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# Melatonin triggers tissue-specific changes in anthocyanin and hormonal contents during postharvest decay of Angeleno plums



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## ABSTRACT

Plum is a stone fruit that stands out for having a short shelf-life because of its high susceptibility to rapid deterioration. Part of this deterioration is explained by fruit overripening. Recently, the role of melatonin in delaying postharvest decay has been investigated but its regulatory function during overripening is still under extensive debate. In this study, to understand physiological events taking place in plums overripening and elucidate the role of melatonin on the postharvest quality of these fruits and its relationship to other plant hormones, Angeleno plums were sprayed with  $10^{-4}$  M of melatonin solution immediately after harvest. We carried out tissue-specific (mesocarp and exocarp) analysis of total phenols and anthocyanin quantification, as well as the evaluation of different phytohormones by LC-MS/MS and fruit quality parameters. Results showed that during postharvest, endogenous melatonin contents decreased both in the mesocarp and the exocarp of Angeleno plums. Likewise, plum firmness also decreased and a strong correlation was found for this parameter with jasmonic acid (JA) and cytokinins. Conversely, after exogenous melatonin application, endogenous melatonin content increased both in mesocarp and exocarp but it had a differential effect depending on the plum tissue. Indeed, total phenol and anthocyanin contents arose by 21% and 58%, respectively, in the mesocarp after melatonin treatment but no variations were found in the exocarp of Angeleno plums. Hormonal analysis of Angeleno mesocarp also revealed an increase in the JA and its precursor, 12-oxo-phytodienoic acid (OPDA), on the fourth day after melatonin application as well as a positive correlation between melatonin and gibberellin 1 (GA<sub>1</sub>). These results suggest that melatonin may be acting as a signal molecule increasing phenolic compounds contents through direct regulation and by signaling with other phytohormones. Therefore, this research provides valuable information for understanding the regulatory role of melatonin and its relationship with plant hormones during overripening to contribute to improve the postharvest quality of plums.

#### 1. Introduction

Plum (*Prunus salicina* L.) is a popular fruit worldwide belonging to the genus *Prunus* and the Rosaceae family. Plums are stone fruits, as their morphology consists of an outer exocarp (skin), a fleshy mesocarp (pulp) and a lignified endocarp (pit) in the middle of the seed-containing fruit (Kader and Mitchell, 1989). Consumers appreciate plum fruits for their organoleptic properties (color, aroma, sweetness) as well as nutritional properties. Likewise, its richness in water, fiber, carbohydrates, vitamins (C, E, A, K) and antioxidants (phenolic compounds, anthocyanins and carotenoids) along with very low-fat contents provides great benefits for human health (Kim et al., 2003a; Lozano et al., 2009). However, stone fruits have a very short shelf-life during storage, being one of the most perishable foods (Kader, 1993). After fruit harvest, a shortage of water and nutrition from tree occurs, resulting in rapid postharvest deterioration that leads to greater waste.

Fruit overripening is one of the main causes of postharvest decay and entails a general loss of organoleptic properties, including flavor deterioration, peel darkness, firmness loss, dehydration and high pathogen susceptibility (Pott et al., 2020; Alkan and Fortes, 2015). Moreover, overripening is a complex process regulated by various endogenous signals, where an intricate interactive hormonal network determines the final quality of fruits and thus, commercial value (Xiang et al., 2021). Hence, elucidate the main causes that contribute to the short shelf-life once the fruit has been harvested has a high impact on both economics and environmental resources because extending fruit quality

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Received 27 December 2021; Received in revised form 7 March 2022; Accepted 11 April 2022 Available online 15 April 2022 0168-9452/© 2022 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/). would prevent from important food losses (Riva et al., 2020). In this sense, several phytohormones have been described to modulate fruit postharvest performance. For instance, postharvest ripening is dependent upon abscisic acid (ABA) and ethylene contents in fruits, both known to fine-tune quality-trait attributes that strongly influence fruits shelf-life (Jia et al., 2016; Farcuh et al., 2018; Siebeneichler et al., 2020; García-Pastor et al., 2021). At the same time, anti-senescence properties have been attributed to auxins, cytokinins or gibberellins (Figueroa et al., 2012a; Li et al., 2016a; do Amarante et al., 2020), even though these phytohormones are usually at very low contents in fruit postharvest (Tijero et al., 2019). Other phytohormones, such as jasmonic acid and salicylic acid have also been described to participate in biotic stress tolerance during postharvest (Yao and Tian, 2005; Babalar et al., 2007) and, in the case of jasmonic acid, several authors have also highlighted its role in sugar and phenolic acid accumulation (Flores et al., 2017; Liu et al., 2017; Zhao et al., 2021). In plums, there has been a huge effort to elucidate ripening elicitors during fruit development, especially because of the presence of both climacteric and non-climacteric cultivars (Minas et al., 2015; Farcuh et al., 2019), but there is scarce information about the involvement of other phytohormones in plum postharvest performance.

