the effect of the interaction between storage periods and melatonin treatment was not significant. Fruit firmness reduced in Physalis fruit during the 21 days of storage, however this reduction was efficiently postponed in response to melatonin treatments (Fig. 3c, d). Retarding the firmness of Physalis fruits due to the melatonin application can be attributed to the fact that the melatonin might be decrease the activity of the softening enzymes, which in turn reduce the ethylene production. In previous studies, maintaining the fruit firmness of mango [14], peach [18] and nectarines [19] have been investigated. Given to the role of melatonin in delaying the disassembling cell wall by hindering polygalacturonase and cellulase gene expression in fruit and changes by hindering ethylene synthesis during storage, maintaining fruit firmness and less tissue deformability are expected [14, 18, 19].

Antioxidant activity

Fruits as rich antioxidant sources consist of variety of different antioxidant constituents. The influence of different treatment on DPPH-radical scavenging activity and reducing power was shown in Fig. 3e, f. The antioxidant activity was significantly ($P < 0.05$) affected by the melatonin treatment and storage periods. However, the effects of the interaction between storage periods and melatonin treatment was not significant. DPPH-radical scavenging activity determines non-enzymatic antioxidant activity in crops. In our study, DPPH-radical scavenging activities showed an increasing trend with the storage period. Additionally, melatonin treatments increased the DPPH-radical scavenging activities, so that, the highest value was observed in fruit treated with 300 μmol L^{-1} melatonin.

