

Article

Melatonin Treatment of Apricot Trees Leads to Maintenance of Fruit Quality Attributes during Storage at Chilling and Non-Chilling Temperatures

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Abstract: The effects of preharvest melatonin treatment on apricot crop yield and fruit quality properties at harvest and during storage have not yet been investigated. Apricot trees, of the ‘Colorado’ and ‘Mikado’ cultivars, were sprayed with 0.1 mM melatonin at three key points of fruit development. Fruit were harvested at commercial ripening stage and yield was higher in melatonin treated trees than in the controls. Fruit were stored at 1 and 8 °C for 21 and 28 days, respectively. Samples were taken weekly and left at 20 °C for 1 day. Weight losses, as well as reduction in firmness and acidity, were delayed in fruits from melatonin treated trees, showing an effect of treatment on delaying the postharvest ripening process, which was attributed to a reduced ethylene production in both cultivars and at both storage temperatures. In addition, chilling injury symptoms were observed in apricots stored at 1 °C, which were reduced by preharvest melatonin treatment. Moreover, apricot from melatonin-treated fruit retained higher total phenolic content than the controls after 14 days of storage, although the phenolic profile was not affected by treatment. Thus, melatonin could be a useful tool for practical purposes to improve apricot crop yield and maintain fruit quality properties during storage.

Keywords: *Prunus armeniaca*; yield; firmness; acidity; soluble solids; phenolics



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1. Introduction

Apricot (*Prunus armeniaca* L.) is a stone fruit highly appreciated by consumers due to its pleasant taste and flavor; nutritive properties; and its content of bioactive compounds with antioxidant activity, such as phenolics, vitamins, and carotenoids (Egea et al., 2007; Fan et al., 2018). Apricot is a climacteric fruit that is usually harvested at the pre-climacteric stage and undergoes a rapid ripening process during storage, leading to quality losses and deterioration in 3–4 weeks at cold storage, depending on cultivar and storage temperature, which are accelerated upon transference to ambient temperature [1–3]. Thus, storage at low temperature is not enough for apricot delivery to distant markets. In this sense, additional postharvest treatments, such as coating with aloe vera gel [4], storage at a controlled atmosphere [5], and 1-methylcyclopropene [6] or polyamine treatments [1], among others, combined with cold storage, have been assayed to delay ripening and maintain fruit quality.

Melatonin was first identified in 1995 in mono and dicotyledonous plant families [7] and, nowadays, it is considered as a multifunctional plant growth regulator, having effects in a wide range of plant physiological processes, including alleviation of the oxidative damages caused by different biotic and abiotic stresses [8,9]. In addition, recent reports have shown a role of melatonin on fruit ripening, although most of them are focused on

postharvest treatments [10]. Thus, 0.5 mM melatonin dipping for 1 h delayed changes of ripening parameters in ‘Guifei’ mangoes through inhibition of ethylene and ABA biosynthesis [11]. Accordingly, Hu et al. [12] reported delayed ripening and ethylene inhibition in banana fruit after postharvest melatonin treatment, which was dose-dependent in the range of 0.05 to 0.5 mM. Similar results have been reported in nectarines and peaches [13,14]. However, the effects of melatonin preharvest treatment on fruit ripening on trees have been evaluated in a few papers, showing different effects depending on fruit species, concentration, or application time. Thus, melatonin 0.1 and 0.01 mM applied at pit hardening inhibited ripening in sweet cherry fruits [15], while irrigation of tomato plants with 0.1 mM melatonin increased sugar and lycopene concentration in fruits, showing a positive effect on fruit ripening [16]. In apricot, foliar spray melatonin treatment increased yield and fruit weight, although no effect on on-tree ripening was observed [17] (Abd El-Naby et al., 2019). However, to the best of our knowledge, no literature is available regarding the effect of preharvest melatonin treatment on fruit quality properties at harvest and during storage. Thus, the present experiment was aimed at evaluating the effects of preharvest melatonin treatment on the on-tree apricot ripening process as well as on the evolution of quality, nutritional, and functional properties during storage at 1 and 8 °C by using two cultivars, ‘Colorado’ and ‘Mikado’.

2. Materials and Methods

2.1. Plant Material and Experimental Design

Experiments were performed in a commercial field plot located at Cieza (Murcia, Spain) with apricot trees (*Prunus armeniaca* L.) of cultivars ‘Colorado’ and ‘Mikado’. Trees were treated with freshly prepared solutions of melatonin 0.1 mM containing 1 mL L⁻¹ Tween or distilled water with 1 mL L⁻¹ Tween as the control. Three replicates of three trees were used for each treatment and cultivar. Treatments were applied by foliar spray of 3 L per tree by using a manual sprayer machine at pit hardening, final fruit growth, and 4 days before harvest. Fruit were harvested at commercial ripening, based on the characteristic skin color of each cultivar. Total production per tree was weighed and fruit counted to obtain data of yield per tree and fruit weight average. Then, a sample of ca 25 kg of each replicate was taken and transferred to the laboratory in 2 h, and 10 lots of ten fruits, homogenous in size and color and without visual defects, were performed from each replicate and treatment, 5 of them being stored at 1 °C and the remaining 5 lots at 8 °C and 85% RH. After 0, 7, 14, 21, and 28 days of storage, one lot was taken at random for each replicate, treatment, and storage temperature and stored for 1 day at 20 °C and 70% RH, and then the following parameters were measured.

2.2. Ethylene Production, Respiration Rate, and Quality Parameters

The weight of each apricot lot was measured at day 0 and after each storage period, and weight loss was expressed as a percentage with respect to weight at day 0. To quantify ethylene production and respiration rate, each fruit lot was hermetically sealed in a 3 L jar for 60 min. After that, 4 mL from the holder atmosphere were withdrawn with a syringe. Two milliliters was used to quantify, in duplicate, ethylene by using a Hewlett-PackardTM 5890A gas chromatograph, and the remaining 2 mL was used to quantify, in duplicate, CO₂ by using a Shimadzu TM 14A gas chromatograph (Kyoto, Japan), equipped with a thermal conductivity detector. Chromatographic conditions have been previously described [11], and ethylene production and respiration rate were expressed as nL g⁻¹ h⁻¹ and mg of CO₂ kg⁻¹ h⁻¹, respectively.