Melatonin (N-acetyl-5-methoxytryptamine) is a natural indolamine synthesized from tryptophan (Back et al., 2016). Since its identification in vascular plants in 1995 (Dubbels et al., 1995), it has been found in a wide number of species being ubiquitous in plants highlighting Rosaceae, Poaceae, Vitaceae, Apiaceae and Brassicaceae families (Nawaz et al., 2016). In recent years, melatonin has been considered both an excellent antioxidant and a signal molecule (Hu et al., 2016) involved in a wide range of physiological processes such as germination (Xiao et al., 2019), plant development and growth (Sun et al., 2021), fruit development, ripening (S.Y. Wang et al., 2020), senescence (S. Li et al., 2019) and responses to biotic and abiotic stresses (Nawaz et al., 2021). In fruits, endogenous melatonin contents differ between different species and varieties, even between different tissues of the same fruit (S.Y. Wang et al., 2020). Additionally, it has been reported that melatonin is involved in delaying postharvest decay and senescence during fruit storage and contributes to quality maintenance (X. Wang et al., 2019). Previous studies found that exogenous melatonin kept firmness, delayed cell wall degradation, reduced weight loss and respiration rate in different fruits such as pear (J. Liu et al., 2019), sweet cherry (F. Wang et al., 2019), peach (Gao et al., 2016), plum (Bal, 2019) and tomato (Aghdam et al., 2019). Moreover, it has been described that melatonin application increases the antioxidant system during postharvest fruits both by itself and by increasing the content of other antioxidants such as ascorbic acid, glutathione, phenols and anthocyanins as well as enzymatic antioxidants (Zhang et al., 2020).

Nevertheless, the regulatory role of melatonin together with its interaction with other phytohormones during overripening is still poorly understood and there is no information about its tissue-specific effects in delaying postharvest decay and maintaining the quality of fruit. Therefore, the aim of this study was (i) to understand the physiological events taking place in Angeleno plums overripening, which have been described as suppressed-climacteric cultivars (Minas et al., 2015), (ii) to evaluate the tissue-specific role of exogenous melatonin on postharvest quality as well as (iii) to elucidate its relationship to other phytohormones during plum postharvest shelf-life.

### 2. Materials and methods

### 2.1. Plant material, treatment and sampling

Plums (cv. 'Angeleno') were collected at commercial harvest maturity from a commercial orchard in Extremadura (Spain) and they were immediately brought to the laboratory at the University of Barcelona (Barcelona, Spain). Plums were selected for uniformity according to their size and color and were excluded those with mechanical damage or pathogen symptoms. Then, fruits were distributed randomly into two groups (i) control and (ii) melatonin consisting of 5 replicates each (10 fruits/replicate) and were stored at room temperature (25 °C) for 8 days. For melatonin treatments, plums were sprayed immediately with  $10^{-4}$  M melatonin, whereas the control group was not subjected to any treatment.

Samplings were performed at 0, 2, 4, and 8 days after treatment application. For each sampling time, the firmness of one fruit for replicate was measured and then, the fruit was cut into two pieces. In both pieces, the endocarp was removed and in one of them, the peel and pulp were separated for subsequent tissular analysis. Then, both the entire fruit, the mesocarp and exocarp were immediately separately frozen in liquid nitrogen and kept at -80 °C until analysis.

### 2.2. Plum quality parameters

Fruit firmness was determined by using a FT 327 penetrometer (QA Supplies, Norfolk, VA, USA) and pushing the cone into fresh plum mesocarp avoiding seed contact.

For total soluble solids (TSS) and total acidity (TA) analysis, 5 g of entire fruit samples were ground to a fine powder using a bead mill (Qiagen TissuLyzer II) and homogenized with MilliQ water using a vortex. TSS estimation was measured from °Brix in 1 mL of the mix using a refractometer (Hannah Instruments, Italy) following the methodology described by Boulton et al. (1999). For TA measures, 10 mL of the mix were diluted in 100 mL of MilliQ water and used for its determination with 0.1 M NaOH and 1% phenolphthalein as an indicator to estimate predominant plum acid content (g malic acid·g fresh weight<sup>-1</sup>) as described in Latimer (2012). Finally, pH was estimated using a pH-meter (MicropH 2002, Crison Instruments, S.A, Alella (Barcelona), España).

Total anthocyanins were determined as described by Gitelson et al. (2001). Mesocarp and exocarp samples (100 mg) were ground to a fine powder using a bead mill (Qiagen TissuLyzer II) and extracted with 750  $\mu$ l methanol (100%, v/v) using vortex and ultrasonication (Branson 2510 ultrasonic cleaner, Bransonic, USA). Extracts were centrifuged at 13,000 rpm for 10 min at 4 °C and then, supernatants were collected and the pellets were re-extracted following the same procedure. Supernatants were pooled followed by acidification with 1% HCL. Total anthocyanins were measured spectrophotometrically (CE Aquarius UV/Visible, Cecil Instruments Ltd, Cambridge, United Kingdom) at 530 nm and they were calculated using cyanidin-3-glucoside as a reference as described in Siegelman and Hendricks (1958).

In order to estimate total phenols content (TPC), methanolic extracts were performed following the same procedure as in total anthocyanins. Subsequently, methanolic extracts were mixed with pure methanol (1:2 dilution, v/v) and 1 mL Folin-Ciocalteu reagent (1:10 diluted with MilliQ water) was added. Then, the mix was incubated for four minutes and 800  $\mu$ l Na<sub>2</sub>CO<sub>3</sub> 5% (75 g/L) were added. After 30 min at room temperature and darkness, absorbances were measured spectrophotometrically (CE Aquarius UV/Visible, Cecil Instruments Ltd, Cambridge, United Kingdom) at 765 nm. TPC was calculated as described in Singleton and Rossi (1965) and Li et al. (2007) expressed in mg gallic acid equivalents (GAE)·g dry weight<sup>-1</sup>.