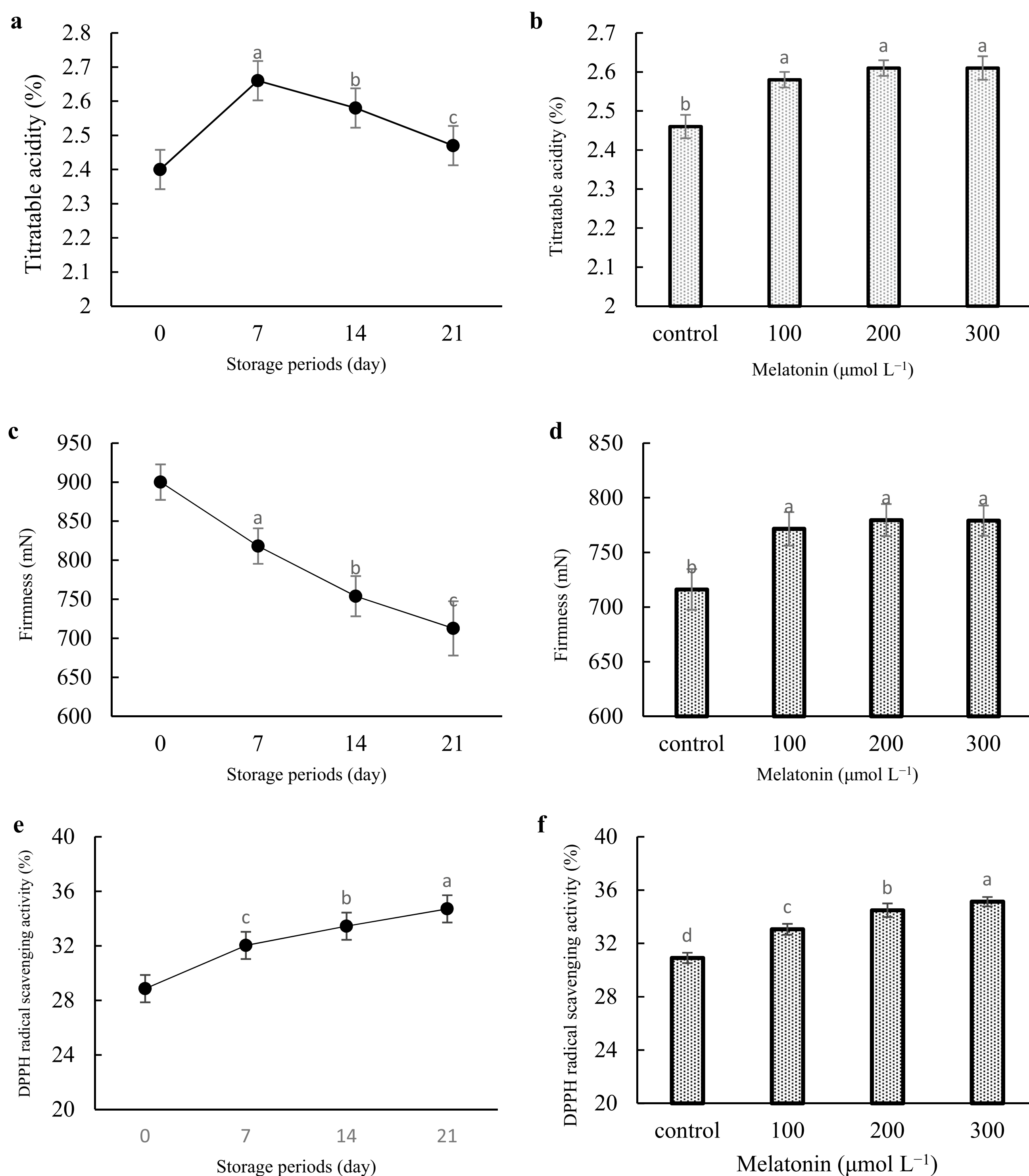

Fig. 3 Effect of storage periods (**a**) and melatonin treatment on titratable acidity (**b**). Effect of storage periods (**c**) and melatonin treatment on firmness (**d**). Effect of storage periods (**e**) and melatonin treatment on DPPH scavenging activity (**f**) of Physalis fruit during 21 days' storage at 10 ± 1 °C and 90% RH 85 ± 1%. Values are means ± SD from three replicates (n = 3). Statistical analysis was performed using LSD test

The increase in antioxidant activity in melatonin-treated fruit can be attributed to the fact that the melatonin plays an important role in increasing secondary metabolites such as phenolics, flavonoids, and ascorbic acid in treated fruits. Similarly, the antioxidant activity enhanced through exogenous application of melatonin in tomato [41], strawberry [22] and mango [14] during storage. Given to the effect of melatonin on antioxidant capacity, one of the reasons for the increase of this activity is the role of this material in enhancing antioxidant enzymes, non-enzymatic antioxidants, and enzymes related to oxidized protein repair, which leads to scavenge excess reactive oxygen species from postharvest fruits [19]. It seems that melatonin increases the antioxidant system of treated fruits through its effect on stimulating biosynthesis of endogenous melatonin, enhancing antioxidant enzymes activity and improving the bidirectional recovering of antioxidants for instance ASA-GSH cycling (Ascorbate-Glutathione cycle) [25]. Previous studies have shown that melatonin treatment effectively led to an increase in DPPH scavenging capacity in strawberry fruit [42]. Also, there was a direct correlation between antioxidant activity and phenolic compounds [22], which is in consistent with the results of this research.

Antioxidant enzymes activity

The generation of reactive oxygen species (ROS) and accumulation of free radicals increased in fruit during harvest, postharvest and aging process. Therefore, antioxidant enzymes including SOD, CAT, APX and GPX play an important role in reducing ROS and protecting the membrane integrity and vital macromolecules [43]. SOD as the first line of the defence system catalyses the superoxide anion to H_2O_2 and prevents the formation of destructive Hydroxyl radicals. CAT, APX and GPX enzymes also decompose it by converting H_2O_2 into water and oxygen. During oxidative stress, the increase in SOD enzyme activity along with the increase in hydrogen peroxide concentration can be regarded as stimulating defence responses, that increase the activity of other antioxidant enzymes such as CAT, APX and GPX, that in turn can be recognised as the inhibitors of hydrogen peroxide concentration [43].

As shown in Fig. 4a, SOD activity in control fruit was stable within the first 14 days of storage and then decreased until the end of storage, reaching final value of 3.36 U g^{-1} FW, while that in melatonin-treated fruit was higher, reaching the maximum level at the end of the storage in fruit treated with 300 μmol L^{-1} melatonin.

As shown in Fig. 4b, the control and the 100 μmol L^{-1} melatonin-treated samples showed a similar pattern of CAT activity during the whole storage. Though, treated samples had significantly higher enzyme activity than the control fruits so that the maximum enzyme activity in treated fruits with 300 or 200 μmol L^{-1} was recorded on day 14, respectively.

The APX activity in the control fruits decreased during the first two weeks, then sharply increased at the end of storage. The melatonin- treated fruits showed a different pattern of enzyme activity changes. In treated fruits, the enzyme activity was higher compared to the control, and decreased throughout the storage (Fig. 4c).

