UNIVERSIDAD MIGUEL HERNÁNDEZ DE ELCHE
ESCUELA POLITÉCNICA SUPERIOR DE ORIHUELA
PROGRAMA DE DOCTORADO EN RECURSOS Y TECNOLOGÍAS AGRARIAS, AGROAMBIENTALES Y ALIMENTARIAS

TESIS DOCTORAL

EFECTO DEL TRATAMIENTO PRECOSECHA Y POSCOSECHA CON MELATONINA SOBRE LA CALIDAD DE FRUTAS Y HORTALIZAS

2023

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Director: Antonio Fabián Guillén Arco

Codirectora: María Serrano Mula

Todas las imágenes presentadas en este manuscrito no relacionadas con la experimentación propiamente dicha son de uso libre y se obtuvieron de las fuentes www.freepik.es y www.freepng.es

Esta tesis doctoral, titulada “Efecto del tratamiento precosecha y poscosecha con melatonina sobre la calidad de frutas y hortalizas”, se presenta bajo la modalidad de **tesis por compendio** de las siguientes **publicaciones**:

Medina-Santamarina, J., Serrano, M., Lorente-Mento, J.M., García-Pastor, M.E., Zapata, P.J., Valero, D. y Guillén, F., Melatonin treatment of pomegranate trees increases crop yield and quality parameters at harvest and during storage. Agronomy 2021, 11, 861. https://doi.org/10.3390/agronomy11050861

Medina-Santamarina, J., Zapata, P.J., Valverde, J.M., Valero, D., Serrano, M. y Guillén, F., Melatonin treatment of apricot trees leads to maintenance of fruit quality attributes during storage at chilling and non-chilling temperatures. Agronomy 2021, 11, 917. https://doi.org/10.3390/agronomy11050917

Guillén, Fabián., **Medina-Santamarina, J**., García-Pastor, M.E., Chen, N.J., Uruu, G. y Paull, R.E., Postharvest melatonin treatment delays senescence and increases chilling tolerance in pineapple, LWT - Food Science and Technology (2022), doi: https://doi.org/10.1016/j.lwt.2022.113989

Medina-Santamarina, J., Serrano, M., Ruiz-Aracil, M.C., Ilea, M.I.M., Martínez-Romero, D. y Guillén, F., A synergistic effect based on the combination of melatonin with 1-methylcyclopropene as a new strategy to increase chilling tolerance and general quality in zucchini fruit. Foods 2022, 11, 2784. https://doi.org/10.3390/foods11182784

Medina-Santamarina, J., Ilea, M.I.M., Ruiz-Aracil, M.C., Valero, D., Castillo, S., Serrano, M. y Guillén, F., Melatonin treatments reduce chilling injury and delay ripening leading to maintenance of quality in cherimoya fruit. Int. J. Mol. Sci. 2023, 24, 3787. https://doi.org/10.3390/ijms24043787

El Dr. D. Antonio Fabián Guillén Arco, director, y la Dra. Dña. María Serrano Mula, codirectora de la tesis doctoral titulada "**Efecto del tratamiento precosecha y poscosecha con melatonina sobre la calidad de frutas y hortalizas**",

CERTIFICAN:

Que D. Jorge Medina Santamarina ha realizado bajo nuestra supervisión el trabajo titulado "**Efecto del tratamiento precosecha y poscosecha con melatonina sobre la calidad de frutas y hortalizas**" conforme a los términos y condiciones definidos en su Plan de Investigación y de acuerdo al Código de Buenas Prácticas de la Universidad Miguel Hernández de Elche, cumpliendo los objetivos previstos de forma satisfactoria para su defensa pública como tesis doctoral.

Y para que conste a los efectos oportunos se firma el presente certificado en Orihuela a 25 de abril de 2023.

Director de la tesis	Codirectora de la tesis
Dr. D. Antonio Fabián Guillén Arco	**Dra. Dña. María Serrano Mula**

Dr. Dña. Juana Fernández López, Catedrática de Universidad y Coordinadora del Programa de Doctorado en Recursos y Tecnologías Agrarias, Agroambientales y Alimentarias (ReTos-AAA) de la Universidad Miguel Hernández de Elche (UMH),

CERTIFICA:

Que la Tesis Doctoral titulada "**Efecto del tratamiento precosecha y poscosecha con melatonina sobre la calidad de frutas y hortalizas "**de la que es autor el graduado en Ciencia y Tecnología de los Alimentos **D. Jorge Medina Santamarina**, ha sido realizada bajo la dirección del **Dr. D. Antonio Fabián Guillén Arco** y la codirección de la **Dra. Dña. María Serrano Mula**, actuando como tutor de la misma el Dr. D. Domingo Jesús Martínez Romero. Considero que la Tesis es conforme, en cuanto a forma y contenido, a los requerimientos del Programa de Doctorado ReTos-AAA, siendo por tanto apta para su exposición y defensa pública.

Y para que conste a los efectos oportunos firmo el presente certificado en Orihuela a 25 de abril de 2023.

Dra. Dña. Juana Fernández López

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Índice de Calidad de las Publicaciones

Artículo 1

Medina-Santamarina, J., Serrano, M., Lorente-Mento, J.M., García-Pastor, M.E., Zapata, P.J., Valero, D. y Guillén, F., Melatonin treatment of pomegranate trees increases crop yield and quality parameters at harvest and during storage. Agronomy 2021, 11, 861. https://doi.org/10.3390/agronomy11050861

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Factor de Impacto (año 2021): 3.949

Factor de Impacto (5 años): 4.117

Artículo 2

Medina-Santamarina, J., Zapata, P.J., Valverde, J.M., Valero, D., Serrano, M. y Guillén, F., Melatonin treatment of apricot trees leads to maintenance of fruit quality attributes during storage at chilling and non-chilling temperatures. Agronomy 2021, 11, 917. https://doi.org/10.3390/agronomy11050917

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Artículo 3

Guillén, Fabián., **Medina-Santamarina, J.,** García-Pastor, M.E., Chen, N.J., Uruu, G. y Paull, R.E., Postharvest melatonin treatment delays senescence and increases chilling tolerance in pineapple, LWT - Food Science and Technology 169, 2022, 113989. doi: https://doi.org/10.1016/j.lwt.2022.113989.

Revista: LWT

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Factor de Impacto (5 años): 6.295

Artículo 4

Medina-Santamarina, J., Ilea, M.I.M., Ruiz-Aracil, M.C., Valero, D., Castillo, S., Serrano, M. y Guillén, F., Melatonin treatments reduce chilling injury and delay ripening leading to maintenance of quality in cherimoya fruit. Int. J. Mol. Sci. 2023, 24, 3787. https://doi.org/10.3390/ijms24043787

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Factor de Impacto (5 años): 6.628

Artículo 5

Medina-Santamarina, J., Serrano, M., Ruiz-Aracil, M.C., Ilea, M.I.M., Martínez-Romero, D. y Guillén, F., A synergistic effect based on the combination of melatonin with 1-methylcyclopropene as a new strategy to increase chilling tolerance and general quality in zucchini fruit. Foods 2022, 11, 2784. https://doi.org/10.3390/foods11182784

Revista: Foods

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Cuartil: Q1

Rango: 35/144

Factor de Impacto (año 2021): 5.561

Factor de Impacto (5 años): 5.940

ÍNDICE

ESTRUCTURA DE LA TESIS DOCTORAL

El contenido de esta memoria se ha redactado de acuerdo con la normativa vigente de la Universidad Miguel Hernández de Elche para defender esta Tesis Doctoral bajo la modalidad de tesis por compendio de publicaciones. Por ello, esta memoria se ha estructurado de acuerdo con los siguientes puntos:

- **Resumen:** breve descripción de los resultados más relevantes y conclusiones obtenidas en esta tesis doctoral.
- **Introducción:** breve introducción de los antecedentes científicos y objeto de la Tesis Doctoral, que relaciona el estado del arte de los problemas de conservación poscosecha de distintos productos vegetales con vidas poscosecha breves. Además, se ha estudiado la importancia de los cultivos para justificar el uso de estos cultivos.
- **Objetivos:** objetivo global y objetivos parciales de la investigación.
- **Resumen de la metodología:** explicación breve del material vegetal utilizado, diseño experimental de los tratamientos estudiados y los métodos analíticos utilizados para llevar a cabo los experimentos incluidos en esta Tesis Doctoral.
- **Publicaciones científicas:** transcripción literal de las cinco publicaciones científicas por las que se compone esta tesis: “Melatonin Treatment of Pomegranate Trees Increases Crop Yield and Quality Parameters at Harvest and during Storage”, “Melatonin Treatment of Apricot Trees Leads to Maintenance of Fruit Quality Attributes during Storage at Chilling and Non-Chilling Temperatures”, “Synergistic Effect Based on the Combination of Melatonin with 1-Methylcyclopropene as a New Strategy to Increase Chilling Tolerance and General Quality in Zucchini Fruit.”, “Postharvest melatonin treatment delays senescence and increases chilling tolerance in pineapple” y “Melatonin treatments reduce chilling injury and delay ripening leading to maintenance of quality in cherimoya fruit”.
- **Resumen de los resultados y discusión:** en esta sección los principales resultados obtenidos en esta Tesis Doctoral se explican y se discuten. Además, se realiza un análisis comparativo entre los efectos

de los tratamientos ensayados sobre la calidad y tolerancia al daño por frío de los productos vegetales estudiados en esta Tesis Doctoral.

- **Conclusiones:** exposición de las principales conclusiones obtenidas en esta Tesis Doctoral.
- **Futuras líneas de investigación:** breve descripción de futuras líneas de investigación que pueden desarrollarse a partir de las conclusiones obtenidas de esta Tesis.
- **Referencias:** bibliografía empleada para escribir y justificar esta Tesis Doctoral.

Resumen / Abstract

RESUMEN

La aplicación de sustancias protectoras o conservadoras de origen artificial con el objetivo de mejorar las producciones o la calidad del fruto en la cosecha, así como durante la posterior poscosecha, genera preocupación en la población. Su potencial tóxico residual, impacto sobre la salud y la contaminación medioambiental fomenta el creciente interés tanto de los consumidores como de productores e investigadores por los alimentos conservados a partir de tecnologías que empleen sustancias de origen natural. Las sustancias bioactivas procedentes del metabolismo de las plantas son capaces de estimular diferentes rutas bioquímicas y diferentes aspectos de la fisiología de la planta. De hecho, es muy difícil determinar la función exacta de cada una de estas sustancias naturales ya que son sustancias que participan en múltiples y variadas actividades metabólicas. La melatonina es una de estas sustancias capaces de generar una respuesta en los tejidos vegetales tanto en la germinación, desarrollo y senescencia de los mismos.

En esta Tesis Doctoral se ha realizado un estudio de las posibilidades de la melatonina de aportar soluciones como estrategia precosecha y poscosecha en diferentes productos vegetales de elevado interés por parte de los consumidores. Las granadas 'Mollar de Elche' y los albaricoques mikado y colorado incrementan su coloración externa a lo largo de su desarrollo en el árbol, la cual es muy apreciada en estos frutos, aunque en estas especies vegetales la coloración rojiza es escasa. Por otro lado, las producciones de estas distintas especies sufren mermas importantes debido a factores bióticos pero también al cambio climático que recrudece la acción de los factores abióticos. Además, estos frutos durante la poscosecha pierden sus atributos de calidad principalmente debido a pérdidas de peso y de firmeza, cambios en la acidez y de color entre otros. Para retrasar la pérdida de calidad se suelen almacenar en condiciones de refrigeración lo cual puede generar daños por el frío. En este sentido, encontramos una situación similar en otras especies como la piña, el calabacín o las chirimoyas. Estos productos vegetales desarrollan fisiopatías relacionadas con la exposición a las bajas temperaturas durante tiempos prolongados, además de perder atributos de calidad rápidamente durante el almacenamiento. Por tanto, el objetivo de esta Tesis Doctoral ha sido evaluar la acción de la aplicación de melatonina en precosecha y poscosecha sobre estos problemas, tanto en el momento de la cosecha como durante el posterior almacenamiento poscosecha.

Los tratamientos precosecha con melatonina se realizaron mediante pulverización foliar con distintas concentraciones entre 0.1 y 1 mM en dos ciclos productivos consecutivos tanto en las granadas (2017-2018) como en las diferentes variedades de albaricoque estudiadas (2019-2020). Los tratamientos poscosecha se realizaron mediante inmersión en soluciones de melatonina con bajas concentraciones a las que se sometió a los frutos durante distintos

tiempos de inmersión. Este primer experimento se realizó sobre todas las especies vegetales de esta Tesis Doctoral de forma que, tras un primer experimento con piñas (0,1 y 0.5 mM), chirimoyas (0,1, 0,3, 0,5 y 1 mM) y calabacines (0,1, 0,5 y 1 mM) sumergidos a distintos tiempos de inmersión, se aplicaron de nuevo en un segundo experimento usando la combinación óptima entre estos dos parámetros (concentración/tiempo de inmersión). En el caso de los calabacines, las condiciones óptimas se combinaron además con 2400 ppb de 1-MCP.

Las aplicaciones precosecha con melatonina incrementaron las producciones de granada y albaricoque. Las granadas tratadas con melatonina incrementaron tanto el volumen como el número de frutos en ambos ciclos productivos. Sin embargo, el incremento de la producción en los albaricoques de ambas variedades se relacionó únicamente con el incremento del peso de los frutos sin afectar al número de frutos. Además, los tratamientos precosecha consiguieron retrasar la maduración tanto de granadas como de albaricoques lo cual fue determinado al evaluar los distintos parámetros de calidad (firmeza, color, contenido en sólidos solubles y acidez) en ambas especies. Así, en el momento de la cosecha pudimos comprobar la efectividad de la melatonina retrasando tanto la maduración del fruto en el árbol como el deterioro durante la conservación poscosecha de granadas y albaricoques atendiendo a diversos parámetros de calidad estudiados. De hecho, durante el almacenamiento refrigerado, los frutos tratados con melatonina mostraron una menor respiración, pérdida de firmeza, y una mayor acidez en general tanto en la recolección como durante su posterior almacenamiento. Además, los tratamientos precosecha con melatonina fueron efectivos incrementando el color rojo de los frutos en granadas y albaricoques almacenados a 10 y 1 ºC respectivamente. Las granadas no sufrieron daños por frío a 10 ºC pero los albaricoques sí mostraron esta fisiopatía al estar almacenados a temperaturas subóptimas. En este sentido, las aplicaciones precosecha con melatonina fueron exitosas a la hora de reducir el impacto de los daños por frío, lo que estuvo en consonancia con la mayor integridad de los tejidos en los frutos tratados con melatonina.

En esta Tesis Doctoral, los tratamientos poscosecha realizados con melatonina retrasaron la maduración y senescencia de los productos vegetales climatéricos (chirimoya) y no climatéricos (piña y calabacín) estudiados en esta Tesis. Las aplicaciones con melatonina en poscosecha retrasaron las pérdidas de peso en chirimoyas y calabacines. En todos los productos vegetales estudiados fueran climatéricos o no, los tratamientos con melatonina mantuvieron los valores de firmeza de los frutos. Este mantenimiento de la calidad en los frutos pudo estar asociada a una menor respiración en los frutos no climatéricos tratados con melatonina y a una menor producción de etileno en las chirimoyas. Estos y otros resultados confirmaron el retraso en la maduración de todos los lotes tratados con melatonina en poscosecha. Este

retraso en la evolución de la maduración estuvo acompañado en el caso de las chirimoyas también por la evolución de la actividad antioxidante y el contenido en polifenoles que mostró un patrón similar. Este retraso también se observó en chirimoyas y calabacines tratados con melatonina con respecto a la degradación de las clorofilas. En esta Tesis Doctoral estos efectos se han relacionado con un menor estrés oxidativo y balance energético dado los menores niveles de MDA y de fuga de electrolitos que se encontraron en las especies vegetales evaluadas. Todas ellas tras el tratamiento con melatonina en poscosecha, mostraron una importante reducción de los daños por frío que pudo estar directamente relacionado con la menor pérdida de agua de los frutos y por tanto mayor firmeza e integridad de los tejidos. Además, se determinó que el menor metabolismo celular observado en los lotes tratados con melatonina fue un factor muy importante en la reducción de los daños por frío en todas estas especies vegetales estudiadas.

Pese a que la melatonina por sí sola es capaz de retrasar la pérdida de calidad en los frutos tratados tras la cosecha, en esta Tesis se ha demostrado por primera vez las buenas perspectivas que ofrece la combinación de sustancias comerciales de origen artificial como el 1-metilciclopropeno utilizadas comercialmente en la actualidad con sustancias de origen natural como la melatonina. La combinación de tratamientos mostró un efecto sinérgico en el retraso en la senescencia de los calabacines. De hecho, los tratamientos aplicados por separado no obtuvieron mejores resultados que los observados cuando ambas tecnologías se aplicaron de forma conjunta. Este efecto fue especialmente importante sobre los daños por frío ya que el tratamiento combinado redujo de forma muy eficiente esta fisiopatía durante la conservación refrigerada.

Por tanto, concluimos que las aplicaciones precosecha y poscosecha con melatonina realizadas en esta Tesis Doctoral han demostrado ser una estrategia eficaz para incrementar las producciones, retrasar la maduración y la senescencia de las especies vegetales evaluadas, prolongando así la vida útil y manteniendo la calidad de las frutas y hortalizas estudiadas. Además, la melatonina es un compuesto natural que se ha descrito como seguro para el consumo humano, por lo que podría ser una alternativa adecuada a los productos químicos sintéticos o en la reducción de la concentración aplicada de los mismos.

ABSTRACT

The application of preservative substances of artificial origin with the aim of improving yields or fruit quality at harvest, as well as during the subsequent postharvest management, generates concern among the population. Their residual toxic potential, impact on health and environmental contamination has led to the growing interest of consumers, producers and researchers in food science with using substances of natural origin as an emerging technology. Bioactive compounds from plant metabolism are able to stimulate different biochemical pathways and different aspects of plant physiology. In fact, it is very difficult to determine the exact function of each of these compounds as they are substances involved in multiple and different metabolic activities. Melatonin is one of these substances capable of generating a response in plant tissues during germination, development and senescence.

In this PhD Thesis, the possibilities of melatonin to provide solutions as a pre-harvest and post-harvest strategy in different vegetable products of high consumer interest has been carried out. Pomegranates 'Mollar de Elche' and apricots ‘Mikado’ and ‘Colorado’ increase their external coloration along their development on trees which is very appreciated in these fruits, although in these species the reddish coloration is scarce. On the other hand, the production of these different fruits suffers significant losses due to biotic factors but also to climate change, which intensifies the action of abiotic factors. In addition, these fruits lose their quality attributes during postharvest storage mainly due to weight and firmness losses, changes in acidity and color, among others. To delay quality losses, these species are usually stored under cold storage, which can cause chilling injury In this sense, we found a similar situation in other studied species in this PhD Thesis such as pineapple, zucchini or Cherimoya. These products develop physiological disorders related to its exposure to suboptimal temperatures for long periods of time, especially after fruits are tempered at warmer temperatures. Therefore, the objective of this PhD Thesis has been to evaluate the action of pre-harvest and post-harvest melatonin application on these problems both at the time of harvest and during the subsequent post-harvest storage.

Pre-harvest treatments with melatonin were performed by foliar spraying with different concentrations between 0.1 and 1 mM in two consecutive growing seasons on both, pomegranates (2017-2018) and the different apricot cultivars studied (2019-2020). Postharvest treatments were carried out by immersion in melatonin solutions with low concentrations during different immersion times. This first experiment was carried out on all the plant species of this Doctoral Thesis so that after a first experiment with pineapples (0.1 and 0.5 mM), cherimoya (0.1, 0.3, 0.5 and 1 mM) and zucchini (0.1, 0.5 and 1 mM) immersed at different immersion times, the optimal combination between these two parameters (concentration/immersion time) was applied again in a second

experiment. In the case of zucchini, the optimum conditions were also combined with 2400 ppb of 1-MCP.

Pre-harvest applications of melatonin increased pomegranate and apricot yield. Pomegranates treated with melatonin increased both fruit volume and number of fruits in two growing seasons. However, the increase in yield in both apricot cultivars was related only to the increase in fruit weight without affecting the number of fruits. In addition, the pre-harvest treatments were able to delay pomegranate and apricot ripening, which was determined by evaluating the different quality parameters (firmness, color, soluble solids content and acidity) in both species. Thus, at harvest time, we were able to evaluate melatonin effectiveness in delaying both, fruit ripening on tree and senescence during postharvest storage of pomegranates and apricots according to various quality parameters studied. In fact, during cold storage, melatonin-treated fruits showed lower respiration, loss of firmness, and higher overall acidity both at harvest and during subsequent storage. In addition, pre-harvest melatonin treatments were effective in increasing fruit red color in pomegranates and apricots stored at 10 and 1 ºC, respectively. Pomegranates did not suffer chilling injury at 10 ºC but apricots did show this disorder when stored at suboptimal temperatures. In this regard, pre-harvest melatonin applications were successful in reducing the impact of chilling injury, which was consistent with higher tissue integrity in melatonin-treated fruit.

In this PhD Thesis, postharvest treatments with melatonin delayed ripening and senescence of climacteric (cherimoya) and non-climacteric (pineapple and zucchini) species studied in this Thesis. Postharvest melatonin applications delayed weight losses in cherimoya and zucchini. In all species studied, whether climacteric or not, melatonin treatments maintained fruit firmness values. This maintenance of fruit quality could be associated with lower respiration in non-climacteric fruits and vegetables treated with melatonin and lower ethylene production in cherimoyas. These and other results confirmed the delay in ripening of all melatonin-treated lots during postharvest storage. This delay in ripening evolution was accompanied in the case of cherimoyas also by the evolution of antioxidant activity and polyphenol content which showed a similar pattern. This delay was also observed in melatonin-treated cherimoya and zucchini with respect to chlorophyll degradation. In this PhD Thesis these effects have been related to a lower oxidative stress and energy balance given the lower levels of MDA and electrolyte leakage found in the plant species evaluated. All the species studies after post-harvest treatments with melatonin, showed a significant reduction in chilling injury, which could be directly related to the lower fruit water loss and therefore greater firmness and tissue integrity. In addition, it was determined that the lower cellular metabolism observed in the melatonin-treated lots was a very important factor in the reduction of chilling injury in all the plant species studied.

Although melatonin by itself is capable of delaying quality loss in treated fruit after harvest, this PhD Thesis has demonstrated for the first time the good expectations offered by the combination of commercial substances of artificial origin such as 1-methylcyclopropene currently used commercially, with substances of natural origin such as melatonin. The combination of treatments showed a synergistic effect in delaying senescence of zucchini. In fact, the treatments applied separately did not obtain better results than those observed when both technologies were applied together. This effect was especially important on chilling injury since the combined treatment reduced this parameter very efficiently during refrigerated storage.

Therefore, we conclude that melatonin pre-harvest and post-harvest applications carried out in this PhD Thesis have proven to be an effective strategy to increase yield, delay ripening and senescence of the plant species evaluated, thus prolonging the shelf life and maintaining the quality of the fruits and vegetables studied. In addition, melatonin is a natural compound that has been described as safe for human consumption, so it could be a suitable alternative to synthetic chemicals or in reducing the concentration of these chemicals commercially applied.

1 Introducción

1. INTRODUCCIÓN

1.1. Importancia del cultivo

1.1.1. Granada (*Punica granatum* L.)

La granada es una de las frutas comestibles más antiguas que se conocen y que ha ganado popularidad e interés científico en los últimos años por su valor nutricional y sus beneficios para la salud, ya que es muy rica en compuestos bioactivos con actividad antioxidante.

En la actualidad, la granada se cultiva en todo el mundo en condiciones climáticas mediterráneas, subtropicales y tropicales. Se pueden encontrar huertos en Oriente Medio y la región del Cáucaso, África septentrional y tropical, el subcontinente indio, Asia central, las zonas más secas del sudeste asiático, la cuenca mediterránea, América del Norte y del Sur y Australia.

En los últimos años, este fruto se puede encontrar en los mercados de Europa y los países occidentales. En muchas zonas del mundo, sobre todo en Asia, la granada también está ampliamente comercializada, además de utilizarse con diferentes fines medicinales (Ferrara et al., 2021).

En España la producción de granada se concentra principalmente en dos comunidades autónomas. La Comunidad Valenciana se sitúa como la principal región productora con un 79% de la producción total del país, seguida por Murcia, con un 13,52%. La provincia de alicante aporta el 69,42% de la producción estatal (**Figura 1**) (MAPA, 2018).

Figura 1: *Producción de granada en toneladas por CC.AA. y provincia. Fuente: MAPA, 2018.*

Entre las variedades comerciales españolas de granada, la más cultivada es la "Mollar de Elche" que se cultiva en la Región de Elche. Esta variedad se encuentra amparada por una Denominación de Origen Protegida (DOP) desde 2016 [R (UE) 2016/83]. Otros cultivares con producciones importantes son: 'Wonderful', 'Acco' y 'Smith'.

1.1.2. Albaricoque (*Prunus armeniaca* L.)

El albaricoque (*Prunus armeniaca* L.) es una fruta de clima templado perteneciente a la familia de las rosáceas, originaria de China y Asia Central. Se cultiva actualmente en todo el mundo, siendo los mayores productores los países de Oriente Medio y del Mediterráneo; España ocupa el sexto lugar en la producción mundial según los datos más recientes de la FAO con 145.830 toneladas (**Figura 2**).

Figura 2 *Top 15 mayores productores de albaricoque a nivel mundial. Fuente: FAOSTAT, 2019*

Esta fruta de hueso es muy apreciada por los consumidores debido a su agradable sabor, marcado por el contenido en azúcar, la relación azúcares/acidez y su aroma (Ali et al., 2020; Batool et al., 2021). En los últimos años también ha aumentado el interés por sus propiedades nutritivas y su contenido en sustancias bioactivas con actividad antioxidante, como los compuestos fenólicos, vitaminas y carotenoides (Egea et al., 2007; Fan et al., 2018a; Velardo-Micharet et al 2021).

En el año 2018 se produjeron 176.289 toneladas de esta fruta en España, de las cuales el 55,45% las produjo la Región de Murcia y en segundo lugar la Comunidad Valenciana con el 13,86% de la producción estatal (**Figura 3**).

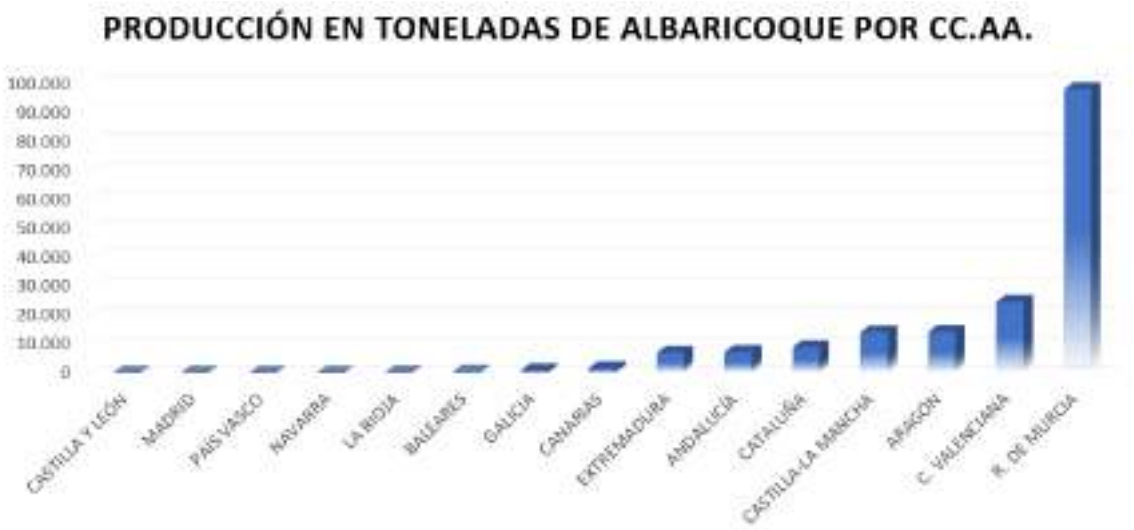


Figura 3: *Producción en toneladas de albaricoque en España por Comunidad Autónoma. Fuente: MAPA, 2018.*

1.1.3. Piña (*Ananas comosus* L.)

La piña o *Ananas comusus* es una fruta perteneciente a la familia de las Bromeliáceas, que se consume en España desde que los conquistadores españoles y portugueses la introdujeron en Europa procedente de América.

La piña es originaria de Sudamérica y fue introducida en la Península Ibérica en el siglo XVI, de la mano de los conquistadores españoles del Nuevo Mundo. Actualmente se cultiva en regiones subtropicales y tropicales representando en la actualidad el segundo fruto tropical más cultivado del mundo (aproximadamente el 20% de la producción comercial de estos productos), tan sólo por detrás de la banana.

Los principales productores a nivel mundial son: Costa Rica, Filipinas, Brasil, Indonesia, China, India, Tailandia, Nigeria, México, Colombia, Angola, Ghana, Vietnam, Perú y Venezuela (**Figura 4**).

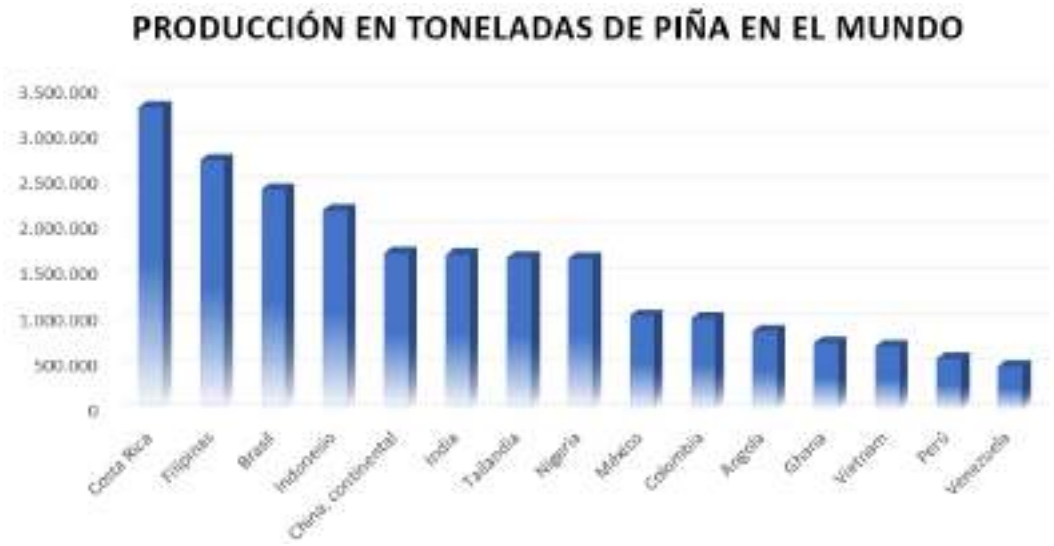


Figura 4: *Producción mundial de piña en toneladas. Fuente: FAOSTAT, 2019*

En España el mayor cultivo de piña se encuentra en las islas canarias, donde se alcanzan a producir hasta 2.327,5 toneladas según datos de 2020 del instituto canario de estadística. Además, en los últimos años se han instalado fincas experimentales con 6000 plantones produciendo piñas con éxito en el campo de Cartagena.

1.1.4. Chirimoya (*Annona cherimola* Mill.)

La chirimoya (familia *Annonaceae*) es originaria del sur de Ecuador y del norte de Perú, donde puede encontrarse en masas forestales silvestres. En el sur de Ecuador, la chirimoya no se cultiva a escala comercial. Sin embargo, a nivel mundial, España es el principal productor, mientras que Perú, Chile, Bolivia y Australia son otros importantes países productores de chirimoya (Scheldenman 2002).

Las frutas de *Annona* son de las más sabrosas del mundo, debido a su pulpa dulce y cremosa y su fragante sabor cuando están completamente maduras. De las 100 especies de *Annona* sólo 5 especies, *A. cherimola* Mill., *A. muricata* L., *A. reticulata* L., *A. senegalensis* Pers. y *A. squamosa* L., son de gran importancia comercial (Pareek et al 2011).

En España el cultivo de la chirimoya se concentra casi en exclusiva en Andalucía, con 43.677 toneladas producidas en 2018 según datos del Ministerio de Agricultura, Pesca y Alimentación, y mayoritariamente en la provincia de Granada. La variedad “Fino de Jete” supone más del 90% de la superficie de chirimoya cultivada en la Costa Tropical de Granada-Málaga, por lo que, al ser España el primer productor mundial, esto convierte a la variedad “Fino de Jete” como la más importante a nivel productivo de todo el mundo (Pinillos et al., 2013). Esta variedad se encuentra dentro de la Denominación de

Origen Protegida (DOP) Chirimoya de la Costa Tropical de Granada-Málaga (DOUE L 80/34 de 26.03.2010), constituida por los terrenos ubicados en la comarca natural del mismo nombre, entre las provincias de Málaga y Granada, de la Comunidad Autónoma de Andalucía.

1.1.5. Calabacín (*Cucurbita pepo* L.)

La familia de las cucurbitáceas incluye una serie de cultivos muy apreciados, como el pepino, el melón, la sandía y la calabaza, que se producen y consumen en todo el mundo. El morfotipo calabacín de *Cucurbita pepo* L. es una de las más importantes variedades de calabacín por su distribución y valor económico (Megías et. al. 2018).

El calabacín es una fruta no climatérica, cosechada en un estado de desarrollo inmaduro, y por tanto muy perecedera. La vida útil de esta fruta es de gran importancia, especialmente en el sur de España, donde la mayor parte de la producción hortícola se exporta a Europa en camiones refrigerados. Es en esta zona donde se concentra casi toda la producción de este producto, siendo Andalucía la comunidad productora por excelencia con 494.374 toneladas producidas en 2018 de las 596.315 toneladas producidas en toda en España (**Figura 6**). A su vez, la provincia de Almería concentra el 92,25% de la producción andaluza de calabacín, seguida por Granada con un 3,44%, Málaga con 1,81% y Cádiz con 1,79% (MAPA, 2018).

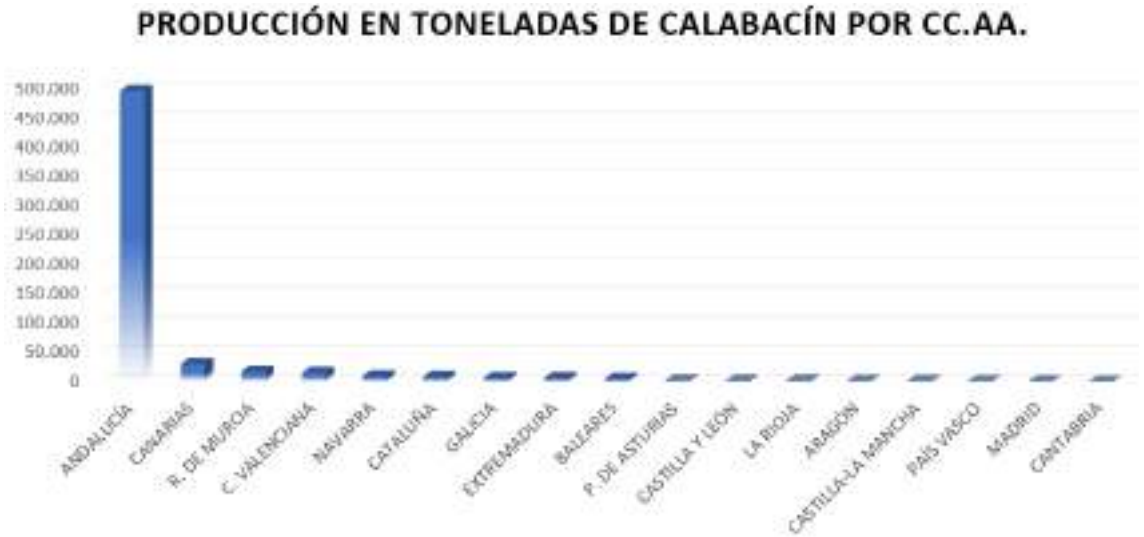


Figura 5: *Producción en toneladas de calabacín en España por Comunidad Autónoma. Fuente: MAPA, 2018.*

1.2. Factores Precosecha que afectan a la calidad

A lo largo de la producción y desarrollo en los campos de productos hortofrutícolas deben tenerse en cuenta múltiples factores que afectan tanto a la productividad de los cultivos como a la calidad en el momento de la

recolección que, de no ser debidamente controlados, pueden producir grandes pérdidas económicas y de calidad en los frutos durante un posterior almacenamiento.

La pudrición de la fruta es el principal problema poscosecha al que deben hacer frente los productores y comercializadores, ya que el deterioro por hongos puede causar grandes pérdidas económicas, aunque la aparición de la podredumbre y su gravedad dependen del tipo de fruto (Zapata et al., 2013).

Por este motivo el uso de productos químicos con acción fungicida está muy extendido durante la producción de los productos hortofrutícolas para controlar la acción de patógenos o plagas que merman la calidad y aceptación de los frutos por el consumidor. Sin embargo, debido a la creciente preocupación por el uso de productos de origen artificial en los alimentos, el incremento de las restricciones en su aplicación y la preocupación por su impacto sobre el medio ambiente existe una tendencia mundial a explorar nuevas alternativas para reducir el uso de fungicidas sintéticos (Zapata et al., 2013; Wang et al., 2014).

Esta concienciación por el cuidado del medio ambiente no solo viene dada por el uso de productos agroquímicos de origen artificial, sino también por los efectos negativos inducidos al planeta por el cambio climático generado por el ser humano, que deterioran los ecosistemas, merman los recursos hídricos y la calidad de la nutrición humana. La escasez de agua por el incremento de la población, las superficies de cultivo extensivas y la contaminación de recursos hídricos es un problema emergente para nuestro planeta. Por lo tanto, para superar estos desafíos, es crucial considerar diferentes tipos de adaptación agrícola, incluyendo cultivos con alta eficiencia en el uso del agua y el uso de compuestos de origen natural que no afecten al ecosistema ni dejen residuos perjudiciales para la salud del consumidor (Kahramanoglu y Usanmaz, 2019).