# 2.3. Analysis of regulatory cis-elements of chalcone synthase (CHS) and phenylalanine ammonia lyase (PAL) from Prunus salicina

To elucidate putative cis-elements on the CHS and PAL sequences of *Prunus salicina* from NCBI accessions CM035341.1:289590–290885 and CM035342.1:10256063–10259379. Melatonin responsive elements were retrieved based on work by Liang et al. (2017), excluding regions related to ARFs and non-fruit related elements. Likewise, jasmonic acid (JA) responsive elements were also analysed according to Fernández-Calvo et al. (2011) and Moore et al. (2022).

### 2.4. Hormonal profiling

Endogenous hormones content, including melatonin, abscisic acid (ABA), salicylic acid (SA), indoleacetic acid (IAA), the gibberellins GA<sub>1</sub>, GA<sub>3</sub>, GA<sub>4</sub> and GA<sub>7</sub>, JA, its precursor 12-*oxo*-phytodienoic acid (OPDA) and its conjugates (JA-Ile, JA-Met, JA-Val, JA-Leu, JA-Phe), the cytokinins *trans*-zeatin (*t*-Z), its riboside *trans*-zeatin (*t*-ZR), 2-isopentenyl adenine (2iP) and its riboside isopentenyl adenosine (IPA), and the ethylene precursor 1-amynocyclopropane-1-carboxylic acid (ACC), were determined by ultrahigh-performance liquid chromatography coupled to tandem mass spectrometry (UHPLC–MS/MS) as described previously in Müller and Munné-Bosch (2011). In short, 100 mg of each mesocarp and exocarp samples were extracted with 250  $\mu$ l methanol: isopropanol:acetic acid, 50:49:1 (v/v/v) and deuterium-labeled internal standards (d4-Mel, d6-ABA, d4-SA, d5-IAA, d2-GA<sub>1</sub>, d2-GA<sub>4</sub>, d2-GA<sub>7</sub> d5-JA, d5-tZ, d6–2iP, and d6-IPA) using vortex and ultrasonication (Branson 2510 ultrasonic cleaner, Bransonic, USA). Extracts were centrifuged at 13,000 rpm for 10 min at 4 °C, supernatants were collected and the pellets were re-extracted with 250  $\mu$ l of the same solvent. Then, collected supernatants were merged and filtered through



**Fig. 1.** Evaluation of plum quality parameters. (A) Total Soluble Solids (TSS), (B) Total Acidity (TA), (C) TSS/TA ratio, (D) pH, (E) Firmness and (F) plum phenotype of control and  $10^{-4}$  M melatonin treated Angeleno plums during days after treatment. Data represent the means  $\pm$  standard error of n = 5. Different lower case letters indicate significant differences over time and asterisks indicate significant differences (p < 0.005) between control and treated at that point in time. See Table 1 for statistics.

a 0,22  $\mu$ l PTFE filter (Phenomenex, United States) before UHPLC-ESI MS/MS analysis. High-performance liquid chromatography was coupled to a triple quadrupole mass spectrometer (QTRAP 4000, AB Sciex, Concord, Ontario, Canada) and a LUNA C18 column (Phenomenex Inc., United States (1,6  $\mu$ m, 100  $\times$ 2,1 mm)) was used. Solvent A was water with 0.05% acetic acid and solvent B was acetonitrile with 0.05% acetic acid. The flow rate was set at 0.6 mL/min. Quantification was made considering recovery rates for each sample by using the deuterium-labeled internal standards and calibration curves for each analyte were generated using MultiQuantTM 3.0.1 software.

### 2.5. Statistical analysis

All data were analyzed by one-way factorial analysis of variance (ANOVA). Multiple comparisons tests were carried out using Tukey's HSD post hoc tests. Differences were considered significant at a probability level of  $p \leq 0.05$  and statistical analyses were performed using the IBM SPSS Statistics 19 statistical package. For the correlations, a Pearson correlation was performed between the multiple parameters analyzed using RStudio® software.

### 3. Results

### 3.1. Tissue-specific physiological events during plum overripening

To characterize the overripening process and its physiological changes during fruit storage at room temperature, TSS, TA, TSS/TA, pH and firmness were evaluated (Fig. 1). A significant decrease of firmness (Fig. 1E-F) was observed with prolonging storage time (0–8 days) with a reduction between dates of 27%, having an abrupt decrease (50%) at day 2 and a negative impact on plum texture and appearance (Fig. 1F). Furthermore, TA only showed slight increases (around 15%) at day 8 compared to the TA value at the beginning of the experiment (Fig. 1B). In contrast, TSS values were constant (Fig. 1A), which together with the

increase of acidity resulted in a 20% decrease in the TSS/TA ratio (Fig. 1C). Additionally, pH measurements also showed variations during postharvest days, having an increase at day 2 and therefore, a slight decrease at day 8 (Fig. 1D).