Chilling injury damage was evaluated by five trained judges according to a scale from 0 to 5. To measure fruit color, one image of each cheek side of the 10 fruits of each of the 3 replicates for each treatment were captured, saved as a JPEG file, and analyzed using the software ImageJ v1.52a (NIH Image, National Institutes of Health, Bethesda, MD, USA). The CIELab model was used to express color as L*, a*, and b* parameters. Fruit firmness was measured using a TX-XT2i Texture Analyzer (Stable Microsystems, Godalming, UK)

equipped with a flat probe that applied a force to achieve a 3% deformation of the fruit diameter. Results were expressed as the relation between the applied force and the travelled distance (N mm^{-1}) and are the mean \pm SE. After that, fruit were peeled and the flesh cut into small pieces to obtain a homogeneous sample of each replicate. About 50 g were squeezed through two layers of cotton cloth, and the juice was used to measure total soluble solids (TSS) and titratable acidity (TA). TA was determined in duplicate in each sample by automatic titration (785 DMP Titrino, Metrohm) of 1 mL of juice diluted in 25 mL of distilled H_2O with 0.1 N NaOH up to pH 8.1, and results were expressed as g malic acid equivalent 100 g^{-1} on a fresh weight basis. TSS were also measured in duplicate in the juice of each sample using a digital refractometer (Atago PR-101, Atago Co. Ltd., Tokyo, Japan) at 20°C , and results were expressed as $\text{g } 100 \text{ g}^{-1}$ in fresh weight basis.

2.3. Total and Individual Phenolic Quantification

Total phenolic compounds were extracted by homogenizing 5 g of fruit pulp samples with 15 mL of water:methanol (2:8, *v/v*) containing 2 mM NaF using an Ultraturrax (T18 basic, IKA, Berlin, Germany) for 30 s. The extracts were centrifuged at $10,000 \times g$ for 10 min at 4°C and total phenolics were quantified in the supernatant, in duplicate, using the Folin-Ciocalteu reagent, as previously described [18]. Results were expressed as mg gallic acid equivalent (GAE) g^{-1} on a dry weight basis and are the mean \pm SE. To quantify individual phenolics, 1 mL of the above supernatant was filtered through a $0.45 \mu\text{m}$ PVDF filter (Millex HV13, Millipore, Bedford, MA, USA) and used for HPLC analyses using an Agilent HPLC 1100 series machine equipped with a photodiode array detector (Agilent Technologies, Waldbronn, Germany). The HPLC was equipped with a C18 column (Mediterranea Sea 18, Teknokroma, Barcelona, Spain) of $25 \text{ cm} \times 0.46 \text{ cm}$ i.d. and $5 \mu\text{m}$ particle size and a C18 security guard of $1 \text{ cm} \times 0.32 \text{ cm}$ i.d. (Ultraguard Sea 18, Teknokroma, Barcelona, Spain). Mobile phases A and B were water:formic acid (99.9:0.1, *v/v*) and acetonitrile, respectively, with a flow rate of 1 mL min^{-1} . The linear gradient started with 1% of solvent B, reaching 30% of solvent B at 30 min, 50% at 40 min, and 95% at 45 min, which was maintained up to 50 min and then returned to initial conditions after 5 min. The injection volume was $20 \mu\text{L}$ and the temperature of column was 30°C . Chromatograms were recorded at 320 nm, and neochlorogenic acid, chlorogenic acid, and rutin were quantified by comparison with calibration curves performed with authentic standards purchased from Sigma-Aldrich (Darmstadt, Germany).

2.4. Statistical Analysis

The experiments were performed over two years (2019 and 2020) and in both years, a factorial design with melatonin treatments (0 and 0.1 mM) and storage time (0, 7, 14, 21, and 28 days) with three triplicates ($n = 3$) of three trees per replicate for melatonin treatment and of three lots of ten fruit for each sampling date during storage was performed. For all the measured parameters, data are the mean \pm SE ($n = 6$) of the results from both years. An analysis of variance (ANOVA) was performed using the SPSS software version 20 (SPSS Inc., Chicago, IL, USA) and means were compared by Tukey's test. Differences at $p < 0.05$ were considered significant. Least Significance Differences (LSD), at 5% level of probability, were calculated when significant differences among treatments were detected. In addition, a *t*-test was performed by comparison between the control and the melatonin treated fruit for each cultivar, storage temperature, and sampling date.

3. Results

3.1. Fruit Weight and Crop Yield

Apricot fruit were harvest when fruit reached their commercial ripening stage, based on color of fruit surface, so that two harvestings were performed for both cultivars in the control or in the treated trees. Melatonin tree treatment led to a significant increase ($p < 0.05$) of fruit weight, ca. 8.5 and 9.2% in 'Colorado' and 'Mikado' cultivars, respectively, although no significant effect was observed on the number of fruit harvested per tree. Thus,

yield, expressed as kg harvested per tree was significantly higher ($p < 0.05$) in melatonin treated trees than in the controls (Table 1).

Table 1. Tree yield (kg), fruit weight (FW, g) at harvest, and color parameters at harvest and after 21 days of storage at 1 or 8 °C in ‘Colorado’ and ‘Mikado’ apricots from the control and melatonin 0.1 mM treated trees.

	Days	‘Colorado’		‘Mikado’	
		Control	Melatonin	Control	Melatonin
Yield		26.25 ± 0.71 a	28.35 ± 0.75 b	19.65 ± 0.85 a	21.42 ± 0.83 b
FW	0	54.77 ± 1.75 a	59.46 ± 1.08 b	59.84 ± 1.11 a	67.38 ± 1.54 b
Colour a*	0	36.35 ± 0.99 aA	37.22 ± 0.48 aA	27.53 ± 1.02 aA	26.24 ± 1.08 aA
	21 at 1 °C	40.73 ± 1.34 aB	42.81 ± 0.60 aB	34.74 ± 1.06 aB	34.62 ± 0.59 aB
	21 at 8 °C	44.84 ± 0.49 aC	45.35 ± 0.41 aC	36.4 ± 1.12 aB	35.68 ± 0.93 aB
Colour b*	0	61.01 ± 1.10 aB	60.72 ± 0.56 aA	61.91 ± 1.27 aA	64.32 ± 1.38 aB
	21 at 1 °C	62.8 ± 0.72 aB	62.37 ± 0.40 aA	62.97 ± 1.22 aA	59.01 ± 1.02 aA
	21 at 8 °C	58.95 ± 0.60 aA	60.06 ± 1.03 aA	59.52 ± 1.79 aA	61.68 ± 1.45 aA
Colour L*	0	61.02 ± 1.01 aC	60.46 ± 0.63 aC	61.71 ± 0.98 aC	63.61 ± 1.14 aB
	21 at 1 °C	58.84 ± 0.58 aB	58.18 ± 0.49 aB	58.69 ± 1.09 aB	54.77 ± 1.06 aA
	21 at 8 °C	52.71 ± 0.55 aA	53.88 ± 0.97 aA	53.95 ± 1.56 aA	56.09 ± 1.35 aA

Data are the mean ± SE of fruits harvested from three replicates of three trees for 2019 and 2020 experiments. For each cultivar and sampling date, different lowercase letters show significant differences ($p < 0.05$) between the control and the melatonin treatments (t -test). For each cultivar and treatment, different uppercase letters show significant differences ($p < 0.05$) during storage.