Cada vez más consumidores exigen una producción de alimentos más natural y respetuosa con el medio ambiente, de gran calidad, con una vida útil prolongada y sin conservantes químicos (Saavedra et al., 2016). Este hecho ha incrementado el interés por el uso de compuestos como jasmonatos, salicilatos o melatonina, compuestos de origen natural capaces de activar los mecanismos de defensa de las plantas frente al estrés abiótico y biótico, además de acumular en los frutos compuestos beneficiosos para la salud de los consumidores. Las plantas responden a estos estreses activando una serie de mecanismos de defensa frente a las infecciones de patógenos o diferentes tipos de estrés ambiental. Estos mecanismos estructurales y bioquímicos están implicados en las defensas naturales pasivas y activas, contribuyendo a aumentar su resistencia frente a futuros ataques de patógenos y teniendo también efecto en la mejora de la composición fitoquímica de las plantas, como

es el caso del contenido en antocianinas y sustancias de tipo fenólico (Martínez-Esplá et al, 2017; Apolinar-Valiente et al., 2018; Viacaba et al., 2018).

Diversos estudios sobre los efectos de la aplicación de elicitores en precosecha muestran el potencial de estas sustancias para afrontar los actuales retos de la agricultura y de la industria, al producir incrementos en la producción, calidad del fruto y en la vida útil durante la poscosecha en productos vegetales como la granada (García-Pastor et al., 2019), el albaricoque (Abd-El Naby et al., 2019) y la cereza (Carrión-Antolí et al, 2022), o inducir mayor resistencia a las enfermedades en tomate (Sheng et al., 2020) y espinacas (Asif et al., 2020) entre otros.

1.3. Problemática poscosecha y daños por frío

El almacenamiento a baja temperatura es el método más utilizado para prolongar la vida útil y mantener la calidad poscosecha de productos hortofrutícolas. Sin embargo, el almacenamiento a largo plazo bajo temperaturas subóptimas induce varios tipos de daños en numerosos frutos tropicales, subtropicales o de zonas templado-cálidas denominados daños por frío (Carvajal et al., 2011; González-Agüero et. al. 2011; Cui et al., 2019).

Los daños por frío se producen en un amplio rango de bajas temperaturas, dependiendo de la susceptibilidad de los cultivos y de la concentración de solutos. Se cree que las respuestas de la fruta a este tipo de daño se activan por el daño de la membrana celular, lo que desencadena una cascada de reacciones que incluyen la degradación de la estructura celular, producción de etileno, aumento de la respiración, interferencias en la producción de energía, reducción de la fotosíntesis, eventos oxidativos como el pardeamiento enzimático, depresiones en la piel, harinosidad, pérdida de firmeza y la acumulación de compuestos tóxicos como el etanol y el acetaldehído (González-Agüero et. al. 2011; Jayarajan y Sharma, 2021).

1.3.1. Granada (*Punica granatum*, L.)

Los frutos del granado (*Punica granatum*, L.) presentan en algunas de sus variedades importantes problemas de intensidad de color, tanto en la piel como en los arilos, en el momento de su recolección. Este hecho dificulta a los agricultores su venta a los mercados nacionales e internacionales, ya que este atributo es muy apreciado por los consumidores.

Durante el almacenamiento poscosecha la granada presenta además importantes pérdidas de calidad debido a varios trastornos fisiológicos y enzimáticos. Estas pérdidas se deben principalmente a las pérdidas de peso, firmeza, color y acidez, lo que reduce la aceptabilidad de los consumidores al

percibir que han perdido su frescura, jugosidad y sabor (Ehteshami et al., 2021). Para evitar estos cambios indeseables y prolongar la capacidad de almacenamiento, la mejor herramienta poscosecha ha sido el almacenamiento a baja temperatura. Sin embargo, la granada es sensible al daño por frío cuando se almacena a temperaturas inferiores a 5-10 °C dependiendo de la variedad, siendo los principales síntomas la deshidratación de la piel, el pardeamiento y el *pitting*, la depresión de la superficie de la fruta, y una mayor susceptibilidad a las podredumbres (Valero y Serrano, 2010; Pareek et al, 2015; García-Pastor et al., 2020a; García-Pastor et al., 2020b).

Las técnicas utilizadas para mantener o incrementar la calidad y las propiedades de la granada son diversas, pudiéndose aplicar durante la precosecha o en poscosecha previamente al almacenamiento. En la precosecha de la granada se han aplicado tecnologías exitosas como el riego salino de los árboles para incrementar la acumulación de compuestos bioactivos (Borochov-Neori et al., 2014). Además, la aplicación foliar de minerales como el cloruro de calcio y elicitores como el metil jasmonato, ácido oxálico y la melatonina incrementan el rendimiento de los árboles, mejoraron la calidad y la coloración de la fruta en la recolección, incrementando el contenido en compuestos bioactivos, retrasando la maduración y la aparición de los síntomas de daño por frío durante el almacenamiento refrigerado (Kamal et al., 2017; Garcia-Pastor et al., 2019; García-Pastor et al., 2020a; García-Pastor et al., 2020c; Lorente-Mento et al., 2021). Otras tecnologías basadas en el embolsado de la fruta en los árboles mejoraron la calidad de los frutos y redujeron la incidencia de la quemadura solar (Asrey et al., 2020). Además, combinando el embolsado con riego deficitario retrasó la maduración y también incrementó la acumulación de compuestos antioxidantes (Griñan et al., 2019). En poscosecha se han aplicado tecnologías como los recubrimientos comestibles que incrementan la vida útil del fruto (Malekshahi y Kaji 2021). La inmersión de los frutos en ácido málico, salicílico, oxálico y putrescina ha mostrado ser eficiente a la hora de reducir los síntomas de daño por frío y el deterioro de los parámetros de calidad, alargando la vida útil del producto durante el almacenamiento convencional o combinado con atmósferas controladas (Yildiz et al., 2020; Koyuncu et al., 2019; Ehteshami et al., 2021). También se han realizado aplicaciones intermitentes de ozono para mantener el color en la piel (Buloc y Koyuncu 2020) así como recubrimientos con ácido γ-aminobutírico (GABA) mejorando la firmeza, actividad antioxidante, composición fenólica y el color del fruto, aliviando los síntomas del daño por frío (Nazoori et al., 2020) entre otros.

1.3.2. Albaricoque (*Prunus armeniaca*, L.)

El albaricoque (*Prunus armeniaca,* L.) es un fruto climatérico muy perecedero con una vida poscosecha corta, ya que es muy susceptible a sufrir deshidratación, pérdida de firmeza, daños mecánicos y podredumbres, por lo

que suele cosecharse prematuramente. La recolección se realiza cuando el fruto ha alcanzado un buen desarrollo del color y un valor de firmeza óptimo para garantizar que la firmeza del fruto sea suficiente para soportar el periodo de comercialización (Velardo-Micharet et al., 2021). Sin embargo, el estado de maduración en el que se recolectan no suele ser el óptimo, no llegando a alcanzan en muchas ocasiones las características organolépticas exigidas por los consumidores (Koushesh et. al*.,* 2012).

Además, las frutas de hueso son muy sensibles a las bajas temperaturas y presentan daños por frío tras un periodo de almacenamiento refrigerado. Los principales síntomas del daño por frío en albaricoques incluyen el desarrollo de depresiones en la piel, decoloración, aspecto acuoso, rotura celular interna, pardeamiento, maduración desigual, mal sabor y deterioro de la calidad (Valero y Serrano, 2010). Dado que el desarrollo de estos trastornos por frío reduce la aceptación del consumidor, la aparición de los síntomas de daño por frío determina el potencial de almacenamiento de la fruta tras la cosecha (Koushesh et al*.,* 2012). Con el objetivo de paliar las pérdidas de calidad durante el almacenamiento e incrementar los atributos de interés que demandan los consumidores, se aplican distintas tecnologías precosecha y poscosecha.

Durante la precosecha, la aplicación de quitosano y ácido salicílico redujeron la pérdida de calidad causada por la senescencia del fruto y los síntomas de daño por frío, mientras que incrementó el metabolismo fenólico (Cui et al., 2020). Por otro lado, la aplicación foliar de compuestos como la benziladenina, calcio, cloruro cálcico, nitrato potásico o ethephon en combinación con ácido oleico produjeron frutos de mayor peso, tamaño y firmeza en la recolección, retrasando en algunos de estos tratamientos la tasa de respiración del fruto (Tzoutzoukou y Bouranis 1997; Farag et al., 2012; Canli et al., 2014; Moradinezhad y Dorostkar, 2021).

Algunas de las tecnologías poscosecha aplicadas en los últimos años para mantener la calidad de estos frutos son el almacenamiento a temperaturas cercanas a la congelación, capaces de retrasar la senescencia, reducir la pérdida de firmeza y disminuir la tasa de respiración (Cui et al., 2019; Cui et al., 2021). También se han aplicado recubrimientos en albaricoques con emulsiones de nanoquitosano, extracto de piel de granada, emulsiones de menta y aceite de comino o goma tragacanto y quitosano redujeron las pérdidas de peso y retrasaron las pérdidas de calidad y redujeron el crecimiento de mohos y levaduras durante el almacenamiento, alargando la vida útil (Ali et al., 2020; Gull et al., 2021; Abd El-Gawad y El-Moghazy, 2021; Ziaolhagh y Kanani, 2021). Asimismo, la inmersión en soluciones de elicitores, como los ácidos orgánicos oxálico y salicílico combinados con el almacenamiento en atmósfera controlada, manteniendo los atributos de calidad a lo largo del almacenamiento, reduciendo el índice del daño por frío en los

frutos tratados (Batool et al., 2021). Además, el cloruro de calcio aplicado en poscosecha sobre los albaricoques redujo los daños por frío causados durante el almacenamiento a temperaturas subóptimas (Koushesh Saba et al., 2016) entre otros.

1.3.3. Piña (*Annanas comosus*, L.)

Las piñas (*Annanas comosus*, L.) tienen un sabor y un aroma únicos, además de ser ricas en nutrientes y antioxidantes, lo que las hace muy atractivas para los consumidores por sus grandes beneficios para la salud. Sin embargo, la piña es un fruto perecedero con una corta vida útil tras la cosecha. Esto se debe principalmente a una alta tasa de pérdida de agua, respiración, senescencia y producción de etileno, lo que da lugar a una rápida maduración y deterioro (Basumatary et al., 2021). Estas rápidas pérdidas poscosecha y el deterioro de la calidad de la piña son los principales retos para productores y manipuladores durante el almacenamiento y transporte a los mercados de la fruta.

El daño por frío es un problema importante que afecta a la calidad y a la vida útil de las piñas, especialmente durante el almacenamiento y el transporte. Los síntomas causados por el daño por frío comienzan en la zona basal del fruto, que muestra un aspecto acuoso. Más tarde, este síntoma de daños por frío afecta a la zona central de la piña y los tejidos acuosos translúcidos que se pardean lentamente. En casos severos las áreas afectadas por el pardeamiento se unen y oscurecen, progresando transversal y longitudinalmente. Estos síntomas están relacionados con la pérdida de la integridad de la membrana y la actividad de las enzimas inducidas por el estrés, especialmente la fenilalanina amonio liasa (PAL) y polifenol oxidasa (PPO), estimuladas por el estrés del frío (Lobo y Paull, 2017; Youryon et. al., 2021).

Las variedades de piña se clasifican en dos grupos, “*Smooth Cayenne*”, menos susceptible a los daños por frío, y “*Queen*”, más sensible a los daños por frío. Las causas del daño por frío pueden ser múltiples, pero mayormente se cree que podría deberse a un efecto sobre la composición lipídica de las membranas o sobre los sistemas antioxidantes de los frutos. Por lo general, los cultivares del grupo "*Queen*" se producen para el consumo en fresco, ya que tienen un gusto dulce, un sabor deseable y una textura crujiente (Nukuntornprakit et al., 2015; Boonyaritthongchai y Supapvanich 2017; Dolhaji et al.,2020).

Los estudios realizados sobre la aplicación de tecnologías precosecha para paliar los daños por frío y mejorar la calidad del fruto son limitados. Entre ellos se encuentra la incorporación de potasio al suelo de cultivo (Gomes-Soares et al., 2005), su combinación con etefón (Nanayakkara et al., 2005) y la aplicación foliar de silicio (Weerahewa y Wicramasekara, 2020) o calcio (Naradisorn et al., 2022).

Por el contrario, las aplicaciones poscosecha de estas tecnologías son más habituales, como es el caso de los recubrimientos de quitosano (Basumatary et al., 2021), tratamientos con cloruro de calcio (Gu et al., 2020), inmersiones de ácido salicílico y metil jasmonato (Boonyaritthongchai y Supapvanich 2017; Sangprayoon et al., 2019). Algunos autores incluso han comenzado a combinar estos tratamientos para incrementar estos efectos como, por ejemplo, la aplicación de ácido salicílico tanto pre como poscosecha (Cano-Reinoso et al., 2022), así como de boro y sulfato de calcio en precosecha seguido de un tratamiento poscosecha de cloruro de calcio (Youryon y Supapvanich, 2021).

1.3.4. Chirimoya (*Annona cherimola*, Mill.)

La chirimoya (*Annona cherimola*, Mill.) es un fruto climatérico de origen subtropical perteneciente a la familia *Annonaceae*. Este fruto requiere un almacenamiento a baja temperatura para retrasar el ablandamiento después de la cosecha. Sin embargo, pese a que el almacenamiento en frío se considera el método más eficaz de preservar la calidad de la fruta, la chirimoya es altamente susceptible a sufrir daños por frío que deprecian su valor comercial del producto e incluso imposibilita su comercialización (González-Agüero et. al. 2011).

Se han realizado estudios sobre los cambios poscosecha que se producen en los frutos del género *Annona*, siendo varios los problemas inherentes a estos frutos aún pendientes por resolver. El ablandamiento rápido de las chirimoyas durante el transporte y en las tiendas de venta al por menor es el mayor problema actual. Su gran susceptibilidad a sufrir daños por frío imposibilita el almacenamiento prolongado de esta fruta y, por lo tanto, se requiere el uso de temperaturas de mantenimiento relativamente altas (Pareek et al 2011). Dado que la maduración de los frutos de *Annona* se caracteriza por una alta tasa de respiración y de producción de etileno, la necesidad de usar temperaturas de refrigeración elevadas para su conservación, con el fin de evitar los daños por frío, reduce enormemente su vida poscosecha y convierte a la chirimoya en un producto altamente perecedero (Pinillos et al., 2013).

La aplicación de tecnologías precosecha capaces de reducir los efectos negativos del almacenamiento a temperaturas subóptimas en chirimoya es un campo aún por explorar, no siendo posible encontrar estudios que las hayan aplicado con éxito. En cambio, tras la cosecha se han aplicado tratamientos efectivos como los recubrimientos de ácido cítrico combinado con quitosano (Liu et al., 2016), aplicación de 1-metilciclopropeno en atmósfera modificada (Silva et al., 2016) y tratamientos con agua caliente e inmersión en ácido salicílico y cloruro de calcio (Vyas et al., 2015).

1.3.5. Calabacín (*Cucurbita pepo* L.)

Los calabacines (*Cucurbita pepo* L.) se cosechan cuando se encuentran en un estado inmaduro, sin embargo, este estado fenológico presenta un elevado índice metabólico debido a la fase de crecimiento rápido en la que se encuentra. Esto a su vez da lugar a que este fruto tenga una corta vida útil poscosecha. (Megías et al., 2016). La aplicación de bajas temperaturas es un buen sistema para reducir el metabolismo del fruto y con ello conseguir incrementar la vida útil del calabacín. Sin embargo, al igual que ocurre en otros frutos el calabacín es sensible a sufrir daños por frío durante el almacenamiento en frío (Carvajal et al., 2011).

Los daños por frío en el calabacín se pueden observar en forma de depresiones en la parte externa del fruto, asociadas a un aumento de la peroxidación lipídica y generación de especies reactivas de oxígeno (ROS). Estas depresiones afectan a la pulpa además de la epidermis, siendo conocido esta alteración como *pitting*. Además, los daños por frío dan lugar a que los frutos sufran elevadas pérdidas de peso y una pérdida de firmeza prematura ya que los tejidos pierden integridad celular (Carvajal et al., 2011; Mejías et al., 2016). Los daños por frío se pueden observar más rápido y con mayor intensidad cuando los frutos son trasladados a condiciones de temperatura ambiente tras ser almacenados a temperaturas subóptimas (Megías et al., 2014). Esta situación suele darse tras el transporte refrigerado y su exposición a temperatura ambiente para la venta al consumidor final en las superficies de distribución, limitando las opciones de comercialización a zonas no muy lejanas a las áreas de producción.

Al igual que ocurre en chirimoya, no se encuentran referencias de la aplicación con éxito de tecnologías precosecha que ayuden a aliviar los daños por frío e incrementar la calidad de los frutos tras la recolección. Sin embargo, en poscosecha se han aplicado tratamientos como los recubrimientos basados en polisacáridos (Castro-Cegrí et al., 2023a), la inmersión de los frutos en soluciones de ácido abscísico (Castro-Cegrí et al., 2023b) y agua caliente (Zhang et al., 2019). También han resultado exitosos los tratamientos poscosecha con óxido nítrico (Jiménez-Muñoz et al., 2021) y ácido γ-aminobutírico (Palma et al., 2019), capaces de reducir las pérdidas de calidad durante el almacenamiento, aumentar la vida útil de los frutos, así como reducir los síntomas de daño por frío durante el almacenamiento a temperaturas subóptimas.

1.4. Melatonina como herramienta precosecha y poscosecha para mejorar la producción y conservación de frutos

La melatonina, también conocida como N-acetil-5-metoxitriptamina, es una molécula derivada del triptófano que se localiza de forma natural tanto en humanos como animales y en todos los órganos de las plantas. Entre sus diversas funciones en las plantas se le atribuye principalmente actividad antioxidante, siendo capaz de eliminar especies reactivas de oxígeno (ROS), así como otros compuestos oxidativos y radicales libres que son nocivos para las plantas y que se encuentran en las células vegetales. De entre las diversas funciones fisiológicas atribuidas a la melatonina podemos destacar su relación con el desarrollo de raíces y de la propia planta, germinación de semillas, así como su capacidad estimulando la actividad fotosintética y la resistencia al estrés abiótico y biótico (Arnao y Hernández- Ruíz, 2020; Asif et al., 2020; Lorente-Mento et al., 2021).

La ruta de biosíntesis de fitomelatonina (**Figura 6**) comienza a partir del triptófano y se desarrolla en cuatro pasos enzimáticos catalizados por al menos seis enzimas: triptófano descarboxilasa (TDC), triptófano hidroxilasa (TPH), triptamina 5-hidroxilasa (T5H), serotonina N-acetiltransferasa (SNAT), N-acetilserotoninmetiltransferasa (ASMT) y ácido cafeico O-metiltransferasa (COMT). El primer paso de esta ruta es la descarboxilación del triptófano, catalizada por la TDC, produciendo triptamina. A continuación, la hidroxilación catalizada por la T5H forma serotonina. El penúltimo paso en la biosíntesis de melatonina es la acetilación de serotonina, que es catalizada por SNAT en plantas. Por último, intervienen en dos fases las enzimas ASMT y COMT, que presenta afinidad de sustrato hacia la serotonina y la N-acetilserotonina. En primer lugar, metilan la serotonina para formar 5-metoxitriptamina, seguido de una reacción con SNAT para formar melatonina (Park et al., 2012; Arnao y Hernández-Ruiz, 2015; Back et al., 2016; Sun et al., 2021). Para su aplicación práctica, la melatonina se puede sintetizar químicamente en un proceso sencillo y barato, convirtiendo así el uso de la melatonina en una estrategia sostenible.

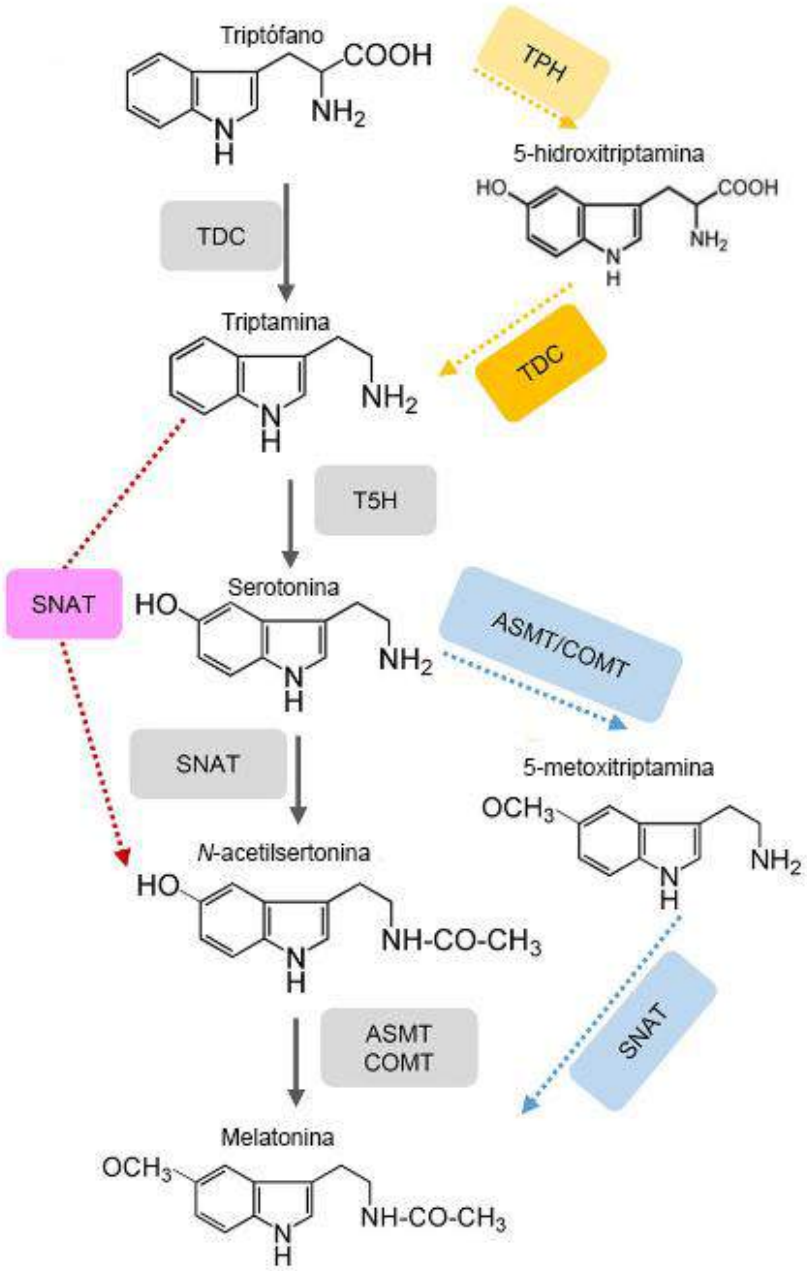


Figura 6: *Ruta de biosíntesis de la melatonina catalizada por las enzimas triptófano descarboxilasa (TDC), triptófano hidroxilasa (TPH), triptamina 5-hidroxilasa (T5H), serotonina N-acetiltransferasa (SNAT), N-acetilserotoninmetiltransferasa (ASMT) y ácido cafeico O-metiltransferasa (COMT). Fuente: adaptado de Sun et al., 2021.*

Son varios los estudios que han demostrado los efectos positivos que tiene la melatonina mediante la aplicación en precosecha o en poscosecha a la hora de incrementar la tolerancia al frío, incrementando la calidad poscosecha en varias especies vegetales tales como melocotón, tomate, pimiento, lichi y cereza (Cao et al., 2016; Jannatizadeh, et al., 2019; Kong et al., 2020; Liu et al., 2020b; Carrión-Antolí et al., 2022).

En precosecha se ha comprobado que las aplicaciones foliares podrían afectar al contenido de los ROS, malondialdehído (MDA) y la permeabilidad relativa de la membrana mediante la activación del sistema antioxidante, reduciendo las podredumbres de los frutos e incluso el agrietado (Sheng et al., 2020; Carrión-Antolí et al., 2022). Esta aplicación exógena también es capaz de mejorar algunos parámetros de calidad en la recolección como el peso, el color, la firmeza, los sólidos solubles, la acidez titulable y los niveles de compuestos bioactivos (Abd-El Naby et al., 2019; García-Pastor et al., 2019). Asimismo, fue capaz de retrasar la senescencia poscosecha y aumentar la resistencia a estreses abióticos al incrementar la concentración de compuestos antioxidantes y controlar la maduración de los frutos retrasando la degradación de la firmeza, los cambios de color, así como las pérdidas de acidez y contenido total de sólidos solubles (Asif et al., 2020; Lorente-Mento et al., 2021; Carrión-Antolí et al., 2022).

También se ha demostrado su potencial como tratamiento poscosecha al retrasar la senescencia de frutas y hortalizas, actuando como intermediario en la vía del metabolismo del ácido γ-aminobutírico en fresa (Aghdam y Fard, 2017), como regulador de la acumulación de antocianinas en el tomate (Sun et al., 2016) y del metabolismo del etileno en plátano (Wang et al., 2021). Además, un estudio reciente sugiere que la melatonina mantiene el normal funcionamiento de las estructuras mitocondriales en semillas de loto, a través de la regulación de la producción de óxido nítrico mediante el aumento de la actividad de la enzima óxido nítrico sintasa (Sun et al., 2022). Esta actividad mitocondrial de la célula es clave a la hora de mantener el metabolismo celular y proveer de energía a las células.

Hasta la fecha se han publicado una gran cantidad de resultados por distintos autores de todo el mundo sobre los efectos de la aplicación de este elicitor en múltiples productos hortícolas. La mayor parte de estos resultados han sido evaluados cuando la aplicación exógena fue en poscosecha, mientras que en aplicación precosecha el número de estudios disponibles es bastante limitado. En las tablas 1 y 2 se muestran algunas referencias bibliográficas en las que se ha observado los efectos positivos de la aplicación de melatonina en poscosecha y precosecha:

Tabla 1: *Revisión bibliográfica sobre los efectos de la melatonina aplicada en poscosecha. Fuente: Elaboración propia.*

POSCOSECHA					
Reducción de los daños por frío		**Control de la maduración y retraso de la senescencia**		**Control de la pudrición**	
Melocotón	Cao et al., 2016	Fresa	Liu et al., 2018	Fresa	Aghdam y Fard, 2017
Melocotón	Cao et al., 2018	Cereza	Wang et al., 2019	Tomate Cherry	Li et al., 2019
Tomate	Aghma et al., 2019	Cereza	Feng et al., 2019	Jengibre	Huang et al., 2021
Tomate	Jannatizadeh et al., 2019	Tomate Cherry	Tijero et al., 2019	Naranja	Ma et al., 2021
Pimiento	Kong et al., 2020	Pera	Liu et al., 2019	Semillas de loto	Sun et al., 2022
Lichi	Liu et al., 2020a	Fresa	El-Mogy et al., 2019		
Mango	Dong et al., 2021	Ciruela	Bal, 2019		
Naranja	Ma et al., 2021	Mango	Liu et al., 2020b		
Semillas de loto	Sun et al., 2022	Uva de mesa	Sun et al., 2020		
Mango	Bhardwaj et al., 2022	Mango	Dong et al., 2021		
		Naranja	Ma et al., 2021		
		Kiwi	Cheng et al., 2022		
		Ciruela	Yan et al., 2022		
		Clavel	Lezoul et al., 2022		

Tabla 2: *Revisión bibliográfica sobre los efectos de la melatonina aplicación en precosecha. Fuente: Elaboración propia.*

PRECOSECHA							
Control de la maduración y retraso de la senescencia		**Control de la pudrición y rajado del fruto**		**Incremento de la productividad y calidad del fruto en la recolección**		**Resistencia a estrés abiótico**	
Granada	García-Pastor et al., 2019	Tomate	Sheng et al., 2020	Albaricoque	Abd-El Naby et al., 2019	Espinaca	Asif et al., 2020
Granada	Lorente-Mento et al., 2021	Cereza	Carrión-Antolí et al., 2022	Granada	García-Pastor et al., 2019		
				Tomate	Sheng et al., 2020		
				Cereza	Carrión-Antolí et al., 2022		

1.5. Acción del 1-metilciclopropeno sobre la calidad y vida útil de los frutos durante su conservación poscosecha.

El etileno es una hormona vegetal encargada de regular importantes aspectos del crecimiento y desarrollo de las plantas, así como las respuestas a estímulos procedentes del medio, como los estreses bióticos y abióticos (Valero et al., 2016). Además, el etileno es conocido como hormona de la maduración y en función de la respuesta a esta hormona los frutos se clasifican en dos grupos: climatéricos y no climatéricos.

La maduración de los frutos climatéricos viene acompañada de un aumento de la respiración a causa del etileno endógeno o aplicado de forma exógena, mientras que en los frutos no climatéricos la maduración es un proceso que se da de forma independiente a la producción de etileno. En las frutas climatéricas, como los tomates, los melones, las manzanas, los plátanos y la mayoría de las frutas de hueso, el inicio de la maduración coincide con un aumento climatérico de la respiración y un incremento de la producción de etileno. El etileno exógeno estimula la respiración e induce la biosíntesis endógena de etileno, lo que acelera el proceso de maduración. En la fruta no climatérica, como las naranjas, las uvas y las sandías, el etileno exógeno aumenta la actividad respiratoria como en la fruta climatérica y estimula algunos procesos de maduración y senescencia.

Debido a la gran influencia de esta fitohormona en los mecanismos de maduración y senescencia de los frutos climatéricos, el control del etileno es uno de los puntos clave a tratar en la tecnología poscosecha, ya que estos frutos responden tanto al etileno endógeno como exógeno en la mayoría de los casos.

El 1-metilciclopropeno (1-MCP) pertenece a una clase de compuestos conocidos como ciclopropenos con una excelente acción como antagonista del etileno, que puede prevenir el efecto del etileno endógeno y exógeno y retrasar eficazmente la maduración y la senescencia mediada por el etileno. El 1-MCP se une a los receptores de etileno de forma irreversible de forma que el etileno no pueda unirse a ellos y ejercer su acción. Esto reduce el número de receptores libres para el etileno, reduciendo así la acción del etileno sobre el fruto (Valero et al., 2016; Brasil y Siddiqui, 2018; Guo et al., 2021).

La cascada de señalización del etileno comienza con la unión del etileno a sus receptores. Los receptores de etileno existen como una familia que en *Arabidopsis* está compuesta por ETR1, ERS1 ETR2, ERS2 y EIN4. Estos receptores funcionan como reguladores negativos de la vía, reprimiendo activamente la respuesta al etileno en ausencia de la hormona. En ausencia de

etileno, los receptores (ETR1, ETR2, EIN4, ERS1 y ERS2) están activados (efecto inhibitorio). Algunos de estos receptores, por ejemplo, ETR1 y ERS1, actúan directamente sobre la proteína CTR1, mientras que otros receptores, por ejemplo, ETR2 EIN4 y ERS2, actúan de forma más indirecta. En este estado activo, permiten que la proteína CTR1 inactive la proteína EIN2, que es esencial para promover las respuestas conocidas del etileno. Cuando el etileno está presente se une a sus receptores y los inactiva, así como a CTR1.

Con CTR1 inactivado la proteína EIN2 puede volver a su forma activa y producir las conocidas respuestas de etileno en la planta. Cuando sólo hay receptores ETR1 y ERS1 o sólo ETR2, EIN4 y ERS2 no hay suficiente activación de CTR1 y las respuestas al etileno se producen sin que éste se una a los receptores. Utilizando este modelo de acción del etileno (**Figura 7**), se propone que 1-MCP suprime la vía de respuesta al etileno al unirse permanentemente a un número suficiente de receptores de etileno (ETR1, ETR2, EIN4, ERS1, y/o ERS2), que mantiene a CTR1 en su estado activo (inhibidor) (Binder et al., 2012; Valero et al., 2016; Días et al., 2021).

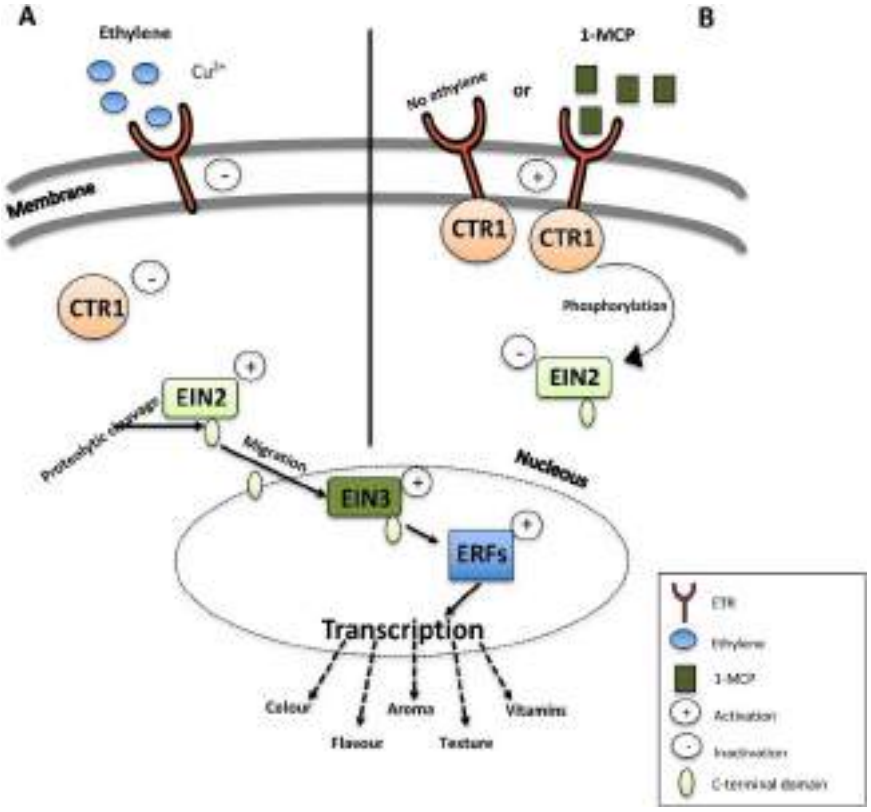


Figura 7: *Vía de señalización del etileno. A) La unión del etileno a los receptores los inactiva, permitiendo la señalización a través del desprendimiento proteolítico de EIN2, transcribiendo los genes que codifican las enzimas responsables de los cambios de calidad de la maduración. B- En ausencia de unión al etileno, o en presencia de un inhibidor (1-MCP), CRT1 se une al receptor bloqueando la señalización descendente del etileno. Fuente: Días et al., 2021.*

Se ha descrito que el 1-MCP tiene una afinidad con los receptores 10 veces mayor que la del etileno, aunque muchos estudios han encontrado que el efecto del 1-MCP desaparece con el tiempo, lo que se ha atribuido a la síntesis de nuevos receptores (Lurie, 2008; Días et al., 2021). Sin embargo, el efecto que produce está ligado a las diferentes especies y cultivares sobre los que se aplica, al estado de maduración previo al tratamiento y a la dosis aplicada (Guillén et al., 2007; Manenoi et al., 2007; Gaikwad et al., 2020; Huan et al.,2020)

El 1-MCP ha mostrado una gran eficacia tanto tras su aplicación en precosecha como en poscosecha. En precosecha, las soluciones de 1-MCP fueron capaces de retrasar la maduración de caqui y manzana (Vilhena et al., 2022; Liu et al., 2022), manteniendo la firmeza de los frutos. Algo similar se ha observado en tratamientos precosecha de pera y melocotones (Yoo et al., 2019; Hayama et al., 2022). Por otro lado, múltiples estudios han demostrado que el tratamiento con 1-MCP retrasa la senescencia de los frutos en poscosecha al potenciar el sistema de producción y eliminación de ROS, lo que podría alargar la vida útil de la fruta (Gwanpua et al., 2017), así como reducir los síntomas de daño por frío en una amplia gama de frutos, tanto climatéricos como no climatéricos, incluida la granada (Valero et al., 2016). El 1-MCP ha sido utilizado comercialmente en todo el mundo en frutos como el mango (Xu et al., 2017), el caqui (Salvador et al., 2004), la manzana (Tatsuki et al., 2007), calabacín (Megías et al., 2015), albaricoque (Fan et al., 2018b), chirimoya (Silva et al., 2016), piña (Setha et al., 2013) y el melocotón (Huan et al., 2018). En general, estos estudios han demostrado las bondades del 1-MCP aplicado en poscosecha a la hora de retrasar la maduración y senescencia de los frutos, así como reducir la incidencia de fisiopatías tales como aquellas observadas durante el almacenamiento a temperaturas subóptimas.

2 Objetivos

2. OBJETIVOS

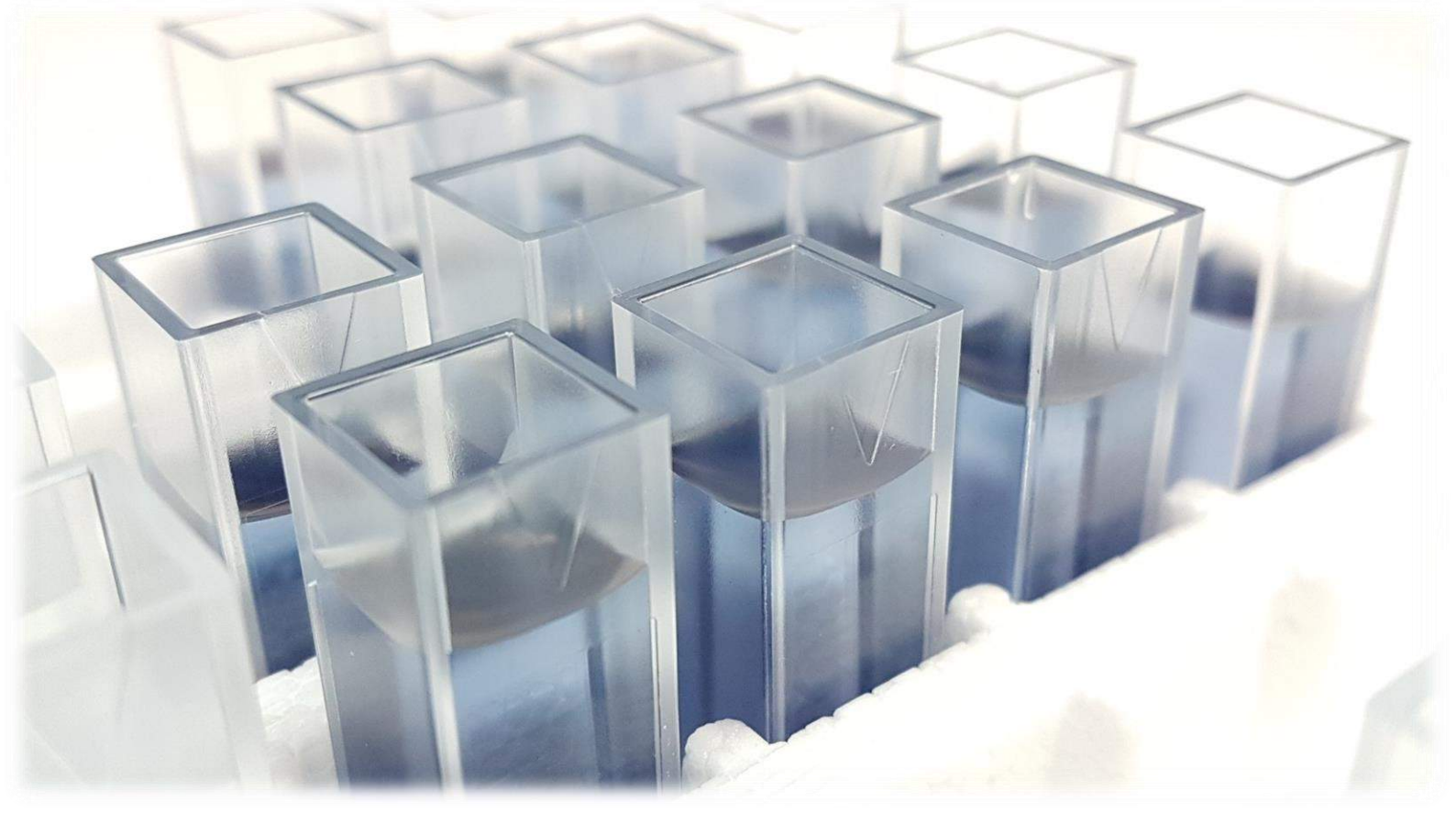
La competitividad de los mercados y las demandas de los consumidores obligan a los productores a alcanzar los mayores niveles de calidad de la fruta en el momento de la recolección y a mantener esta calidad durante la conservación y distribución hasta su llegada al consumidor. Al mismo tiempo, se deben tener en cuenta los importantes problemas poscosecha que presenta cada una de las especies descritas en esta Tesis Doctoral. Por ello, nos planteamos la necesidad de encontrar nuevas tecnologías capaces de suministrar tanto a productores como a consumidores soluciones de origen natural en la producción y conservación de estos frutos.