Enhancement of antioxidant enzyme activity is in agreement with the redox network model (a model that confirms an equilibrium between the possibly oxidizing power of cells and antioxidant agents), which elucidated that melatonin enhances the enzyme activities of SOD, CAT, and APX [23]. In previous studies, the positive effect of melatonin has been reported on increasing the redox enzyme activities in other fruits, such as sweet cherry [23], litchi [16], peaches [44], pears [37], strawberries [22], eggplants [45] and cassava [46].

The reason for the increase of the antioxidant enzyme by application of melatonin in Physalis fruit might be the ability of exogenous melatonin in retarding senescence and death-related protein destruction through enhancing activities and/or gene expression of antioxidant enzyme [16, 18, 47].

Phenol content, carotenoid, and PAL enzyme activity

According to Fig. 5a, a decrease in total phenol content was observed in control samples within the storage period. Melatonin treatments increased the total phenol content, and the difference between melatonin-treated and control was significant throughout the storage. Fruit treated with 300 μmol L^{-1} melatonin showed the highest total phenol with the value of 10.57 mg GA g^{-1}FW at the end of the storage period. Phenolic compounds as the most important secondary metabolites extensively found in fruits that represented antioxidant activity and also preserved sensory quality of fruits such as astringency, flavour, colour and bitterness [14].

The reduction in phenolic content of the control fruits throughout the storage seems to be associated with increases in polyphenol oxidase enzyme activity during senescence. Given to the role of melatonin in retarding oxidation of phenolics, a reduction in loss of phenolic compounds is expected [19]. There has been observed that the effect of melatonin treatment in enhancement of phenolic compounds in Jumbo blackberry species [20], litchi fruit [16, 42], nectarine [19], pomegranate [48], and litchi [16] was due to the increased transcription of genes upregulating main enzymes in the phenylpropanoid pathway, containing *PAL*, *C4H*, *CHS*, *CHI* and *F_3H*. Also, a great content of phenolic compounds was demonstrated in Physalis fruit [49]. Therefore, in this study, the enhancement of these compounds through