Analysis of total phenol contents on plums exocarp showed a progressive increase during overripening (Fig. 2A), with maximum contents at day 8, being 28% higher than initial contents. Meanwhile, no changes were observed in the mesocarp, where total phenol contents remained constant and were twice lower than exocarp contents (Fig. 2A). Moreover, anthocyanin contents also increased in the exocarp of Angeleno plums (Fig. 2B), being 58% higher at day 8 than the initial day. Nonetheless, no variations on mesocarp anthocyanin contents were found along the time of evaluation (Fig. 2B). Additionally, anthocyanin contents in the exocarp of plums were 188 and 351-fold higher than in the mesocarp at the beginning and the end of the study, respectively, indicating lower anthocyanin contents in the mesocarp and tissue-specific physiochemical changes during overripening. In this sense, when the values were expressed by 100 g of fruit FW (Fig. 3), total phenol and anthocyanin contents in the mesocarp of plums after 8 days of storage, only represented 1/3 and half of the exocarp amounts, which has important nutritional implications.

Endogenous melatonin contents had a strong decrease during postharvest shelf-life, both in mesocarp and exocarp of Angeleno plum (Fig. 4). Initially, plum tissues showed high values of melatonin contents ( $14 \pm 3,6$  and  $20 \pm 6,4$  ng·g DW<sup>-1</sup> in mesocarp and exocarp, respectively). Thereafter, its contents abruptly decreased in both tissues from day 2–8, showing a 90% decrease to almost undetectable contents, less than 2 ng·g DW<sup>-1</sup> (Fig. 4). Likewise, in plum mesocarp a significant decrease in the content of indole-3-acetic acid (IAA) from day 0–2 were observed and thereafter, its levels remained constant (Fig. S1). Conversely, JA showed a transient increase of 20% at day 2 (Fig. S2), the same as 2iP and IPA (Fig. S3). Instead, jasmonic acid precursor, OPDA, showed an abrupt decrease from day 0–2, just as all other conjugated jasmonates (JA-Ile, JA-Met, JA-Leu, JA-Val), which also showed



**Fig. 2.** (A) Total phenol contents (TPC) and (B) anthocyanin contents in the mesocarp and exocarp of control and  $10^{-4}$  M melatonin treated Angeleno plums during days after treatment. Data represent the means  $\pm$  standard error of n = 5. Different lower case letters indicate significant differences over time and asterisks indicate significant differences (p < 0.005) between control and treated at that point in time. See Table 1 for statistics.



**Fig. 3.** (A) Total phenol contents (TPC) and (B) anthocyanin contents in the mesocarp and exocarp of control and  $10^{-4}$  M melatonin treated Angeleno plums during days after treatment. Data represent the means  $\pm$  standard error of n = 5. Data represent the means  $\pm$  standard error of n = 5. Different lower case letters indicate significant differences over time and asterisks indicate significant differences (p < 0.005) between control and treated at that point in time. See Table 1 for statistics.



**Fig. 4.** Endogenous contents of melatonin in the mesocarp and exocarp of control and  $10^{-4}$  M melatonin treated Angeleno plums during days after treatment. Data represent the means  $\pm$  standard error of n = 5. Different lower case letters indicate significant differences over time and asterisks indicate significant differences (p < 0.005) between control and treated at that point in time. See Table 1 for statistics.

decreasing contents during plums postharvest (Fig. S2). Additionally, a decrease in *t*-ZR and GA<sub>7</sub> contents to values less than 1 ng·g DW<sup>-1</sup> was also observed (Fig. S3 and S4), and, in contrast, ABA, SA and ACC levels remained constant (Fig. S1). Therefore, in general, a degradation of a wide variety of phytohormones was occurring during overripening in the mesocarp of Angeleno plums.

Finally, a strong positive correlation was found for firmness with OPDA, JA-Ile and JA-Met but a negative one with JA, *t*-Z, 2iP and IPA (Table S1).

### 3.2. Exogenous melatonin induces tissue-specific changes in fruit quality

Plum quality was assessed to evaluate the effects of  $10^{-4}$  M exogenous melatonin treatment. No significant differences were observed for TSS, TA, TSS/TA, pH and firmness in treated plums compared to control group (Fig. 1).

In the analysis of nutritional properties, total phenol contents showed significant differences between control and melatonin-treated plums, presenting an increase of 21% in the mesocarp phenol contents of Angeleno-treated fruits after 4 days of application (Fig. 2A). Despite endogenous melatonin contents increased both in mesocarp and exocarp (97,21% and 99,46% respectively) (p < 0.001) after melatonin treatments of Angeleno plums (Fig. 4), no significant differences were found for total phenols in the exocarp of these treated fruits (Fig. 2A). Likewise, anthocyanin contents presented a rise of 58% in the mesocarp of fruits after melatonin treatment mainly from day 4, while the exocarp presented no differences (Fig. 2B). This increase of phenolic compounds was also observed in 100 g of an edible portion (Fig. 3), reaching three times more anthocyanins at the end of the experiment. Moreover, a high correlation with total phenolic compounds (TPC) and melatonin as well as with TPC and anthocyanin were found (Table S1). Therefore, exogenous melatonin showed a differential effect depending on the plum tissue regarding phenolic and anthocyanin composition.

An analysis of regulatory cis-elements (Fig. S5 and S6) on the sequences of CHS and phenylalanine ammonia lyase PAL of *Prunus salicina* showed that both genes had upstream and downstream regions with responsive elements to both melatonin and JA, which could be an additional evidence to elucidate the mechanism behind greater contents on TPC and anthocyanins after melatonin treatments.