El rendimiento de las producciones, así como la sensibilidad que presentan las especies estudiadas en esta Tesis Doctoral a los daños por frío, son problemas clave que requieren de estudios en profundidad y para los que se siguen necesitando soluciones. Existe muy poca información de los efectos precosecha que tiene la melatonina, y tampoco se han estudiado sus efectos en la poscosecha de los frutos propuestos en este estudio. Por ello y con el fin de dilucidar las posibilidades de la melatonina como herramienta precosecha y poscosecha se ha realizado esta Tesis Doctoral que abarca un variado espectro de frutos tanto climatéricos como no climatéricos.

Por tanto, esta Tesis Doctoral tiene como **Objetivo General** estudiar los efectos beneficiosos que las aplicaciones con melatonina podrían aportar a niveles productivos, y tanto en la cosecha como durante su posterior almacenamiento poscosecha. Para ello planteamos además los siguientes **objetivos parciales**:

I. Estudiar el efecto de las aplicaciones precosecha con melatonina sobre las producciones de granadas y albaricoques.
II. Incrementar la coloración en la cosecha de granadas y albaricoques.
III. Aumentar la vida útil poscosecha mediante tratamientos precosecha.
IV. Reducir la incidencia de los daños por frío en frutos climatéricos y no climatéricos mediante tratamientos precosecha y poscosecha con melatonina
V. Estudiar los beneficios de aplicar esta tecnología natural de forma aislada o combinada con otras tecnologías comerciales utilizadas habitualmente.

3 Materiales y Métodos



3. MATERIALES Y MÉTODOS

En este apartado se incluyen las principales características del material vegetal, las condiciones experimentales, los tratamientos realizados en precosecha y poscosecha, las determinaciones analíticas y el diseño estadístico utilizado en esta Tesis Doctoral. Para más detalles, pueden consultarse las publicaciones que constituyen la sección de resultados.

3.1. Material Vegetal

Los estudios precosecha realizados en esta Tesis Doctoral se han realizado en los ciclos productivos de 2017 y 2018 en el caso de las granadas cv. Mollar de Elche (*Punica granatum* L.), mientras que en los ciclos productivos 2019 y 2020 se realizaron los estudios con albaricoques (*Prunus armeniaca* L.) cv. Colorado y Mikado. Las parcelas de estudio se localizaban en Elche (granadas) y Cieza (albaricoques). Los árboles bajo estudio se distribuyeron en un diseño de bloques al azar. Los granados se trataron en 5 diferentes momentos del desarrollo del fruto, separados 30 días desde la plena floración, mientras que en los albaricoqueros de ambas variedades se aplicó melatonina en el momento de endurecimiento del hueso, en la fase final de crecimiento y 4 días antes de la recolección. El tratamiento consistió en la aplicación por pulverización foliar de 3 litros de cada concentración de melatonina por árbol.

Con respecto a los estudios poscosecha, las piñas (*Ananas comosus* (L.) Merr.) del híbrido comercial PRI 73-050 fueron recolectadas en las parcelas de *Dole Fresh Fruit* localizadas al noroeste de la isla de Oahu (Hawái) aunque la optimización de las concentraciones más efectivas se realizó con piñas obtenidas en la lonja de Orihuela y fueron posteriormente ensayadas de nuevo en las piñas recolectadas en Oahu. Las chirimoyas (*Annona cherimola* Mill) cv. Fino de Jete fueron recolectadas en estado de madurez comercial en una parcela localizada en Granada mientras que los calabacines (*Cucurbita pepo* spp. *pepo*) cv. Cronos utilizados en esta Tesis Doctoral se recolectaron en un invernadero comercial de Orihuela.

Las distintas actividades agronómicas de poda, aclareo, fertilización y riego realizadas en las distintas especies vegetales fueron las recomendadas y estandarizadas por los productores en los distintos cultivos. Todos los frutos de esta Tesis Doctoral se recolectaron en base a criterios comerciales estándar para cada especie vegetal en relación con el tamaño del fruto, color y el contenido total de sólidos solubles de cada variedad.

Los tratamientos con melatonina aplicados, tanto en precosecha como en poscosecha, se prepararon de forma similar y siguiendo el mismo procedimiento. Las soluciones de melatonina a las distintas concentraciones de

ensayo se aplicaron junto con un agente dispersante (tween 20 al 0.05 ó 0.01 % dependiendo la especie vegetal a tratar) en todos los experimentos. La melatonina fue obtenida de Sigma (Sigma-Aldrich, Madrid, España) preparándose cada una de las soluciones por triplicado en cada uno de los experimentos. De forma específica (**Diagrama 1**) las concentraciones ensayadas en cada especie vegetal fueron las siguientes.

- Árboles o frutos control: Los árboles control en los estudios precosecha y los frutos controles en los estudios poscosecha fueron tratados con agua destilada conteniendo tween 20 a una concentración concreta en cada experimento con aplicaciones o tiempos de inmersión similares a los aplicados en cada especie vegetal con los tratamientos que contenían melatonina.
- Granadas: las concentraciones aplicadas en precosecha con melatonina durante el ciclo productivo de 2017 fueron 0.1 y 1 mM. En el ciclo productivo posterior (2018) se repitió la concentración con mejores resultados con respecto al incremento de la producción y los parámetros de calidad evaluados en el ciclo productivo previo que fue la de 0.1 mM.
- Albaricoques: los albaricoques fueron tratados en precosecha durante dos ciclos productivos consecutivos con una única concentración de melatonina 0.1 mM que fue seleccionada en un estudio previo en el que se aplicaron 3 distintas concentraciones de melatonina (0.1, 0.5 y 1 mM) cuyos datos no se han incluido en esta Tesis Doctoral.
- Piña: en estudios previos realizados tanto en Orihuela como en Oahu se estudió el efecto de distintas concentraciones de melatonina en poscosecha (0.1 y 0.5 mM) aplicadas a diferentes tiempos de inmersión (10 y 60 minutos). Tras evaluar la incidencia de los daños por frío, así como los distintos parámetros de calidad en un segundo experimento se volvieron a aplicar únicamente las condiciones experimentales óptimas evaluadas en los experimentos iniciales, que fueron de 10 minutos de inmersión en melatonina 0.1 mM.
- Chirimoya: un primer experimento realizado con el objetivo de determinar las condiciones óptimas de tratamiento se basó en el tratamiento por inmersión a 4 distintas concentraciones de melatonina (0,1, 0,3, 0,5 y 1 mM) de las chirimoyas sometidas a distintos tiempos de inmersión (10, 60 y 180 minutos) siendo seleccionada la dosis más baja aplicada durante el tiempo de inmersión más corto (0.1 mM durante 10 minutos). Así, en un segundo experimento se aplicaron en estos frutos concentraciones inferiores a las ya aplicadas (0,01, 0.05 y 0.1 mM) con inmersiones de 10 minutos de duración.
- Calabacín: mediante un estudio inicial se consideraron diferentes concentraciones de melatonina (0,1 0,5 y 1 mM) que se aplicaron a diferentes tiempos de inmersión en los calabacines (10, 30, 60, 120 y 180 minutos).

Además, todas estas concentraciones se ensayaron combinadas con aplicaciones gaseosas de 1-MCP a 2400 nL L^{-1} durante un tiempo de 48 horas. En un segundo experimento y una vez seleccionadas las condiciones óptimas de aplicación (1 mM durante 60 minutos de inmersión) se aplicaron tanto de forma aislada como combinadas con aplicaciones de 1-MCP.

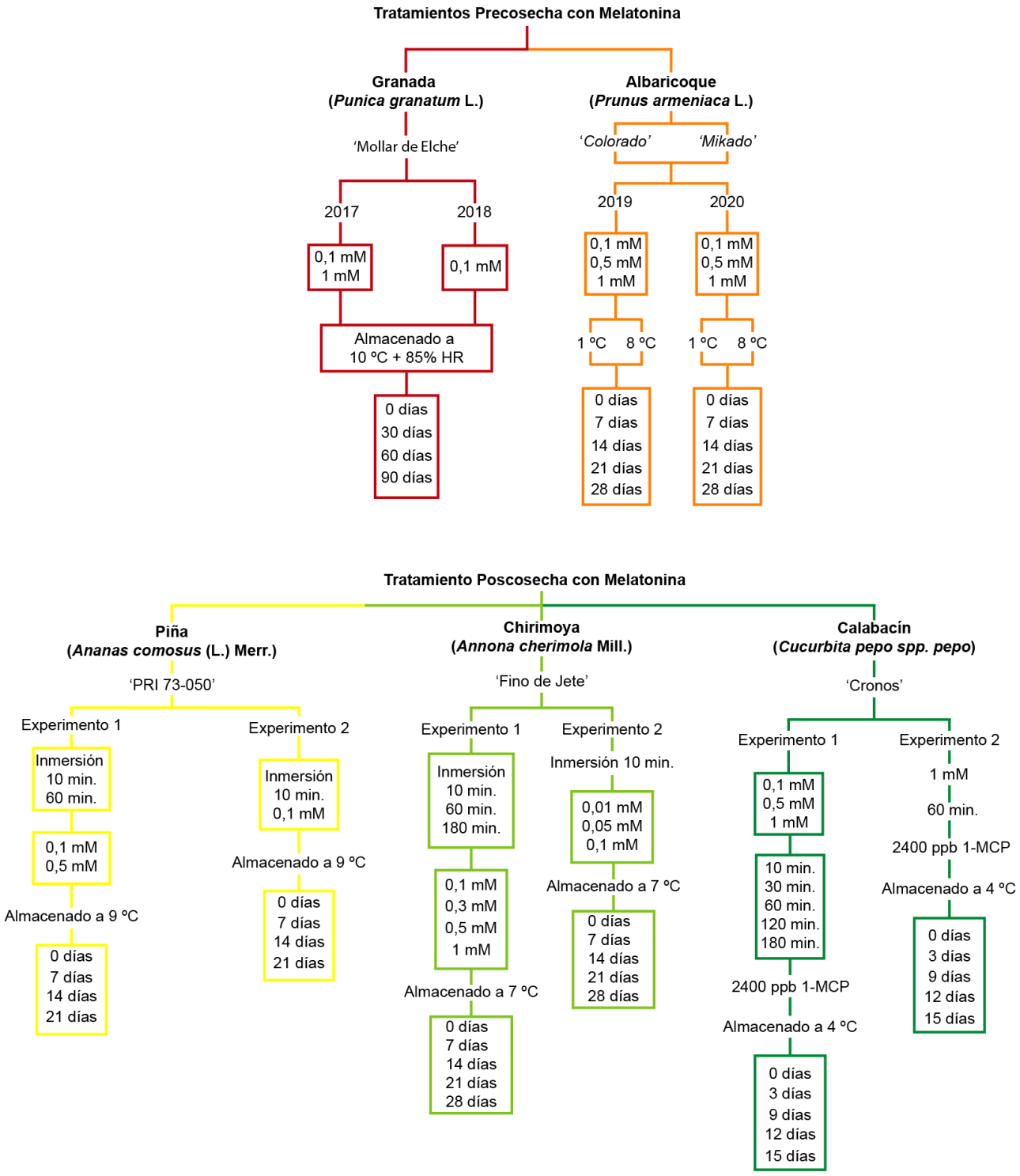


Diagrama 1: *Diseño experimental de los tratamientos con melatonina en distintas especies vegetales. Fuente: elaboración propia.*

3.2. Crecimiento de los frutos y rendimiento del cultivo

La evolución del crecimiento de la granada se determinó en frutos etiquetados en el árbol desde el tratamiento hasta la primera fecha de cosecha midiendo el diámetro del fruto (mm) a intervalos de 7-10 días utilizando un calibre digital Vernier. Para cada fecha de cosecha, el rendimiento del cultivo se calculó y expresó como kg árbol^{-1} y número de frutos árbol^{-1}. El número total de frutos y el total de kg árbol^{-1} se utilizaron para calcular el peso de los frutos en las dos fechas de cosecha. Los resultados se expresaron como la media ± ES.

Por otro lado, los albaricoques se cosecharon en el momento de la maduración comercial, según el color característico de la piel de cada cultivar. Se pesó la producción total por árbol y se contó el número de frutos por árbol para obtener los datos de rendimiento en cada árbol y el peso medio de los frutos.

3.3. Parámetros de calidad de la fruta

3.3.1. Pérdida de peso

Las pérdidas de peso se midieron en cada lote registrando el peso del fruto en la cosecha (día 0) y en las diferentes fechas de muestreo durante el almacenamiento. La acumulación de pérdidas de peso se expresó en forma de porcentaje (%) o como g de peso perdido por cada 100 g de muestra con respecto al peso del fruto en el día 0.

3.3.2. Tasa de respiración y producción de etileno

Para cuantificar la producción de etileno y la tasa de respiración, cada especie vegetal se cerró herméticamente en un frasco de 2,2 L, 3 L o 7,5 L durante 30 o 60 min, dependiendo del experimento. Después, se extrajeron 4 mL de la atmósfera del envase hermético con una jeringa de 1 mL. Dos mililitros se utilizaron para cuantificar, por duplicado, el etileno mediante un cromatógrafo de gases Hewlett-PackardTM 5890A, y los 2 mL restantes se utilizaron para cuantificar, por duplicado, el CO_2 mediante un cromatógrafo de gases Shimadzu 14B (Shimadzu Europe Gmbh, Duisburg, Germany), equipado con un detector de conductividad térmica. Las condiciones cromatográficas se han descrito previamente (Martínez-Romero et al, 2002).

3.3.3. Color externo e interno

Los parámetros de color en granada, calabacín, piña y chirimoya se midieron individualmente en tres o seis puntos, dependiendo del experimento, del perímetro longitudinal externo (ambos lados) e interno del fruto utilizando un

colorímetro Minolta colorímetro Minolta (CRC200, Minolta Camera Co.; Kanto, Tokio, Japón) y el color se expresó como tono CIE* ($180 + \tan^{-1} b^*/a^*$, si $a^* < 0$) según las coordenadas CIELab.

Para medir el color de los albaricoques se capturó una imagen de cada lado de los 10 frutos de cada una de las 3 réplicas para cada tratamiento con una cámara de fotos digital (Nikon D3400) en una caja de luz con fondo negro. Las condiciones de configuración de la cámara fueron las siguientes: fuente de luz LED que simula la luz diurna (temperatura de color igual a 5600 K), velocidad del flash de 1/5 s, ISO-200, una apertura focal (f) de 20 y longitud de 35 mm. La imagen guardad en formato JPEG se analizó utilizando el software ImageJ v1.52a (NIH Image, National Institutes of Health, Bethesda, MD, USA). Se utilizó el modelo CIELab para expresar el color como parámetros *L**, *a** y *b**.

3.3.4. Firmeza

La firmeza en granada, albaricoque y calabacín se midió con un analizador de textura TX-XT2i (Stable Mycrosystems, Godalming, Reino Unido) equipado con una sonda plana para producir sobre el fruto una fuerza de deformación del 3% del diámetro del fruto, mientras que en calabacín y chirimoya se usó una deformación del 5%. Los resultados se expresaron como la relación entre la fuerza aplicada y la distancia recorrida (N mm^{-1}) y son la media del SE.

La firmeza de la piña se midió en la pulpa de cada piña utilizando una sonda de acero de 1 cm de diámetro acoplada a un banco de pruebas manual (ZPS-DPU-110 Digital Force Gauge. Imada, Japón) conectado a un ordenador. Para cada piña, la distancia de penetración aplicada en la pulpa fue de 3,5 cm. La firmeza se registró como una presión de carga máxima en Newtons (N) aplicada en tres puntos equidistantes de las rodajas ecuatoriales de la piña de 7 cm de grosor.

3.3.5. Sólidos Solubles Totales (SST) y Acidez titulable (AT)

Los sólidos solubles totales (SST) se determinaron por duplicado en el zumo obtenido de la pulpa de la mezcla de aproximadamente 50 g de pulpa o arilos, según el experimento, de cada réplica por lote tomada con un refractómetro digital Atago PR-101 (Atago Co. Ltd.; Tokio, Japón) a 20 ºC, y se expresaron como porcentaje (g 100 g^{-1}). Además, en el mismo zumo de cada réplica se determinó la acidez total (AT) mediante valoración automática con NaOH 0,1 N hasta alcanzar un valor de pH 8,1, utilizando 1 mL de zumo diluido en 25 mL o 30 mL de H_2O destilada, según el experimento, y expresado en el

ácido mayoritario. El índice de maduración (IR) se evaluó como el cociente de SST y AT.

3.3.6. Cuantificación de fenoles individuales y totales

La extracción de los compuestos fenólicos totales se realizó homogeneizando con un Ultraturrax (T18 basic, IKA, Berlín, Alemania) 5 g de muestras de pulpa de fruta con 15 mL de una mezcla de agua:metanol (2:8, v/v) a la que se le añadieron 2 mM de NaF durante 60 s. Los extractos se centrifugaron a 10.000 *g* durante 10 min a 4 ºC y se cuantificaron los fenoles totales en el sobrenadante, por duplicado, utilizando el reactivo Folin-Ciocalteu, como han descrito previamente García-Pastor et al. (2020c). Los resultados se expresaron como mg de ácido gálico equivalentes (GAE) g^{-1} en base al peso seco o en g $100g^{-1}$ con respecto al peso fresco y son la media ± ES.

Para cuantificar los fenoles individuales, se filtró 1 mL del sobrenadante anterior a través de un filtro de PVDF de 0,45 µm (Millex HV13, Millipore, Bedford, MA, EE.UU.) y se utilizó para los análisis por HPLC utilizando un equipo Agilent HPLC 1100 equipado con un detector de matriz de fotodiodos (Agilent Technologies, Waldbronn, Alemania). El HPLC estaba equipado con una columna C18 (Mediterranea Sea 18, Teknokroma, Barcelona, España) de 25 cm x 0,46 cm i.d. y 5 µm de tamaño de partícula y un C18 de 1 cm x 0,32 cm de diámetro interior (Ultraguard Sea 18, Teknokroma, Barcelona, España). Las fases móviles A y B fueron agua:ácido fórmico (99,9:0,1, v/v) y acetonitrilo, respectivamente, con un flujo de 1 mL min^{-1}. El gradiente lineal comenzó con un 1% de disolvente B, alcanzando 30% de disolvente B a los 30 min, 50% a los 40 min y 95% a los 45 min, que se mantuvo hasta 50 min y luego se volvió a las condiciones iniciales después de 5 min. El volumen de inyección fue de 20 µL y la temperatura de la columna fue de 30 ºC. Los cromatogramas se registraron a 320 nm, y el ácido neoclorogénico, el ácido clorogénico y la rutina se cuantificaron por comparación con curvas de calibración realizadas con estándares auténticos adquiridos en Sigma-Aldrich (Madrid, España).

3.3.7. Azúcares y ácidos orgánicos

Para las determinaciones de ácidos orgánicos y azúcares, se extrajeron 5 g de arilos de cada réplica con 10 mL de tampón fosfato (50 mmol L^{-1}, pH = 7,8) y luego se centrifugaron a 15.000 *g* durante 15 min a 4 ºC. El sobrenadante se utilizó para la cuantificación de azúcares y ácidos orgánicos por duplicado, tal como lo describieron previamente Mirdehghan et al. (2006), 1 mL del extracto se filtró a través de un filtro Millipore de 0,45 µm y luego se inyectó en un HPLC Hewlett-Packard serie 1100. El sistema de elución se basó en ácido fosfórico al 0,1% como fase móvil constante con un flujo de 0,5 mL min^{-1}. Los ácidos orgánicos se hicieron pasar a través de una columna Supelco

(Supelcogel C-610H, 30 cm 7,8 mm, Supelco Park, Bellefonte, PA, USA), midiendo la absorbancia a 210 nm. Previamente a la cuantificación se determinaron los ácidos orgánicos puros (ácidos L-ascórbico, málico, cítrico, oxálico y succínico) con una curva de estándares obtenidos en Sigma-Aldrich (Madrid, España). Los resultados se expresaron en g 100 g^{-1}. Para las concentraciones de azúcares se utilizó el mismo equipo, sistema de elución, velocidad de flujo y columna, y se detectaron utilizando un detector de índice de refracción. Al igual que en el caso de los ácidos, se usó una curva de estándares puros (glucosa, fructosa y sacarosa) con reactivos adquiridos en Sigma para su posterior cuantificación en las muestras. Los resultados se expresaron en g 100 g^{-1}.

3.3.8. Malondialdehido

El contenido de malondialdehído (MDA) se ensayó en la piel de calabacín siguiendo el método de Zhang et al. (2019) con modificaciones. La muestra de tejido (1,0 g) se homogeneizó en 10 mL de solución de ácido tricloroacético al 10% y luego se centrifugó a 10.000 *g* durante 10 min. Se añadieron 2 mL de sobrenadante a un tubo de ensayo con 6 mL de ácido tiobarbitúrico al 0,6% por duplicado y se mezcló vigorosamente. Los tubos de ensayo se mantuvieron a 95 ºC durante 20 min. Las muestras se enfriaron rápidamente, se atemperaron a temperatura ambiente y se evaluaron en un espectrofotómetro (1900 UV/Vis, Shimadzu, Kioto, Japón) donde se midió la absorbancia a 450, 532 y 600 nm. El contenido de MDA se calculó siguiendo las indicaciones establecidas por Zhang et al. (2019) y se expresó como µmol kg^{-1}. Cada evaluación se realizó por triplicado en cada réplica.

3.3.9. Fuga de electrolitos

La fuga de electrolitos se determinó siguiendo el método descrito por Mao et al. (2007) con algunas modificaciones. De cada tratamiento se evaluaron tres réplicas, cada una de las cuales consistía en 20 discos en calabacín o 15 discos en chirimoya de la piel de las frutas de 0,5 mm de diámetro obtenidos con un perforador metálico, a partir de tiras longitudinales de 2 mm de grosor de piel tomadas de lados opuestos del fruto. Después de 3 enjuagues con agua desionizada de 3 min cada uno, los discos se incubaron en 50 mL de agua desionizada a temperatura ambiente con agitación constante durante 30 minutos. A continuación, se midió la conductividad eléctrica (CE) (C1). Por último, las muestras se hirvieron a 100 ºC durante 15 minutos y se midió de nuevo su conductividad total (C2). La fuga de electrolitos se expresó como porcentaje, utilizando la siguiente fórmula: EL = (C1/C2) 100.

3.3.10. Determinación de clorofilas

El contenido de clorofila en las hojas de la corona de piña se midió utilizando un medidor atLEAF Chl (FT Green LLC, Wilmington, DE) y se expresó como unidades relativas atLEAF. El contenido total de clorofila se obtuvo convirtiendo los valores de atLEAF ChL en unidades SPAD según los cálculos de Zhu et al. (2012) y considerando la relación entre el contenido total de clorofila y las unidades SPAD establecida por Richardson et al. (2002). Los resultados se expresaron como mg cm^{-2} y fueron la media ± SE (n= 3).

Para la medida del contenido total de clorofilas en la piel de calabacín y chirimoya, se perforaron seis discos, cada uno de 6,25 mm de diámetro, de las mismas capas de la piel cortadas para la fuga de electrolitos. Los discos se pesaron y se colocaron inmediatamente en 8 mL de metanol al 100%. Los pigmentos se dejaron extraer en la oscuridad a 30 ºC durante 24 h. La absorbancia del extracto se midió utilizando un espectrofotómetro (1900 UV/Vis, Shimadzu, Kioto, Japón) a 652 y 665 nm. Se evaluaron dos extracciones por cada una de las réplicas.

3.3.11. Daños por frío

Los daños por frío en albaricoque y chirimoya fueron evaluados por cinco jueces entrenados según una escala de 0 a 5 en relación al área superficial afectada por esta fisiopatía. La evaluación del daño en calabacín consistió en la calificación del área superficial afectada por el daño por frío y la severidad del daño. Las calificaciones se basaron en una escala hedónica de 6 puntos, en la que la superficie del fruto afectada se utilizó para clasificar cada fruto de forma similar a Megías et al. (2014) con la siguiente escala: 0 = sin picado, 1 = 5% de picado, 2 = 6-15% de picado, 3 = 16-25% de picado, 4 = 26-50% de picado y 5 = 50% del área superficial afectada por el picado. Por otra parte, para evaluar la gravedad de los síntomas de depresiones superficiales o picadura, la escala fue 0 = sin daños, 1 = daños muy superficiales, 2 = daños superficiales, 3 = daños moderados, 4 = daños graves, 5 = daños muy graves. El índice de daño por frío se obtuvo al hacer la media de ambas evaluaciones.

La translucidez y el índice de pardeamiento en la piña se estimó subjetivamente en un corte longitudinal corte longitudinal de medio fruto. La gravedad de la translucidez de la pulpa se determinó basándose en el porcentaje de superficie afectada por este trastorno (0%; opaca, no translúcido; a 100%; totalmente translúcido) (Murai et al., 2021) y para evaluar el índice de pardeamiento, la escala de gravedad fue: 0 (sin síntomas) hasta 4 (síntomas graves) (Selvarajah et al., 2001).

3.3.12. Análisis estadístico

Los resultados de esta Tesis Doctoral se expresan como media ± ES de tres réplicas. Los datos de todos los experimentos se sometieron a un análisis de varianza (ANOVA), seguidos de una comparación mediante la prueba de Tukey para determinar las diferencias de los parámetros entre los tratamientos, días de almacenamiento y variedades en la publicación 2, 3, 4 y 5. Por otro lado, se realizó la prueba t de Student en las publicaciones 1 y 2. Las diferencias se consideraron estadísticamente significativas a una $p < 0,05$ y se indicaron utilizando diferentes letras minúsculas o mayúsculas en cada parámetro. Todos los análisis se realizaron utilizando el paquete de software SPSS para Windows.

4 Publicaciones

4. PUBLICACIONES

4.1. Publicación 1 (Transcripción Literal)

Artículo 1

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Melatonin Treatment of Pomegranate Trees Increases Crop Yield and Quality Parameters at Harvest and during Storage

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Abstract: With the aim to study the effect of melatonin treatment of pomegranate trees on crop yield and fruit quality at harvest and during storage, two experiments were carried out in two consecutive years: 2017 and 2018. In the first year, trees were treated with melatonin (at 0.1 and 1 mM) along the developmental growth cycle and fruit quality parameters were evaluated at harvest and during storage at 10 °C for 90 days. Treatments with melatonin led to an increase of crop yield (number of fruits per tree and kg per tree), as well as higher fruit quality attributes, such as fruit size (diameter and weight), color, total soluble solids (TSS), and total acidity (TA), especially with the 0.1 mM dose. Then, in the second year, melatonin at 0.1 mM was selected for repeating the pre-harvest treatments with similar results in terms of crop yield and fruit quality parameters. During storage, pomegranate fruit treated with 0.1 mM melatonin maintained higher quality attributes than controls, such as TSS, TA, and firmness and lower weight losses were observed in fruit from treated trees, in both trials. In addition, the content of the major sugars (glucose and fructose) and organic acids (malic, succinic and ascorbic acid) were higher in melatonin-treated than in non-treated fruit. These results suggest that pre-harvest melatonin treatment could be a useful tool to increase pomegranate crop yield as well as fruit quality parameters at harvest and their maintenance during storage due to an effect of melatonin on reducing the postharvest ripening process.

Keywords: Punica granatum; firmness; color; sugars; organic acids; ripening

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1. Introduction

Melatonin (N-acetyl-5-methoxytryptamine) is a natural-occurring compound derivative of tryptamine, and plays important roles in plant growth, such as delay of senescence process and increased tolerance to both biotic and abiotic stress [1]. These effects have been attributed to the melatonin action as a natural antioxidant molecule scavenging free radicals either in vitro as in vivo studies using different vascular plants [2]. The melatonin content in edible fruit has been associated with health-benefits, since high levels of this compound in foods are beneficial for consumers. Thus, the consumption of a Jerte Valley cherry product enhances mood and increases 5-hydroxyindoleacetic acid but reduces cortisol levels in urine, which may protect against stress and act as a mood enhancer by increasing serotonin availability in the organism, particularly with advancing age [3].

As postharvest treatments, melatonin delayed physiological deterioration of cassava roots and reduced the accumulation of H_2O_2 while increasing the activity of antioxidant enzymes, and thus maintaining homeostasis of cellular reactive oxygen species (ROS) through increasing the endogenous melatonin levels [4]. In tomato fruit, postharvest treatment with melatonin at 50 µM stimulated the content of anthocyanins, and might be positively related to fruit ripening but negatively related to fruit senescence, since the proteins related

with senescence were downregulated while an increase of catalase and peroxidase was observed in treated fruit [5]. In peaches, postharvest application of melatonin at 0.1 mM reduced weight loss, decay incidence, and respiration rate as well as maintained firmness, total soluble solids and ascorbic acid contents during seven days of storage at 25–28 °C, with a concomitant increase in antioxidant enzymes and maintenance of membrane integrity, which might be a part of the mechanism implicated on delaying senescence in peach fruit [6].

As pre-harvest treatment, very little evidence exists about the role of melatonin on fruit growth and ripening, and different effects have been reported depending on fruit species, concentration, or time of application. Thus, melatonin was naturally found in grape tissues (skin, flesh and seed), and, during ripening, melatonin content decreased in skin, while it increased in both seed and flesh tissues [7]. Melatonin at 100 mg L^{-1} (0.43 mM), applied once or twice to grapevines at pre-veraison stage, significantly increased (6.6%) the grape berry size, which was correlated with increased concentration of endogenous melatonin [8]. In two cherry cultivars ('Hondeng' and 'Rainier'), melatonin was evaluated during the growth cycle, and a maximum peak at stage 2 was found, which coincided with endocarp lignification in both cherry cultivars [9] and in 'Prime Giant' sweet cherry, melatonin 0.1 and 0.01 mM applied at pit hardening inhibited fruit ripening [10]. However, irrigation of tomato plants with 0.1 mM melatonin increased sugar and lycopene concentration in fruits, showing a positive effect on fruit ripening [11], while, in apricots, foliar spray melatonin treatment increased yield and fruit weight, although no effect on on-tree ripening was observed [12].

Pomegranate is one of the oldest known edible fruits which has gained popularity and scientific interest in the last several years due to its nutritional value and health benefits, since it is very rich in bioactive compounds with antioxidant activity [13,14]. The quality parameters for pomegranate are based on external attributes such as size, shape, and color of the skin, as well as on internal ones such as aril color, sugar and acid content, and the presence of small and soft seeds [15,16]. The color of the pomegranate increases in both skin and arils during ripening, due to the accumulation of anthocyanins, the major in the sweet varieties being cyanidin 3-glucoside, followed by delphinidin 3,5-diglucoside and pelargonidin 3-glucoside, as well as sugar content, while decreases occur on acid content and fruit firmness [16–19]. However, during postharvest storage, pomegranate exhibits important quality losses due to several physiological and enzymatic disorders, the major ones being weight loss, together with loss of firmness, aril color, and acidity, which lead to a reduction of consumers' acceptability in terms of freshness, juiciness, and taste [15,16,19–21].

However, no literature is available about the effect of pre-harvest melatonin treatment on pomegranate growth and ripening on-tree, or on quality attributes at harvest and during postharvest storage, which has been the main objective of this paper. In addition, the effect of melatonin treatment on fruit yield was evaluated.

2. Materials and Methods

2.1. Plant Material and Experimental Design

Pomegranate (*Punica granatum* L. cv. Mollar de Elche) trees (10 years-old) were used for this study. The experiment was carried out during the developmental cycle of the 2017 and 2018 spring−summer periods, in a commercial plot located at Elche (Alicante, Spain), and full blossom (FB) was established on 30 April and 9 May, in the 2017 and 2018 experiments, respectively. During 2017, 5 trees were selected for each treatment: control (distilled water) and melatonin (purchased from Sigma, Sigma-Aldrich, Madrid, Spain) at 0.1 mM or 1 mM concentrations. Freshly prepared solutions (containing 0.05% Tween 20) were foliar sprayed with a mechanical mist sprayer and repeated at five dates of the growth cycle (30, 60, 90, 105, and 120 days after full blossom: T1, T2, T3, T4, and T5). Five fruits were labeled around the equatorial perimeter of each tree, in which fruit growth was followed by measuring cheek diameter. Fruits were harvested according to commercial criteria, when external color and size characteristic of this cultivar were acquired, and ca. 15° Brix. However, the ripening process of pomegranate fruit is heterogeneous in the tree and, therefore, some fruit reaches their

commercial ripening stage before others. Thus, two harvests (first and second harvest) were carried out ten days apart, in which yield (kg tree^{-1} and number of fruit tree^{-1}), and fruit weight were determined. Immediately after harvest, pomegranate fruit of the first harvest (about 200 fruit from each treatment) were transferred to the laboratory, sorted, and 120 homogeneous fruit were grouped in lots of 10 pieces of fruit. Fruit was stored at 10 °C (a non-chilling temperature for pomegranate fruit) and 85% relative humidity in cardboard boxes covered with perforated films. Three lots of 10 pieces of fruit were taken at random for each treatment, and sampling date (0, 30, 60 and 90 days) in which fruit firmness, weight loss, total soluble solids (TSS), total acidity (TA), the ratio between TSS/TA (ripening index) and color (external and internal) were determined. During 2018, and considering the best results obtained, the 0.1 mM concentration was chosen for repeating the experiment by choosing 5 different trees for each treatment in the same commercial plot. As previously mentioned, in the 2017 experiment, two different harvests were performed and, for each yield (kg tree^{-1} and number of fruit tree^{-1}), fruit weight was again determined. Fruit from the second harvest date was transferred to the laboratory for storage at 10 °C, as performed in the 2017 experiment. After 0, 30, 60, and 90 days of storage, three lots from each treatment were taken at random, in which the above-mentioned parameters were evaluated. In addition, the composition of individual sugars and organic acids was analyzed in control and melatonin-treated pomegranates at harvest time.

2.2. Fruit Growth and Crop Yield

The evolution of fruit growth was determined in the labeled fruit from T1 treatment until the first harvest date by measuring the fruit diameter (mm) at 7–10 day intervals by using a Vernier digital calliper. For each harvest date, crop yield was expressed as kg tree^{-1} and number of fruit tree^{-1}. The total number of fruit and total kg tree^{-1} were used to calculate fruit weight at the two harvest dates. Results were expressed as the mean ± SE.

2.3. Fruit Quality Parameters

Fruit quality parameters were measured according to Mirdehghan et al. [22] and García-Pastor et al. [20]. Weight loss was measured for each individual lot by recording the fruit weight at harvest (0 day) and at the different sampling dates during storage. Cumulative weight losses were expressed as a percentage with respect to fruit weight at day 0. External color was determined in three equidistant points along the equatorial perimeter of 10 fruits from each replicate, by using a Minolta colorimeter (CRC200, Minolta Camera Co., Tokyo, Japan), and the CIELab coordinates and color were expressed as Hue angle (h°). The pomegranate was cut into 2 halves and again 3 readings were performed in the arils from each fruit to measured internal color [20]. Fruit firmness was determined independently in the 10 fruits of each replicate using a TX-XT2i Texture Analyzer (Stable Microsystems, Godalming, UK) interfaced to a personal computer, with a flat steel plate mounted on the machine. For each fruit, the cheek diameter was measured and then a force that achieved a 3% deformation of the fruit diameter was applied. Results were expressed as the force–deformation ratio (N mm^{-1}). The arils from 10 fruit of each replicate were combined to obtain a homogeneous sample for each replicate. TSS were determined in duplicate in the juice obtained from 50 g of each sample with a digital refractometer Atago PR-101 (Atago Co. Ltd., Tokyo, Japan) at 20 °C, and expressed as g 100 g^{-1}. Titratable acidity (TA) was determined in duplicated in the same juice by automatic titration (785 DMP Titrino, Metrohm) with 0.1 N NaOH up to pH 8.1, using 1 mL of diluted juice in 25 mL distilled H_2O, and results expressed as g 100 g^{-1} malic acid equivalent. The ratio of TSS/TA (ripening index) was then calculated.