3.2. Weight Loss, Ethylene Production and Respiration Rate

Weight loss increased during storage in both apricot cultivar and both storage temperatures, reaching final values of 29.65 ± 2.07 and $26.62 \pm 1.00\%$ in ‘Colorado’ control fruits after 21 and 28 days of storage at 8 and 1 °C, respectively (Figure 1A) and 29.43 ± 0.72 and 21.98 ± 2.30 and in ‘Mikado’, respectively (Figure 1B). However, weight losses were significantly lower ($p < 0.05$) in fruits from melatonin treated trees, with reductions of 36 and 25% in ‘Colorado’ and 19 and 13% in ‘Mikado’, taking into account the data of all sampling dates during storage at 1 and 8 °C, respectively (Figure 1A,B). Ethylene production rate increased sharply from harvest day to day 7 + 1 in the control fruit, reaching maxima values of 33.77 ± 1.128 and $43.59 \pm 1.34 \text{ nL g}^{-1} \text{ h}^{-1}$ in the ‘Colorado’ and ‘Mikado’ control fruits stored at 8 °C, respectively, and were significantly lower, ($p < 0.05$) 12.42 ± 3.33 and $18.53 \pm 1.41 \text{ nL g}^{-1} \text{ h}^{-1}$, respectively, in those stored at 1 °C. After that, ethylene production decreased in the fruits of both cultivars and storage temperatures (Figure 2A,B). Ethylene production in fruits from treated trees followed a similar pattern, although values were significantly lower than in the controls in all sampling dates for both cultivars and storage temperatures (Figure 2). Respiration rate increased steadily in both cultivars during storage at 8 °C, with values significantly lower ($p < 0.05$) in fruits from treated trees than in the controls (Figure 3). Respiration rate of fruit stored at 1 °C was also significantly reduced ($p < 0.05$) in treated fruits with respect to the controls in both cultivars, although stabilization and decrease trends were observed after 14–21 days of storage.

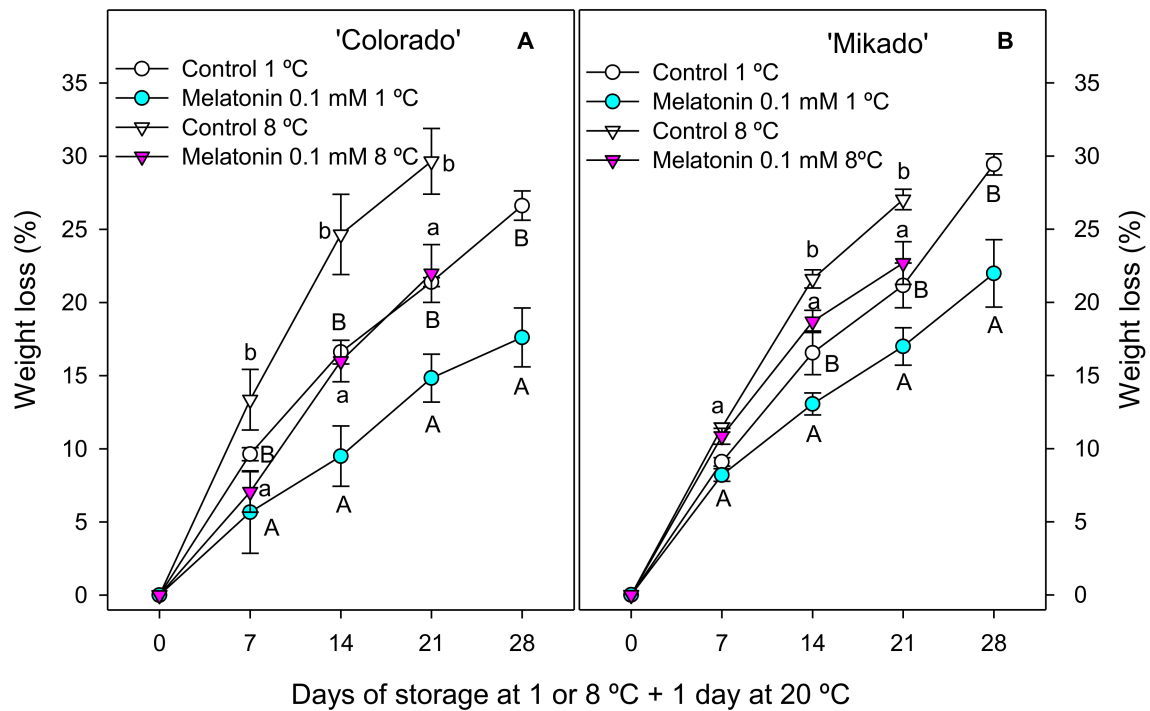


Figure 1. Weight loss of 'Colorado' (A) and 'Mikado' (B) apricots from the control and melatonin treated trees during storage at 1 and 8 °C. Data are the mean \pm SE of three replicates of ten fruits from 2019 and 2020 experiments. LSD values were 1.196 and 0.943 for (A,B), respectively. Different capital and lowercase letters show significant differences (*t*-test, $p < 0.05$) between treatments for each sampling date during storage at 1 and 8 °C, respectively.