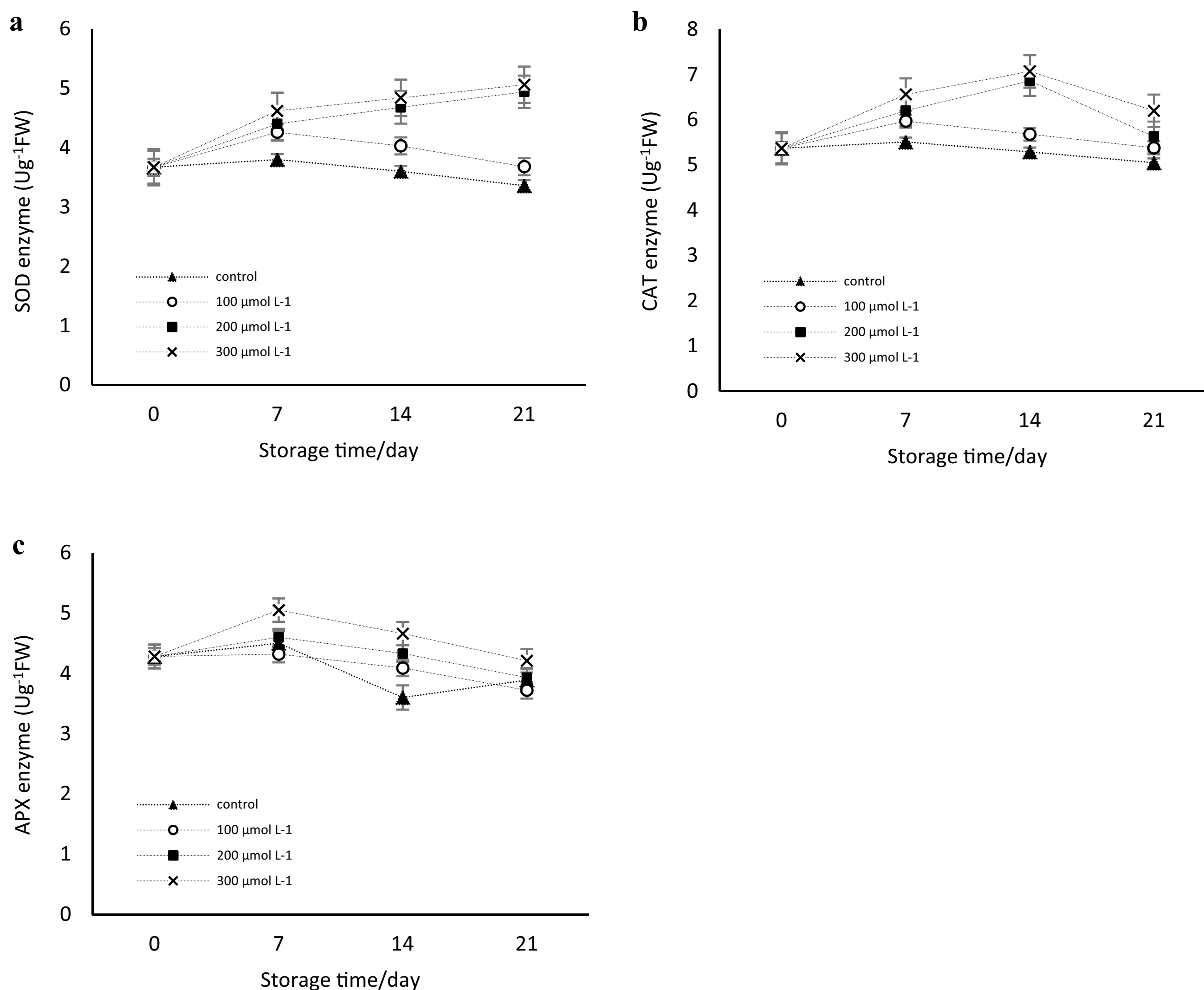

Fig. 4 The interaction between melatonin treatment and storage periods on antioxidant enzymes activity of Physalis fruit during 21 days' storage at 10 ± 1 °C and 90% RH 85 ± 1%. Values are means ± SD from three replicates (n = 3). Statistical analysis was performed using LSD test

melatonin treatment seems to result in promoting high levels of nutritional and antioxidant compounds in this fruit.

Carotenoids as the fruit pigments play an important role in the formation of fruit color as a crucial food quality feature, that indirectly influences taste, flavor perception, and consumer acceptance [50]. As shown in Fig. 5b, the control and the 100 µmol L^{-1} melatonin-treated samples showed a similar pattern of carotenoid content throughout the storage. However, treated samples had significantly higher carotenoid content than the control fruits. The treated fruits with 200 or 300 µmol L^{-1} melatonin showed a different pattern of carotenoid changes. In these treated fruit, the carotenoid content showed an upsurge trend during the whole storage period, so that in the final stage of storage, the concentration of 300 µmol L^{-1} melatonin showed the highest carotenoid value (8.06 µg g^{-1}FW). This carotenoid-promoting biosynthesis by melatonin can be explained because melatonin controls and encodes several carotenogenesis genes, thereby enhances the levels of some carotenes, consisting α and β carotene, zeaxanthin, lycopene and lutein. Moreover, the carotenoid biosynthesis is mediated by ethylene. Therefore, the role of melatonin in upregulating ethylene biosynthesis enzyme transcripts and ripening factors should be considered [51]. This carotenoid-promoting biosynthesis by melatonin has been also reported in tomato and microalgae [50, 52].

As shown in Fig. 5c, PAL activity was decreased gradually during storage, reaching a minimum level at the last storage in the control fruits. Melatonin-treated fruit showed a similar trend except in 300 µmol L^{-1} samples. The PAL activity in the 300 µmol L^{-1} melatonin-treated fruit reached the highest value at end of the storage (16.65 U g^{-1} FW). The increase in PAL activity due to the melatonin consumption