2.4. Sugars and Organic Acids

For organic acid and sugar determinations, 5 g of arils from each replicate were extracted with 10 mL phosphate buffer (50 mmol L^{-1}, pH = 7.8) and then centrifuged at 15,000× g for 15 min at 4 °C. The supernatant was used for sugars and organic acids quantification in duplicate as previously described by Mirdehghan et al. [22]. One mL of the extract was filtered through a 0.45 µm Millipore filter and then injected into a Hewlett-Packard HPLC series 1100. The elution system consisted of 0.1% phosphoric acid running isocratically with a flow rate of 0.5 mL min^{-1}. Organic acids were eluted through a Supelco column (Supelcogel C-610H, 30 cm 7.8 mm, Supelco Park, Bellefonte, PA, USA) and detected by absorbance at 210 nm. A standard curve of pure organic acids (L-ascorbic, malic, citric, oxalic, and succinic acids) purchased from Sigma (Madrid, Spain) was used for quantification. Results were expressed as g 100 g^{-1}. For sugar concentrations, the same HPLC, elution system, flow rate, and column were used and they were detected by using a refractive index detector. A standard curve of pure sugars (glucose, fructose, and sucrose) purchased from Sigma was used for quantification. Results were expressed as g 100 g^{-1}.

2.5. Statistical Analyses

All data are represented as means ± standard error of the mean (S.E.M.). Statistical analyses were performed using SPSS software, version 21.0 (SPSS Inc., Chicago, IL, USA). The data were subjected to an analysis of variance (ANOVA), the means were compared using Student's *t*-tests, and the differences were considered significant at $p < 0.05$.

3. Results

3.1. Effect of Pre-Harvest Melatonin Treatment on Crop Yield

Fruit diameter was recorded during on-tree fruit growth and ripening and results are shown in Figure 1, in which a simple-sigmoid growth curve from full blossom (FB) to harvest (155 d after full blossom, DAFB) can be observed. In addition, results showed that melatonin treatment at 0.1 mM concentration stimulated fruit growth from the second application (T2), leading to pomegranates with significantly higher size ($p < 0.05$) in 0.1 mM melatonin-treated trees (≈84 mm) than in 1 mM or control ones (≈79 and 77 mm, respectively) at the first harvest date. The increase of fruit size as a consequence of 0.1 mM melatonin treatment was not due to an increased peel width but to an increase in the portion of the arils, as can be observed in the photograph of cut pomegranate fruit detailed below.

With respect to yield, the results of the 2017 experiment are presented in Table 1, showing that both the number of fruit harvested by tree and kg of fruit harvested by tree were significantly higher ($p < 0.05$) in 0.1 mM melatonin-treated trees than in controls, although these increases were not significant in 1 mM melatonin treated trees. In addition, fruit ripening was delayed in treated trees, since yield at the first harvest date was significantly ($p < 0.05$) higher in control trees (28.65 ± 3.16 kg $tree^{-1}$) than in those treated with 0.1 and 1 mM melatonin doses (ca. 21 kg $tree^{-1}$), as well as the number of fruit per tree (78.0 ± 7.9, 61.40 ± 4.01 and 65.00 ± 3.83 for control and 0.1 and 1 mM melatonin treated trees, respectively). The average fruit weight, taking into account data from the two harvest dates, was significantly higher ($p < 0.05$) in fruit from 0.1 mM melatonin treated than from controls, although no significant effect was observed with 1 mM melatonin treatment. However, since total yield per tree was only significantly increased in 0.1 mM melatonin treated trees, this concentration was used in the next year, in which these effects were confirmed. Thus, higher values of kg $tree^{-1}$, the number of fruits per tree and average fruit weight were obtained in 0.1 mM-melatonin treated trees compared with control ones (Table 2).

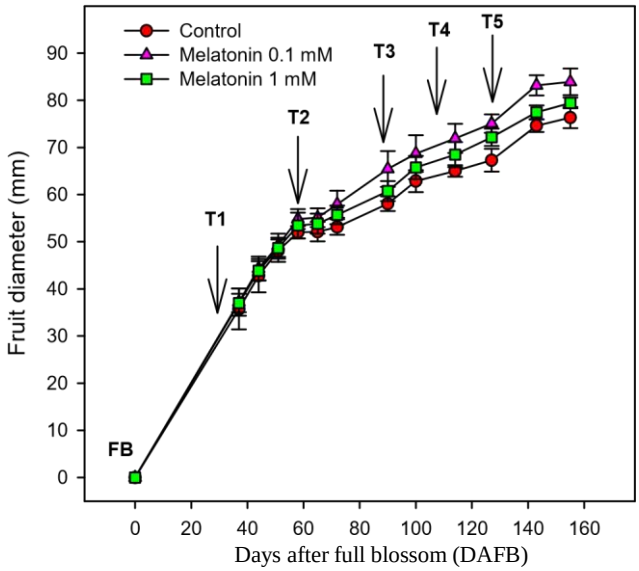

Figure 1. Evolution of fruit size (diameter) during pomegranate development from full blossom (FB) to harvest, in fruit from control and 0.1 or 1 mM melatonin-treated trees. T1–T5 represent the day of treatment. Data are the mean ± SE.

Table 1. Data for yield (kg tree^{-1} and number of fruit tree^{-1}), and fruit weight in control and melatonin-treated pomegranate trees (0.1 and 1 mM) at two harvest dates (1 and 2). Year 2017.

Parameter	Control	Melatonin 0.1 mM	Melatonin 1 mM
Yield (kg tree^{-1})			
Harvest 1	28.65 ± 3.16 a	21.68 ± 2.02 b	20.66 ± 2.84 b
Harvest 2	9.10 ± 0.98 a	20.36 ± 3.60 b	18.05 ± 2.29 b
Total	37.75 ± 3.28 a	42.04 ± 1.06 b	38.71 ± 2.49 a
Yield (number of fruit tree^{-1})			
Harvest 1	78.0 ± 7.9 a	61.40 ± 4.01 b	65.00 ± 3.83 b
Harvest 2	29.8 ± 3.21 a	58.00 ± 3.98 c	46.60 ± 5.65 b
Total	107.8 ± 5.16 a	119.40 ± 3.96 b	111.60 ± 4.15 ab
Fruit weight (g)			
Harvest 1	367.31 ± 11.29 a	353.09 ± 9.14 a	317.84 ± 10.21 b
Harvest 2	305.37 ± 7.62 a	351.03 ± 9.95 b	387.33 ± 8.45 c
Average	350.18 ± 6.19 a	352.03 ± 12.97 a	346.86 ± 9.15 b

Data are the mean ± SE. For each parameter and harvest date, different letters within a row show significant differences at $p < 0.05$.

Table 2. Data for yield (kg tree^{-1} and number of fruit tree^{-1}), total number of fruits in 5 trees and fruit weight in control and 0.1 melatonin-treated pomegranate trees at two harvest dates (1 and 2). Year 2018.

Parameter	Control	Melatonin 0.1 mM
Yield (kg tree^{-1})		
Harvest 1	26.73 ± 1.72 a	22.91 ± 2.34 b
Harvest 2	10.37 ± 1.37 a	20.34 ± 3.19 b
Total	37.10 ± 1.56 a	43.25 ± 1.57 b
Yield (number of fruit tree^{-1})		
Harvest 1	72.40 ± 3.64 a	60.90 ± 3.85 b
Harvest 2	36.75 ± 4.02 a	60.40 ± 5.92 b
Total	109.15 ± 3.92 a	121.30 ± 4.96 b
Fruit weight (g) Harvest 1	369.20 ± 9.55 a	376.19 ± 7.45 a
Harvest 2	282.17 ± 6.46 a	336.75 ± 6.17 b
Average	339.90 ± 5.15 a	357.43 ± 8.07 b

Data are the mean ± SE. For each parameter and harvest date, different letters within a row show significant differences at $p < 0.05$.

3.2. Effect of Melatonin on Fruit Quality Parameters

Weight losses increased during storage in pomegranate fruit from control and treated trees, although these increases were significantly reduced by melatonin treatment, with significant differences ($p < 0.05$) after 60 days of storage (Table 3). With respect to TSS, levels at harvest in the 2017 experiment were 15.17 ± 0.26 g 100 g^{-1} in fruit from control trees and significantly higher ($p < 0.05$) in those from 0.1 mM treated trees, although no significant differences were observed due to 1 mM melatonin treatment (Table 3). TSS content increased during storage in fruit from control and treated trees and these increases occurred later in fruit from melatonin treated trees with respect to those from controls (Table 3). Acidity levels (TA) at harvest were also significantly increased at harvest by melatonin treatments and they decreased during storage, but the decrease was much lower in melatonin-treated fruit (both 0.1 and 1 mM) than in controls (Table 3). The ratio between TSS/TA, also known as ripening index (RI), increased during storage, but it was reduced by the pre-harvest application of melatonin (Table 3). Melatonin treatments also affected pomegranate skin and aril color, since significantly lower ($p < 0.05$) values of Hue angle were recorded in fruit from melatonin treated trees than in controls and those differences were maintained during the whole storage period, although Hue angle of skin and arils decreased in all pomegranate fruit (Table 3).

Table 3. Physiological and biochemical parameters in pomegranate fruits from control and melatonin treated trees (0.1 and 1 mM) at harvest (day 0) and during 90 days of storage at 10 °C. Year 2017.

Parameter	Days	Control	Melatonin 0.1 mM	Melatonin 1 mM
Weight los (%)	0	-	-	-
	30	3.94 ± 0.19 aA	3.67 ± 0.15 aA	3.75 ± 0.20 aA
	60	4.82 ± 0.49 bA	3.83 ± 0.48 aB	4.38 ± 0.11 bB
	90	6.87 ± 1.09 cA	4.86 ± 0.26 bC	5.59 ± 0.17 cB
TSS (g 100 g^{-1})	0	15.17 ± 0.26 aA	16.37 ± 0.22 aB	15.38 ± 0.35 aA
	30	16.68 ± 0.12 bA	16.38 ± 0.10 aA	16.40 ± 0.12 aA
	60	16.93 ± 0.23 bcA	16.48 ± 0.16 aA	17.18 ± 0.26 a, bA
	90	17.42 ± 0.15 cA	17.25 ± 0.19 bA	17.32 ± 0.12 bA
TA (g 100 g^{-1})	0	0.45 ± 0.03 aA	0.56 ± 0.01 aA	0.56 ± 0.05 aA
	30	0.38 ± 0.01 bA	0.51 ± 0.02 bB	0.57 ± 0.03 aB
	60	0.32 ± 0.02 bcA	0.46 ± 0.01 bB	0.45 ± 0.02 bB
	90	0.21 ± 0.01 cA	0.38 ± 0.02 cB	0.30 ± 0.01 cB
RI (TSS/TA)	0	35.04 ± 0.51 aA	29.23 ± 0.52 aB	27.46 ± 0.54 aB
	30	43.88 ± 0.73 bA	32.01 ± 0.54 bB	28.77 ± 0.38 aB
	60	52.91 ± 0.84 cA	35.82 ± 0.74 cB	38.18 ± 0.56 bB
	90	82.95 ± 1.22 dA	45.39 ± 0.41 dB	57.73 ± 0.62 cC
External Color Hue	0	68.30 ± 1.01 aA	58.84 ± 1.47 aC	62.69 ± 1.12 aB
	30	64.71 ± 1.07 abA	55.89 ± 1.88 abB	57.71 ± 1.03 abB
	60	61.53 ± 1.09 bcA	53.66 ± 1.88 bcB	55.80 ± 1.68 bB
	90	59.93 ± 1.57 cB	52.26 ± 1.89 cB	54.48 ± 1.42 bB
Internal Color Hue	0	34.13 ± 1.71 aA	30.34 ± 1.18 aB	31.92 ± 1.29 aAB
	30	31.78 ± 1.04 abA	28.34 ± 0.73 abB	28.77 ± 1.22 abB
	60	28.91 ± 0.53 bA	26.00 ± 0.64 bB	26.93 ± 0.65 bcB
	90	26.91 ± 0.83cA	23.84 ± 0.55 cB	24.74 ± 0.69 cAB

Data are the mean ± SE. For each parameter, different lowercase letters within a column show significant differences at $p < 0.05$ during storage, while capital letters show significant differences at $p < 0.05$ among treatments for each sampling date.

Fruit firmness at harvest, in the 2017 experiment, was significantly higher ($p < 0.05$) in fruit from 0.1 mM melatonin treated trees than in controls (26.52 ± 0.50 and 28.93 ± 0.710 N mm^{-1}, respectively), while no significant effect was observed with 1 mM melatonin treatment. During storage, fruit firmness decreased in pomegranates from control and treated trees, although firmness levels were the highest in 0.1 mM melatonin treated fruit until the last sampling date (Figure 2).

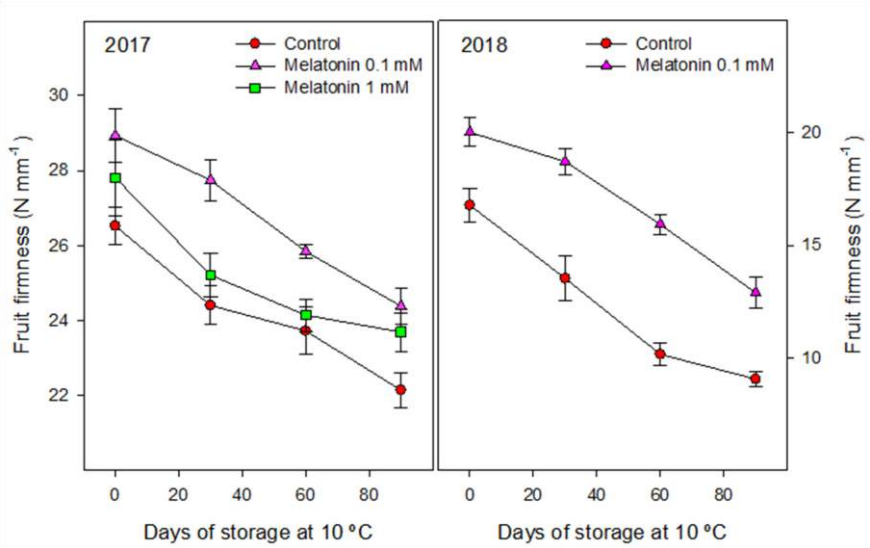

Figure 2. Fruit firmness evolution (Nmm^{-1}) during refrigerated storage in two consecutive production cycles (2017 and 2018), in control fruit and 0.1 or 1mM melatonin-treated trees. Data are the mean ± SE.

Similar results with respect to firmness (Figure 2), weight loss, TSS, TA, and RI were obtained in the experiment assayed in 2018 (Table 4), in which 0.1 mM melatonin concentration was chosen, since better results were obtained compared with 1 mM dose in the 2017 experiment. Accordingly, melatonin treatments did not affect skin color, although aril color was affected by treatment, since Hue angle values were significantly lower in melatonin treated than in control fruit, either at harvest or during storage (Table 4), which indicated a darker red color of the arils. These differences are evident in Figure 3, in which the visual aspect of pomegranates from control and melatonin-treated trees (0.1 mM) at harvest and during storage is shown. Small shrivelling symptoms were observed on pomegranate fruit after 90 days of storage in fruits from control and treated ones without differences attributed to melatonin treatment (data not shown).

Table 4. Quality parameters in control and 0.1 mM melatonin-treated pomegranate fruits at harvest (day 0) and during postharvest storage at 10 °C. Year 2018.

Parameter	Treatment	Day 0	Day 30	Day 60	Day 90
Weight loss (%)	Control	-	7.07 ± 0.49 Aa	8.49 ± 0.48 Ab	9.88 ± 0.54 Ac
	Melatonin		6.03 ± 0.14 Aa	7.18 ± 0.25 Bb	7.98 ± 0.24 Bc
TSS (g 100 g^{-1})	Control	16.05 ± 0.10 Aa	17.03 ± 0.15 Ab	17.52 ± 0.16 Ab	17.41 ± 0.22 Ab
	Melatonin	17.18 ± 0.12 Ba	17.02 ± 0.13 Aa	17.13 ± 0.18 Aa	17.25 ± 0.36 Aa
TA (g 100 g^{-1})	Control	0.35 ± 0.03 Aa	0.31 ± 0.03 Ab	0.21 ± 0.02 Ac	0.19 ± 0.04 Ac
	Melatonin	0.42 ± 0.04 Ba	0.37 ± 0.03 Ba, b	0.34 ± 0.02 Bb, c	0.29 ± 0.01 Bc
Ripening index (TSS/TA)	Control	45.85 ± 1.74 Aa	54.93 ± 2.15 Ab	83.42 ± 1.65 Ac	91.63 ± 1.97 Ad
	Melatonin	40.90 ± 1.03 Ba	48.62 ± 0.78 Bb	50.38 ± 2.18 Bb	59.48 ± 2.18 Bc
External Color Hue	Control	66.41 ± 1.22 Aa	64.20 ± 1.52 Aa	60.02 ± 1.34 Ab	58.38 ± 0.99 Ab
	Melatonin	60.02 ± 0.51 Ba	56.70 ± 1.57 Bbc	54.07 ± 1.24 Bcd	52.49 ± 1.61 Bd
Internal Color Hue	Control	33.13 ± 0.58 Aa	30.75 ± 1.90 Aab	29.23 ± 0.56 Ab	27.65 ± 1.07 Ac
	Melatonin	30.56 ± 1.24 Ba	27.22 ± 1.40 Bab	26.02 ± 1.18 Bb	22.79 ± 1.17 Bc

Data are the mean ± SE. For each parameter, different lowercase letters within a row show significant differences at $p < 0.05$ during storage, while capital letters show significant differences at $p < 0.05$ between treatments for each sampling date.

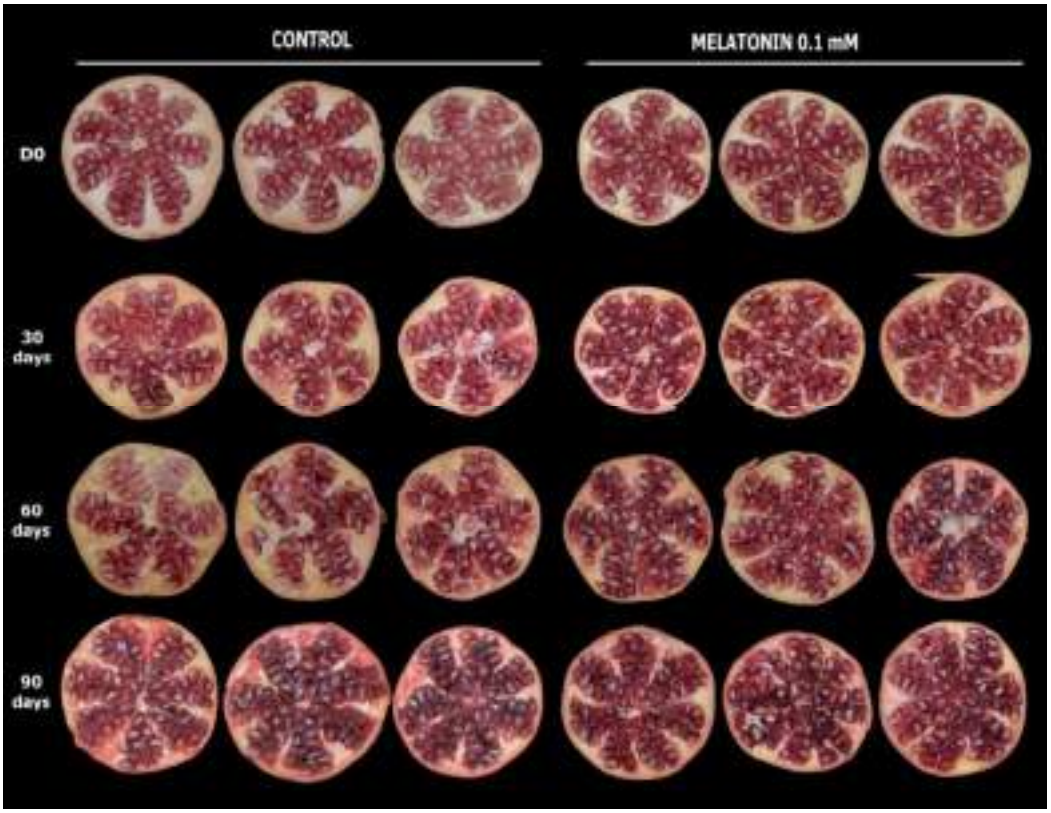

Figure 3. Photography displays the visual aspect of pomegranates from control and 0.1 mM melatonin-treated trees during storage in the 2018 experiment.

Individual sugars are mainly responsible for TSS in pomegranate and their quantification by HPLC showed that the major one was fructose, followed by glucose, while sucrose was found at very low concentrations (Table 5). Interestingly, fruit from melatonin treated trees had significantly higher $p < 0.05$ content of fructose and glucose at harvest than controls. With respect to organic acids, the major one found in 'Mollar de Elche' pomegranate was malic acid, followed by succinic and citric acids, which were also found at higher concentration in fruit from melatonin-treated trees, while no significant differences were observed in the minor ones, citric, fumaric, and oxalic acid. However, it is worth mentioning the effect of pre-harvest melatonin treatment on increasing ascorbic acid content in the arils, with concentrations being 1.5 higher in treated than in control fruit (Table 5).

Table 5. Reducing sugars and organic acids in fruits from control and 0.1 mM melatonin-treated trees at harvest. Year 2018.

Parameter	Control	Melatonin 0.1 mM
Sugars (g 100 g^{-1})		
Sucrose	0.06 ± 0.01 a	0.06 ± 0.02 a
Glucose	4.17 ± 0.10 a	4.81 ± 0.24 b
Fructose	11.61 ± 0.33 a	12.54 ± 0.26 b
Organic acids (g 100 g^{-1})		
Malic acid	0.32 ± 0.03 a	0.36 ± 0.04 b
Succinic acid	0.07 ± 0.01 a	0.12 ± 0.01 b
Citric acid	0.09 ± 0.01 a	0.09 ± 0.01 a
Ascorbic acid	0.04 ± 0.01 a	0.06 ± 0.01 b
Fumaric acid	0.03 ± 0.01 a	0.03 ± 0.01 a
Oxalic acid	0.01 ± 0.01 a	0.01 ± 0.01 a

Data are the mean ± SE. For each parameter, different letters within a row show significant differences at $p < 0.05$.

4. Discussion

As far as we know, this is the first report showing the effects of melatonin, applied as pre-harvest treatment, in pomegranate yield and fruit quality properties, although some evidence exists in other plant species. In a recent study performed on Arabidopsis treated with melatonin and grown at 4 °C, melatonin-treated plants had significantly greater fresh weight, primary root length, and shoot height compared with untreated plants, the effect being both time and concentration dependence [23]. However, the yield increase found in the present study was higher with 0.1 mM melatonin treatment than with 1 mM dose, and due to an increase in fruit size (diameter and weight). This effect was due to an increased aril portion but to an increase in peel width as can be observed in the photograph of cut pomegranates in Figure 3. On the other hand, the amount of fruit harvested by tree was also increased by melatonin treatment. Given the fact that treatments were applied when fruit was in its active phase of growth, the higher amount of fruit harvested from treated fruit could be attributed to the effect of melatonin on decreasing the normal fruit abscission that occurs during fruit development due to environmental factors, such as wind or rain. In addition, the effect of melatonin on alleviating biotic and abiotic stress in plants has been reported [23,24]. Thus, giving the semi-arid climate conditions of Southern Spain, the melatonin treatment could increase net photosynthesis rate and productivity throughout enhancement of tree tolerance to heat and drought stresses. Accordingly, grape berries treated with melatonin at pre-veraison exhibited higher endogenous melatonin accumulation and increased berry size and weight [8]. These authors attributed this effect to an increase of the sink strength of the berry, leading the fruit to uptake more sugars and develop a larger size at harvest. On the other hand, melatonin foliar spray treatment increased fruit weight and yield for 'Canino' apricot [12]. In addition, we have found increased crop yield 'Colorado' and 'Mikado' apricot cultivars after melatonin treatment (unpublished data), which were attributed to increases in tree net photosynthesis, due to enhanced total chlorophyll and leaf area.

'Mollar de Elche' pomegranate is very much appreciated by consumers due to the high content of sugars and low acidity, which confer a sweet taste while also being very aromatic [15–18]. In this sense, the higher values of TSS, TA, sugars, and organic acids found at harvest in pomegranates of melatonin treated trees show that they had higher quality attributes than controls. However, this cultivar is characterized by having a pale aril color compared with other cultivars [15,17,25] and thus several research papers have been performed with the aim to increase aril color while enhancing anthocyanin synthesis, by applying water restrictions in summer, during the linear phase of fruit growth [26], as well as treatment with methyl jasmonate or salicylates during on-tree pomegranate fruit development [16,27]. Results of the present research show that melatonin-treated fruit had a deeper red color than controls (lower Hue angle) at harvest and during storage, showing a stimulation of the anthocyanin biosynthesis by melatonin treatment, which is the pigment contributing to the red color of pomegranates [15,16,20,27]. On the other hand, no effects of melatonin treatments on fruit taste and flavour were appreciated either at harvest or during storage, although a proper sensorial analysis would be useful to a scientific validation of this observation.

On the other hand, results show that pre-harvest melatonin treatment delayed the postharvest ripening process during storage, since weight, firmness, and acidity losses were delayed in fruit from melatonin-treated trees with respect to controls, these effects being higher for 0.1 mM dose than for 1 mM. 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 about postharvest treatments. Thus, in banana, exogenous application of melatonin (at 0.05, 0.2 and 0.5 mM) resulted in a delay of postharvest ripening, although in this report the effect was dose dependent [28]. The lower weight losses observed in fruits from melatonin treated trees with respect to those of controls might be attributed to an effect of melatonin on increasing cuticle thickness, as recently proposed for nectarines [29] and mangos [30] after postharvest melatonin treatment. Accordingly, postharvest 0.1 mM melatonin treatment delayed the postharvest ripening process during cold storage in apples [31], peaches

[32], nectarines [29], pears [33], and mangos [34], which was attributed to a inhibition of ethylene production, although similar effects have been reported in non-climacteric fruits such as sweet cherries [35].

5. Conclusions

This is the first report in which melatonin (at 0.1 or 1 mM) has been applied as preharvest treatment with a significant effect on increasing crop yield and pomegranate fruit quality at harvest and during storage. The best results were found for the 0.1 mM dose. In addition, the concentration of sugars (glucose and fructose) and ascorbic acid were also higher after melatonin treatment. During postharvest storage, reduced softening and weight and acidity losses were found in melatonin-treated fruit. Overall, melatonin could be a reliable, feasible, and cost-effective tool to be used as plant bio-stimulant in order to increase pomegranate crop yield and fruit quality parameters at harvest and to maintain them during storage. In the future, the possible role of melatonin on bioactive compounds responsible for their beneficial health effects should be investigated.

Author Contributions: M.S. and D.V. conceived and designed the experiment in association with the other authors. P.J.Z. and F.G. performed the field treatments. J.M.-S., J.M.L.-M. and M.E.G.-P. performed most of the analytical determination in collaboration with the other authors. D.V., M.S., and F.G. analyzed the data and wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

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4.2. Publicación 2 (Transcripción Literal)

Artículo 2

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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–Packard™ 5890A gas cromatograph, and the remaining 2 mL was used to quantify, in duplicate, CO_2 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 CO_2 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 Mycrosystems, 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 ± 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 g 100 g^{-1} in fresh weight basis.

2.3. Total and Individual Phenolic Quantification

Total phenolis 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× *g* for 10 min at 4 °C and total phenolics were quantified in the supernatant, in duplicate, using the FolinCiocalteu 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 ± SE. To quantify individual phenolics, 1 mL of the above supernatant was filtered through a 0.45 µm 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 cm × 0.46 cm i.d. and 5 µm particle size and a C18 security guard of 1 cm × 0.32 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 µL 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 ± 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.

		'Colorado'		**'Mikado'**	
	Days	**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 ± 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 ± 1.34 nL g^{-1} 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 ± 1.41 nL g^{-1} h^{-1}, respectively, in those stored at 1 °C. After that, ethylene production deceased 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.

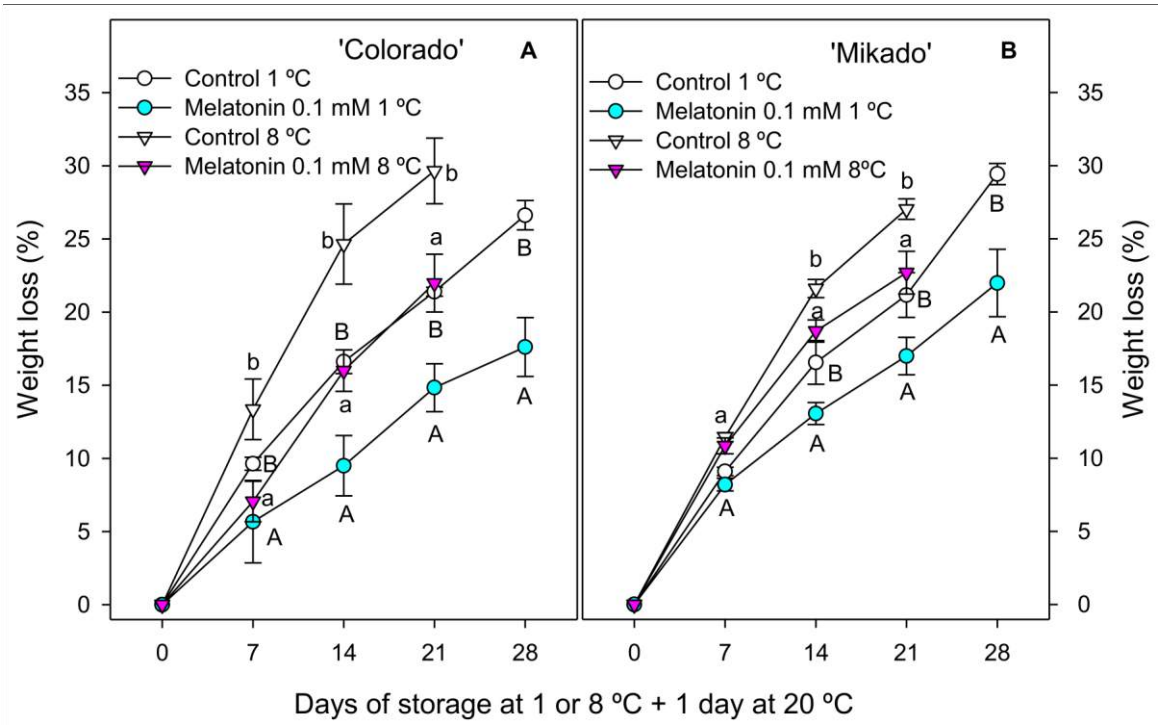


Figure 1. Weight loss of 'Colorado' (**A**) and 'Mikado' (**B**) apricots from the control and melatonin treated trees during storage at 1 and 8 °C. Data are the mean ± SE of three replicates of ten fruits from 2019 and 2020 experiments. LSD values were 1.196 and 0.943 for (**A**,**B**), respectively. 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.

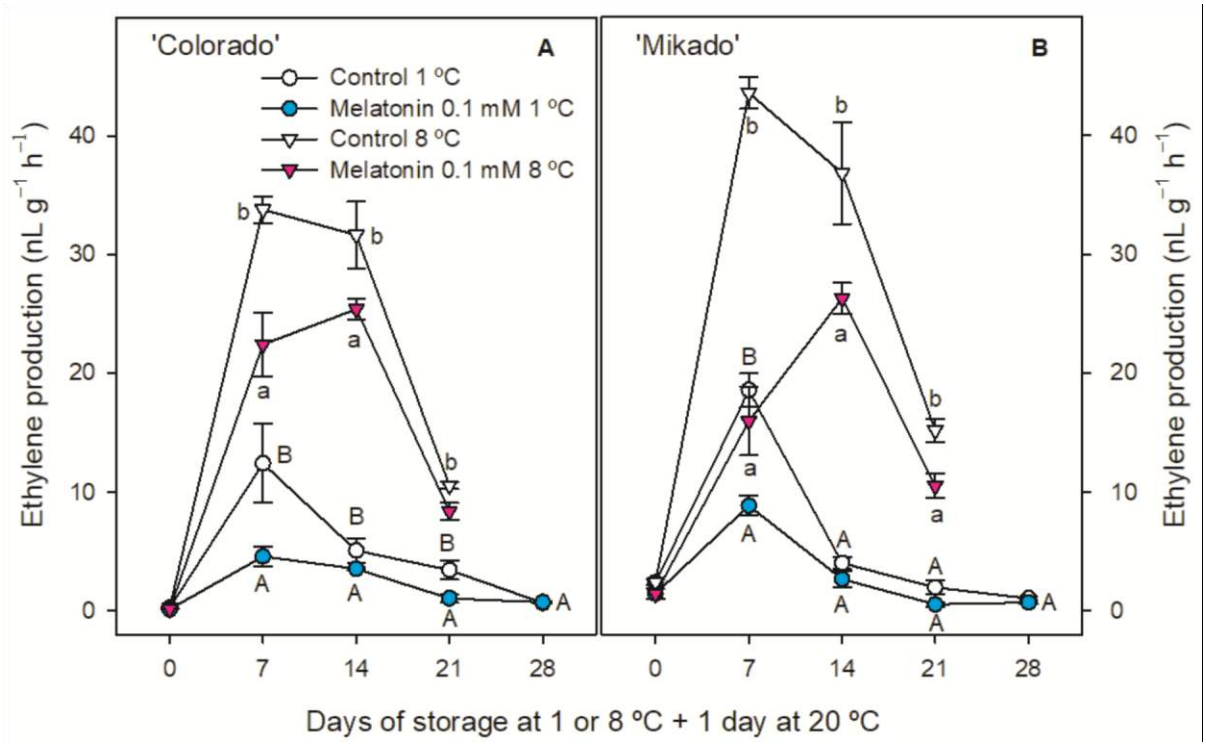


Figure 2. Ethylene production rate of 'Colorado' (**A**) and 'Mikado' (**B**) apricots from the control and melatonin treated trees during storage at 1 and 8 °C. Data are the mean ± SE of three replicates of ten fruits from 2019 and 2020 experiments. LSD values were 0.90 and 1.14 for (**A**,**B**), respectively. 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.

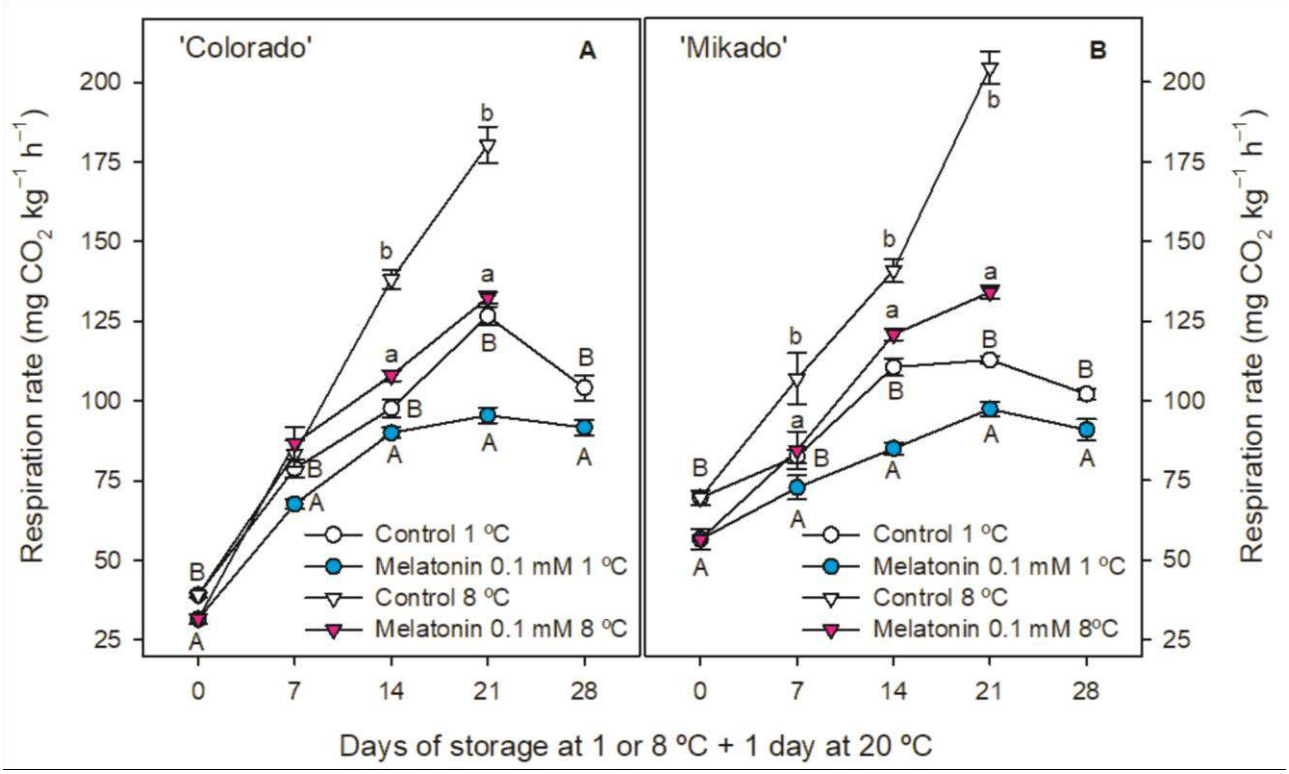


Figure 3. Respiration rate of 'Colorado' (**A**) and 'Mikado' (**B**) apricots from the control and melatonin treated trees during storage at 1 and 8 °C. Data are the mean ± SE of three replicates of ten fruits from 2019 and 2020 experiments. LSD values were 2.32 and 2.73 for (**A**,**B**), respectively. 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).