# 3.3. Exogenous melatonin promotes endogenous changes of other hormonal groups in plums

In order to study the possible relationship between melatonin and other phytohormones during postharvest overripening, the endogenous contents of different developmental and ripening-related plant hormones in the exocarp and mesocarp of Angeleno plums were analyzed (Fig. 5). After melatonin treatment, the contents of IAA, SA, ABA and ACC (Fig. S1) did not differ significantly but a transient increase of 28,74% in the contents of OPDA at day 4 were observed (p = 0.012), 4fold more than those registered in control fruits (Fig. S2a). Moreover, JA increased concomitantly with the increase of OPDA at day 4 in melatonin-treated plums, being 14% higher compared to the control fruits, but thereafter, its contents decreased and were 18% lower than non-treated plum fruits (Fig. S2a). Nevertheless, no variations were found in the studied conjugated jasmonates (Fig. S2b). The content of cytokinins, including 2iP, t-Z and t-ZR, did not show significant differences in treated fruits (Fig. S3). However, IPA contents showed a decrease of 17% on day 8 of the study (Fig. S3). Furthermore, no differences were observed in the content of GAs (Fig. S4) but GA1 had a sharp increase at day 4 in melatonin treated fruits, and even there were no statistical differences at this point between treatments (p = 0.065), the mean values of the contents were 5-fold higher than those in control plums (Fig. S4). Nevertheless, a sturdy correlation between GA1 and melatonin was found (p < 0.01) in Angeleno plums (Table S1).

Overall, quality parameters and hormonal profiling indicated a differential response to postharvest decay for an endogenous condition and exogenous melatonin treatment. While for the first, overripening was mainly characterized by a firmness reduction in the mesocarp as well as an increase of phenolic compounds in the exocarp and a general phytohormones degradation, including melatonin. The second one was characterized by an increase of melatonin in both exocarp and mesocarp in addition to an increase of antioxidant phenolic compounds in the



**Fig. 5.** (A) Variations in the endogenous phytohormones in the mesocarp of Angeleno plum after  $10^{-4}$  M melatonin application during days after treatment. Gray boxes indicate no variations in their content, green boxes indicate an increase, red boxes indicate a decrease and yellow boxes indicate a strong trend to increase. Significant variations were considered when p < 0.05. (B) 12-oxo-phytodienoic acid (OPDA), jasmonic acid (JA) and gibberellin 1 (GA<sub>1</sub>) endogenous content in the mesocarp of control and  $10^{-4}$  M melatonin treated Angeleno plums during days after treatment. Data represent the means  $\pm$  standard error of n = 5 and asterisks indicate significant differences (p < 0.005) between control and treated at that point in time. See Table 1 for statistics.

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### Table 1

P\_values of one way ANOVA performed for the time and treatment factors.

	P <sub>Time</sub>	P <sub>Treatment</sub>
TSS (°Brix·gFW $^{-1}$ )	NS	NS
TA (g malic $gFW^{-1}$ )	0.025	NS
TSS/TA	0.046	NS
pH	< 0.001	NS
Firmness (N)	< 0.001	NS
TPC (mg GAE·gDW <sup><math>-1</math></sup> ) mesocarp	0.048	0.017
TPC (mg GAE·gDW <sup><math>-1</math></sup> ) exocarp	0.016	NS
TPC (mg GAE $100$ gFW <sup>-1</sup> ) mesocarp	NS	0.019
TPC (mg GAE $\cdot 100$ gFW <sup>-1</sup> ) exocarp	0.024	NS
Anthocyanin content ( $\mu$ g cyanidin gDW <sup>-1</sup> ) mesocarp	NS	0.045
Anthocyanin content ( $\mu$ g cyanidin gDW <sup>-1</sup> ) exocarp	0.011	NS
Anthocyanin content (µg cyanidin 100gFW <sup>-1</sup> ) mesocarp	NS	0.045
Anthocyanin content (µg cyanidin 100gFW <sup>-1</sup> ) exocarp	0.015	NS
Melatonin (ng $\cdot$ g DW <sup>-1</sup> ) mesocarp	NS	< 0.001
Melatonin (ng·g DW <sup>-1</sup> ) exocarp	NS	< 0.001
IAA (ng·g DW <sup>-1</sup> ) mesocarp	< 0.001	NS
SA (ng·g DW <sup>-1</sup> ) mesocarp	NS	NS
ABA (ng $\cdot$ g DW <sup>-1</sup> ) mesocarp	NS	NS
ACC (ng·g $DW^{-1}$ ) mesocarp	NS	NS
OPDA (ng·g $DW^{-1}$ ) mesocarp	< 0.001	NS
JA (ng·g $DW^{-1}$ ) mesocarp	< 0.001	NS
JA-Ile (ng·g $DW^{-1}$ ) mesocarp	< 0.001	NS
JA-Met (ng·g DW <sup>-1</sup> ) Mesocarp	0.004	NS
JA-Leu (ng·g $DW^{-1}$ ) mesocarp	< 0.001	NS
JA-Phe (ng·g DW <sup>-1</sup> ) Mesocarp	NS	NS
JA-Val (ng·g $DW^{-1}$ ) mesocarp	< 0.001	NS
2-ip (ng·g $DW^{-1}$ ) mesocarp	< 0.001	NS
IPA (ng·g $DW^{-1}$ ) mesocarp	0.003	NS
Z (ng·g $DW^{-1}$ ) Mesocarp	0.001	NS
ZR (ng·g DW <sup><math>-1</math></sup> ) mesocarp	< 0.001	NS
$GA_1$ (ng·g DW <sup>-1</sup> ) mesocarp	NS	NS
$GA_3$ (ng·g DW <sup>-1</sup> ) mesocarp	0.008	NS
$GA_4$ (ng·g DW <sup>-1</sup> ) mesocarp	NS	NS
$GA_7 (ng \cdot g DW^{-1})$ mesocarp	0.013	NS