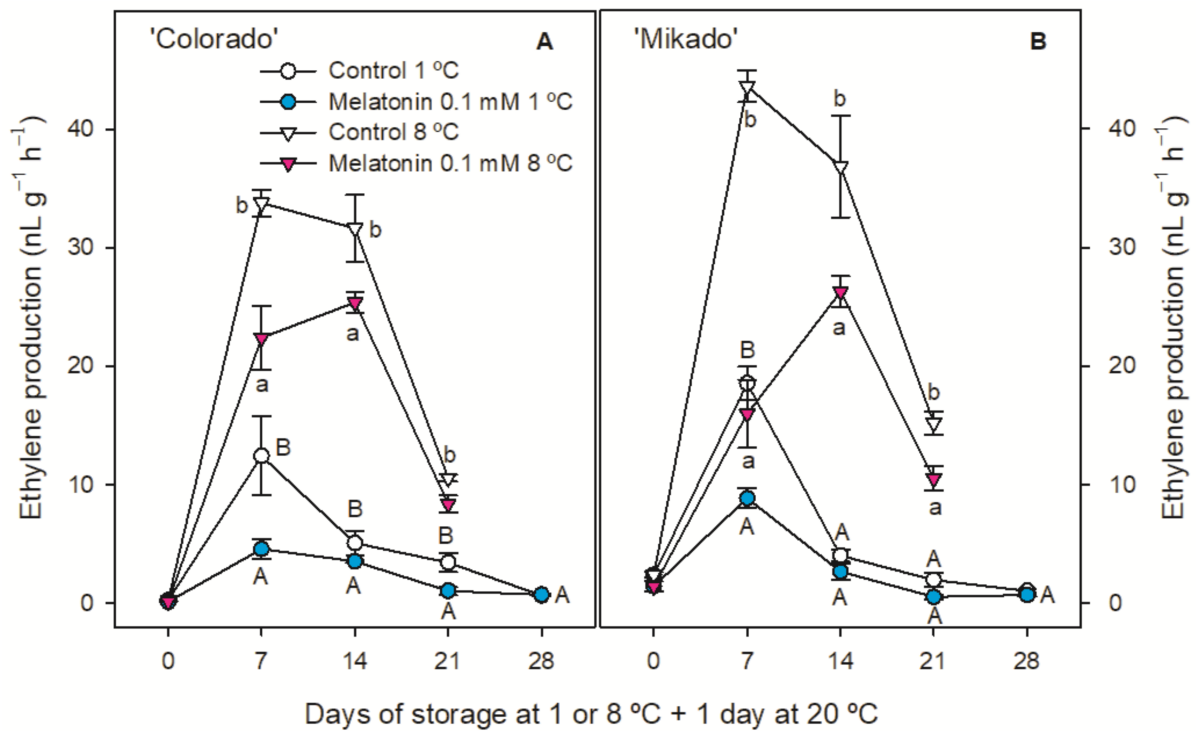


Figure 2. Ethylene production rate of 'Colorado' (A) and 'Mikado' (B) apricots from the control and melatonin treated trees during storage at 1 and 8 °C. Data are the mean \pm SE of three replicates of ten fruits from 2019 and 2020 experiments. LSD values were 0.90 and 1.14 for (A,B), respectively. Different capital and lowercase letters show significant differences (*t*-test, $p < 0.05$) between treatments for each sampling date during storage at 1 and 8 °C, respectively.

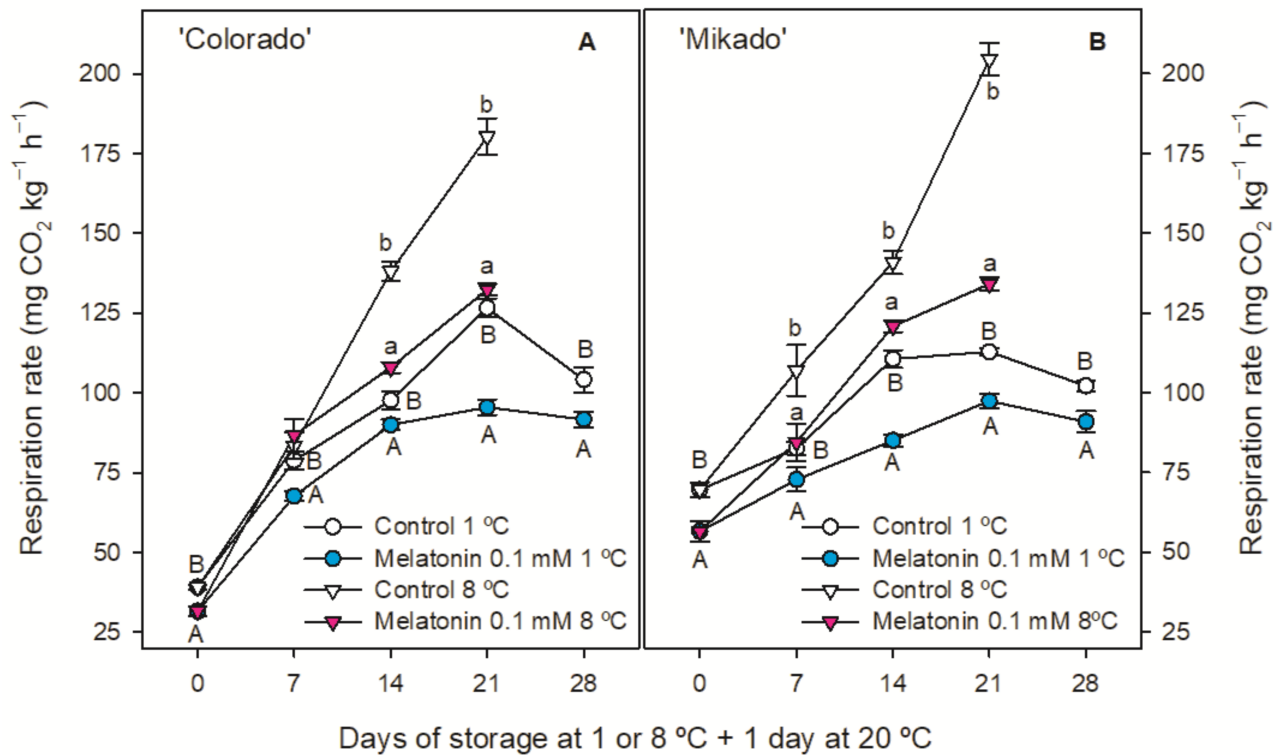


Figure 3. Respiration rate of ‘Colorado’ (A) and ‘Mikado’ (B) apricots from the control and melatonin treated trees during storage at 1 and 8 °C. Data are the mean \pm SE of three replicates of ten fruits from 2019 and 2020 experiments. LSD values were 2.32 and 2.73 for (A,B), respectively. Different capital and lowercase letters show significant differences (*t*-test, $p < 0.05$) between treatments for each sampling date during storage at 1 and 8 °C, respectively.