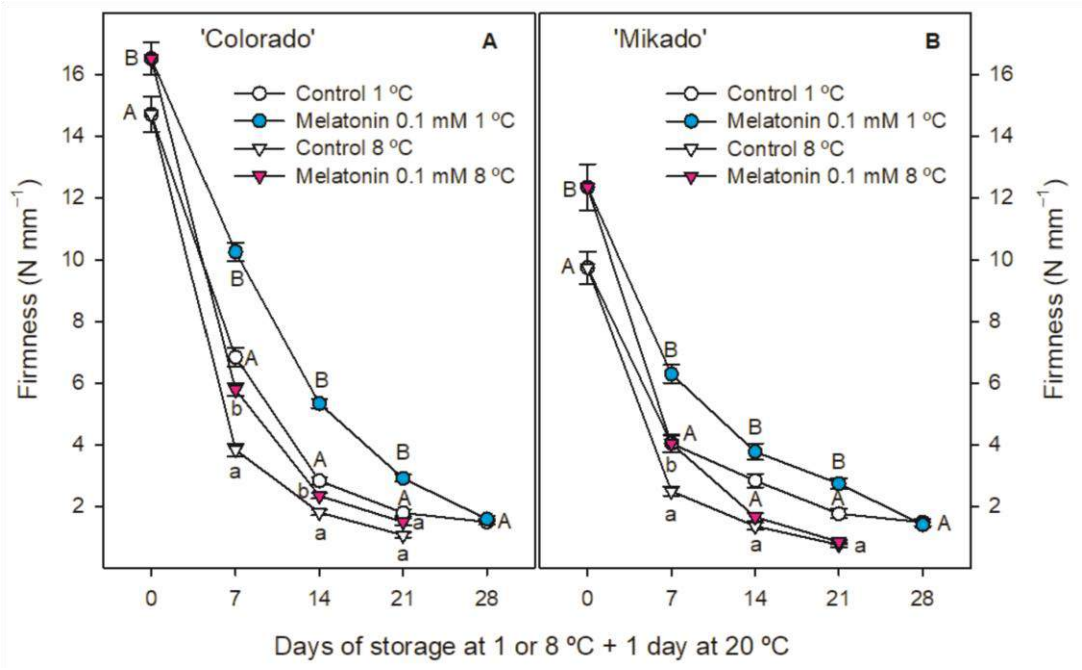


Figure 4. Fruit firmness of 'Colorado' (**A**) and 'Mikado' (**B**) apricots from the control and melatonin treated trees during storage at 1 and 8 °C. Data are the mean ± SE of three replicates of ten fruits from 2019 and 2020 experiments. LSD values were 0.23 and 0.37 for (**A**,**B**), respectively. 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 ± 0.21 aA	10.25 ± 0.31 aA	9.02 ± 0.25 aA	9.23 ± 0.08 aA
	21 d 1 °C + 1 d 20 °C	13.88 ± 0.26 aB	12.60 ± 0.15 bB	10.15 ± 0.14 aB	9.92 ± 0.12 bB
	21 d 8 °C + 1 d 20 °C	14.53 ± 0.19 aC	13.30 ± 0.26 bC	11.47 ± 0.18 aC	10.55 ± 0.04 bC
TA	Day 0	2.55 ± 0.03 aC	2.66 ± 0.04 aB	1.83 ± 0.05 aC	1.78 ± 0.07 aB
	21 d 1 °C + 1 d 20 °C	2.32 ± 0.04 aB	2.69 ± 0.06 bB	1.65 ± 0.04 aB	1.82 ± 0.06 bB
	21 d 8 °C + 1 d 20 °C	1.70 ± 0.06 aA	2.13 ± 0.02 bA	1.32 ± 0.11 aA	1.68 ± 0.05 bA

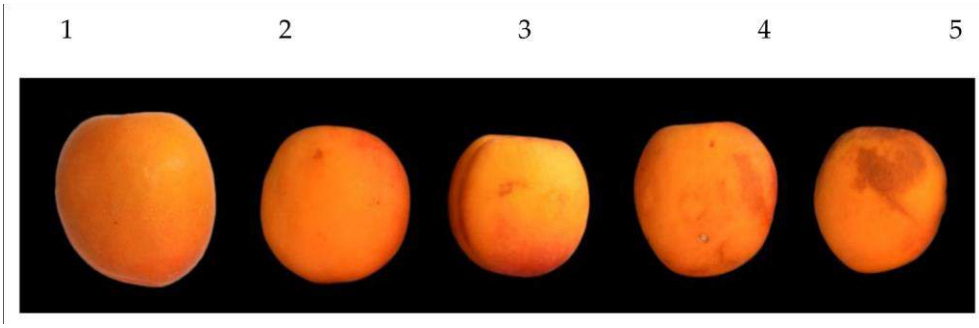
Data are the mean ± SE of three replicates from 2019 and 2020 experiments. For each cultivar, different lowercase letters show significant differences ($p < 0.05$) between the control and the melatonin treatments (*t*-test), and different uppercase letters show significant differences ($p < 0.05$) during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



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).

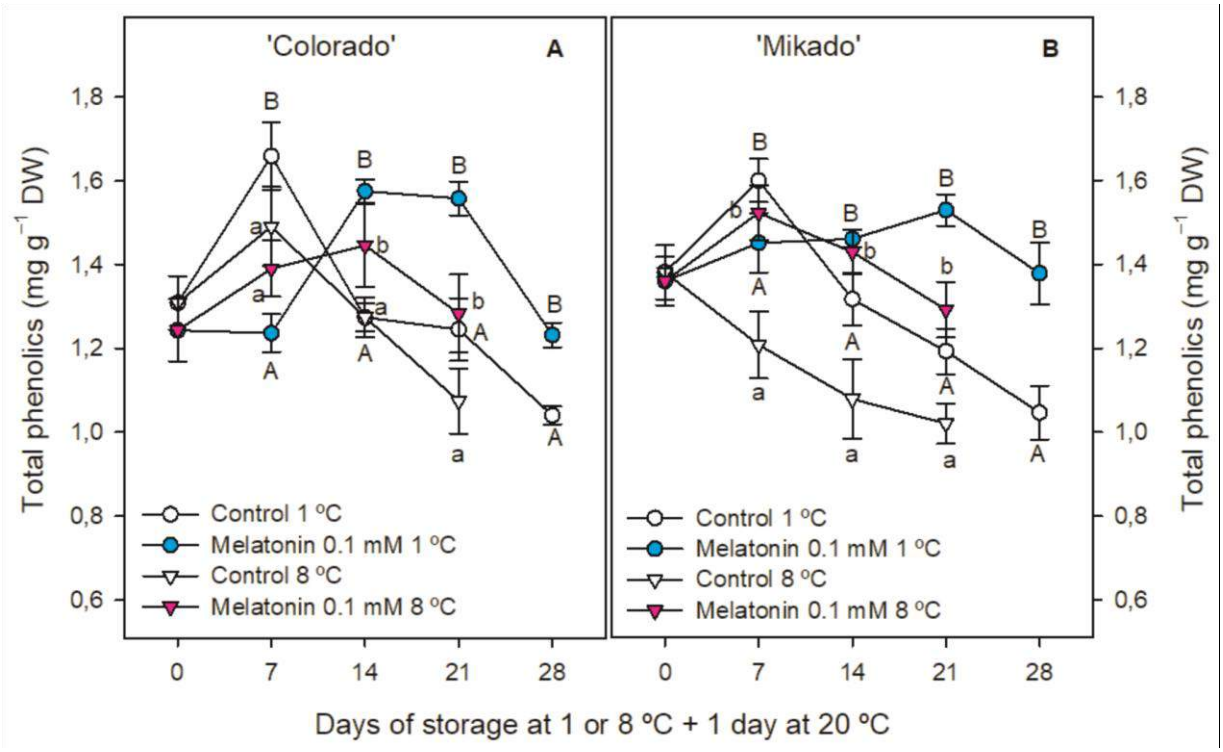


Figure 5. Total phenolic content on the flesh of 'Colorado' (**A**) and 'Mikado' (**B**) apricots from the control and melatonin treated trees during storage at 1 and 8 °C. Data are the mean ± SE of three replicates of ten fruits from 2019 and 2020 experiments. LSD values were 0.09 and 0.07 for (**A**, **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 exits 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 postharvest 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 tress 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 ± 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 ± 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.

Author Contributions: P.J.Z., M.S., D.V. and F.G. conceived and designed the work in association with other authors. J.M.-S., J.M.V. and F.G. performed the field treatments. J.M.-S. and F.G. performed most of the analytical determination in collaboration with J.M.V. Finally, M.S. and D.V. analyzed the data and wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

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4.3. Publicación 3 (Transcripción Literal)

Artículo 3

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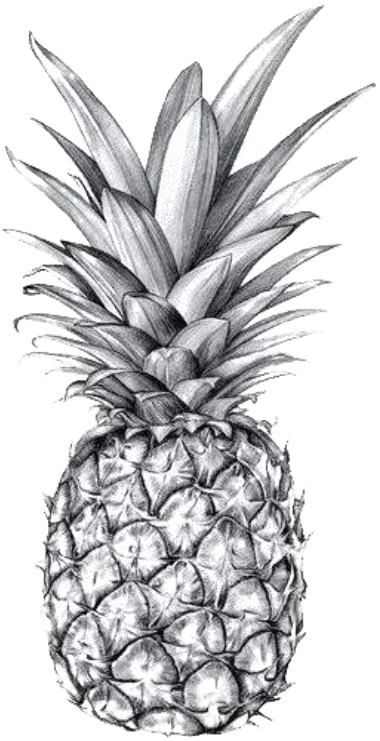

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Postharvest melatonin treatment delays senescence and increases chilling tolerance in pineapple.

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Chilling injury

ABSTRACT

Pineapple (*Ananas comosus* (L.) Merr.) is currently the third most important tropical fruit in world trade after banana and mango. Melatonin (MT) is found in different plant organs and tissues and is described as an endogenous elicitor with a signalling role that alleviates fruit chilling symptoms during postharvest storage. Melatonin effects on the overall quality of pineapple was investigated in fruit dipped in a 0.1 mM solution for 10 min and then stored at 9 °C for 21 days followed by 4 days at 22 °C. MT application delayed the over-ripening process in pineapple during storage. The respiration rate was reduced (~ 47.76%) after 7 days of cold storage, and shell color and flesh firmness losses were delayed in treated fruit compared to untreated fruit. Flesh translucency and internal browning due to chilling injury were higher in untreated control versus treated fruit. This is the first study that showed the potential of MT fruit immersions to alleviating internal browning symptoms maintaining the external quality of pineapple during postharvest storage.

1. Introduction

Pineapple (*Ananas comosus* (L.) Merr.) is one of the most appreciated tropical fruit worldwide. The new low-acid pineapple hybrids developed in Hawaii for the fresh fruit market have been introduced in Central and South America, Thailand, Philippines, Australia, Africa, Malaysia, and Taiwan. These newer cultivars (PRI 73-050 (MD-1, CO-2) & PRI 73–114 (MD2)) present new challenges in production and in the maintenance of postharvest quality (Taniguchi, Sanewski, Bartholomew, & Paull, 2008). Low acid types have become the preferred and the acreage has expanded rapidly to supply the fresh fruit markets of USA, Japan, and Europe. The two low acid hybrid, 'PRI 73-050' and 'PRI 73–114', were bred by the Pineapple Research Institute in Hawaii, both having similar sugars levels to the older canning types of Smooth Cayenne with lower titratable acidity and higher ascorbic acid levels. Moreover, these two hybrids have a greater pigmentation and flesh fiber content, and also are sensitive to cold induced internal browning (IB) at suboptimal temperatures (Chen & Paull, 2017; Taniguchi et al., 2008). Specifically, the hybrid PRI 73-050 is better suited to the cooler tropics and is sold as 'Maui Gold' and 'Dole Premium Select'.

Translucency is a physiological disorder that affects pineapple flesh, in which the flesh shows water soaking and is associated with greater fruit sensitivity to mechanical injury. This disorder begins before harvest and continues after harvest (Paull & Reyes, 1996). Membrane permeability in translucent pineapple flesh is higher than in non-translucent flesh and this disorder has also been reported when heat and irregular irrigation or rainfall occur at the same time when the fruit is ripening on the plant. Nitrogen supplied in excess enhances translucency occurrence. Pineapple translucency has also been suggested to be related to an increase in cell wall hydrolases and membrane permeability (Paull & Chen, 2019).

Pineapple deteriorates quickly at ambient temperature. Cold storage is used to reduce the high metabolic activity reducing ripening process although pineapple fruit is very sensitive to chilling injury (CI). The symptoms associated with chilling induced IB start under the shell around the base of the fruitlets adjacent to the fruit core and spread throughout the fruit flesh, especially when the storage temperature is lower than 12 °C (Paull & Chen, 2019; Raimbault et al., 2011). In this sense, it has been suggested that chilling stress could affect the membrane fluidity or could induce damage to mitochondria through the production of excessive reactive oxygen species (Nukuntornprakit, Luengwilai, & Siriphanich, 2020).

Melatonin (N-acetyl-5-methoxytryptamine, MT) is an endogenously produced indoleamine in all plant species (Liu, C., Zheng, H., Sheng, Liu, W. & Zheng, L., 2018) and a healthy component in the diet since many fruits and vegetables provide natural MT (Feng, Wang, Zhao, Han, & Dai, 2014; Sun et al., 2015). MT

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plays a major role in regulating plant developing and is involved in root morphology, senescence, seed germination, crop yield, and fruit ripening (Palma, Freschi, Rodríguez-Ruiz, Gonzalez-Gordo, ´ & Corpas, 2019; Reiter et al., 2015; Tan, Manchester, Esteban-Zubero, Zhou, & Reiter, 2015). As a safe and beneficial indoleamine, MT acts as a signaling molecule for enhancing the resistance of plants to biotic and abiotic stresses but also as a powerful free-radical scavenger which has a direct antioxidant activity (Arnao & Hernandez-Ruiz, 2020´ ; Tan et al., 2015) that may contribute to the maintenance of cellular redox homeostasis in fruits (Zhang et al., 2018). Exogenous MT treatment has been tested as an effective postharvest tool to promote ripening, improve quality of tomato (Sun et al., 2015), delay postharvest senescence and increase chilling tolerance of peach (Cao et al., 2016; Gao et al., 2016), and attenuates postharvest decay and maintain nutritional quality of strawberry (Liu, Zheng, Sheng, Liu, & Zheng, 2018). However, there is no available information regarding the effects of melatonin on the shelf life and quality of pineapple. In this study, melatonin was applied as a postharvest treatment to evaluate pineapple postharvest quality and resistance to chilling injury (CI) following storage at 9 ◦C for 21 days plus 4 days at 22 ◦C.

2. Experimental design

2.1. Plant material and postharvest treatments

Pineapples (*Ananas comosus* (L.) Merr. hybrid 'PRI 73-050'), were harvested from a commercial planting (Dole Fresh Fruit, Hawaii) located in central Oahu. All pineapples were harvested at the commercial ripening stage stage (with a visual appearance of around a 25% peel yellow) and immediately after harvest transferred to the laboratory. Fruit weighing about 2.2 kg were selected for uniformity of fruit size (20–22 cm) and absence of any visual defects. In our previous experiments (6 homogeneous fruit per treatment and sample time), different MT (Sigma-Aldrich, USA, > 98% M5250) concentrations (0 mM (control), 0.1 mM and 0.5 mM) and immersion times (10 and 60 min) were evaluated. Preliminary results showed that 0.1 mM MT for 10 min gave the great affect on reducing pineapple CI symptoms. Thus, 2 batches of 3 replicates with 5 homogeneous fruit for each treatment and sampling date were dipped in distilled water and freshly prepared MT solutions (both treatments containing 0.5% Tween 20) for 10 min. After treatment, pineapples were allowed to surface dry at room temperature for 2 h and then stored at ~80% of relative humidity (RH) in commercial pineapple cartons for 0, 7, 14 and 21 days at 9 ◦C + 4 days at 22 ◦C (Paull, Chen, & Saradhuldhat, 2017).

2.2. Postharvest quality parameters

Weight loss of individual pineapple was calculated as percentage with respect to the weight on day 0. Pineapple firmness was measured in the flesh of each pineapple using a steel probe with 1 cm of diameter coupled with a manual test stand (ZPS-DPU-110 Digital Force Gauge. Imada, Japan) interfaced to a computer. For each pineapple, the penetration distance applied in the pulp was 3.5 cm. Firmness was recorded as a maximum load pressure in Newtons (N) applied in three equidistant points of the pineapple equatorial slices of 7 cm. Results were the mean ± SE (n = 3). Respiration rate was measured by placing each pineapple in a 7.5 L glass jar hermetically sealed with a rubber stopper for 60 min following the static method proposed to measure CO_2 concentration (Kader, 1992). After that, 1 mL gas sample was taken by duplicate from head space and carbon dioxide was quantified with an infrared gas analyzer detector (LiCor Inc NB: Li-820 Gas Analyzer) in a 30 mL min^{-1} nitrogen gas stream (Clegg, Sullivan, & Eastin, 1978). Results were the mean ± SE (n = 3) and expressed as nmol CO_2 kg^{-1} s^{-1}. Color parameters (CIE *L**, CIE *a** and CIE *b**) were determined individually in each pineapple in both shell and flesh (Internal and external color) as well as in crown using the CIE Lab System (Minolta Camera Co., Japan; Chromameter CR200). Six determinations were performed in opposite sides of each pineapple. CIE *hue**$(180 + \tan^{-1} b^*/a^*$, if $a^* < 0)$ was calculated and results were expressed as the mean ± SE (n = 3). Chlorophyll content of the crown leaves was measure using an atLEAF Chl meter (FT Green LLC, Wilmington, DE) and expressed as atLEAF relative units. The total chlorophyll content is obtained by converting the atLEAF ChL values into SPAD units according to the calculations of Zhu, Tremblay, and Liang (2012) and considering the relationship among total chlorophyll content and SPAD units stablished by Richardson, Duigan, and Berlyn (2002). Results were expressed as mg cm^{-2} and were the mean ± SE (n = 3).

Total soluble solids (TSS) were determined by triplicate in the juice obtained from 10 longitudinal fruit slices of 1 cm of each replicate (~ 25 g each slice) taken from opposite sides with a digital refractometer Atago PR-101 (Atago Co. Ltd., Tokyo, Japan) at 20 ◦C, and expressed as percentage (g 100 g^{-1}) (n = 3). Also, for each replicate total acidity (TA) was determined by triplicate in the same juice by automatic titration with NaOH 0.1 N up to pH 8.1, using 1 mL of diluted juice in 30 mL distilled H_2O, and results were expressed as the percentage of citric acid (meq. citric acid = 0.064) (n = 3). Ripening index (RI) was evaluated as the quotient of TSS and TA (Vilaplana, P´erez-Revelo, & Valencia-chamorro, 2018).

Translucency and IB were subjectively estimated on a longitudinal cut half fruit. The severity of flesh translucency was determined based upon the percentage of affected area by this disorder (0%; opaque, not translucent; to 100%; fully translucent) (Murai, Chen, & Paull, 2021) and to evaluate IB, the severity scale was: 0 (without symptoms) up to 4 (severe symptoms) (Selvarajah, Bauchot, & John, 2001).

2.3. Statistical analysis

Results were expressed as mean ± SE. Data were subjected to analysis of variance (ANOVA). Sources of variation were treatment and storage days. Mean comparisons were carried out using a multiple range test (Tukey's HSD test) to find significant differences ($P < 0.05$) among storage days for each parameter and these differences were expressed in each graph or table as lowercase letters. For each sampling date, significant differences at $P < 0.05$ between both treatments were analysed according to Student's *t*-test and represented with * symbol. All analyses were performed using SPSS software package, version 22 (IBM Corp., Armonk, NY, USA).

3. Results and discussion

3.1. Optimization of postharvest melatonin treatment

Previously, to select the optimal MT treatment, we carried out a screen test with different MT doses. Thus, 6 pineapples per MT dose (0, 0.1 and 0.5 mM) during 2 different immersion times (10 and 60 min) were individually evaluated after 10 days at 9 ◦C + 1 day at 22 ◦C. External and internal quality was maintained specially for 0.1 mM MT dose assayed during the shortest immersion time (Table 1).

Although all MT treatments were able to maintain different quality parameters with respect to the harvest day as compared to control fruit, only the lowest MT dose (0.1 mM) significantly delayed all parameters studied ($P < 0.05$). On the other hand, no significant effect ($P > 0.05$) was observed on pineapple weight losses (data not shown) during the period studied. Furthermore, no additional benefits were observed by increasing the immersion time. For these reasons, immersions during 10 min with 0.1 mM MT were the conditions applied in the present study.

Table 1
Quality parameters of pineapples on the harvest day and after storage of 10 days at 9 ◦C + 1 day at 22 ◦C in fruit treated with melatonin (0, 0.1 and 0.5 mM) during 10 min or 60 min

		Nmol CO_2 kg^{-1} s^{-1}	CIE *a** (Crown)	CIE *a** (Shell)	CIE *hue** (Shell)	Flesh Firmness (N)	Internal Browning
At harvest		80.19 ± 1.16a	-12.16 ± 0.42a	1.05 ± 0.08b	88.60 ± 0.98c	75.85 ± 3.61b	-
		10 days of storage at 9 ºC + 1 day at 22 ºC					
		Nmol CO_2 kg^{-1} s^{-1}	**CIE *a** (Crown)**	**CIE *a** (Shell)**	**CIE *hue** (Shell)**	**Flesh Firmness (N)**	**Internal Browning**
Control	**10 imn**	131.25 ± 2.97b	-8.29 ± 0.85c	3.63 ± 0.45c	80.13 ± 0.78ab	56.28 ± 1.10a	2.50 ± 0.28bc
	60 min	171.83 ± 7.71c	-8.95 ± 0.87c	3.92 ± 0.01c	82.14 ± 1.01b	61.35 ± 2.12a	2.75 ± 0.25c
MT 0.1 mM	**10 min**	88.28 ± 9.93a	-11.60 ± 0.45ab	0.24 ± 0.06a	89.45 ± 1.51c	71.75 ± 3.16b	0.40 ± 0.14a
	60 min	165.85 ± 12.94c	-11.55 ± 0.51ab	0.28 ± 0.34ab	90.66 ± 0.81c	67.63 ± 4.12ab	0.50 ± 0.19a
MT0.5 mM	**10 min**	73.19 ± 6.60a	-12.37 ± 0.68ab	3.17 ± 0.41c	83.26 ± 0.79b	70.74 ± 2.13b	0.45 ± 0.25a
	60 min	146.72 ± 5.01bc	-10.58 ± 0.35bc	5.04 ± 0.28d	78.59 ± 0.57a	64.49 ± 2.10ab	1.80 ± 0.20b

Data are the mean ±SE. For each parameter, different letters, within the same column, show significant differences at P <0.05 among treatments, according to Tukey's HSD multiple range test.

3.2. Effect of postharvest melatonin treatment on weight loss and fruit firmness

Weight loss in pineapple is due to transpiration from the crown leaves and from the fruit body with the crown leaves showing the greater loss in quality (Chen & Paull, 2001). Weight losses increased in all fruit during storage regardless of whether pineapples were treated with MT or with distilled water (control) (Fig. 1A). After cold storage, weight loss in those pineapples treated with MT was not significantly ($P \geq 0.05$) reduced with respect to control fruit. This small difference after 21 days storage in weight loss may be related to a greater cell membrane integrity in MT treated pineapples (Liu et al., 2018) or to a possible impact of the treatment on closing the crown leaf stomata. Reduced water losses during storage following MT postharvest treatment have been reported in peach, strawberry and plum (Bal, 2019; Gao et al., 2016; Liu et al., 2018) and recently also after a preharvest treatment on pomegranate and apricot trees (Medina-Santamarina, Serrano, et al., 2021, Medina-Santamarina, Zapata, et al., 2021).

Fruit firmness loss is directly related with the maintenance of overall fruit quality and the postharvest life of pineapple (Hu, Li, Dong, & Chen, 2011). This loss in firmness is ascribed to cell wall deterioration and decrease of turgor in the fruit cells (Vilaplana et al., 2018), and has an important impact in fruit acceptance by consumers. Flesh firmness of MT treated pineapples was significantly higher (P < 0.05) during storage at 9 ◦C plus 4 days at room temperature as compared to control fruit (Fig. 1B). In previous studies, MT up-regulated cell wall structure-related genes (Sun et al., 2015; Zhai et al., 2018), reducing the depolymerization of pectic substances responsible of fruit softening (Brummell & Harpster, 2001). MT is also able to reduce reactive oxygen species (ROS), increasing peroxidase (POD), superoxide dismutase (SOD) and other antioxidant enzymes, reducing membrane lipid peroxidation during postharvest storage (Zhao et al., 2013; Gao et al., 2016), that could account for the reduction in loss of fruit firmness after MT treatment observed in this study.

3.3. Effect of postharvest melatonin treatment on respiration rate

The respiration rate of treated and control fruit increased during storage at 9 ◦C, this increase being higher when pineapples were transferred to 22 ◦C. Respiration rate was significantly lower (P < 0.05) for MT treated pineapples, both at 9 ◦C and at room temperature (Fig. 1C and D). Postharvest MT treatments do enhance GABA shunt activity

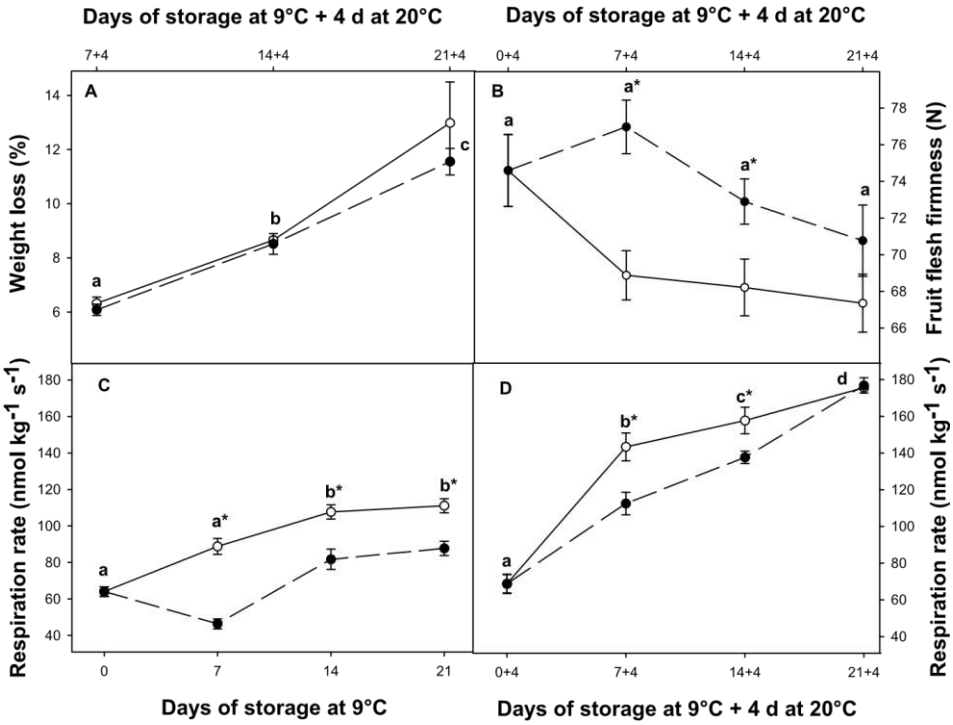

Fig. 1. Evolution of weight losses (%) (A) fruit flesh firmness (N mm^{-1}) (B) and respiration rate (nmol kg^{-1} s^{-1}) (C and D) in pineapples treated with melatonin at 0.1 mM (MT) (–●–) or distilled water (control) (─○─) during cold storage (C) and after cold storage plus 4 days at 22 ◦C (A, B and D). Data are the mean ± SE (n = 3). Different lowercase letters show significant differences among storage days for each parameter, according to Tukey's HSD test. The * symbol shows significant differences between both treatments at P < 0.05, according to Student's t-test.

increasing ATP supply in strawberry (Aghdam & Fard, 2017) and mitochondria electron transport increasing the energy status of broccoli (Zhu, Tremblay & Liang, 2018) reducing the respiration rate. The lower respiration rate in MT treated fruit could also be responsible for the reduced weight loss found in those pineapple samples. It is also possible that MT may stimulate nitric oxide synthesis that inhibits ethylene synthesis (Palma et al., 2019) that leads to a reduction in the respiration rate as it has been described for other non-climacteric fruits (Tian et al., 2000).

3.4. Effect of postharvest melatonin treatment on color and chlorophyll content of the crown

The loss of crown leaf appearance is a major marketing and consumer acceptance concern for pineapple fruit. Reasons for loss of crown appearance are mechanical injury, water loss and color changes due to chlorophyll degradation (Liu, He, Shen, Zhang, & Zhu, 2017; Londers, Ceusters, Godts, De Proft, & Van De Poel, 2011). In a number of commercial marketing systems, the crown is removed before shipping although crown removal can lead to increase internal browning and the level of ROS in pineapple leading to acceleration of senescence process and reduction of fruit quality (Liu et al., 2017). Thus, the maintenance of crown appearance is an important goal, and the increased of CIE *a**, which represents changes from green to yellow, of MT treated crowns was significantly delayed ($P < 0.05$) compared to untreated control fruit during storage (Fig. 2A). MT treated crowns had a lower CIE *a** parameter specially when samples were held at room temperature for 4 days after 14 days of cold storage. Mirza, Senthilkumar, and Singh (2016), described the symptoms of CI in pineapple including wilting, drying and discoloration of crown leaves. In the present experiment, it was observed that the chlorophyll content of MT treated crowns was significantly higher ($P < 0.05$) as compared to control during storage at room temperature (Fig. 2B). A similar effect of maintaining chlorophyll content has been observed in rice and broccoli after MT treatment indicating that MT delays natural leaf senescence (Liang et al., 2015; Wu et al., 2020). Chloroplasts are easy targets of ROS-induced damage during cold storage and natural senescence since ROS detoxification systems decrease postharvest in leaves and other green parts of the plant (Khanna-Chopra, 2012). Zhao et al. (2016) observed changes in photosynthetic electron flux that is capable of suppressing ROS production in MT treated cucumber seedlings leaves. The level of ROS in chloroplasts is not only dependent on ROS generating rate but also on the ROS scavenging ability. In this sense, the stimulation of the antioxidant system in leaves and other plant parts after MT applications has been described (Wu et al., 2021).

3.5. Effect of postharvest melatonin treatment on external and internal color of pineapples

The development of external yellow peel in pineapple is a quality attribute appreciated by international markets and consumers. However, during commercial storage shell chlorophyll content changes very little until the final 10–15 d before full ripeness through the chlorophyll degradation when the chloroplast evolves to chromoplast (Paull, 1993). Coincident with chlorophyll degradation, shell carotenoids pigments accumulate during fruit ripening (Paull, 1993). The degreening of the shell was indicated by increases of CIE *a** and decreases of CIE *hue** that was delayed by MT treatment during cold storage at 9 °C ($P < 0.05$) (Fig. 3A and B). The internal color change in pineapple flesh, associated with carotenoid synthesis and flesh yellowing, was also significantly retarded by MT treatment manifested by a maintenance of CIE *L** and CIE *hue** while sharp decreases occurred in control fruit (Fig. 3C and D). Final CIE *L** values at the end of the postharvest period were 73.56 ± 0.52 and 67.95 ± 0.98 for MT and control fruit, respectively (Fig. 3C). Pineapple respiration was lower in MT-treated fruit so that changes in the physical quality of the shell color would be delayed until the end of storage. In this sense, the pineapple does not reach the maximum of maturity (100% of shell color). This means the fruit is still fresh and with a higher quality level based on a good appearance for longer time in order to sell in the market (Rahmadhanni et al., 2020). Accordingly, MT treatment also delays color evolution in strawberry, litchi and broccoli (Liu et al., 2018; Wu et al., 2020; Zhang et al., 2018). The delay in carotenoids increase following MT application led to a paler and whiter flesh showing a reduction in carotenoid biosynthesis, specially cryptoxanthin which is the major carotenoid in pineapple flesh (Paull, 1993).

3.6. Effect of postharvest melatonin treatment on total soluble solids (TSS), titratable acidity (TA) and ripening index

TSS is frequently used as an indicator of pineapple and other fruits sweetness and as well as a harvest maturity index. The TSS level gradually increases in pineapple during the last six weeks prior to the full ripe stage, further increasing through to senescence, unless the fruit is harvested (Chen & Paull, 2000; Paull, 1993). The accumulation of sucrose is concomitant with a decrease on the activities of acid, neutral and cell-wall invertase, and sucrose synthase (Paull & Chen, 2015). Similarly, TA in low acid hybrids pineapple, initially increases during development then rapidly declines as the fruit approaches the ripening stage, being citric and malic acids the two major organic acids in pineapple (Saradhuldhat & Paull, 2007). In this study, MT treatment did not significant affect TSS content ($P \geq 0.05$) with values at the end of the storage of 15.45 ± 0.21% in control fruit and 15.70 ± 0.22% in MT treated pineapples (Fig. 4A). However, a marked effect was observed between control and MT treated fruit in TA, with pineapples treated with MT maintaining similar initial levels of TA at the end of the

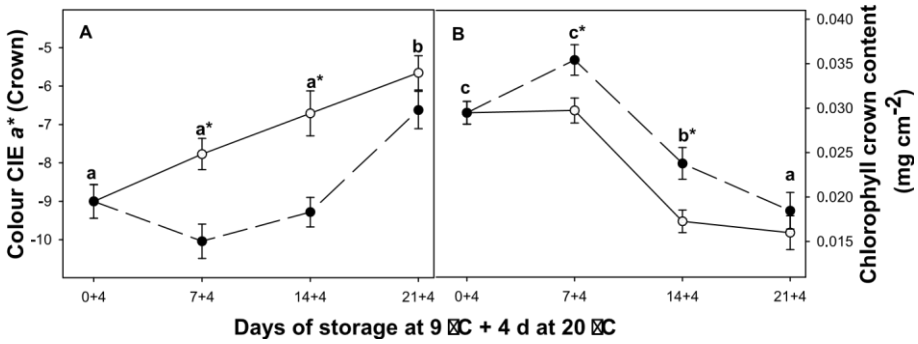

Fig. 2. Evaluation of color *a** of the crown (A) and chlorophyll content in the crown (mg cm^{-2}) (B) after cold storage plus 4 d at 22 °C in pineapples with melatonin at 0.1 mM (MT) (–●–) or distilled water (control) (—○—). Data are the mean ± SE (n = 3). Different lowercase letters show significant differences among storage days for each parameter, according to Tukey's HSD test. The * symbol shows significant differences between both treatments at $P < 0.05$, according to Student's *t*-test.

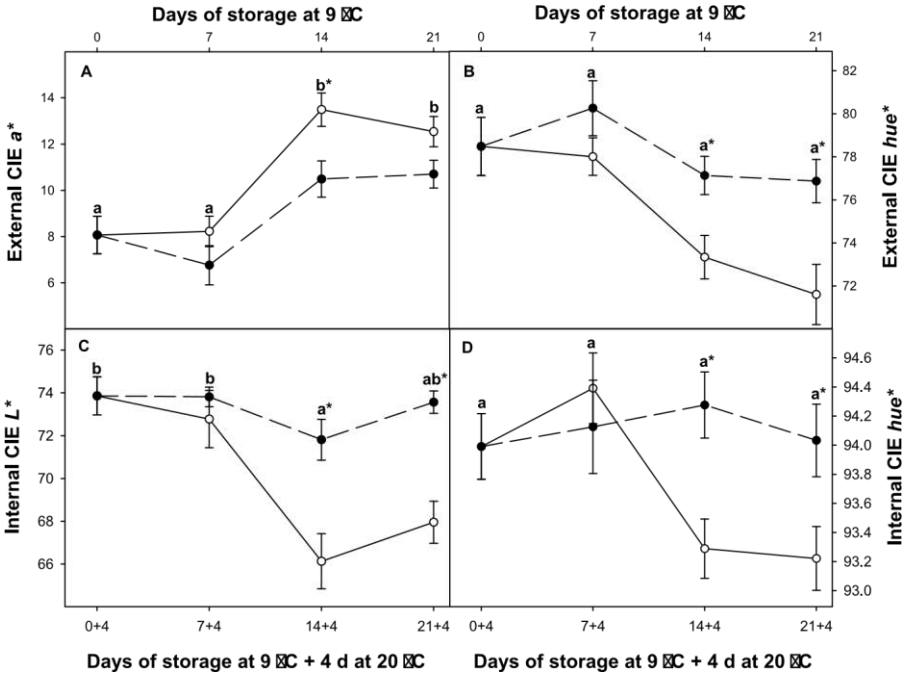

Fig. 3. Evaluation of external color parameters (CIE *a** (A) and CIE *hue** (B)) and internal color parameters (CIE *L** (C) and CIE *hue** (D)) in pineapples with melatonin at 0.1 mM (MT) (–●–) or distilled water (control) (–○–) during cold storage (external color) and after cold storage plus 4 d at 22 °C (internal color). Data are the mean ± SE (n = 3). Different lowercase letters show significant differences among storage days for each parameter, according to Tukey's HSD test. The * symbol shows significant differences between both treatments at $P < 0.05$, according to Student's *t*-test

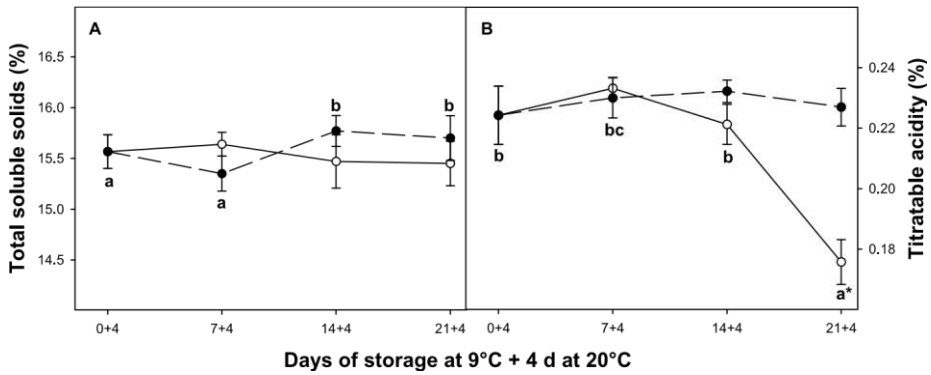

Fig. 4. Evolution of total soluble solids (TSS, %) (A) and titratable acidity (TA, %) (B) in pineapples with melatonin at 0.1 mM (MT) (–●–) or distilled water (control) (–○–) after cold storage plus 4 d at 22 °C. Data are the mean ± SE (n = 3). Different lowercase letters show significant differences among storage days for each parameter, according to Tukey's HSD test. The * symbol shows significant differences between both treatments at $P < 0.05$, according to Student's *t*-test.

experiment. In control fruit, TA significantly decreased ($P < 0.05$) after 14 days of storage at 9 °C plus 4 days at 22 °C reaching final values of 0.17% (Fig. 4B). The delay in the expected decline of TA level following by MT treatment suggested an overall delay in the ripening process, leading to a lower TSS/TA ratio in treated fruit as compared to control ones The increase in TA during fruit growth and subsequently decline during ripening are associated with the activities of citrate synthase and aconitase enzymes which play a major role in pineapple acidity changes (Saradhuldhat & Paull, 2007). The higher TA levels in treated pineapple could impact consumer acceptance since it affects the sugar-acid ratio (Bal, 2019). The low acid hybrids often have very low acid level during the warm season and the application of MT could maintain the TA and hence give a more uniform year-round level of sugars to acids. Similar results regarding maintenance of acidity levels without affecting sugar content have been found as a consequence of MT treatment in other fruits such as strawberries and plums (Bal, 2019; Liu et al., 2018).