mesocarp, including anthocyanins leading to a color change in the pulp, while an increase of OPDA and JA was also observed (Fig. 6).

### 4. Discussion

# 4.1. Fruit softening and hormonal degradation are events tightly associated with overripening of Angeleno plums

Plums are an important and well-liked stone fruit worldwide valued for their color, palatability and aromatic properties as well as their richness in bioactive compounds, including vitamins, phenols, and anthocyanins (Gil et al., 2002). However, ripe plums are highly perishable and predisposed to a rapid deterioration triggering several physiological and visible changes resulting in a loss of edible quality during their postharvest life, mainly because of overripening (Pott et al., 2020). Fruit softening is a needed and complex regulated process that includes cell wall degradation to become an edible fruit. However, an excessive degree of softening leads to texture decay and has a negative impact on fruit quality (Wang et al., 2018). In our study, plums stored at room temperature for 8 days showed an immediate decline in firmness during storage time and a later recovery, probably due to a typical overripening desiccation process once the fruit is separated from the mother plant, which also adversely affected the appearance and texture of plum fruits. Other quality parameters like TSS, TA, and the ratio TSS/TA are also determining for fruit acceptance and usually, these parameters present several changes after harvested and stored at room temperature (Kappel et al., 2002). Here, postharvest Angeleno plums showed variations in TSS/TA as well as pH alterations through the days of evaluation, which could modify organoleptic properties of plum fruits and compromise their quality, as it has been described for other fruits such as pomegranate, peach, tomato and apple (Hussein et al., 2020; Gohari et al., 2021; Al-Dairi et al., 2021; Nyamende et al., 2021).

Plums are a rich source of antioxidants such as anthocyanins and phenols. In this sense, a high level of phenolic compounds has been reported in plums (Kim et al., 2003b), which have important beneficial



**Fig. 6.** Proposed model of the different physiological changes during both endogenous and after exogenous melatonin overripening in the mesocarp and exocarp of plum. TPC: total phenol content; ANT: anthocyanin content; Mel: melatonin; JA: jasmonic acid, JAs: jasmonates; OPDA: 12-*oxo*-phytodienoic acid; JA-Met: jasmonoyl-methionine; JA-Ile: jasmonoyl-isoleucine; 2iP: 2-isopentenyl adenine; Z: *trans*-zeatin; ZR; *trans*-zeatin riboside; IAA: indole-3-acetic acid; GA: gibberellin. Discontinuous lines indicate putative relationships. This figure was created with the help of a vector taken from the vecteezy account at https://www.vecteezy.com /free-vector/nature.

health-promoting effects since these bioactive compounds are known to promote free radical scavenging (Di Carlo et al., 1999). Likewise, anthocyanins stand out for being both potent antioxidants and plant pigments responsible for the red, blue and purple colors of many fruits. Angeleno plum is characterized by a high anthocyanin content in the exocarp while very low contents can be found in the mesocarp, acquiring a red-purple coloration in the peel and a vellow one in the pulp. However, from the present study, it was visible that during postharvest storage at room temperature, the content of anthocyanins as well as total phenols in the exocarp was increased, which resulted in a darkening of the exocarp up to almost black color. In plums mesocarp, both anthocyanins and total phenols remained constant until the end of postharvest evaluation. Throughout fruit storage, it is described that there is also accumulation of reactive oxygen species (ROS), being one of the main factors causing fruit decay because their accumulation can lead to cell damage and ultimately cell death (Vicente et al., 2006). Hence, the high content of phenolic compounds in Angeleno exocarp could prevent from oxidative damage given the antioxidant nature of these compounds, especially since fruit exocarp is the most exposed part to abiotic and biotic pressure during fruit postharvest.