3.3. Quality Parameters

Fruit firmness at harvest was significantly higher ($p < 0.05$) in fruits from treated trees than in the controls, 16.50 ± 0.53 and 14.69 ± 0.56 N mm⁻¹, respectively, for ‘Colorado’ (Figure 4A) and 12.33 ± 0.75 and 9.74 ± 0.52 N mm⁻¹, respectively, for ‘Mikado’ (Figure 4B). A sharp decrease in fruit firmness was observed from day 0 to day 7 + 1 of storage for both cultivars and storage temperatures, the rate of softening being lower thereafter. Fruit firmness was maintained at significantly higher values in melatonin treated fruits than in controls at 7 + 1 and 14 + 1 days of storage at 1 °C and at 7 + 1 days in storage at 8 °C (Figure 4), showing that the effect of preharvest melatonin treatment on reducing fruit softening was higher in fruits stored at lower temperature. Color parameters (L^* , a^* and b^*) of apricot fruit at harvest were not affected by melatonin treatment, and their evolution during storage was similar in apricots from the control and treated fruits, although it was higher at 8 than at 1 °C (Table 1).

TSS and TA were similar ($p > 0.05$) in fruits from the control and treated trees, with values of TSS ca. 10.5 and 9.2 g 100 g⁻¹ for ‘Colorado’ and ‘Mikado’, respectively, and ca. 2.6 and 1.6 g 100 g⁻¹ for TA in ‘Colorado’ and ‘Mikado’, respectively (Table 2). TSS increased during storage at 1 and 8 °C in the control and treated fruits for both cultivars, although these increases were significantly lower ($p < 0.05$) in fruits from melatonin treated trees than in the controls from most sampling dates (Table 2). However, taking into account the great fruit weight losses that occurred during storage, the increase in TSS could be due to sugar concentration in fruit tissues. In fact, when TSS were calculated on a dry weight basis, no significant changes ($p > 0.05$) were observed during the whole storage time, with values in the range of 0.69–0.76 and 0.55–0.65 g g⁻¹ dry weight for ‘Colorado’ and ‘Mikado’, respectively (Figure S1). On the contrary, TA values, expressed on a fresh weight basis, decreased steadily during storage at 8 °C in ‘Mikado’, while decreases were only observed from day 21 + 1 to day 28 + 1 during storage at 1 °C, and no significant

changes ($p > 0.05$) were observed in 'Mikado' fruits during storage at 1 or 8 °C (Table 2). Nevertheless, when TA was expressed on a dry weight basis, decreases were observed during the whole storage time, although values were significantly higher ($p < 0.05$) for apricots from melatonin treated trees than in the controls (Figure S2). Thus, TA values were increased by 18 and 15% in 'Colorado' and 13 and 11% in 'Mickado' fruits from melatonin treated trees stored at 1 and 8 °C, respectively, with respect to the control fruits (Figure S2).

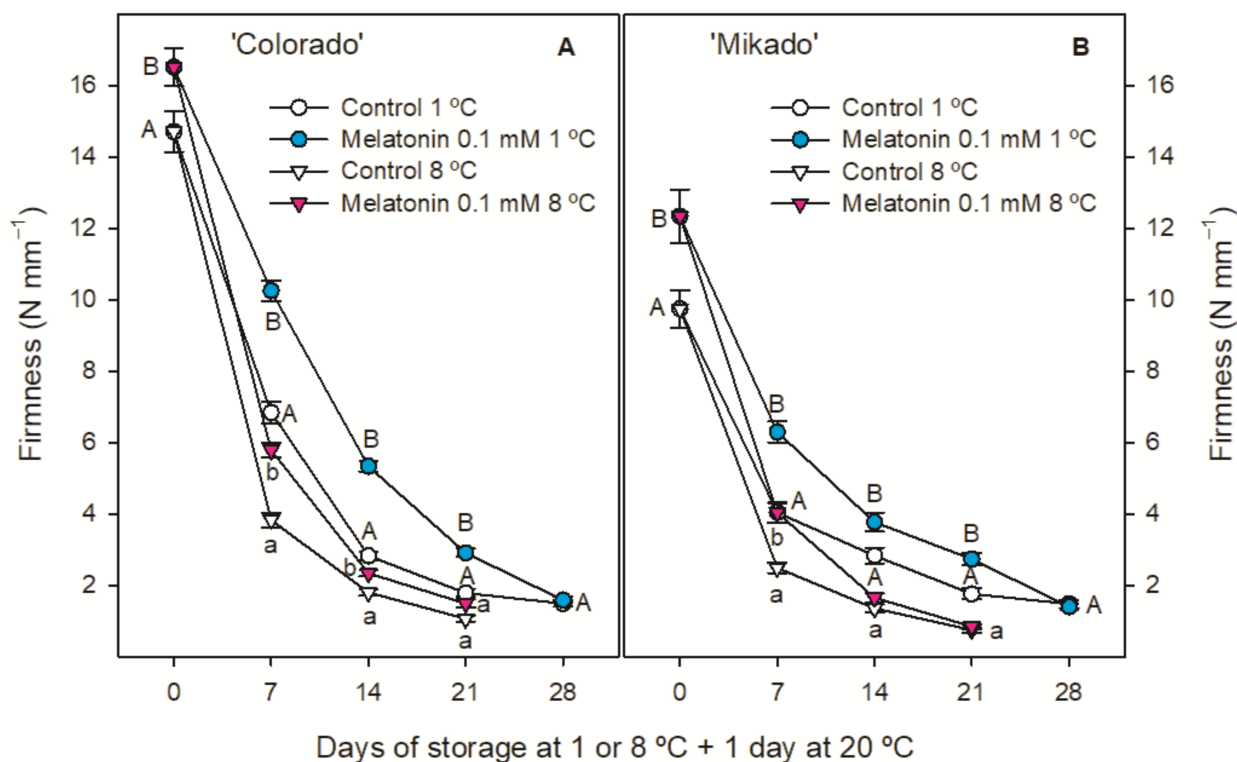


Figure 4. Fruit firmness of 'Colorado' (A) and 'Mikado' (B) apricots from the control and melatonin treated trees during storage at 1 and 8 °C. Data are the mean \pm SE of three replicates of ten fruits from 2019 and 2020 experiments. LSD values were 0.23 and 0.37 for (A,B), respectively. Different capital and lowercase letters show significant differences (t -test, $p < 0.05$) between treatments for each sampling date during storage at 1 and 8 °C, respectively.