3.7. Effect of postharvest melatonin treatment on translucency and internal browning

The evaluation of translucency or water soaking in the pineapple flesh can be used to establish the physiological maturity of this fruit (Bartolome, Ruperez, & Fuster, 1995). This disorder first appears at the basal end of the fruit flesh with a sensory impact that can be compared with water core in other fruits such as apples and pears and is tied to the earliest manifestation of the IB process in pineapple (Paull & Chen, 2015). The melatonin effect on internal disorders can be clearly observed in the photographs obtained (Fig. S1). The level of translucency in pineapple fruit was significantly reduced ($P < 0.05$) by MT treatment (~ 55.54%), since control fruit did show a doubling in translucency index at the end of storage while no significant increases ($P \geq 0.05$) occurred in MT treated fruit (Fig. 5A). MT treatment also reduced the occurrence of CI related IB symptoms that were significantly reduced ($P < 0.05$) in MT treated fruit (Fig. 5B). After 3 weeks of cold storage plus 4 days at 22 °C, MT IB index was half that of the control fruit 2.25 ± 0.33 and 1.12 ± 0.25 for control and

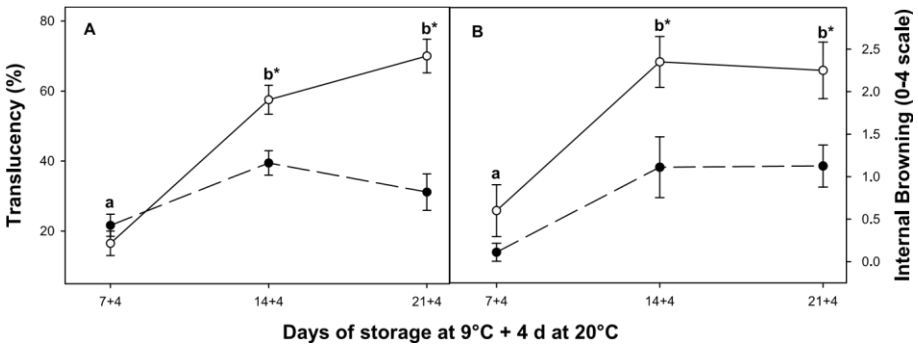

Fig. 5. Evaluation of translucency (%) (A) and internal browning (0–4 scale) (B) in pineapples with melatonin at 0.1 mM (MT) (–●–) or distilled water (control) (–○–) after cold storage plus 4 days at 22 °C. Data are the mean ± SE (n = 3). Different lowercase letters show significant differences among storage days for each parameter, according to Tukey's HSD test. The * symbol shows significant differences between both treatments at $P < 0.05$, according to Student's *t*-test.

melatonin treated pineapples, respectively (Fig. 5B). The increase of membrane permeability in the flesh cells is proposed as the main factor involved in pineapple translucency in this cultivar (Paull & Chen, 2015) while IB appearance in pineapple is mainly due to the impact of the oxidation of soluble phenolic compounds which normally are located inside the vacuole (Paull & Rohrbach, 1985), increasing the PPO activity through the chilling-induced membrane damage. Nukuntomprakit et al. (2020) demonstrated that chilling stress in pineapple leads to mitochondrial dysfunction increasing ROS, and MT has antioxidant activity that could limit the phenol oxidation to quinones in analogous consonance with that observed controlling chlorophyll oxidation in MT-treated pineapple crowns during cold storage. On the other hand, MT-treated fruit showed a higher level of TA, in this sense it is commonly recognized that a higher level in organic acids as ascorbic or citric acid may contribute to a reduced CI impact (Boonyaritthongchai & Supapvanich, 2017; Sangsoy, Beckles, Terdwongworakul, & Luengwilai, 2022). MT has been described as an alleviator of the membrane damages in several fruits and vegetables such as peach, strawberry and sapota fruit, via a reduction of lipid peroxidation and increasing the unSFA/SFA ratio leading to a higher membrane integrity, thus maintaining cell homeostasis (Aghdam & Fard, 2017; Gao et al., 2016; Mirshekari, Madani, Yahia, Golding, & Vand, 2020) providing a higher pulp firmness as we observed in this study. Further research will be necessary to elucidate the different pathways in which melatonin affects senescence and alleviates CI in pineapple fruit.

4. Conclusion

This is the first study in which the effect of MT on increasing cold tolerance in pineapple fruit has been demonstrated. The present study confirmed that a 0.1 mM MT postharvest dip treatment can extend the storage life of pineapple by reducing respiratory metabolism and maintaining fruit firmness but did not affect weight loss. The overall fruit appearance was improved by maintaining crown chlorophylls and reducing shell color evolution. MT dip application also delayed IB symptoms, translucency development and maintained the sugar-acid ratio close to that found in recently harvested fruit. These results suggest that application of MT at low concentration (0.1 mM) may be a promising commercial tool to increase the storability of pineapple, while maintaining the overall fruit quality demanded by consumers during cold storage.

CRediT authorship contribution statement

Fabián Guillén: Conceptualization, Investigation, Methodology, Formal analysis, Data curation, Visualization, Writing – original draft, Writing – review & editing, Validation. Jorge Medina-Santamarina: Investigation, Methodology, Data curation, Formal analysis, Visualization, Software. María E. García-Pastor: Data curation, Formal analysis, Visualization, Software. Nancy J. Chen: Investigation, Methodology, Data curation, Visualization. Gail Uruu: Investigation, Methodology, Data curation, Visualization. Robert E. Paull: Conceptualization, Investigation, Methodology, Data curation, Validation, Writing – review & editing, Resources, Supervision.

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.

Data availability

Research data are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2022.113989.

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4.1. Publicación 4 (Transcripción Literal)

Artículo 4

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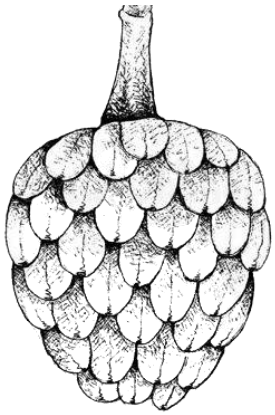

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Article

Melatonin Treatments Reduce Chilling Injury and Delay Ripening, Leading to Maintenance of Quality in Cherimoya Fruit

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Abstract: Spain is the world's leading producer of cherimoya, a climacteric fruit highly appreciated by consumers. However, this fruit species is very sensitive to chilling injury (CI), which limits its storage. In the present experiments, the effects of melatonin applied as dipping treatment on cherimoya fruit CI, postharvest ripening and quality properties were evaluated during storage at 7 °C + 2 days at 20 °C. The results showed that melatonin treatments (0.01, 0.05, 0.1 mM) delayed CI, ion leakage, chlorophyll losses and the increases in total phenolic content and hydrophilic and lipophilic antioxidant activities in cherimoya peel for 2 weeks with respect to controls. In addition, the increases in total soluble solids and titratable acidity in flesh tissue were also delayed in melatonin treated fruit, and there was also reduced firmness loss compared with the control, the highest effects being found for the 0.05 mM dose. This treatment led to maintenance of fruit quality traits and to increases in the storage time up to 21 days, 14 days more than the control fruit. Thus, melatonin treatment, especially at 0.05 mM concentration, could be a useful tool to decrease CI damage in cherimoya fruit, with additional effects on retarding postharvest ripening and senescence processes and on maintaining quality parameters. These effects were attributed to a delay in the climacteric ethylene production, which was delayed for 1, 2 and 3 weeks for 0.01, 0.1 and 0.05 mM doses, respectively. However, the effects of melatonin on gene expression and the activity of the enzymes involved in ethylene production deserves further research.

Keywords: *Annona cherimola*; ion leakage; chlorophyll; firmness; total soluble solids; titratable acidity; ethylene

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1 . Introduction

Cherimoya (*Annona cherimola* Mill), also named chirimoya, is an edible fruit tree belonging to the family Annonaceae, and although its origin is still under discussion, it is considered that it is native from the Mesoamerican region, being already cultivated in 1200 BCE in the Inca empire [1,2]. The cherimoya was introduced from America to Spain between the 16th and 18th centuries, with the primary available record in 1757, and then it was distributed to tropical and subtropical areas of Europe, Africa and Asia. The main worldwide producer of cherimoya fruit is Spain, but also Peru and Chile are important cherimoya producers, and the most widely spread cultivar around the world is 'Fino de Jete'.

Cherimoya fruit is highly appreciated by consumers due to this excellent taste and flavour, and also because of its phytochemical content with health beneficial effects, such as phenolics and vitamins C and E as well as its high concentration in essential minerals, mainly K, Ca, Fe, and Zn [2–5]. However, the quality parameters of cherimoya fruit change quickly after harvest due to its fast climacteric ripening process [6,7], leading to rapid quality losses and a short shelf life, which limits its market potential.

Cold storage is the most used technique to preserve fruit quality, but cherimoya, as other tropical and subtropical fruit species, is susceptible to chilling injury (CI), with symptoms such as abnormal ripening and browning [8,9]. The safe temperatures for prolonged cold storage of cherimoya without affecting organoleptic properties and avoiding development of chilling injury-related symptoms range from 8 to 15 °C depending on the cultivar [7,8,10].

Thus, there have been various attempts to avoid CI during cherimoya storage at low temperature in order to increase its storage period while maintaining its high quality attributes. In this sense, Alique and Oliveira [11] showed that the combination of 3 and 6 KPa CO_2 with 3 KPa O_2 had an additive effect on reducing CI and extended the maintenance of quality properties in 'Fino de Jete' cherimoya during storage at 9 °C for two weeks with respect to fruit stored in air. CI was also reduced in this cherimoya cultivar by a treatment with 20% CO_2 for 3 days before storage at 6 °C [12]. Accordingly, Tinebra et al. [13] have recently reported that modified atmosphere packaging (MAP with 21% O_2 and 1% CO_2) and active-MAP (10% O_2 and 30% CO_2) maintained quality of cherimoya during storage at 10 °C compared with control fruit stored in open air. Heat treatment at 55 °C for 5 h was also reported to alleviate CI symptoms when cherimoya fruit were stored at 4 °C, due to the synthesis of small molecular size heat-shock proteins (sHSPs) involved in preventing and/or repairing stress induced damage [14].

Melatonin is an endogenous indole compound with multiple biological functions in plants [15–17] from seed germination to stress tolerance and fruit growth and ripening. It is synthesised from tryptophan, which is converted into melatonin in a two-step pathway. Then, serotonin is converted into melatonin through two different pathways involving the intermediates N-acetyl serotonin and 5-methoxy-tryptamine [18]. Melatonin has been reported to reduce CI in a wide range of fruit species when applied as a postharvest treatment, with additional effects on delaying ripening and senescence processes, leading to maintenance of fruit quality traits [19–21]. For instance, CI symptoms were delayed by 1 and 2.5 mM melatonin dipping treatment for 15 min in 'Friar' plum [22], and delays were also achieved with 0.1 mM melatonin dipping for 2 h in several mango cultivars [23]. Accordingly, CI symptoms, manifested as flesh browning and translucency, were reduced in pineapple after 21 days of storage at 9 °C followed by 4 days at 22 °C as a consequence of 0.1 mM melatonin dipping treatment for 10 min [24]. Melatonin treatment has been also reported to be effective in increasing zucchini fruit tolerance to CI, although this effect was enhanced when melatonin and 1-methylcyclopropene treatments were combined [25]. In addition, melatonin has been reported to extend the shelf life of fruit when stored at non-chilling temperatures in a wide range of fruit species, including mango, banana and pear, among other [19–21,26–28]

Based on these previous reports, it was hypothesised that postharvest melatonin treatment could reduce CI in cherimoya fruit and delay the postharvest ripening process leading to maintenance of fruit quality traits during storage. Thus, the main goal of the present research was to increase CI tolerance of cherimoya fruit by melatonin treatment, which is a new approach since as far as we know, no previous reports about this issue are available in the literature. The effects of melatonin treatments on fruit quality attributes and ripening process were also analysed.

2. Results

2.1. Experiment 1—Selection of the Most Appropriate Melatonin Treatment

Melatonin treatments, applied at 0.1, 0.3, 0.5 and 1 mM as dipping for 10, 60 and 180 min, reduced weight losses after 14 days of storage at 7 °C + 2 days at 20 °C, although significant ($p < 0.05$) differences were observed among melatonin concentrations and dipping times. The lowest weight losses were observed for 10 min of dipping treatments for all the melatonin concentrations tested, and a 0.1 mM concentration was the most effective dose (Figure S1). Fruit firmness at harvest was 36.45 ± 2.42 N mm^{-1} and decreased sharply during storage, reaching values lower

than 10 N mm^{-1} in control fruit after 14 days of cold storage + 2 days at 20 °C. However, melatonin treatments reduced significantly ($p < 0.05$) the softening process, and the highest firmness levels were found in 0.1 mM melatonin dipping treatment for 10 min (Figure S2). Thus, 0.1 mM melatonin concentration and dipping treatment for 10 min was selected for the next experiment, in which two additional melatonin concentrations were assayed, 0.05 and 0.01 mM.

2.2. Effects of Melatonin Treatment on Cherimoya CI, Quality Parameters and Ripening

2.2.1. Chilling Injury and External Quality Parameters

Chilling injury (CI) symptoms, manifested as peel browning, increased during storage, although melatonin-treated fruit showed significantly ($p < 0.05$) lower CI symptoms than controls from the first week of storage, especially with 0.1 and 0.05 mM doses (Figure 1A). A similar trend was observed for ion leakage (IL), that is to say, it increased with storage with significant ($p < 0.05$) higher values in the control and 0.01 mM melatonin-treated fruit than in the 0.05- and 0.1 mM-treated fruit (Figure 1B).

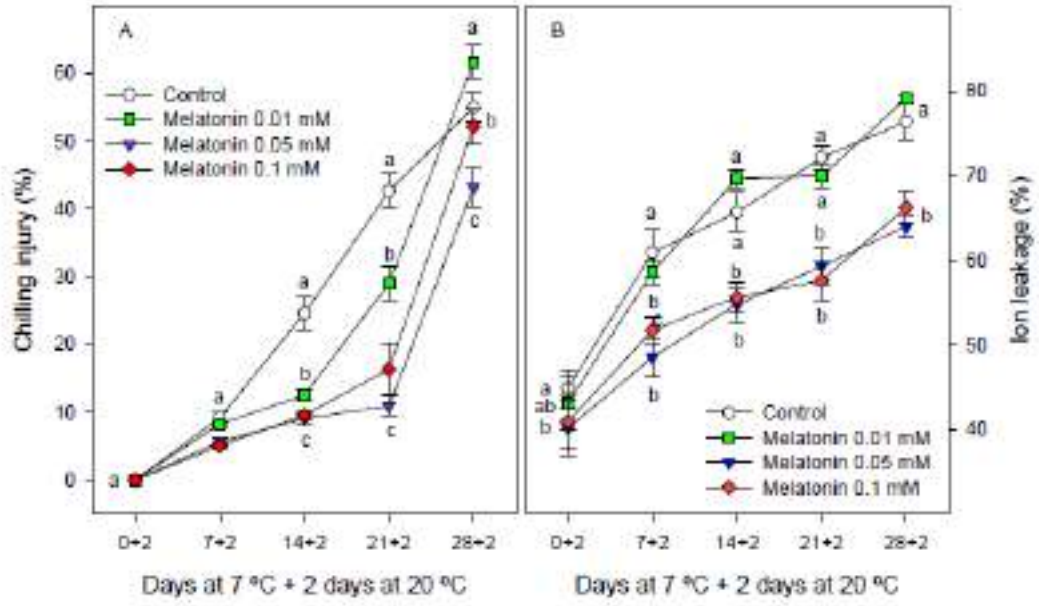

Figure 1. Chilling injury (**A**) and ion leakage (**B**) in cherimoya peel as affected by melatonin (0.01, 0.05 and 0.1 mM) treatments. Data are the mean ± SE of three replicates. Different letters show significant differences ($p < 0.05$) among treatments for each sampling date.

Significant differences in peel colour, measured as CIElab coordinates, were also found between the control and melatonin-treated fruit, the highest being observed for the L^* and b^* parameters, which decreased during storage from the first sampling date in control fruit, whereas these changes were significantly ($p < 0.05$) delayed in melatonin-treated fruit, mainly for the 0.05 and 0.1 mM doses (Figure 2A,B). The chlorophyll content in fruit peel showed values of ca. 7.5 mg 100 g^{-1} at the first sampling date, without significant difference among treatments, and remained stable during the first two weeks of storage in the control and all treated fruit, whereas significant decreases ($p < 0.05$) occurred in the control and 0.01 mM melatonin-treated fruit from the second week of storage. This decreasing trend was observed after three weeks of storage in 0.05 and 0.1 mM melatonin treated fruit, and it is worth noting that chlorophyll concentration at the last sampling date was significantly higher ($p < 0.05$) in these fruit (5.1–5.2 mg 100 g^{-1}) than in the control and 0.1 mM melatonin-treated fruit with ca. 4.3 mg 100 g^{-1} (Figure 3A).

Weight loss increased during storage, although values were significantly lower ($p < 0.05$) in the 0.05 mM melatonin-treated fruit than in the control and the remaining melatonin treated fruit (10.5–10.9%) until the third week of storage. However, at the last sampling date, weight loss reached values of 13–14%, without significant differences among the control and melatonin-treated fruit (data not shown). With respect to fruit firmness, significant decreases ($p < 0.05$) were observed during storage, from values of ≈35 N mm^{-1} at the first sampling date to 7.10 ± 1.51 N mm^{-1} in control fruit after 28 days of cold storage + 2 days at 20 °C. However, firmness levels were significantly higher ($p < 0.05$) in all melatonin-treated fruit than in the control after the first week of storage and during the whole storage period for the 0.05 mM dose (Figure 3B).

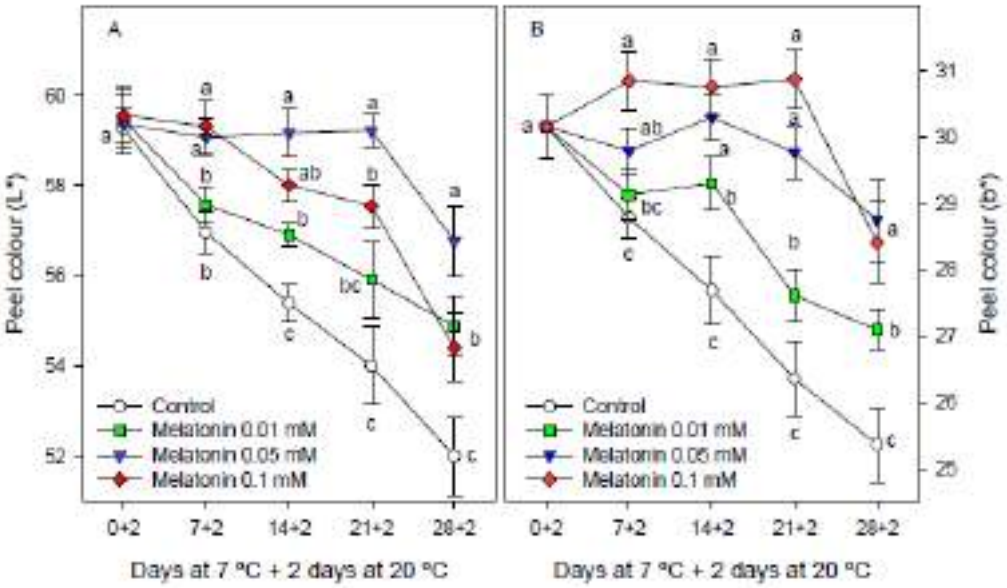

Figure 2. *L** (**A**) and *b** (**B**) colour parameters in cherimoya peel as affected by melatonin (0.01, 0.05 and 0.1 mM) treatments. Data are the mean ± SE of three replicates. Different letters show significant differences ($p < 0.05$) among treatments for each sapling date.

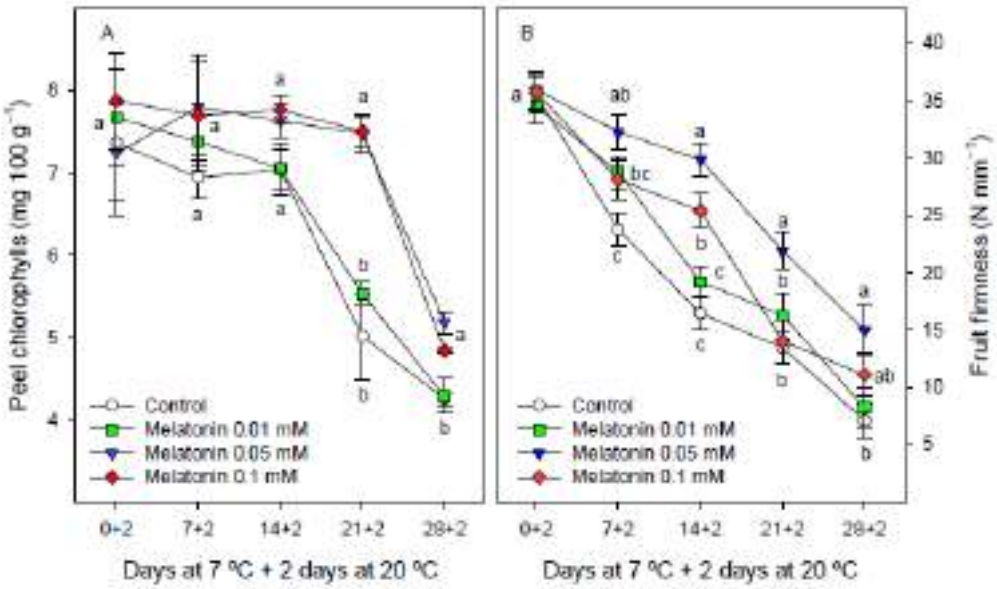

Figure 3. Peel chlorophyll concentration (**A**) and fruit firmness (**B**) as affected by melatonin (0.01, 0.05 and 0.1 mM) treatments. Data are the mean ± SE of three replicates. Different letters show significant differences ($p < 0.05$) among treatments for each sapling date.

2.2.2. Total Phenolic and Antioxidant Activity in Cherimoya Peel

The total phenolic content in the peel of cherimoya fruit ranged from 156 to 176 mg 100 g^{-1} at the first sampling date and increased during storage, reaching final values of 461.26 ± 8.69 and ca. 350 mg 100 g^{-1} in the control and melatonin-treated fruit, respectively (Figure 4A). Similarly, hydrophilic antioxidant activity (H-TAA) increased during ripening, although the values were significantly higher ($p < 0.05$) in the control than in melatonin treated fruit (Figure 4B). Lipophilic antioxidant activity (L-TAA) in the peel of control fruit increased sharply during the first week of storage, and thereafter remained almost stable. However, in the 0.01 and 0.1 mM melatonin-treated fruit, L-TAA in the peel increased during the whole storage period, whereas no changes occurred in the 0.05 mM-treated fruit (Figure 4C).

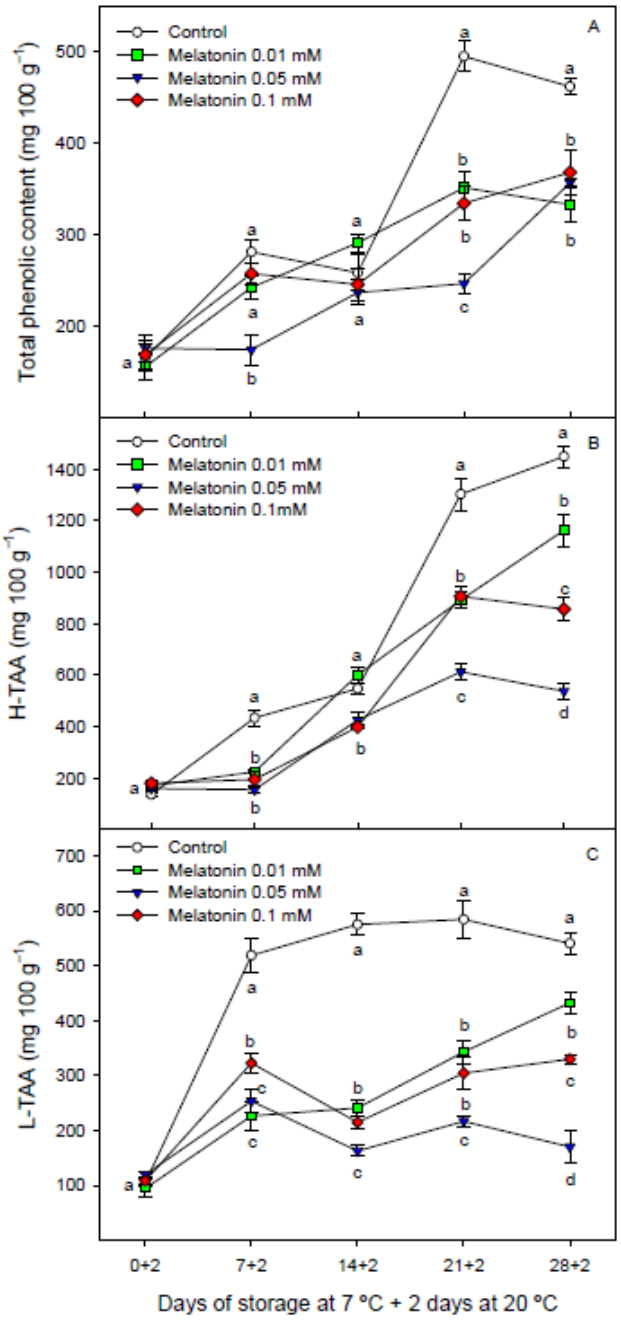

Figure 4. Total phenolic content (**A**), hydrophilic antioxidant activity (H-AA, (**B**)) and lipophilic antioxidant activity (L-AA, (**C**)) in cherimoya peel as affected by melatonin (0.01, 0.05 and 0.1 mM) treatments. Data are the mean ± SE of three replicates.

Different letters show significant differences ($p < 0.05$) among treatments for each sampling date.

2.2.3. Internal Fruit Quality Parameters

The total soluble solids (TSS) concentration in cherimoya pulp tissue at the first sampling date was similar in the control and melatonin-treated fruit, ca. 15.5 g 100 g^{-1}, and a steady increase was observed during storage in the control and treated fruit, with values being, in general, significantly lower ($p < 0.05$) in melatonin-treated fruit than in the control, especially for 0.1 mM concentration (Figure 5A). In a similar way, increases were observed in titratable acidity (TA) from the first sampling date to the third week of storage, and then, no changes or slight decreases occurred. Nevertheless, it is worth mentioning that significantly higher ($p < 0.05$) values of TA were observed for control fruit with respect to melatonin-treated fruit during the whole storage time, these effects being dose-dependent (Figure 5B).

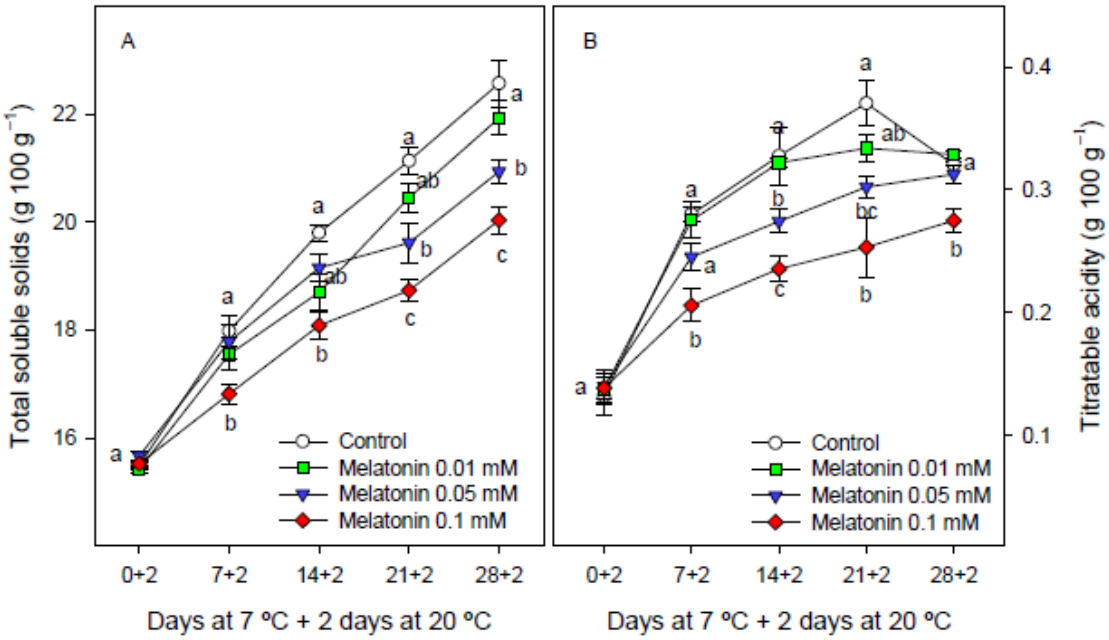

Figure 5. Total soluble solids (**A**) and titratable acidity (**B**) as affected by melatonin (0.01, 0.05 and 0.1 mM) treatments. Data are the mean ± SE of three replicates. Different letters show significant differences ($p < 0.05$) among treatments for each sampling date.

2.2.4. Respiration Rate and Ethylene Production

The respiration rate at harvest was 30.95 ± 3.46 mg CO_2 kg^{-1} h^{-1} and decreased sharply to 16–19 mg CO_2 kg^{-1} h^{-1} when cherimoya fruit were stored at 7 ˚C in both the control and in treated fruit, remaining stable during the whole cold storage period, without significant differences among treatments (Figure 6A). Ethylene production was very low in cherimoya fruit during the first week of storage at 7 ˚C, and increased thereafter in the control and 0.01 mM melatonin-treated fruit, whereas in the 0.1 and 0.05 mM treatments, the respiration rate remained at significantly lower levels ($p < 0.05$) until the end of storage (Figure 6B). However, the respiration rate and ethylene production increased in all cherimoya fruit when they were transferred for 2 days at 20 ˚C after cold storage. Considering the respiration rate, a peak was reached in the control and 0.01 mM melatonin treated fruit after 7 days at 7 ˚C + 2 days at 20 ˚C, with values of ca. 75–80 mg CO_2 kg^{-1} h^{-1}, whereas this peak in respiration was reached after 21 days at 7 ˚C + 2 days at 20 ˚C in the 0.05 and 0.1 mM treatments (Figure 6C). Increases in ethylene production were also found when cherimoya fruit were transferred to 20 ˚C after cold storage. In the control fruit, a peak of 34.17 ± 2.91 nL g^{-1} h^{-1} was reached after 7 days at 7 ˚C + 2 days at 20 ˚C. A decrease then occurred from the second to the third week, and thereafter, an increasing trend was observed until the last sampling date. In melatonin-treated fruit,

the peak in ethylene production was delayed for 1, 2 and 3 weeks at the 0.01, 0.05 and 0.1 mM melatonin concentrations, respectively, although the maxima values were similar and independent of the treatment, ranging from 33 to 38 nL g^{-1} h^{-1} (Figure 6D).

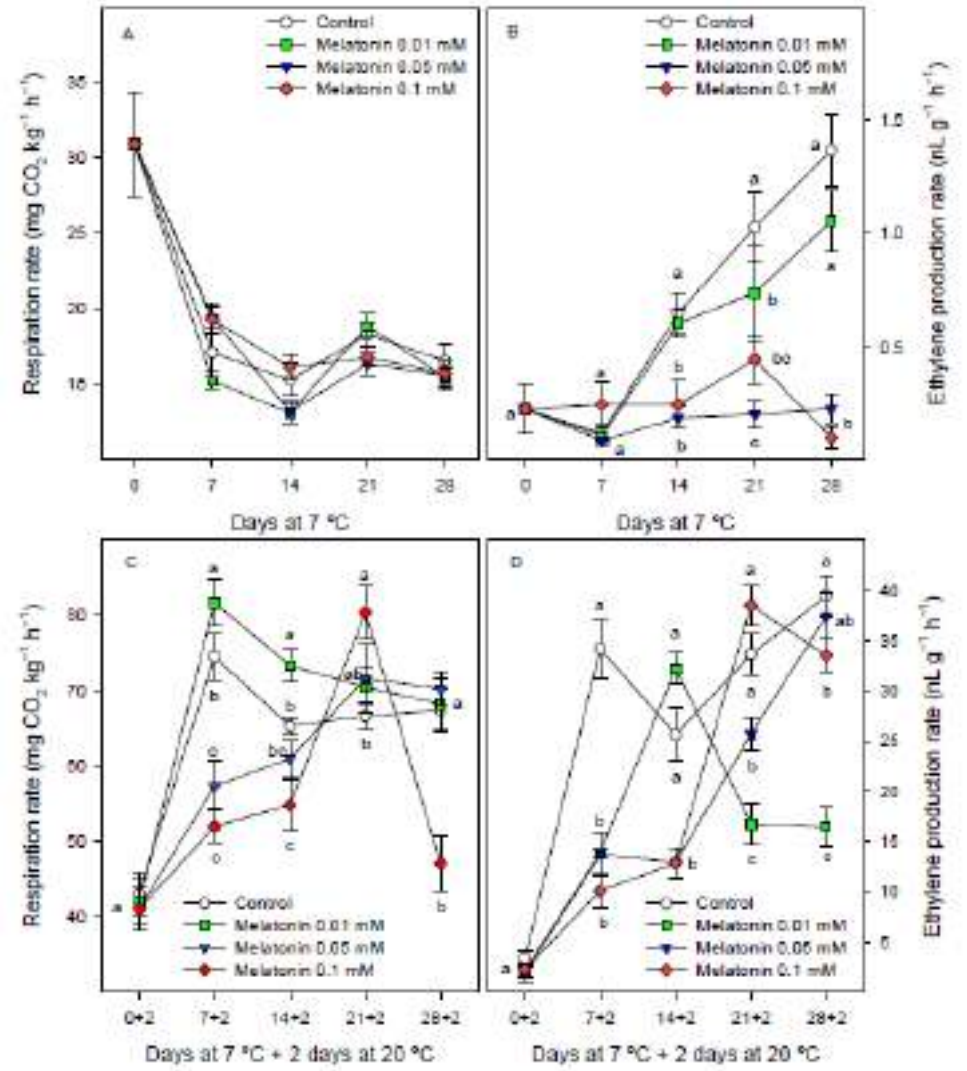

Figure 6. Respiration rate (**A**,**C**) and ethylene production (**B**,**D**) in control and 0.01, 0.05 and 0.1 mM melatonin-treated fruit during storage at 7 °C (**A**,**B**, for respiration and ethylene, respectively) and subsequent shelf life at 20 °C (**C**,**D**, for respiration and ethylene, respectively). Data are the mean ± SE of three replicates. Different letters show significant differences ($p < 0.05$) among treatments for each sampling date.

3. Discussion

Cherimoya fruit are generally harvested when the peel colour changes from dark green to light green or greenish-yellow, with *L** values close to 60 and *b** ca. 30–32 in the 'Fino de Jete' cultivar [29], which are similar to the values at harvest in the present experiments. CI symptoms in cherimoya fruit, manifested as peel browning, increased during storage, although they were significantly reduced by melatonin treatment, especially with 0.05 and 0.1 mM doses (Figure 1A), and the ion leakage in peel tissues was likewise reduced (Figure 1B). In fact, a high correlation was found between ion leakage and CI ($y = 0.48x + 49$; $r^2 = 0.723$) when considering data of all cherimoya fruit and sampling dates during storage. The increase in ion leakage in CI-damaged fruit is due to an increased action of membrane lipid-degrading enzymes, leading to reductions in the concentration of unsaturated fatty acids and the unsaturated:saturated fatty acid ratio, increased cell membrane permeability and disruption of the compartmentalised function of the cell membranes [30–32]. Similar to the present results, melatonin postharvest treatments have been shown to increase

chilling tolerance in several fruit species, such as litchi [31], pepper [32], guava [33], peach [34], longan [35] and banana fruit [36]. This effect has been attributed to the maintenance of cell membrane structure and permeability, due to a higher content of unsaturated fatty acids and enhanced antioxidant enzyme activities, which lead to lowering the accumulation of malondialdehyde (MDA) and reactive oxygen species (ROS) and maintaining the cellular redox state.

The browning process of cherimoya peel was manifested as decreases in *L** and *b** colour parameters, which decreased during the whole storage period in the control and 0.01 mM melatonin-treated fruit but were maintained until the third week of storage in 0.05 and 0.1 mM melatonin-treated fruit (Figure 2A, B). The *L** and *b** colour parameters were negatively correlated with CI incidence: $y = -0.09x + 51$, $r^2 = 0.791$; and $y = -0.06x + 30.31$, $r^2 = 0.661$, respectively. In Figure S3, it can be observed that 0.05 mM melatonin-treated fruit had a visual quality optimum for consumption after 21 days of storage at 7 °C + 2 days at 20 °C, while the control and 0.01 and 0.1 mM melatonin-treated fruit were highly deteriorated. In addition, it is worth noting that after 28 days 7 °C + 2 days at 20 °C, all the fruit, both the control and treated fruit, showed severe browning symptoms and were unacceptable for consumption. These changes in peel colour could not be attributed to reduced chlorophyll concentration, which was maintained at levels similar to those at the first sampling date until the second week of storage in the control and treated fruit (Figure 3A), whereas significant colour changes were found in the control and 0.01 mM treated fruit (Figure 2A,B). Accordingly, decreases in *L** and *b** colour parameters and visual colour changes were observed in the 'Fino de Jete' cultivar during 7 days of storage at 20 °C, without changes in peel chlorophyll concentration [6]. However, at the last sampling dates of cold storage, significant losses in peel chlorophyll concentration were observed, although they were delayed in fruit treated with 0.05 and 0.1 mM concentrations of melatonin, which could be attributed to the effect of these treatments on delaying senescence processes.

Cherimoya peel is rich in phenolic compounds compared with other fruit species, with 46 different phenolic compounds being identified recently, the major ones being quercetin derivatives, rutin and quinic acid [37,38]. However, there is no available literature about changes in peel phenolic content during storage of cherimoya fruit for comparative purposes. The present results showed an increasing trend in peel phenolic content during storage, as well as for H-TAA and L-TAA, which were delayed in melatonin-treated fruit compared with the control (Figure 4A–C). Similarly, melatonin treatment led to lower increases in total phenolic content in banana peel, which was associated with reduced CI symptoms [36], and similar results were observed in the flesh of 'Friar' plum [22]. In contrast, melatonin treatment reduced CI and increased phenolic and flavonoid content in four mango cultivars, due to higher activity of phenylalanine ammonia lyase and tyrosine ammonia lyase [23], as well as in longan pericarp [35] and in guava fruit [39], leading to enhanced antioxidant activity. Thus, the effects of melatonin treatment of phenolic content could be dependent on fruit tissue and fruit species.