Ripening and overripening are complex processes fine-tuned at different physiological levels, including hormonal regulation, which represents a key factor determining fruit quality (Fenn and Giovannoni, 2021). Here, ABA was the most abundant phytohormone in the mesocarp of plums during postharvest and its levels remained constant during postharvest storage. Nevertheless, hormonal content variations such as a decrease of different phytohormones contents also occurred as fruit deterioration progressed, as it was the case for t-Z, t-ZR and the bioactive gibberellin GA7. A previous study in the postharvest of sweet cherries showed that there was a high degradation of cytokinins and gibberellins during decay of cherry fruits (Tijero et al., 2019), although a higher decrease was observed for free cytokinin bases more than their ribosides, contrary to what happened in plums of the present study. In another study with strawberries, exogenous cytokinin applications induced a defense response against abiotic and biotic stresses, maintaining postharvest fruit quality (Li et al., 2016b). Likewise, preharvest applications of GA<sub>3</sub>in apples improved their quality during storage especially by reducing firmness loss (Souza et al., 2016). These results, together with those found for plums in the present study, strike gibberellins and cytokinin contents as important factors to either determine quality or fruit shelf-life. On the other hand, a decrease in the contents of OPDA, conjugated jasmonates and IAA were also observed during postharvest of plums. In plants, OPDA, which is the precursor of JA, could play a role both by itself and JA-dependent (Böttcher and Pollmann, 2009) in redox signaling (Müller et al., 2017) and plant defense against biotic stress (Gleason et al., 2016; Scalschi et al., 2015; Raacke et al., 2006). Previous studies also reported that exogenous auxin application prevents cell wall degradation in postharvest of strawberries (Chen et al., 2016; Figueroa et al., 2012b). Hence, this wide variety of phytohormones degradation may also contribute to fruit deterioration in plums stored at room temperature although further investigations are required to unravel the possible roles of these plant hormones in plum overripening. Furthermore, although cytokinins and JA have been described to improve quality maintenance and delay softening (Rojas et al., 2021; Roussos et al., 2021; Massolo et al., 2014; Balbontín et al., 2018; Saracoglu et al., 2017), here a negative correlation was found with firmness and JA, Z, 2iP and IPA which showed an increase at day 2 corresponding with the main decrease of firmness. In contrast, a positive correlation with firmness and OPDA, JA-Ile and JA-Met were observed, suggesting that these phytohormones might be involved in the modulation of fruit firmness, although its role is less clear.

# 4.2. Melatonin acts as a signal molecule in plum postharvest quality inducing tissue-specific phenolic compounds and plant hormones

Melatonin is a multifunctional molecule distributed ubiquitously in

diverse plant organs and is responsible for stimulating various physiological processes (Debnath et al., 2019). In fruits, the endogenous melatonin content differs between different species and varieties. In this case, 13 ng·g DW<sup>-1</sup> of endogenous melatonin have been detected in the mesocarp of Angeleno plums at the start of the experiment. These values are similar to those detected in other species such as 3–12 ng·g DW<sup>-1</sup> in some varieties of cherry (Kirakosyan et al., 2009) but higher than others as grapes (<1 ng·g DW<sup>-1</sup>) (Iriti et al., 2006). However, in other fruits like cranberries or tomatoes higher contents have been found (25–96 ng·gDW<sup>-1</sup> and 7–240 ng·g DW<sup>-1</sup> respectively) (Brown et al., 2012; Riga et al., 2014). Nevertheless, during postharvest storage, it is common for fruits to experience a general decrease of endogenous melatonin contents to very low values, as it was observed in Angeleno plums, which could contribute to postharvest decay of plums.

Previous studies have described the effects of exogenous melatonin treatments for maintaining or improving quality properties of fruits as well as nutritional compounds during storage delaying postharvest deterioration by regulating redox metabolism (Gao et al., 2016; Liu et al., 2018), maintaining fruit firmness and TSS levels (Zhai et al., 2018; Rastegar et al., 2020) as well as increasing resistance to pathogens (T. Li et al., 2019). Despite this, its role in delaying postharvest decay and its relationship to other plant hormones during postharvest is still unclear. To elucidate the putative role of melatonin during plum fruit overripening, the tissue-specific effects of  $10^{-4}$  M melatonin treatment were evaluated. In the current study, contrary to the studies aforementioned, exogenous melatonin did not influence the main organoleptic properties of plum fruits such as TSS/TA, pH and firmness. The maintenance of the nutritional quality of fruits after exogenous melatonin improving anthocyanin and phenolic compounds accompanied by an increase of biosynthesis-related genes expressed has also been described in litchi (Zhang et al., 2018), strawberry (Aghdam and Fard, 2017), jujube (Wang et al., 2021), grapes (L. Wang et al., 2020) and recently in plums (Bal, 2019; Xu et al., 2022; Yan et al., 2022). In fact, work from Xu et al. (2022) showed that redness index was strongly variable upon melatonin dosage, where melatonin applications of higher concentrations led to a reduction in redness index and anthocyanin accumulation, while lower melatonin applications at umolar level increased plum postharvest redness index. Similar results have been found in the present experiment, where melatonin applications at  $10^{-4}$  M on plum fruits resulted in increased total phenol and anthocyanin contents in the mesocarp, while the exocarp remained unaffected. In other words, despite melatonin application was exogenously in the exocarp and it increased the content of endogenous melatonin in both mesocarp and exocarp, the increase of phenolic compounds was activated in the mesocarp, not in the exocarp, and most particularly from day 4. Indeed, the concentration of anthocyanins in the mesocarp of treated plums was almost 3-fold higher, leading to higher quality and resulting in a pulp reddish coloration. To our knowledge, this is the first evidence of a differential contribution of melatonin at different tissue levels on these antioxidants during the postharvest shelf-life of plums. Since, phenolic compounds stand out for their antioxidant properties and therefore, their increase could lead to human health benefits and could also participate in the biotic defense response, being able to contribute to the maintenance of the postharvest quality and delaying fruit senescence (Singh et al., 2010; Liu et al., 2014; Meena et al., 2001).