Table 2. Total soluble solids (TSS, g 100 g⁻¹ fresh weight) and titratable acidity (TA, g 100 g⁻¹) at harvest and after storage at 1 or 8 °C for 21 days + 1 day at 20 °C in 'Colorado' and 'Mikado' apricots from the control and melatonin 0.1 mM treated trees.

		'Colorado'		'Mikado'	
		Control	Melatonin	Control	Melatonin
TSS	Day 0	10.88 \pm 0.21 aA	10.25 \pm 0.31 aA	9.02 \pm 0.25 aA	9.23 \pm 0.08 aA
	21 d 1 °C + 1 d 20 °C	13.88 \pm 0.26 aB	12.60 \pm 0.15 bB	10.15 \pm 0.14 aB	9.92 \pm 0.12 bB
	21 d 8 °C + 1 d 20 °C	14.53 \pm 0.19 aC	13.30 \pm 0.26 bC	11.47 \pm 0.18 aC	10.55 \pm 0.04 bC
TA	Day 0	2.55 \pm 0.03 aC	2.66 \pm 0.04 aB	1.83 \pm 0.05 aC	1.78 \pm 0.07 aB
	21 d 1 °C + 1 d 20 °C	2.32 \pm 0.04 aB	2.69 \pm 0.06 bB	1.65 \pm 0.04 aB	1.82 \pm 0.06 bB
	21 d 8 °C + 1 d 20 °C	1.70 \pm 0.06 aA	2.13 \pm 0.02 bA	1.32 \pm 0.11 aA	1.68 \pm 0.05 bA

Data are the mean \pm SE of three replicates from 2019 and 2020 experiments. For each cultivar, different lowercase letters show significant differences ($p < 0.05$) between the control and the melatonin treatments (t -test), and different uppercase letters show significant differences ($p < 0.05$) during storage.


On the other hand, apricot stored at 1 °C manifested chilling injury symptoms, such as brown spot on the fruit surface, which increased, as did storage time. However, scores for chilling injury were significantly lower ($p < 0.05$) in fruit from melatonin treated trees

than in the controls, for both cultivars, with reductions of 23 and 42% in ‘Colorado’ and ‘Mikado’, respectively, after 21 days of cold storage + 1 day at 20 °C (Table 3).

Table 3. Chilling injury scores for the control and melatonin treated fruit after 21 days of storage at 1 °C + 1 day at 20 °C. Chilling injury damage was rated according to a 0–5 scale, as shown in the photograph on the last row.

	Control	Melatonin
Colorado	2.42 ± 0.09 b	1.88 ± 0.08 a
Mikado	2.83 ± 0.11 b	1.63 ± 0.08 a

Scale for chilling injury damage

1	2	3	4	5
				

Data are the mean ±SE of three replicates from 2019 and 2020 experiments. Different letters show significant differences ($p < 0.05$) between the control and treated fruit (t -test) for each cultivar.

3.4. Individual and Total Phenolic Content

Individual phenolics were quantified in both apricot cultivars from the control and the melatonin treated fruits at harvest, and similar phenolic profiles and concentrations were obtained without significant differences ($p > 0.05$) being attributed to melatonin treatment (Table 4). The major phenolic was chlorogenic acid, with values ca. 15 and 12.5 mg 100 g⁻¹ FW in ‘Colorado’ and ‘Mikado’, respectively, followed by neochlorogenic acid and rutin (quercetin-3-O-rutinoside) at lower concentrations; 2–3 and 0.1–0.2 mg 100 g⁻¹ FW, respectively. Total phenolic content at harvest (measured by the Folin–Ciocalteu reagent) was similar ($p > 0.05$) in fruits from the control and melatonin treated trees in both cultivars. During storage, total phenolic content, expressed on a dry weight basis, firstly increased and then decreased after 7 + 1 days of storage in the control fruits at both temperatures, except in ‘Mikado’ apricots stored at 8 °C, in which decreases were found from day 0. Fruits from melatonin-treated trees followed a similar trend, although decreases were significantly delayed ($p < 0.05$) with respect to control fruits, occurring after 14 + 1 and 21 + 1 days in fruits stored at 8 °C and 1 °C, respectively, in both cultivars (Figure 5A,B).

Table 4. Concentration of individual phenolics (mg 100 g⁻¹ FW) at harvest in pulp of ‘Colorado’ and ‘Mikado’ apricots from the control and melatonin 0.1 mM treated trees.

	‘Colorado’		‘Mikado’	
	Control	Melatonin	Control	Melatonin
Neochlorogenic acid	3.60 ± 0.28 a	2.52 ± 0.16 a	2.10 ± 0.06 a	2.16 ± 0.18 a
Chlorogenic acid	15.78 ± 1.08 a	14.73 ± 0.46 a	12.25 ± 0.21 a	12.61 ± 0.60 a
Rutin	0.22 ± 0.01 a	0.21 ± 0.03 a	0.08 ± 0.01 a	0.12 ± 0.01 a

Data are the mean ± SE of three replicates of five fruits for 2019 and 2020 experiments. For each cultivar, different letters show significant differences ($p < 0.05$) between the control and melatonin treatments (t -test).