Early reports used penetration tests to evaluate cherimoya softening [6,40]. However, the flesh of cherimoya fruit is composed of soft segments arranged around its longitudinal axis and contains many hard seeds; thus, the results of the penetration test may be highly biased by the presence of seeds and the segment orientation [40]. Thus, the compression test, as performed in the present experiments, is more suitable to measure cherimoya fruit firmness and is similar to the way that consumers subjectively estimate cherimoya fruit softness by applying a compression force to the fruit surface with the fingers. Cherimoya fruit soften quickly after harvest, the decreases in firmness being quicker at higher temperature and is attributed to cell wall hydrolytic enzymes, such as pectin methylesterase (PME), polygalacturonase (PG), xyloglucon endotransglycosylases and expansins [7,41–43]. This softening process was delayed by melatonin treatments, the largest delay of two weeks being found for the 0.05 mM concentration (Figure 3B). These effects could be due to a low

activity of the cell wall hydrolytic enzymes and/or a delay in the expression of their coding genes, as reported for PG, PME and β-galactosidase activities in mango fruit as a consequence of melatonin treatment [26].

Cherimoya fruit is described as a climacteric fruit but has two particular peaks of respiration during ripening, the first one occurring 2–3 days after harvest and the second one after 7–10 days when stored at ambient temperature and with a peak in ethylene production after the first peak of respiration [6,8,10,11]. In addition, the time of the ethylene climacteric peak was independent of the harvest date [44] and reached different maxima values, up to 50–300 nL g^{-1} h^{-1}, depending on the cultivar and storage temperature [7,41,42,45,46]. These previous papers also reported that changes related to organoleptic quality properties, such as softening and accumulation of sugars, organic acids and aroma compounds, started concomitantly with the increase in ethylene production, and the optimum quality for eating was reached at the ethylene peak. In the present experiment, melatonin treatments delayed the respiration rate and ethylene production peaks in a concentration-dependent manner, and also delayed the reduction in fruit firmness and the increases in TSS and TA. Similarly, 0.05 mM melatonin dipping treatments for 1 h of 'Guifei' mango delayed ethylene production and decreased 1-aminocyclopropane-1-carboxylic (ACC) content and the activities of ACC-synthase (ACS) and ACC-oxidase (ACO) [26]. Similar results were obtained in banana [27] and pear [28] fruit after melatonin dipping treatments at 0.05 mM for 2 h and 0.1 mM for 12 h, respectively, which were attributed to reduced expression of the genes coding for ACS and ACO enzymes. In contrast, Sun et al. [47] reported that melatonin dipping treatment at 0.05 mM for 2 h down-regulated the expression of genes coding for ACS and ACO in tomato fruit. Thus, it is clear that the effect of melatonin treatment on ethylene production and its physiological related process depends on the fruit species, applied dose and time of dipping treatment.

The increase in TSS during cherimoya ripening has been attributed to enhanced fructose and glucose concentrations, due to starch hydrolysis, an ethylene dependent process evolving more quickly with higher storage temperature [6,8,48]. With respect to TA, which usually decreases during postharvest fruit ripening [49], increases were found in cherimoya flesh during storage, according to previous reports, in 'Fino de Jete' and other cherimoya cultivars harvested at different dates and stored at different temperatures, due to enhanced concentrations of malic and citric acids [6,12,29]. However, the increase in TA was delayed in 0.05 and 0.1 mM melatonin-treated fruit with respect to controls. Taking together the results for ripening quality parameters, it could be inferred that melatonin delayed the cherimoya postharvest ripening process during storage, due to delayed ethylene production, as previously reported by other treatments, such as shock CO_2 treatments for three days [12], 500 nL L^{-1} 1-methylcyclopropene (1-MCP) for 16 h [41] or an edible coating based on carnauba wax [50]. Furthermore, melatonin has been reported to delay senescence and ripening processes, leading to maintenance of fruit quality properties in guava [33], rambutan [51], strawberries [52] and peaches [34], among other fruit species. These effects have been attributed to a more efficient system for scavenging reactive oxygen species and reduced membrane lipid peroxidation. In addition, melatonin effects on delaying and/or reducing ethylene biosynthesis have been reported in climacteric fruit, such as mango [26], banana [27] and pear [28], which led to fruit quality maintenance.

Finally, it is worth noting that melatonin is commonly used for insomnia and improving sleep in different conditions, such as jet lag, depression, chronic pain or dementia, among others, in doses up to 8 mg a day for up to 6 months in adults. Melatonin is a dietary supplement approved for the U.S. Food and Drug Administration (US FDA), and there are no dosage restrictions for melatonin since it does not have side effects nor generate dependence [53]. In the present experiment, the best results were obtained with a 0.05 mM dose, which corresponds to 11.6 mg/L. Thus, even 1 L of melatonin solution would be safe for human consumption.

4. Materials and Methods

4.1. Plant Material and Melatonin Treatments

Experiment 1: Optimisation of melatonin concentration and time of dipping. Cherimoya (*Annona cherimola* Mill.) fruit of the "Fino de Jete" cultivar were manually harvested in a commercial plot located at Motril (Granada, Spain, Latitude: 36°45^{0}02^{00} N Longitude: 3°31^{0}04^{00} W). The fruit were harvested early in the morning from 12-year-old trees at the commercial ripening stage (16 November 2021) and immediately transferred to the laboratory in a in a refrigerated truck in 6 h. Fruit with uniform size and colour were randomly divided into 3 replicates of 75 fruit. Then, 15 lots of 5 fruit were allocated to each replicate for the following melatonin treatments: 0 (control), 0.1, 0.3, 0.5 and 1 mM by dipping for 10, 60 and 180 min. The experiment was replicated three times. Melatonin (Sigma-Aldrich, Darmstadt, Germany, purity > 98% M5250) solutions were freshly prepared in distilled water containing 0.05% Tween 20 before treatments. Then, fruit were left to dry at ambient temperature for 2 h and stored at 7 °C for 14 days plus 2 days at 20 °C. Thereafter, analytical determinations were carried out in each fruit (three replicates of five fruit).

Experiment 2: The best results in terms of quality maintenance of cherimoya fruit in the first experiment were obtained with 0.1 mM melatonin concentration and dipping for 10 min. Thus, for the second experiment, the treatment with 0.1 mM melatonin for 10 min was chosen and the other two lower concentrations, 0.05 and 0.01 mM applied for 10 min, were also assayed by using cherimoya fruit harvested on 11 January 2022 (as indicated in Experiment 1). The preparation of melatonin dipping solutions and the application of treatments were performed as previously indicated by using 3 replicates of 25 fruit per replicate for the control and each melatonin concentration (0.01, 0.05 and 0.1 mM). Then, cherimoya fruit were allowed to dry at room temperature, and thereafter, the fruit were stored at 7 °C to induce CI [10] for 0, 7, 14, 21 and 28 days. After each cold storage time, one lot of 5 fruit from each treatment and each one of the three replicates was selected at random and stored at 20 °C for 2 days before the following analytical determinations were performed.

4.2. Respiration Rate, Ethylene Production and Quality Parameters

The weight loss of individual cherimoya fruit was calculated by weighing the fruit before storage (initial weight) and after each sampling date (final weight) and was expressed as a percentage loss. The respiration rate and ethylene production were quantified in each individual fruit in duplicate by placing each fruit in a hermetic 1 L glass container for 1 h. After that, four samples of 1 mL of headspace atmosphere were taken by using airtight syringes. Two of these were injected into a gas chromatograph GC Shimadzu 14B (Shimadzu Europa GmbH, Duisburg, Germany) to measure CO_2 concentration, and the other two were injected into a Shimadzu GC-2010 gas chromatograph (Shimadzu Europa GmbH, Duisburg, Germany) to measure ethylene concentration. The chromatographic conditions were previously described by Martínez-Romero et al. [54]. The respiration rate and ethylene production were expressed as mg of CO_2 kg^{-1} h^{-1} and nL g^{-1} h^{-1}, respectively. The colour parameters (L^*, a^* and b^*) were individually measured in each fruit by using a Minolta colorimeter (CRC400, Minolta Camera Co.; Kanto, Tokio, Japan). Three readings along the fruit's equatorial perimeter were made in each fruit. Fruit firmness was evaluated for each fruit by using a TA.XT-plus Texture Analyzer (Stable Mycrosystems, Godalming, UK) equipped with a force that achieved a 5% deformation at the equator area on the fruit using a flat steel plate probe. The results were expressed as the force-deformation (N mm^{-1}). External CI was assessed visually in each fruit and rated as the percentage of the superficial area showing browning. For all these parameters, the results are the mean ± SE of determinations performed individually in 5 fruit for each of the 3 replicates.

Electrolyte leakage (EL) was determined according to Mao et al. [55] with some modifications. From each treatment and replicate of five fruit, 15 peel discs (3 from each fruit) with 0.5 mm diameter were taken and rinsed 3 times for 3 min each

with 50 mL of deionized water at room temperature with constant shaking. Then, discs were incubated in deionized water for 30 min and the electrical conductivity (EC) was measured (C1).

Finally, the samples were boiled at 100 °C for 15 min and the electrical conductivity (C2) was measured again. EL was calculated using the following formula: EL = (C1/C2) 100, and the data, expressed as percentage, are the mean ± SE of 3 replicates.

For the chlorophyll measurements, one disc of 6.25 mm diameter of the peel tissue of each of the 5 fruit of each replicate were taken, weighed and placed immediately into 8 mL of 100% methanol. The chlorophyll extraction was left to occur in the dark at 30 °C for 24 h. The absorbance of the extracts was measured using a spectrophotometer (1900 UV/Vis, Shimadzu, Kyoto, Japan) at 652 and 665 nm [56], and the total chlorophyll concentration was expressed as mg 100 g^{-1} (mean ± SE).

A 10-g flesh sample of each of the 5 fruit of each replicate was taken and cut into small pieces to obtain a homogenous sample for each replicate. About 20 g of this sample was homogenised with distilled water (50:50 *w/v*) and centrifuged at 5000× *g* for 10 min. The TSS concentration was determined in duplicate in the supernatant with an Atago PR-101 digital refractometer and expressed as g 100 g^{-1} (mean ± SE). TA was determined, also in duplicate in each sample, by automatic titration (785 DMP Titrino, Metrohm, Herisau, Switzerland) and expressed as g of malic acid equivalent per 100 g^{-1} fresh weight (mean ± SE).

4.3. Total Phenolic Quantification and Total Antioxidant Activity

A longitudinal strip of peel tissue of the five fruit of each replicate was taken and ground with liquid N_2 to obtain a homogeneous sample for each replicate, which was stored at −20 °C until further analysis of total phenolics and antioxidant activity. The total phenolics were measured by homogenising 2 g of frozen peel samples with 15 mL of water: methanol (2:8, *v/v*) containing 2.0 mM NaF. After centrifugation of the extracts at 10,000× *g* at 4 °C for 20 min, the total phenolic content (TPC) was measured in duplicate in each extract sample by using the Folin–Ciocalteau reagent, as previously reported [57], and the results (mean ± SE) were expressed as mg of gallic acid equivalent per 100 g^{-1} fresh weight. The total antioxidant activity was measured by using the ABTS test as described by Serna-Escolano et al. [57] with small modifications. In total, 10 mL of 50 mM phosphate buffer solution and 6 mL of ethyl acetate were added to 2 g of cherimoya peel sample, and the mixture was homogenized, as indicated for the phenolic extraction, and then centrifuged at 10,000× *g* for 20 min at 4 °C. The hydrophilic and lipophilic phases were separated, and the hydrophilic and lipophilic antioxidant activities (H-TAA and L-TAA) were measured in duplicate in each extract. The H-TAA determination was carried out with 890 µL of 50 mM glycine buffer solution mixed with 30 µL of 10 mM $2,2^0$-azinobis(3ethylbenzothiazoline-6-sulfonic acid (ABTS) solution, 30 µL of 1 mM H_2O_2 and 25 µL of 10 µM peroxidase. The absorbance of this mixture was measured at 730 nm. Then, 25 µL of water-soluble phase extract was added to the preceding mixture, and the absorbance was measured again at 730 nm after 1 min. The results (mean ± SE) were expressed as mg of (±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) equivalent per 100 g^{-1} of cherimoya peel with reference to the Trolox calibration curve. On the other hand, L-TAA determination was assayed with 30 µL of 10 mM ABTS solution mixed with 30 µL of 1 mM H_2O_2, 25 µL of 10 µm peroxidase and 850 µL of ethanol. The L-TAA was measured as above and the results (mean ± SE) were expressed in mg Trolox equivalent per 100 g^{-1} of peel weight with reference to the Trolox calibration curve.

4.4. Statistical Analysis

All data in this paper are expressed as mean ± standard error (SE) (n = 3). The data were subjected to analysis of variance (ANOVA). Mean comparisons were carried out using a multiple range test (Tukey's HSD test) to find significant

differences ($p < 0.05$). All the analyses were performed using the SPSS software package, version 22 (IBM Corp.; Armonk, NY, USA).

5. Conclusions

Our results show that melatonin treatments before storage at chilling temperatures could be a useful tool to decrease CI damage in cherimoya fruit, since ion leakage, browning and chlorophyll losses in skin tissues of treated fruit were delayed compared with the control. Moreover, additional effects of melatonin treatments on retarding postharvest ripening and senescence processes and on maintaining quality parameters were observed. According to the results of CI damage and quality parameters, the storage of cherimoya control fruit with optimal quality traits for consumption was 7 days at 7 °C + 2 days at 20 °C. Storage could be extended up to 14 days at 7 °C + 2 days at 20 °C for the 0.01 and 0.1 mM melatonin-treated fruit and up to 21 days at 7 °C + 2 days at 20 °C for the 0.05 mM dose. These effects were attributed to a delay in the climacteric ethylene production in treated fruit with respect to control. However, further research is needed in order to clarify the effect of melatonin on the activity and gene expression levels of the enzymes involved in ethylene biosynthesis.

Supplementary Materials: The following supporting information can be downloaded at: https: //www.mdpi.com/article/10.3390/ijms24043787/s1.

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4.2. Publicación 5 (Transcripción Literal)

Artículo 5

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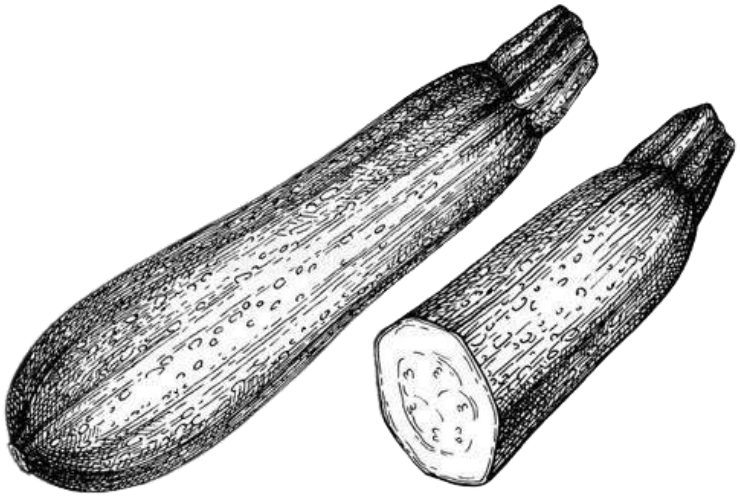

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A Synergistic Effect Based on the Combination of Melatonin with 1-Methylcyclopropene as a New Strategy to Increase Chilling Tolerance and General Quality in Zucchini Fruit

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Abstract: Zucchini fruit are highly sensitive to low temperatures leading to significant peel depressions, increasing weight loss and making them impossible to be commercialized. In this study the effect on the reduction of chilling injury (CI) assaying different postharvest treatments to cv. Cronos was evaluated. We have compared the application of substances such as 1-methylcyclopropene (1-MCP) with the application of a natural origin compound as melatonin (MT), both with demonstrated activity against CI in different vegetal products. The effects of MT (1 mM) by dipping treatment of 1 h and 1-MCP (2400 ppb) have been evaluated on zucchini fruit during 15 days of storage at 4 °C plus 2 days at 20 °C. Treatments applied independently improved some fruit quality parameters in comparison with control fruit but were not able to manage CI even though they mitigated the impact on several parameters. However, when these two separated strategies were combined, zucchini cold tolerance increased with a synergic trend. This synergic effect affected in general all parameters but specially CI, being also the only lot in which zucchini fruit were most effectively preserved. This is the first evidence in which a clear positive effect on zucchini chilling tolerance has been obtained combining these two different strategies. In this sense, the combined effect of 1-MCP and MT could be a suitable tool to reach high quality standards and increasing shelf life under suboptimal temperatures.

Keywords: chilling injury; storage; melatonin; quality; 1-MCP

1. Introduction

Zucchini (*Cucurbita pepo* spp. *pepo*), is considered a non-climacteric fruit as many others unripen vegetal fruit. The commercial harvest stage is coincident with a high fruit metabolism which deteriorates rapidly quality. On the other hand, this fruit is very sensitive to cold storage being a major problem their marketability to European countries and worldwide at suboptimal temperatures. Common postharvest strategies have been demonstrated to be effective reducing chilling injury (CI) in zucchini, as physical treatments managing temperatures [1–3] or relative humidity during storage [4,5] and controlling atmospheres [6,7] also through a minimal packaging [8].

Recently, chemical treatments as nitric oxide [9] or widely used treatments as 1-methylciclopropene (1-MCP) [8,10] have demonstrated a protective role delaying zucchini chilling injury. 1-MCP effectively blocks ethylene action delaying zucchini senescence and different associated physiological changes as softening and CI [10]. Strategies based into plant hormones or natural elicitors as γ-aminobutyric acid (GABA) [11], glycine betaine [12], polyamines [13–15] and methyl jasmonate [16] have also reduced CI impact in zucchini fruit.

In this sense, melatonin has shown a positive effect delaying CI when applied as a postharvest treatment in different non-climacteric species as strawberry and pomegranate [17,18]. With respect to climacteric fruit, MT increased CI tolerance in peach [19] and tomato fruit [20] Also showing effectiveness against CI after preharvest treatments on apricot trees [21]. In these research studies the CI tolerance was associated with a major energy status obtained through the GABA-shunt pathway activation which provides extra ATP. Plant tissues demand ATP especially under metabolic stress conditions. In this sense MT, methyl jasmonate, polyamines or GABA, have been proposed as responsible of GABA shunt pathway stimulation, providing extra ATP, and decreasing ROS accumulation [17,22]. For this reason, MT treated fruit could lead to maintenance membrane permeability and balanced antioxidant system under cold stress, increasing unsaturated/saturated fatty acids ratio in both non-climacteric, and climacteric fruit [17,23].

Several of the above-mentioned strategies have been shown an additional benefit when applied in combination with MCP describing a synergistic effect delaying senescence in comparison with these technologies when applied alone. In this sense, recently, a combination of hot air treatments with 1-MCP lead to delayed softening and quality maintenance of nectarines [24]. With respect to postharvest chemical treatments, 1-MCP in combination with chlorine dioxide postharvest treatment showed a synergistic inhibitory effect on chlorophyll degradation of green pepper fruit [25]. On the other hand, dipping in calcium chloride and then applying 1-MCP was a successful strategy to improve fresh cut strawberries and watermelon quality during storage [26,27]. Other natural origin compounds as carvacrol [28] or elicitors as methyl salicylate [29] and melatonin [30] have increased shelf-life delaying ripening when these treatments were applied in combination with 1-MCP in red pitaya, tomato fruit, and apricot respectively. However, none of these studies have evaluated the chilling tolerance provided by a combined effect of the previous mentioned strategies. For this reason, the aim of this research has been to evaluate for the first time the effect of different strategies as melatonin and 1-MCP combined or alone over zucchini CI tolerance and quality during storage at suboptimal temperatures.

2. Materials and Methods

2.1. Plant Material and Postharvest Treatments

Zucchini fruit (*Cucurbita pepo* spp. *pepo*) commercial hybrid ‘Cronos’, were harvested from a commercial greenhouse located in Orihuela (Spain) and immediately transferred to the laboratory. Fruit of uniform size (20–22 cm) were randomly divided into 3 lots of 5 homogeneous fruit for each treatment and sampling date. Control fruits were submerged in distilled water containing 0.5% Tween 20 while MT treated fruit were dipped in a 1 mM MT solution (Sigma-Aldrich, Germany >98% M5250) for 60 min. In preliminary experiments, 9 zucchini fruit were selected per MT dose and different MT concentrations (0.1, 0.5 and 1 mM) and immersion times (from 10, 30, 60, 120 and 180 min) were evaluated. These treatments were assayed alone and in combination with 2400 ppb of 1-MCP for 48 h at 12 °C following Megías et al. [10] conditions for Cronos cultivar. 1 mM MT for 1 h and combined with 1-MCP showed the best effect on reducing CI symptoms. Thus, 3 replicates of 5 zucchini fruit for each treatment and sample time were selected to repeat the experiment at the optimal conditions observed. freshly prepared MT solutions (0 and 1 mM) with 0.5% Tween 20 were used to dip the different lots for 1 h at 20 °C. Then zucchini fruit were allowed to surface dry and then all the fruit were placed in 4 different 130 L hermetic containers. One lot with MT-treated and another lot with no immersed fruit were exposed to 2400 ppbL^{-1} of 1-MCP for 48 h at 12 °C. The other two lots previously immersed in MT solutions with 0 (Control) or 1 mM MT were treated with air and stored in the same conditions. After this period fruit were taken out from containers and placed under cold storage at 4 °C to induce CI following Megías et al. [10] conditions for 0, 3, 6, 9, 12 and 15 days + 2 days at 20 °C.

2.2. Postharvest Quality Parameters

Three replicates of 5 fruit were randomly selected from each treatment lot at 3 days interval during cold storage +2 days at 20 °C. Weight loss of individual zucchini fruit was calculated as percentage with respect to the weight on day 0. Firmness was determined individually as the force to achieve a 5% fruit diameter deformation in both sides and fruit firmness was expressed as N by using a Texture Analyzer (TX-XT2i, S Microsystems, Godalming, UK). Chilling injury was evaluated visually with a panel of 5 trained judges scoring superficial area affected by pitting damage and the pitting severity. Ratings were based on a 6-point hedonic scale, where the fruit surface affected was used to classify each fruit similarly to Megías et al. [31] with the following scale: 0 = no pitting, 1 = ≤5% pitting, 2 = 6–15% pitting, 3 = 16–25% pitting, 4 = 26–50% pitting, and 5 = ≥50% pitting. On the other hand, to assess the severity of pitting symptoms, the scale was 0 = no damage, 1 = very superficial damage, 2 = superficial damage, 3 = moderate damage, 4 = severe damage, 5 = very severe damage. The final CI index displayed in this manuscript was the average of both assessments.

CO_2 and ethylene production were determined by placing individually 6 randomly selected zucchini from each treatment in a 2.2 L plastic jar hermetically sealed with a rubber stopper for 30 min. After that, 1 mL gas sample per duplicate was taken from head space and carbon dioxide was quantified by using a Shimadzu TM 14A gas chromatograph (Kyoto, Japan) equipped with thermal conductivity detector and ethylene production was evaluated with a Hewlett-Packard™ 5890A gas chromatograph. Chromatographic conditions were previously described [32]. Ethylene production and respiration rate were expressed as nL g^{-1} h^{-1} and mg of CO_2 kg^{-1} h^{-1}, respectively.

Malondialdehyde (MDA) content was assayed in the peel tissue of the zucchini samples following the method of Zhang et al. [33] with modifications. The tissue sample (1.0 g) was homogenized in 10 mL 10% trichloroacetic acid solution, then centrifuged at 10,000× *g* for 10 min. 2 mL of supernatant was added to a testing tube with 6 mL of 0.6% thiobarbituric acid per duplicate and mixed vigorously. Testing tubes were held at 95 °C for 20 min. Samples were cooled rapidly, tempered at room temperature, and evaluated in a spectrophotometer (1900 UV/Vis, Shimadzu, Kyoto, Japan) where absorbance was measured at 450, 532 and 600 nm. MDA content was calculated as described by Zhang et al. [33] and expressed as µmol kg^{-1}. Each assessment was repeated three times.

Electrolyte leakage (EL) was determined following Mao et al. [34] with some modifications. From each treatment, three replicates were measured, each consisting of 20 peel discs with 0.5 mm diameter obtained with a cork borer, from longitudinal 2 mm peel exocarp slices taken from opposite sides of each zucchini. After 3 rinses of 3 min each, discs were incubated in 50 mL of deionized water at room temperature with constant shaking for 30 min. Then electrical conductivity (EC) was measured (C1). Finally, samples were boiled at 100 °C for 15 min and measured to calculate total conductivity (C2). EL was expressed as percentage using the following formula: EL = (C1/C2) 100.

For chlorophyll measurement in peel tissue, six disks, each of 6.25 mm in diameter, were punched from same peel layers sliced for EL. Disks were weighed and placed immediately into 8 mL of 100% methanol. Pigments were allowed to be extracted in the dark at 30 °C for 24 h. Extract absorbance was measured using spectrophotometer (1900 UV/Vis, Shimadzu, Kyoto, Japan) at 652 and 665 nm [35]. Two extractions were evaluated by replicate. Colour parameters (CIE a^* and CIE b^*) were individually measured on three points of the external (both sides) and internal longitudinal fruit perimeter by using a Minolta colorimeter (CRC200, Minolta Camera Co.; Kanto, Tokio, Japan) and colour was expressed¯ as CIE *hue** ($180 + \tan^{-1} b^*/a^*$, if $a^* < 0$) according the CIELab coordinates.

Total soluble solids (TSS) were determined by duplicate in the juice obtained from the pulp of mix of 5 zucchini of each replicate per lot taken with a digital refractometer Atago PR-101 (Atago Co. Ltd.; Tokyo, Japan) at 20 °C, and expressed as percentage (g 100 g^{-1}). Also, for

each replicate total acidity (TA) was determined by duplicate in the same juice by automatic titration with NaOH 0.1 N up to pH 8.1, using 1 mL of diluted juice in 25 mL distilled H_2O, and results were expressed as the percentage of malic acid (meq. malic acid = 0.067).

2.3. Statistical Analysis

All data in this paper are expressed as mean ± standard error (SE). Data were subjected to analysis of variance (ANOVA). Mean comparisons were carried out using a multiple range test (Tukey's HSD test) to find significant differences ($p < 0.05$). Different lowercase letters indicated a significant difference among treatments at the same sampling date. All analyses were performed using SPSS software package, version 22 (IBM Corp.; Armonk, NY, USA).

3. Results and Discussion

CI was evaluated in a previous experiment, carrying out a screen test with four different MT concentrations (0, 0.1, 0.5 and 1 mM) during 5 different immersion times (10, 30, 60, 120 and 180 min), to select the optimal MT treatment conditions (data not shown). Nine zucchini fruit were selected per MT treatment and individually evaluated after 7 d at 4 °C + 1 d at 20 °C. Visually, CI incidence was evaluated with 5 trained judges, and external quality was maintained specially for 1 mM MT dose assayed during 1 h immersion time. On the other hand, no additional benefits were observed by increasing the immersion time. Thus, immersions during 1 h with 1 mM MT were the conditions applied with or without 1-MCP in the present study.

3.1. Effect of Exogenous MT and 1-MCP on Weight Loss, Fruit Firmness and Cold Tolerance

Weight loss of zucchini fruit increased throughout cold storage regardless of the treatment applied. Zucchini weight losses were not significantly ($p \geq 0.05$) affected by MT dips and 1-MCP when applied alone. However, weight losses were significantly lower ($p < 0.05$) when combined treatments (1-MCP + MT) were evaluated during storage (Figure 1A).

In this sense the combined treatment (MT + 1-MCP) reduced weight loss (20.45%) after 6 days of cold storage plus an additional period at 20 °C as compared with the rest of the different lots evaluated. This trend was maintained until the end of the experiment.

Storage of zucchini at 4 °C plus 2 additional days at 20 °C resulted in a decrease in fruit firmness as expected (Figure 1B). However, fruit firmness levels remained higher in MT, 1-MCP and MT + 1-MCP treated fruit as compared to control fruit specially after 6 and 9 days of cold storage. On the other hand, MT and 1-MCP samples did not show significant differences ($p \geq 0.05$) as compared to control fruit at the end of the experiment showing a similar fruit firmness level. On the contrary MT + 1-MCP samples significantly ($p < 0.05$) maintained in general a higher fruit firmness along cold storage compared with the rest of the fruit evaluated.

Zucchini fruit are very sensitive to cold storage displaying CI after 3 days of cold storage in all fruit studied (Figure 1C). The CI index was in general significantly ($p < 0.05$) higher in control fruit as compared to treated fruit with MCP and MT during storage. Although MT and 1-MCP delayed CI symptoms even after 3 and 6 days of cold storage respectively MT + 1-MCP combined treatments showed the lowest CI incidence (42.66% lower as compared to control fruit) after 6 days of refrigerated storage. According to the observations (Figure 1C) only when 1-MCP was applied combined with MT, zucchini fruit still displaying an increased chilling tolerance after 9 days of cold storage showing additional benefits when both substances were applied together. The effect of MT and 1-MCP applied alone or as combined treatment on internal disorders can be clearly observed in the photographs performed (Figure 2).

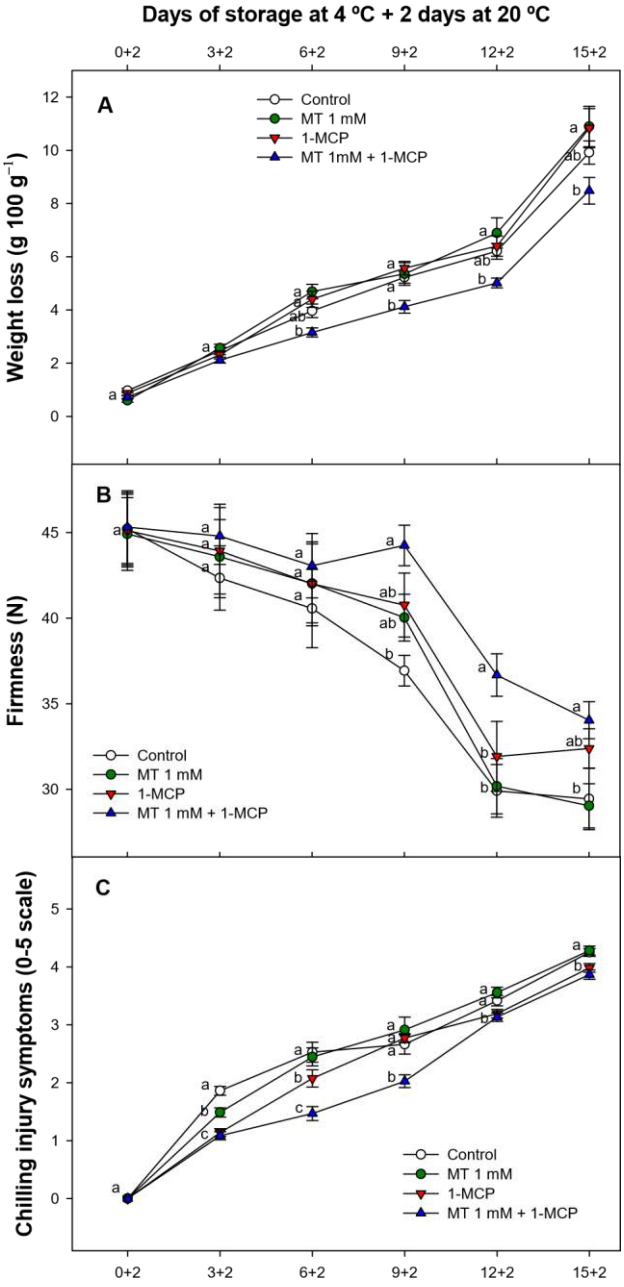

Figure 1. Evolution of weight losses (g 100 g^{-1}) (**A**) fruit flesh firmness (N) (**B**) and chilling injury (0–5 scale) (**C**) of 'Cronos' zucchini fruit treated with melatonin at 1 mM (MT) or distilled water (Control) with or without 1-MCP during cold storage plus 2 days at 20 ˚C. Data are the mean ± SE (n = 3).

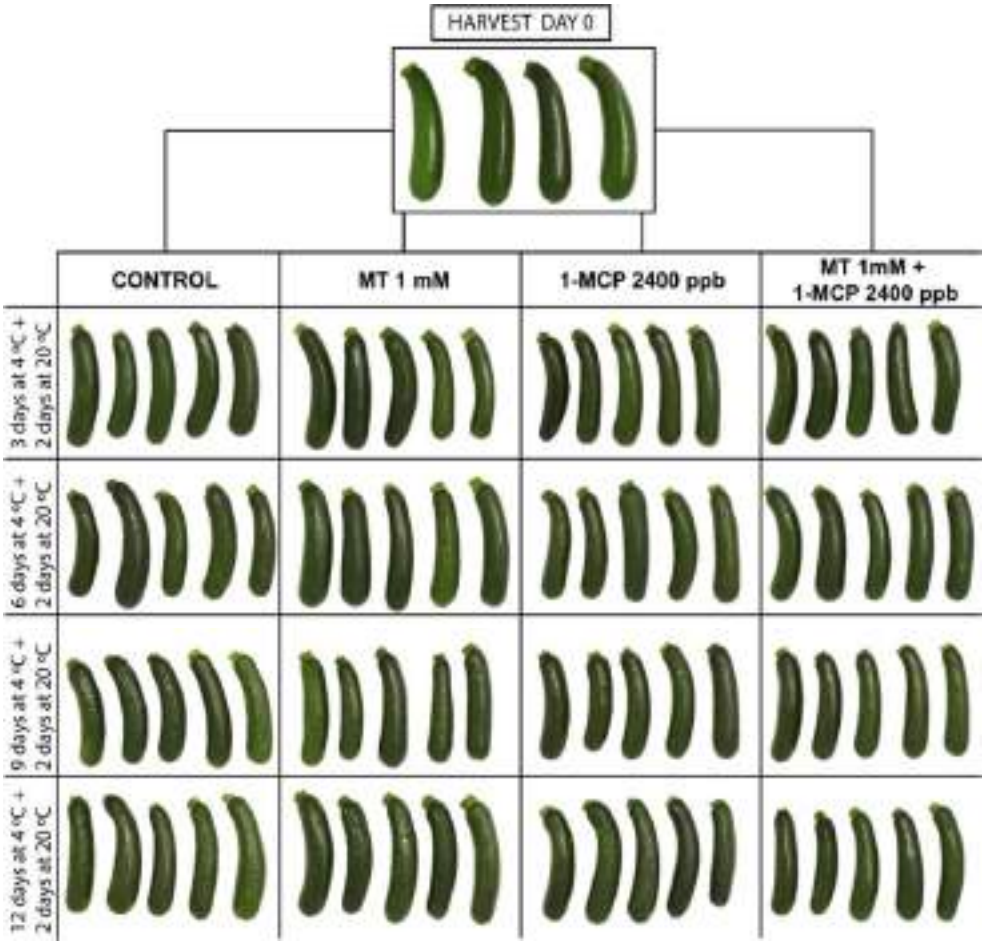

Figure 2. Photography displays the external visual aspect of 'Cronos' zucchini fruit treated with melatonin at 1 mM (MT) or distilled water (Control) with or without 1-MCP after 3, 6, 9 and 12 days of cold storage plus 2 days at 20 ˚C.

In general, in zucchini fruit, weight and fruit firmness decrease along storage specially when this fruit is stored at suboptimal temperatures mainly due to transpiration through a higher membrane permeability increased by pitting incidence. In this sense, weight loss affects cells turgor reducing fruit firmness in different fruit during storage [36,37]. For this reason, these two parameters use to be correlated between them and with CI incidence. Differences in weight loss between control and treatments were significant only when MT was applied in combination with 1-MCP. However, fruit firmness and CI were affected slightly by MT and 1-MCP alone but when were applied as a combined treatment a higher positive effect was exerted as compared to control fruit. For this reason, we inferred that 1MCP combined with MT may have synergistic effect on these traits. 1-MCP can be effective controlling weight loss, fruit firmness and CI incidence in different non-climacteric and climacteric fruit [10,38–41]. In zucchini fruit, this trend could be cultivar dependent as it has been previously demonstrated. In fact, in Cronos cultivar, differences in weight loss were not significant when these parameters were analysed after 1-MCP treatment when stored at 4 ˚C [10]. These results were in consonance with our study, and we also observed an important effect for 1-MCP applied alone reducing CI. On the other hand, MT postharvest treatments have shown a strong effect delaying weight loss in some fruit [42] but a weak effect or even unaffected weight loss in different other fruit [43,44] as we observed when MT was applied alone in zucchini fruit. This slight effect also was observed on zucchini fruit firmness and CI with single MT applications. In previous studies, MT up-regulated cell wall structure-related genes [43,45].

MT also showed antioxidant activity that delayed membrane peroxidation and consequently caused a lower phenol oxidation through an effective inhibition of peroxidase (POD) and PPO activities [46,47]. Storing zucchini at suboptimal temperatures (below 7 ˚C) can lead to serious CI, characterized by an intense surface pitting, and sunken lesions on the skin surface, which could be caused by damage to the cell walls or cell membranes [2,12,48]. For this reason, and based in our results, we proposed that a combined effect of 1-MCP and MT delaying cell wall disassembly could allow MT antioxidant activity to control CI in a synergistic way when zucchini was treated with the combined treatment.

3.2. Effect of Exogenous MT and 1-MCP on Respiration Rate and Ethylene Production

Respiration rate in zucchini fruit for all treatments tended to increase during shelf life after cold storage. However, for 1-MCP and especially for MT + 1-MCP samples respiration just increased slightly during the beginning of the study (Figure 3A) showing significant differences between treatments ($p < 0.05$).