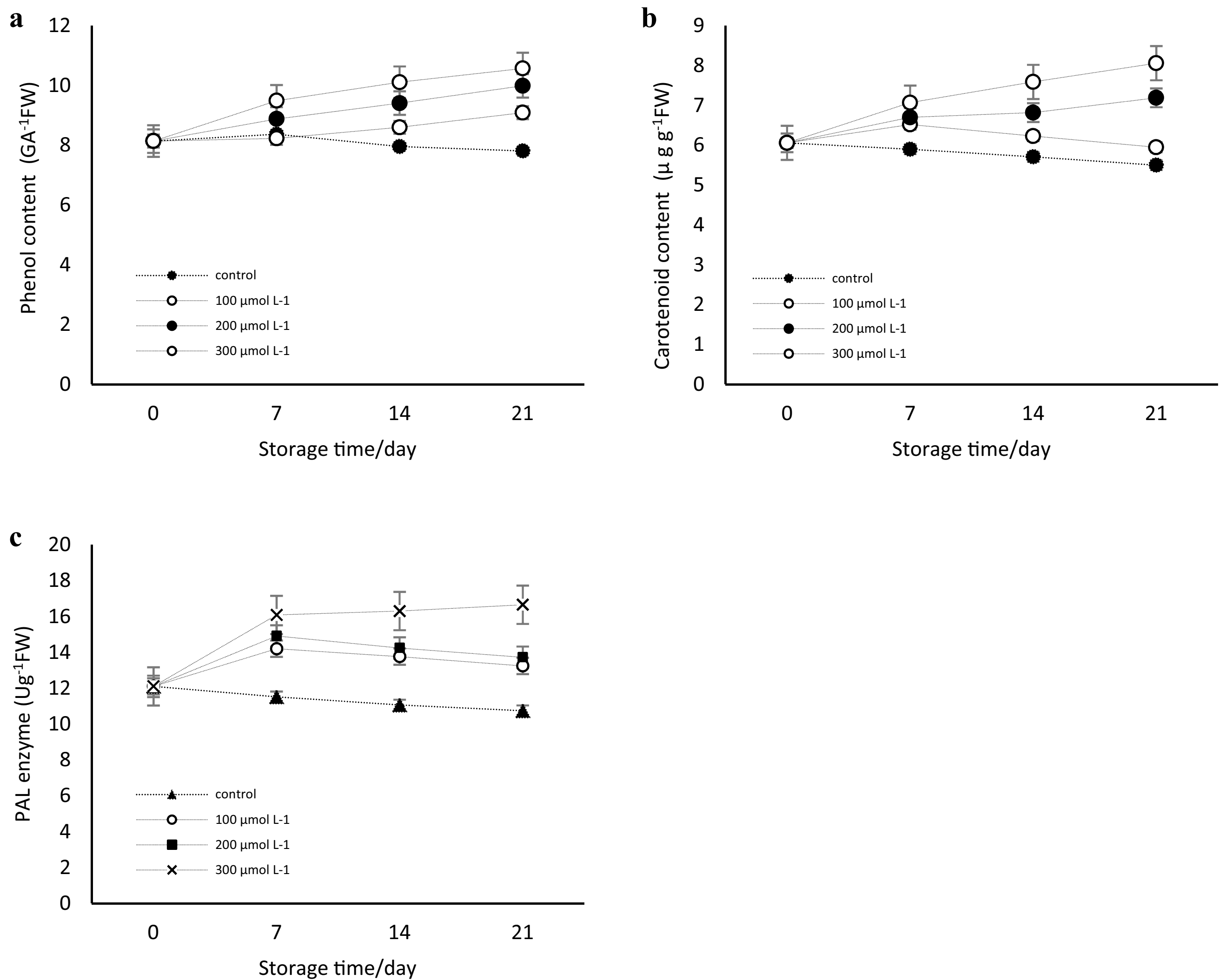

Fig. 5 The interaction between melatonin treatment and storage periods on phenolic content (**a**), carotenoid content (**b**) and PAL enzyme (**c**) of Physalis fruit during 21 days' storage at 10 ± 1 °C and 90% RH 85 ± 1%. Values are means ± SD from three replicates (n = 3). Statistical analysis was performed using LSD test

can be attributed to the fact that PAL plays a principal role in inducing phenylpropanoid pathway activity through the transformation of *L*-phenyalanine into *trans*-cinnamic acid initiating higher phenols generation and improving nutritional values of fruits [53, 54]. Moreover, many authors supported findings [54, 55].

Conclusion

The postharvest application of melatonin on retarding senescence reflects its positive role in reducing respiration, weight loss, maintaining fruit firmness, decreasing the TSS content as well as enhancing antioxidant enzyme activities (SOD, APX, and CAT). Furthermore, 300 µmol L^{-1} melatonin treatment promoted the accumulation of carotenoid and total phenolic, resulting in higher antioxidant capacity and improving nutritional value of fruit. In summary, melatonin treatment at higher concentration had positive effects on the postharvest life and nutritional quality of Physalis fruit. The development of melatonin application as a plant growth regulator can provide new prospects in postharvest technology and the achievement of ripening mechanism.

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Declarations

Conflict of interest The authors declare no conflict of interest.

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