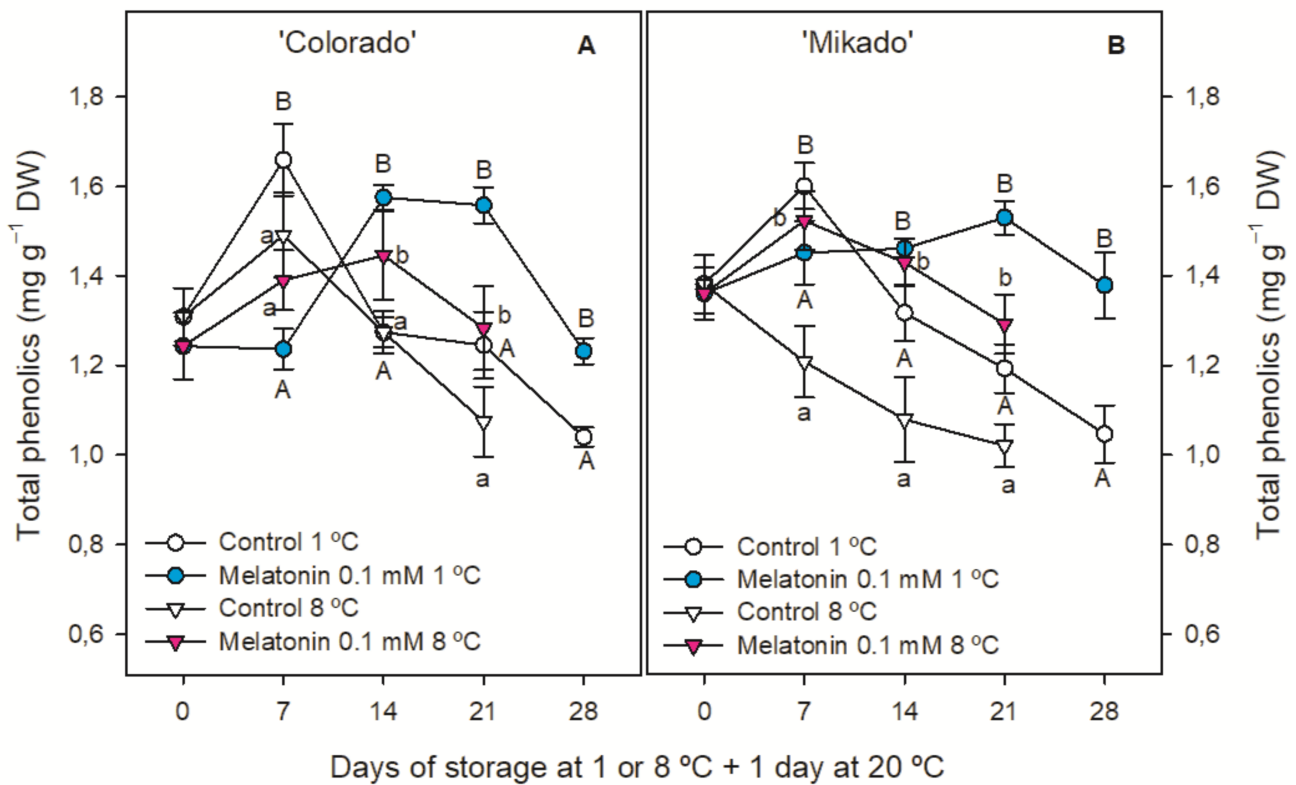


Figure 5. Total phenolic content on the flesh of ‘Colorado’ (A) and ‘Mikado’ (B) apricots from the control and melatonin treated trees during storage at 1 and 8 °C. Data are the mean \pm SE of three replicates of ten fruits from 2019 and 2020 experiments. LSD values were 0.09 and 0.07 for (A,B), respectively. Different capital and lowercase letters show significant differences (*t*-test, $p < 0.05$) between treatments for each sampling date during storage at 1 and 8 °C, respectively.

4. Discussion

Previously published papers have shown that foliar melatonin treatment affects crop yield. Thus, preharvest melatonin treatment of tomato plants ameliorated the reduction in yield occurring when plants were grown under rain acid stress conditions, although no increases were found in the control plants [19]. These results were attributed to the effects of melatonin treatment on boosting the stress tolerance of tomato plants to stress. In addition, a 37% increase in the yield of tomato plants under water deficit stress was observed as a consequence of melatonin treatment at 30 and 50 days after transplanting, although increases of 14% were also observed in well-irrigated plans [20]. Moreover, seed soaking with melatonin or melatonin applied in the irrigation system also increased tomato plant yield [16]. These effects were attributed to an increase in leaf chlorophyll content and photosynthetic rate. Results of the present experiment also show an effect of melatonin spraying of apricot trees on increasing yield in both cultivars due to enhanced fruit weight (Table 1), leading to improving the economical profit of this crop. Accordingly, melatonin foliar spray treatment increased fruit weight and yield on the ‘Canino’ apricot as well as total chlorophyll and leaf area [17].

Weight loss, softening, and TA decreases are the major changes leading to apricot quality losses during storage [4,6,21], which were delayed by preharvest melatonin treatments in both cultivars and storage temperatures. No previous reports are available in the literature regarding the effect of preharvest melatonin treatment on the evolution of fruit quality parameters during storage, although information exists concerning postharvest treatments. Weight loss is mainly due to transpiration rate through the fruit surface. The reduction observed in weight loss of fruits from melatonin treated trees with respect to those of the controls (Figure 1) might be attributed to an effect of melatonin increasing cuticle thickness, as recently proposed for nectarines [14] and mangos [22] after posthar-

vest melatonin treatment. Fruit firmness sharply declined during storage in both apricot cultivars, being higher at 8 than at 1 °C (Figure 4), which has been attributed to increases on polygalacturonase, β -galactosidase, and pectin methyl esterase activities, leading to dissolution of the middle lamella, although degradation of cellulose also occurred due to cellulase activity [23]. However, fruit firmness at harvest was higher in fruit from melatonin treated trees than in the controls, and firmness losses were significantly ($p < 0.05$) delayed by preharvest melatonin treatment until 14 d of storage, this effect being higher at 1 than at 8 °C (Figure 4). Softening during storage was also delayed by postharvest melatonin treatment in nectarine [14], peach [13], pear [24], and mango [11] due to down-regulation of the expression of cell wall degrading enzymes. TSS and TA content in apricots are key factors affecting fruit taste and consumers' acceptance, as has been reported for other stone fruits [2,3,25], and their maintenance during storage is a pivotal task. In 'Colorado' and 'Mikado' apricots, TSS increased and TA decreased throughout storage, these changes being higher at 8 than at 1 °C and significantly delayed in melatonin treated fruits (Table 2). However, fruit color parameters at harvest were not affected by melatonin treatment as expected because the main harvest criterion was fruit color. During storage, they evolved in a similar way to fruit from the control and treated trees, and no effects of preharvest melatonin treatment were observed, neither in the 'Colorado' nor in the 'Mikado' cultivars.