It has been shown that melatonin acts as a general antioxidant, capable of interacting with ROS and RNS (Galano et al., 2011) being a key compound reducing oxidative stress in the biological membranes (García et al., 2014) and a master regulator of redox homeostasis in plants (Arnao and Hernández-Ruiz, 2019). However, the action of melatonin in many physiological processes inducing considerable changes in gene expression and the discovery of its receptor in plants in 2018 indicate that it could be considered as a plant hormone (Arnao and Hernández-Ruiz, 2020). Likewise, whether melatonin is acting as an antioxidant or as a signaling molecule in different physiological processes is still unclear. In this study, the high increase of total phenols in

the mesocarp of plum fruits might be due to both an increase of total phenols resulting from exogenous melatonin and/or an increase of endogenous melatonin, because of its antioxidant properties and the unspecificity limitation of the method. However, the enhanced accumulation of anthocyanins suggest that this increase could be derived by a regulatory role of melatonin acting as a signal molecule by itself. In addition, the possible relationship between melatonin and other plant hormones has been described (Arnao and Hernández-Ruiz, 2018). Mansouri et al. (2021) also showed a higher endogenous accumulation of ABA attributed to exogenous melatonin during strawberry fruit ripening. Despite this, there is a lack of information about the joint contribution of melatonin with other plant hormones in postharvest quality. Thus, in our study, the endogenous content of different phytohormones of Angeleno plums was analysed. The hormonal analysis reveals that an increase of 4-fold OPDA contents occurred in the mesocarp of melatonin-treated plums at day 4, concomitantly with the increase on phenolic compounds in the mesocarp aforementioned. JA also presented higher values at this point of the experiment compared to control plums. It is known that JA is involved in promoting phenylpropanoid compounds biosynthesis such as anthocyanins (Pauwels et al., 2008) by regulating the key WD-repeat/bHLH/MYB complex for its activation (Shan et al., 2009; Qi et al., 2011; Gális et al., 2006). Indeed, Liu et al. (2019a) reported that melatonin treatment increased MeJA contents and JA signaling pathway in tomato fruit disease resistance to B. cinerea resulting in a general increase of phenylpropanoid derivates. Since previous work has related increased JA accumulation after melatonin treatments through downregulation of the JASMONATE-ZIM DOMAIN (JAZ) repressor family proteins, which encode for jasmonate co-receptors and transcriptional repressor in JA signaling pathway (Liu et al., 2019b; Tan et al., 2019), the present study might indicate that melatonin treatments could elicit JA signaling to increase endogenous contents of TPC and anthocyanins. However, there is no previous information about the regulatory role of OPDA by itself in the phenylpropanoid pathway beyond being the precursor of JA and even less during postharvest decay. Additionally, analysis of regulatory cis elements in the sequences of CHS and PAL, revealed that melatonin and JA could be interacting with these enzymes, since different responsive elements for these phytohormones were identified upstream and downstream of their sequence, which adds further evidence to the direct regulation of the phenylpropanoid pathway by melatonin or JAs. Moreover, even if not statistically significant, a strong upward trend of 5-fold more GA<sub>1</sub> was noticed at day 4. This result linked to a high significant correlation between GA1 and melatonin, suggest a close interaction of these phytohormones in plum fruits. In other models such as petunia flowers, gibberellins have been shown to be required for the anthocyanin biosynthesis and accumulation (Weiss et al., 1995), but in other fruits such as strawberries (Martinez et al., 1996; Lin et al., 2021) or Arabidopsis leaves (Loreti et al., 2008) gibberellins inhibited anthocyanin accumulation, indicating contradictory results and an unclear role of GAs on anthocyanin synthesis. Results derived from the present study also suggest that melatonin may be increasing phenolic contents during postharvest storage at room temperature not only through direct regulation but also by signaling with other phytohormones, such as OPDA and JA (Fig. 6). Further studies are however needed to confirm this approach and keep investigating the possible cross-talk between melatonin and other hormones and how these interactions contribute to an improvement of postharvest fruit quality.

### 5. Conclusions

Overall, we have seen that differential and tissue-specific responses to plum postharvest occurred for an endogenous condition and an exogenous melatonin treatment. Endogenous plum overripening is mainly characterized by an immediate loss of firmness while there is an active degradation of melatonin and other phytohormones, and increased darkening of plum exocarp by an accumulation of phenolic compounds. In contrast, during overripening after exogenous melatonin, a high increase of endogenous melatonin in both tissues occurred, leading to tissue-specific effects, including increased anthocyanin and phenol contents in the mesocarp. It is suggested that melatonin may be playing a role as a signal molecule enhancing these antioxidant compounds, which contribute to the maintenance of the fruit quality. However, it is also suggested that melatonin could be acting extending the shelf-life of plums fruits rising these phenolic compounds not only by direct regulation but also through signaling with other plant hormones, increasing their contents during overripening. Therefore, the present study provides valuable information to contribute to the knowledge of the regulatory role of melatonin, which seems to be an interesting molecule to delay senescence and extend the postharvest shelf-life of plum fruits. Further researches are however needed to deeply understand the tissue-specific role of melatonin as well as the hormonal crosstalk and its underlying molecular mechanisms occurring during postharvest overripening.

### **Declaration of Competing Interest**

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

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### Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.plantsci.2022.111287.

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