The effects of preharvest melatonin treatments on the evolution of quality parameters during storage show that the postharvest ripening process was delayed in apricots from melatonin treated trees at both storage temperatures, which could be attributed to their reduced ethylene production as compared with those of the control fruits (Figure 2). No previous reports are available in the literature regarding the effects of preharvest melatonin treatments on ethylene production of climacteric fruits during storage, although some information exists concerning postharvest treatments. Thus, 0.1 mM melatonin applied after 1 month of cold storage to three pear cultivars ('Starkrimson', 'Abbé Fetel', and 'Red Anjou') decreased ethylene production during storage at 20 °C due to a reduced expression of *PcACS* and *PcACO* genes, codifying for ACC-synthase and ACC-oxidase, respectively [26]. Accordingly, postharvest 0.1 mM melatonin treatment reduced ethylene production on apple fruit during cold storage, due to the decreased expressions of *MdACO1*, *MdACS1*, *MdAP2.4*, and *MdERF109* [27]. Lower ethylene production and expression of *MaACO1* and *MaACS1* genes were also observed in banana fruit during storage at ambient temperature as a consequence of postharvest melatonin treatment [12]. On the other hand, melatonin preharvest treatments reduced fruit respiration rate during storage at both temperatures (Figure 3), showing a reduction in fruit metabolism. Accordingly, postharvest 0.1 mM melatonin treatment delayed and reduced the climacteric respiration peak during storage at different temperatures in other climacteric fruits such as peach [28], nectarines [14], pear [24], and mango [11], and even in non-climacteric fruits such as sweet cherry [29], leading to delay the postharvest ripening process.

Phenolic compounds are a wide range of secondary metabolites with beneficial effects on human health because their antioxidant properties have preventive effects on a wide range of chronic and age-related diseases such as hypertension; obesity; diabetes; and cardiovascular, neurodegenerative, and oncologic diseases [30–32]. Apricot phenolic profile and concentration are influenced by several factors, including cultivar, ripening stage, and agronomic and environmental conditions, as well as the part of fruit analyzed, with fruit peel containing relatively higher concentrations than fruit flesh [33–35]. For instance, total phenolic content ranged from 44 to 345 mg 100 g⁻¹ in five orange-fleshed apricot cultivars cultivated in the northeast USA [35] and between 30 and 559 mg 100 g⁻¹ in the study performed by Drogoudi et al. [34] with 29 Greek and American apricot cultivars and using similar methods of analyses. Apricot cultivars of the present research had a total phenolic concentration at harvest of ca. 20 mg 100 g⁻¹ of fresh weight, showing that phenolic content in 'Colorado' and 'Mikado' cultivars is low compared with other apricot cultivars. Catechin, chlorogenic acid, neochlorogenic acid, epigallocatechin, and rutin have been reported as the major phenolics compounds in apricot, although their

relative concentrations depend on cultivar and ripening stage [2,33,35,36] In ‘Colorado’ and ‘Mikado’ apricots, three phenolic compounds were identified and quantified, the major one being chlorogenic acid followed by neochlorogenic acid and rutin (Table 4). It is worth noting that total or individual phenolic concentrations at harvest were not affected by preharvest melatonin treatment, although significant differences were observed during storage. Thus, total phenolic concentration in the control fruits of both cultivars increased during the first 7 days of storage at both temperatures and then decreased, except in the control ‘Mikado’ fruits stored at 8 °C in which a decrease occurred from day 0 (Figure 5) according to previous reports in the ‘Shushanggan’ cultivar [2]. However, in fruit from melatonin treated trees, the decrease of phenolic content was delayed in both cultivars and storage temperatures leading to higher phenolic levels in treated fruit from 14 to 21 and 28 days of storage (Figure 5). Given the antioxidant properties of phenolics and their reported health beneficial effects [30–32], melatonin treated apricots would maintain their health benefits after prolonged storage. Recently, it has been reported that dipping nectarine fruits for 30 min in 0.25, 0.5, and 1 mM melatonin minimized phenolic losses during storage at 1 °C [14] as well as 0.1 mM melatonin dipping for 10 min in peaches [13]. This high level of phenolic concentration has been related to enhancement of chilling tolerance in peaches, apart from the maintenance of a higher ratio of unsaturated to saturated fatty acids [13,28]. Thus, the reduction of CI observed in the present research in apricot fruits as a consequence of preharvest melatonin treatment (Table 3) could be due to the induced maintenance of higher phenolic concentrations (Figure 5). In addition, reduction on polyphenol oxidase (PPO) activity has been related to a lower CI incidence in apricot fruit [37] and, in turn, the higher phenolic content and the lower CI found in apricots from melatonin treated trees could be due to lower PPO enzyme activity. Accordingly, Koushesh Saba et al. [38] reported that postharvest melatonin treatment of pomegranate led to enhanced fruit chilling tolerance, which was related to inhibition of PPO activity. On the other hand, in other climacteric fruits, such as kiwifruit [39] and tomato [40], and even in non-climacteric fruits, such as pomegranate [41], a relationship between reduced ethylene production and lower chilling injury damage during cold storage has been observed. In this sense, the ethylene reduction described previously in this research could suggest that inhibiting ethylene biosynthesis with preharvest melatonin treatments could contribute to reducing chilling injury impact during apricot storage.

5. Conclusions

Results show that melatonin treatment of ‘Colorado’ and ‘Mikado’ apricot trees, at key points of fruit development, increased quality parameters at harvest, such as fruit weight and firmness, as well as crop yield. Chilling injury symptoms were reduced, and the evolution of parameters responsible for fruit quality reduction, such as weight, firmness, and acidity losses, were delayed in fruit from melatonin treated trees with respect to the controls at both storage temperatures, these effects being attributed to the reduced ethylene production found in treated fruits. Thus, melatonin could be a useful tool to improve apricot crop yield and maintain fruit quality properties during storage, as well as their content in bioactive compounds with health beneficial effects, such as phenolics.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/agronomy11050917/s1>, Figure S1: Total soluble solid content of ‘Colorado’ (A) and ‘Mikado’ (B) apricots from control and melatonin treated trees during storage at 1 and 8 °C. Data are the mean \pm SE of three replicates of ten fruits. LSD values were 0.018 and 0.019 for (A,B), respectively. Different capital and lowercase letters show significant differences (*t*-test, $p < 0.05$) between treatments for each sampling date during storage at 1 and 8 °C, respectively. Figure S2: Total acidity content of ‘Colorado’ (A) and ‘Mikado’ (B) apricots from the control and melatonin treated trees during storage at 1 and 8 °C. Data are the mean \pm SE of three replicates of ten fruits. LSD values were 0.006 and 0.005 for (A,B), respectively. Different capital and lowercase letters show significant differences (*t*-test, $p < 0.05$) between treatments for each sampling date during storage at 1 and 8 °C, respectively.

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