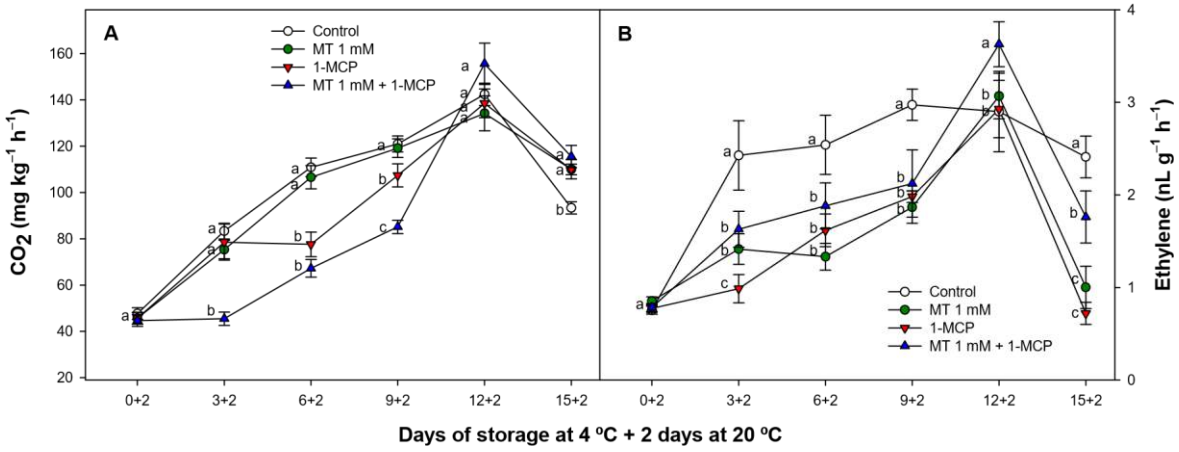

Figure 3. Respiration (mg CO_2 kg^{-1} h^{-1}) (**A**) and ethylene production rates (nL g^{-1} h^{-1}) (**B**) of 'Cronos' zucchini fruit treated with melatonin at 1 mM (MT) or distilled water (Control) with or without 1-MCP during cold storage and after cold storage plus 2 days at 20 ˚C. Data are the mean ± SE (n = 3).

At the end of storage CO_2 concentrations decreased for all zucchini fruit tested but all treatments applied containing 1-MCP delayed this pattern as compared with control and MT treated fruit. On the other hand, ethylene production in zucchini fruit was low during the experiment. Control fruit significantly increased ethylene concentration ($p < 0.05$) from the beginning of the study, but the different treatments applied delayed this pattern as compared with control fruit (Figure 3A).

Respiration is a key factor involved in weight loss process showing in this study a correlation between these two parameters. Megías et al. [10] found that 1-MCP was able to delay respiration process and ethylene production in different zucchini cultivars in consonance with our results. The reduction of respiration and ethylene production has been linked to an increased cold tolerance in zucchini fruit although the impact on fruit quality during cold storage depends on cultivar [10,49]. MT regulates γ-aminobutyric acid (GABA) content in non-climacteric and climacteric fruit [17,50], stimulating GABA-shunt pathway [51]. This increase in GABA provides the cell with an immediate energy substrate that it uses to recover from stress, increasing the net energy balance in plant cells and covering the energy needs of the plant [17]. In this study, MT-treated zucchini displayed lower medium CO_2 values but with no significant differences ($p \geq 0.05$) as compared with the rest of treatments applied, but 1-MCP treatment alone significantly ($p < 0.05$) delayed the respiration process. On the other

hand, a synergistic effect delaying the respiration process was observed when MT and 1-MCP were applied as a combined treatment probably due to an additive effect between both treatments. This additional benefit applying the combined treatment was also observed on ethylene production though MT and 1-MCP reduced cold induced ethylene production when are applied alone (Figure 3B). In this sense all the treatment delayed ethylene production until peak also delaying the decrease of ethylene at the end of storage. MT has been described as a regulator of the expression of different genes reducing ethylene production [52] and 1-MCP is a potent inhibitor of ethylene action [53]. However, the combination of treatments did not reduce ethylene production with an additional effect as compared to these treatments when applied alone. For this reason, the synergistic and beneficial effect observed on cold tolerance of zucchini (Figure 1C; Figure 2) could be explained in relation to the stimulated antioxidant balance that MT exhibits when is applied as a postharvest treatment [52] as we will describe through the following parameters (MDA and total chlorophyll content) evaluated.

3.3. Effect of Exogenous MT and 1-MCP on Membrane Permeability (MDA Content and EL)

According to the results (Figure 4A) although all treatments showed a delay in the MDA accumulation, this parameter was significantly lower ($p < 0.05$) in the cases of 1-MCP and MT + 1-MCP compared to that of control fruit.

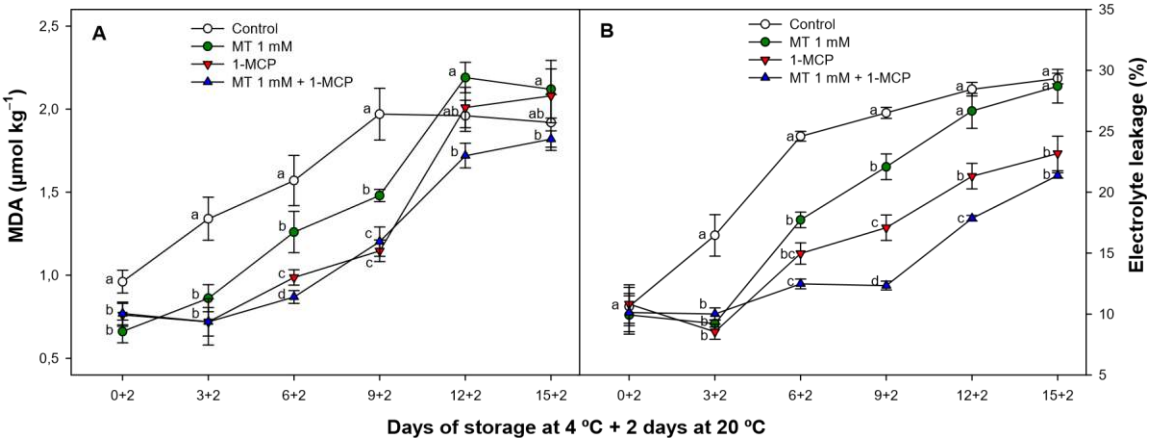

Figure 4. Evolution of malondialdehyde (MDA) content (µmol kg^{-1}) (**A**) an electron leakage (EL) (%) (**B**) of 'Cronos' zucchini fruit treated with melatonin at 1 mM (MT) or distilled water (Control) with or without 1-MCP during cold storage and after cold storage plus 2 days at 20 ˚C. Data are the mean ± SE (n = 3).

MDA in zucchini fruit treated with MT, 1-MCP or MT + 1-MCP was reduced by 19.7, 33.6 and 44.6% on the 6th day of storage respectively, as compared to control. This delay was observed only for MT + 1-MCP after 12 days of cold storage plus 2 days at 20 ˚C.

MT and 1-MCP alone or as a combined treatment (MT + 1-MCP) significantly ($p < 0.05$) delayed EL evolution (Figure 4B). However, a lower EL was observed especially when these compounds were applied as a combined treatment since the lowest EL was observed during storage when MT + 1-MCP were applied. On the contrary MT applied alone was the treatment with a weaker effect on EL. MDA reflects the lipid peroxidation of plasma membranes which directly affects the structural integrity of vegetal tissues [54]. Previous works have suggested that 1-MCP affects the activities of antioxidant enzymes [55,56] as well as MT treatments on different fruit [57,58] reducing MDA content and the impact on EL in consonance with our results. For this reason, the similar effect observed on these parameters after applying 1-MCP or MT alone, could be the reason why the reduction in MDA content and EL evolution was

greater when both substances were applied together displaying an additive cold tolerance effect.

3.4. Effect of Exogenous MT and 1-MCP on Chlorophyll Content and External Colour

Chlorophyll content in treated and untreated samples showed a decreased pattern for all fruit tested as it was expected (Figure 5A).

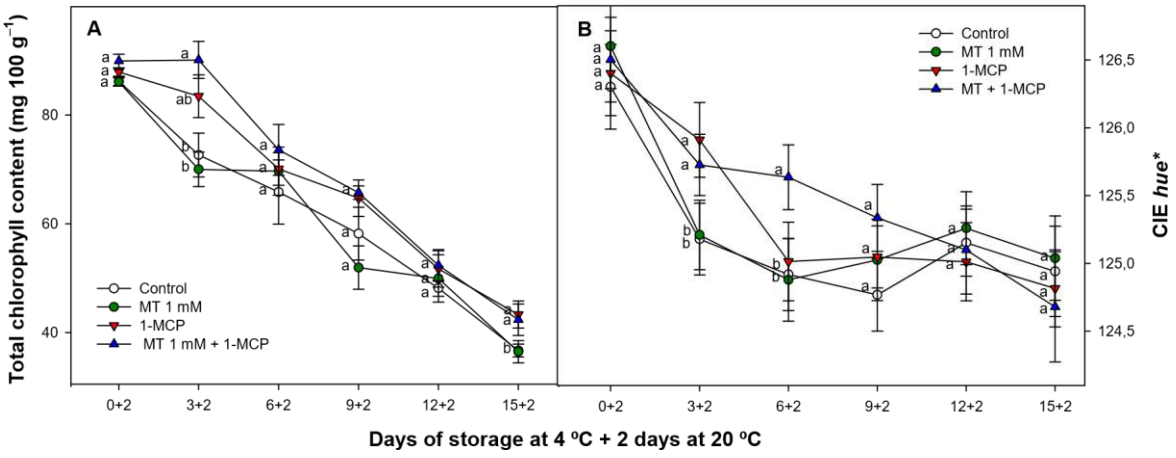

Figure 5. Evolution of total chlorophyll content (mg 100 g^{-1}) (**A**) and CIE *hue** (**B**) of 'Cronos' zucchini fruit treated with melatonin at 1 mM (MT) or distilled water (Control) with or without 1-MCP during cold storage and after cold storage plus 2 days at 20 °C. Data are the mean ± SE (n = 3).

Interestingly there was a clear effect provided by 1-MCP alone or combined with MT showing a positive effect on the maintenance of this parameter after 3 days of storage (83.44 ± 3.93 and 90.11 ± 3.41 mg 100 g^{-1} fw respectively). These values were significant higher ($p < 0.05$) than observed for MT and control fruit (70.00 ± 3.17 and 72.64 ± 4.01 mg 100 g^{-1} fw respectively). This positive effect was in general maintained along the whole experiment. On the other hand, when CIE *hue** slightly decreased during storage in all zucchini fruit tested, 1-MCP and MT + 1-MCP lots maintained significant differences ($p < 0.05$) as compared with the rest of treatments applied (Figure 5B) though these differences were reduced along the experiment. However, when MT and 1-MCP were applied as a combined treatment these differences were maintained delaying the evolution of this parameter for longer time than when these substances were applied alone.

Green colour in zucchini is determined by chlorophyll content and storage impact on pigment degradation is correlated with a reduced CIE *hue** in Cronos cultivar [59]. These authors also observed that zucchini fruit senescence is accompanied by decrease of chlorophyll pigments and CIE *hue** during storage in Cronos cultivar mainly due to loss of cell wall integrity, reducing firmness and contributing to chlorophyll pigment degradation [59]. In this sense 1-MCP treatments have been shown to maintain tissue firmness and chlorophyll content delaying senescence in climacteric and non-climacteric fruit [25,53]. On the other hand, chlorophyll pigments are also affected during postharvest as the main targets of ROS-linked damage since ROS detoxification systems decrease during plant senescence and other different stresses [60]. MT treatments have been shown to delay tissue degreening maintaining chlorophyll content in different other MT-treated vegetal products as broccoli, mango, or cucumber [61–63]. In our study, 1-MCP treatments maintained these parameters, but MT treatment did not affect both of them. For this reason, the synergistic effect observed when

treatments were applied combined, could be due to a better antioxidant MT performance mediated by an improved cell homeostasis since 1-MCP when applied alone, also showed a better control over chlorophyll content, MDA and EL than observed for MT-treated fruit for all these parameters.

3.5. Effect of Exogenous MT and 1-MCP on TSS and TA

TSS in zucchini fruit is shown in Figure 6A exhibiting a decrease in all treatments.

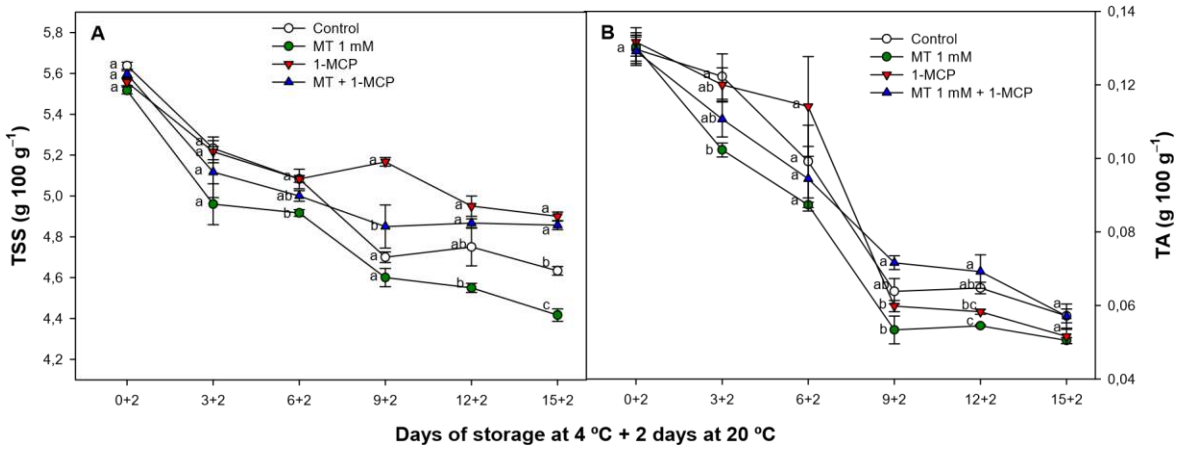

Figure 6. Evolution of total soluble solids (g 100 g^{-1}) (**A**) and titratable acidity (g 100 g^{-1}) (**B**) of 'Cronos' zucchini fruit treated with melatonin at 1 mM (MT) or distilled water (Control) with or without 1-MCP during cold storage and after cold storage plus 2 days at 20 ˚C. Data are the mean ± SE (n = 3).

TSS content in 1-MCP and MT + 1-MCP groups was significantly higher ($p < 0.05$) as compared to control fruit after 9 days of 4 ˚C storage. On the other hand, MT fruit displayed the lowest values during the storage period decreasing to 4.41 ± 0.03 g 100 g^{-1} at the end of the storage.

Similarly to TSS, TA in all the groups evaluated decreased during storage conditions and when MT was applied alone TA levels were lower as compared to the rest of fruit groups studied (Figure 6B). 1-MCP alone retained initial TA levels during 6 days of storage but as compared to control fruit no significant differences ($p \geq 0.05$) were observed. However, fruit treated with MT + 1-MCP exhibited higher TA levels than the rest of treatments after 9 days of cold storage plus an additional period of 2 days at 20 ˚C.

TSS content is an important attribute which reflects the sugar concentration in cells which increases through the conversion of starch to sugar. However, in this study and in consonance with previous studies on zucchini fruit it seems that at 20 ˚C, the sugar consumption is faster than its accumulation leading to senescence [33,64]. On the other hand, is well documented the decreased TA level in zucchini and other different fruits by the use of organic acids as respiration substrates during ripening process [64,65]. Previous reports have revealed the effect of 1-MCP on delaying ripening processes as decreasing the respiration rate in zucchini [10] and maintaining TSS and TA content through this mechanism in different fruit species [53,65]. There are no previous studies of the effect of MT treatments on zucchini fruit though in a recent review MT was described as a substance capable of inducing cold tolerance by the accumulation of sugar and organic acids in different fruit species [66]. However, MT treatments did not increase TSS or TA in zucchini probably due to the similar respiration pattern in MT-treated fruit than observed for control fruit (Figure 3A). For this reason and based in our results, the combined treatment (MT + 1-MCP) though did not increase TSS to a higher concentration than that observed for 1-MCP alone, a synergistic effect maintaining

higher TA levels was displayed when both treatments were applied together showing the highest levels of this parameter. We propose that this additional benefit could be due to a reduction in the ripening process and respiration caused by the 1-MCP but also to an increase in organic acids as have been observed in different fruit species treated with MT [66]. In this sense, a higher solute concentration is a positive factor for maintaining the protoplasm osmoregulation enhancing the cold tolerance. Higher levels in TSS and TA are directly related with a higher cell homeostasis and higher contents in sugars and organic acids as ascorbic or citric acid may also contribute to a reduced CI impact [66–68].

4. Conclusions

The present study confirmed that melatonin at 1 mM concentration as a postharvest dip treatment when combined with 1-MCP can extend the storage life of zucchini by reducing respiratory metabolism and maintaining fruit firmness reducing weight loss. The reduced metabolism observed by 1-MCP, combined with a melatonin treatment with an also displayed antioxidant effect observed maintaining chlorophyll content or reducing MDA accumulation were crucial factors on cell membrane integrity increasing zucchini cold tolerance. Also, an increased solute concentration observed in zucchini exposed to the combined treatment, could determine zucchini cold tolerance during storage at suboptimal temperatures. In this sense, results suggest that application of a combined treatment based on melatonin and 1-MCP could be a promising tool to increase storability of this fruit.

Author Contributions: J.M.-S.: Investigation, Methodology, Data curation, Formal analysis, Visualization, Writing-Original draft preparation, Software. M.S.: Investigation, methodology, Formal analysis, Validation, Visualization, Resources, Supervision. M.C.R.-A.: Investigation, Data curation, Formal analysis. M.I.M.I.: Investigation, Data curation. D.M.-R.: Investigation, Methodology, Data curation, Validation, Visualization. F.G.: Conceptualization, Investigation, Methodology, Formal analysis, Data curation, Validation, Visualization, Writing—Reviewing and Editing, Resources, Supervision. All authors have read and agreed to the published version of the manuscript.

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5 Resultados y Discusión

5. RESULTADOS Y DISCUSIÓN

La obtención de producciones con suficiente calidad es un desafío al que se enfrentan los productores año tras año. Para mejorar el rendimiento de los cultivos, la planta se ha de desarrollar de forma óptima proporcionando frutos de buen calibre y con elevada calidad en el momento de la cosecha. Procesos complejos dirigidos por distintos factores controlan la calidad del producto vegetal tanto en precosecha como durante el posterior almacenamiento poscosecha. Además, son diversos los agentes tanto abióticos como bióticos que pueden comprometer tanto las producciones como la vida útil de los productos vegetales recolectados. Los tejidos vegetales, son capaces de hacer frente en cierta medida a estos agentes mediante una cascada de señalizaciones bioquímicas. Sin embargo, cuanto mejor sea el estado de la planta, el sistema de defensa natural en los frutos y demás órganos de la planta será más eficiente y, por tanto, más tolerante frente a condiciones subóptimas. Una vez recolectado el producto vegetal se inicia un estrés energético determinado por la maduración y la senescencia de los tejidos. Además, la calidad de los frutos en el momento de la cosecha tiene un gran impacto en su vida útil poscosecha. Por ello, factores tales como la firmeza y el contenido nutricional del fruto son claves tanto para soportar los procesos de manipulación y comercialización como para alcanzar los niveles de aceptación que requiere el consumidor final.

Los frutos como la chirimoya y el albaricoque, durante su almacenamiento poscosecha muestran un patrón climatérico y tienen una vida útil muy corta. Por ello ambas especies vegetales se recolectan en estado pre-climatérico alcanzando un máximo de 3-4 semanas de vida útil en refrigeración (Palma et al., 1993; Stanley et al., 2013). Por ello es muy complicada la comercialización de estos productos especialmente hacia mercados lejanos. En este sentido, España es una importante productora tanto de albaricoque como de chirimoya. De esta última, España se posiciona como la principal nación productora de esta fruta a nivel mundial (Gonçalves-Alburquerque et al., 2016). Tampoco la aplicación de bajas temperaturas permite alcanzar estos mercados ya que, pese a que alargan de forma significativa la vida útil de estos frutos, una vez atemperados muestran un metabolismo muy acelerado. Por otro lado, es simplemente una cuestión de tiempo el que el almacenamiento a temperaturas subóptimas termine por provocar daños por frío, ya que ambas especies son muy sensibles a la exposición a bajas temperaturas (Alique et al., 1994; Fan et al., 2018a).

La sensibilidad al frío también es característica de los productos vegetales no climatéricos estudiados en esta Tesis Doctoral como son la granada, la piña y el calabacín. La granada, es uno de los frutos más antiguos conocidos en la historia de la humanidad siendo muy popular tanto por su contenido en compuestos bioactivos como por la calidad de los frutos (García-

Pastor et al., 2020a). Esta calidad suele estar basada en el tamaño, forma y color de la piel, así como otros factores internos como el color de los arilos, el contenido en azúcar y en acidez (Pareek et al., 2015). La variedad 'Mollar de Elche' estudiada en esta Tesis Doctoral, incrementa su pigmentación rojiza tanto internamente en los arilos como externamente en la superficie del fruto durante su maduración en el árbol. Sin embargo, suele presentar una deficiente pigmentación interna y externa en el momento de la cosecha probablemente debido a las altas temperaturas que se registran en las zonas de cultivo de esta variedad (sudeste español) que dificulta la acumulación de antocianinas (García-Pastor et al., 2020c). Las granadas son especialmente sensibles al almacenamiento refrigerado, así como la piña como el calabacín. Esta sensibilidad provoca grandes pérdidas durante la comercialización y almacenamiento poscosecha cuando este se realiza a bajas temperaturas. Además, en estos frutos, distintos aspectos condicionan la pérdida de calidad no sólo en su parte comestible sino también en otras partes no comestibles del fruto, como la pérdida de clorofilas que se observa en la corona de la piña (Liu et al., 2017) y en la piel de los calabacines (Blanco-Díaz et al., 2016) durante su almacenamiento. Asimismo, las bajas temperaturas inducen en los calabacines principalmente daños externos (depresiones y decoloraciones en la piel) mientras que en el caso de las piñas los daños que presentan por el frío son internos, en forma de pardeamientos y translucidez de la pulpa (Mejías et al., 2016; Paull y Chen, 2019).

La comercialización poscosecha de los frutos estudiados en esta Tesis Doctoral, se ve comprometida especialmente cuando los frutos son almacenados a temperatura ambiente tras su conservación refrigerada. El aumento del metabolismo a temperatura ambiente y las condiciones externas aceleran las pérdidas de peso, y por tanto la deshidratación y las pérdidas de firmeza conforme aumenta el tiempo de almacenamiento. Esta desintegración de los tejidos provoca la aparición de pardeamientos externos o internos además de facilitar la incidencia microbiana impidiendo la comercialización de los productos (Valero y Serrano, 2010). En estas condiciones, también se ven afectados los compuestos bioactivos (polifenoles, clorofilas, ácido ascórbico…) de interés en chirimoyas (Almela et al., 2000), calabacines (Palma et al., 2019), albaricoques (Campbell et al., 2013), piñas (Liu et al., 2017), y granadas (García-Pastor et al., 2020a).

Con anterioridad y de forma común y en todos los frutos estudiados en esta Tesis Doctoral se han aplicado estrategias tradicionales de conservación en poscosecha. En este sentido, tratamientos físicos basados en el control de la temperatura (Jomngam et al., 2017; Cui et al., 2019; Taghipour et al., 2021; Maeda et al., 2021; Zuo et al., 2022) o en la modificación de la atmósfera circundante al fruto (Yahia, 2009) han sido ampliamente estudiados. También se ha estudiado el efecto de tratamientos químicos como el uso de inhibidores comerciales de la acción del etileno como el 1-MCP (Blankenship et al., 2003;

Watkins, 2006; Megías et al., 2015; Li et al., 2016; Silva et al., 2016). Más recientemente estrategias basadas en la aplicación de compuestos naturales (hormonas vegetales o elicitores naturales), presentes en la propia planta, han mostrado efectividad a la hora de retrasar la maduración (Romanazzi et al., 2016) y de reducir los daños por frío, tanto en albaricoques (Batoo et al., 2021) como en piñas (Boonyaritthongchai y Supapvanich, 2017), granadas (García-Pastor et al., 2020c) calabacines (Palma et al, 2019) y chirimoyas (Pareek et al., 2011). Aunque se han estudiado en menor medida y la bibliografía disponible es escasa, también se han aplicado compuestos de origen natural en precosecha. Este es el caso de la aplicación de metil salicilato, ácido salicílico, benciladenina y ácido giberélico en albaricoques (Canli et al., 2014; Cui et al., 2020; Fan et al., 2021) o melatonina, ácido γ-aminobutírico y jasmonato de metilo en granadas, incrementando la calidad tanto en el momento de la cosecha (Lorente-Mento et al., 2023), como durante su posterior almacenamiento poscosecha (García-Pastor et al., 2019; García-Pastor et al., 2021; Lorente-Mento et al., 2021).

El interés en la aplicación de compuestos propios de las plantas como estrategias precosecha o poscosecha radica tanto en las restricciones legales con respecto al uso de tratamientos químicos con sustancias de origen artificial, así como por las reservas que tienen los consumidores sobre ellos. Por tanto, el objetivo de esta Tesis Doctoral se centra en el estudio de la aplicación de una sustancia natural presente en los tejidos de las plantas como es la melatonina. Para ello se han evaluado por primera vez los beneficios que puedan acompañar la aplicación de esta nueva tecnología en precosecha o en poscosecha especialmente a la hora de retrasar la maduración y mantener la calidad de los frutos durante el almacenamiento a temperaturas óptimas o subóptimas.

Los principales resultados obtenidos con respecto al efecto de las aplicaciones precosecha con melatonina tanto en granadas como en albaricoques han sido presentadas como las Publicaciones 1 y 2 de la presente Tesis Doctoral. Con ello se pretende evaluar si las aplicaciones precosecha con melatonina son eficaces tanto en el momento de la recolección, mejorando las producciones y parámetros de calidad, como durante el posterior almacenamiento a distintas temperaturas. Estas publicaciones reflejan los estudios precosecha realizados durante dos diferentes ciclos productivos consecutivos tanto en las granadas cv. Mollar de Elche (2017 y 2018) (**Publicación 1**) como en los albaricoques cv. Colorado y cv. Mikado (2019 y 2020) (**Publicación 2**).

Los tratamientos con melatonina se aplicaron mediante pulverización foliar tanto en los granados como en los albaricoqueros a las concentraciones de 0,1 y 1 mM. En ambas especies vegetales, se determinó que los mejores resultados fueron los obtenidos a la menor de las concentraciones (0,1 mM).

Por esta razón al año siguiente, en el siguiente ciclo productivo de los granados y albaricoqueros se aplicó de nuevo únicamente la mejor concentración en términos de producción y calidad general del fruto. En el caso de los albaricoques los resultados fueron expresados como la media de los valores obtenidos en ambos ciclos productivos con la mejor dosis de melatonina ensayada en el primero de ellos.

Dado el potencial interés de la melatonina como alternativa a los tratamientos con sustancias químicas de origen artificial, también se han evaluado las posibilidades de este tratamiento como una tecnología poscosecha capaz de retrasar la maduración y el deterioro resultantes de la importante sensibilidad al frío que muestran piñas y chirimoyas. Los resultados obtenidos de los diferentes estudios poscosecha con melatonina han quedado reflejados en las Publicaciones 3 y 4. Las piñas cv. PRI 73-050, uno de los híbridos comerciales de mayor importancia en las islas Hawái, se evaluó en dos experimentos consecutivos realizados en 2018 (**Publicación 3**). En primer lugar, se comprobó el impacto de distintas concentraciones de melatonina (0,1 y 0,5 mM) sobre piñas sometidas a distintos tiempos de inmersión (10 y 60 minutos). Seguidamente, en un segundo experimento, se volvió a aplicar la dosis y tiempo de inmersión con los mejores resultados observados (frutos sumergidos 10 minutos en melatonina 0,1 mM) confirmando los datos obtenidos en el experimento anterior. Paralelamente, y con el objetivo de alargar la vida útil y reducir el impacto de los daños por frío en las chirimoyas cv. Fino de Jete, se evaluaron distintas concentraciones de melatonina (0,01, 0,05, 0,1, 0,3 y 0,5 mM) sumergidas a diferentes tiempos de inmersión (10, 60 y 180 min). Tras la realización de este estudio se realizó un segundo experimento cuyos resultados se presentan en esta Tesis Doctoral (**Publicación 4**) en los que se volvieron a aplicar únicamente las condiciones óptimas del estudio anterior. Estas condiciones consistieron en la aplicación de las dosis más bajas de melatonina (0,01, 0,05 y 0,1 mM) durante el menor de los tiempos de inmersión ensayados (10 minutos).

Dada la sensibilidad al frío que presentan tanto las piñas como las chirimoyas, estos experimentos se diseñaron aplicando condiciones de almacenamiento a temperaturas subóptimas, con el objetivo de evaluar el impacto de los tratamientos poscosecha con melatonina sobre los daños por frío y sobre la calidad general durante el almacenamiento.

Finalmente, se realizó un estudio en el que hemos tratado de comparar el efecto de compuestos químicos de origen artificial (1-MCP) con la respuesta observada tras realizar aplicaciones de melatonina en calabacines cv. Cronos (**Publicación 5**). La aplicación de 1-MCP fue realizada en las condiciones comerciales recomendadas para esta variedad de 2400 ppb durante 48 horas. El efecto causado por el 1-MCP fue comparado con tratamientos basados en melatonina. En un primer experimento se aplicaron distintas concentraciones

de melatonina (0,1, 0,5 y 1 mM) durante diferentes tiempos de inmersión (10, 30, 60, 120 y 180 minutos). Estas condiciones fueron ensayadas con o sin aplicaciones adicionales de 1-MCP, con el objetivo de evaluar el posible efecto aditivo o sinérgico positivo que manifiestan cuando se aplican por separado.

5.1. Efecto de las aplicaciones precosecha con melatonina en granados y albaricoqueros sobre la producción.

Los tratamientos precosecha con melatonina en granados y albaricoqueros fueron capaces de provocar un retraso de la maduración del fruto en el árbol en ambas especies vegetales (**Publicaciones 1 y 2**). Este hecho se observó en diferentes parámetros observados en el día de la cosecha. El retraso de la maduración se observó especialmente en el caso de las granadas. En esta especie vegetal, al evaluar la producción de las granadas tras el primer corte, se observó que esta fue significativamente mayor en los granados control con respecto a los árboles tratados con melatonina a las distintas concentraciones. Sin embargo, y tras las dos recolecciones realizadas la mayor producción observada fue la recolectada de los árboles tratados con melatonina tanto en el caso de los granados (en ambos ciclos productivos) (**Publicación 1**) como en los albaricoqueros (**Publicación 2**). Ambas variedades de albaricoqueros experimentaron incrementos de la producción similares tras ser tratados con melatonina. El incremento de la producción observado en ambas especies vegetales estuvo relacionado con un mayor peso del fruto. Las granadas incrementaron el volumen de porción comestible como se puede apreciar en las granadas cortadas ecuatorialmente en la fotografía de la Publicación 1. Adicionalmente en el caso de las granadas, este incremento de la producción estuvo condicionado también por un mayor número de frutos recolectados en los árboles tratados con melatonina. En los albaricoqueros este parámetro fue similar entre los distintos árboles de ambas variedades tratados con melatonina o controles.

Artículos publicados anteriormente han demostrado que el tratamiento foliar con melatonina afecta el rendimiento de los cultivos. En el caso de las plantas de tomate, se ha demostrado en diferentes estudios que las aplicaciones precosecha con melatonina son capaces de incrementar la tolerancia al estrés producido por la lluvia ácida y también el estrés soportado por la planta en condiciones de déficit hídrico (Debnath et al., 2018; Ibrahim et al., 2020). Además, la imbibición previa de semillas de tomate en soluciones de melatonina, o la aplicación de esta sustancia mediante irrigación, ha sido eficaz a la hora de incrementar las producciones de tomate (Liu et al., 2016). Estos efectos se atribuyeron a un aumento en el contenido de clorofila foliar y a la mayor tasa fotosintética. Por lo tanto, y dadas las condiciones climáticas semiáridas del sur de España donde se han realizado los estudios precosecha

de esta Tesis Doctoral, el tratamiento con melatonina podría estar aumentando la tasa neta de fotosíntesis y la productividad. De esta forma se mejoraría la tolerancia de los árboles al calor y al estrés por la sequía. Además, estos tratamientos podrían estimular la fuerza de sumidero de los frutos, lo que llevaría a acumular más azúcares y desarrollar un tamaño más grande en la cosecha. Este efecto fue propuesto por Meng et al. (2015) al observar que las bayas de uva tratadas con melatonina en el pre-envero exhibieron una mayor acumulación endógena de melatonina, además de un mayor tamaño y peso de la baya. De hecho, un efecto similar fue observado recientemente tras el tratamiento precosecha con melatonina de albaricoques y de cerezas (Abd El-Naby et al., 2019; Carrión-Antolí et al., 2022).

Por otro lado, y de forma adicional en el caso de las granadas la cantidad de fruta cosechada por cada árbol también se incrementó con el tratamiento con melatonina (**Publicación 1**). Dado que los tratamientos se aplicaron cuando la fruta estaba en su fase activa de crecimiento, el mayor número de frutos cosechados en los árboles tratados con melatonina podría atribuirse al efecto de la melatonina en la disminución de la abscisión normal de los frutos que ocurre durante el desarrollo en el árbol debido a factores ambientales, como el viento o la lluvia. En este sentido, se ha descrito previamente el efecto de la melatonina a la hora de reducir tanto el estrés biótico como el abiótico que sufren las plantas (Kolodziejczyk y Posmyk, 2016; Arnao y Hernández-Ruiz, 2019).

El efecto del tratamiento con melatonina durante el desarrollo del fruto en el árbol sobre el aumento del rendimiento de los cultivos es claro cuando las plantas están bajo distintos tipos de estrés. Sin embargo, en condiciones óptimas de cultivo, este efecto depende de la especie vegetal o de las distintas variedades, de la concentración aplicada o de la etapa de desarrollo de la planta en la que se aplica la melatonina (Debnath et al., 2019). Estos aspectos merecen la atención de los investigadores en estudios futuros.

5.2. Efecto de los tratamientos precosecha con melatonina sobre la calidad de las granadas y albaricoques en la cosecha y durante su conservación a temperaturas óptimas y subóptimas.

Tanto en las granadas como en los albaricoques, el tamaño, color de la piel, la firmeza del fruto y el contenido en azúcares y ácidos son los principales parámetros que determinan su calidad, tanto en el momento de la cosecha como la posterior vida útil poscosecha (Pareek et al., 2015; Rebeaud et al., 2023). En el caso de las granadas, el mercado internacional requiere frutos

brillantes y atractivos, de color rojo, sin lesiones físicas por marcas, arañazos, manchas de enfermedades, etc., (Gadze et al., 2012). Tanto la coloración rojiza interna como la externa aumentan durante la maduración en el árbol debido a la acumulación de antocianinas (García-Pastor et al., 2020b). La ausencia de color rojo en los arilos es una fisiopatía que parece estar intensificándose en diferentes cultivares de granada debido al cambio climático (Moradi et al., 2022). La granada objeto de estudio en esta Tesis Doctoral, además tiene una coloración externa con una baja intensidad rojiza.

Los albaricoques, en general, se recolectan en una etapa de madurez temprana o madurez preclimatérica. Este hecho garantiza la calidad final tras pasar por los diferentes procesos poscosecha (Mencarelli et al., 2006), y se recomienda especialmente cuando van a ser transportados atravesando largas distancias comerciales. Además, existe una alta incidencia en el nivel de pérdidas que causan en Europa nuevas plagas que atacan directamente a los albaricoques maduros como la *Drosophila suzukii* (Mazzetto et al., 2015). Estos factores obligan a los productores a recolectar los albaricoques en un estado de maduración temprano, a pesar del riesgo de que los atributos de calidad del fruto no satisfagan las preferencias de los consumidores (Bruhn et al., 1991).

Los efectos derivados de las aplicaciones precosecha con melatonina en las especies vegetales de esta Tesis Doctoral tuvieron efectos similares en ambos ciclos productivos. Por tanto y para una mejor comprensión del efecto observado en los distintos parámetros evaluados, a continuación, se presentan los resultados como una media de los valores obtenidos de cada parámetro en los distintos ciclos productivos de cada especie y cultivar estudiado (**Tabla 3**).

Tabla 3: *Análisis comparativo porcentual (%) entre los valores medios determinados en los frutos tratados en precosecha con melatonina (0,1 mM) y los frutos control en dos ciclos productivos de granadas y albaricoques en el momento de la cosecha y durante su posterior almacenamiento a temperaturas óptimas y subóptimas.*

PARÁMETROS EVALUADOS EN LA COSECHA			
	GRANADA cv. Mollar de Elche	**ALBARICOQUE**	
		cv. Colorado	**cv. Mikado**
Respiración	↓ 7,28	↓14,96	↓18,75
Firmeza (N mm^{-1})	↑ 11,65	↑ 10,96	↑ 22,1
Acidez Total (%)	↑ 18,15	↑ 4,12	↓ 2,73
SST (%)	↑ 6,95	↓ 5,79	↑ 2,27
Índice de madurez	↓ 13,68	↓ 9,68	↑ 5,01
Ángulo CIE *hue** externo	↓ 11,23	↓ 1,21	↑ 2,62
Ángulo CIE *hue** interno	↓ 9,42	-	-

PARÁMETROS EVALUADOS TRAS EL ALMACENAMIENTO DURANTE 60 DÍAS (GRANADAS) Ó 21 DÍAS (ALBARICOQUES)					
	GRANADA cv. Mollar de Elche 10 ºC	**ALBARICOQUE**			
		cv. Colorado		**cv. Mikado**	
		1 ºC	**8 ºC**	**1 ºC**	**8 ºC**
Etileno	-	↓76,78	↓20,71	↓43,63	↓36,75
Respiración	↓15,57	↓26,90	↓26,65	↓14,07	↓32,94
Firmeza (N mm^{-1})	↑22,69	↑23,60	↑28,85	↑22,46	↑11,76
Pérdida de peso	↓22,22	↓30,70	↓25,86	↓19,71	↓12,36
Acidez Total (%)	↑ 34,33	↑ 13,75	↑ 20,08	↑ 9,34	↑ 21,42
SST (%)	↓ 2,22	↓ 9,22	↓ 8,46	↓ 2,26	↓ 8,02
Índice de madurez	↓ 35,92	↓ 21,74	↓ 26,93	↓ 11,38	↓ 27,65
Ángulo CIE *hue** externo	↓ 9,91	↓ 2,61	↑ 0,37	↓ 2,47	↑ 2,33
Ángulo CIE *hue** interno	↓ 10,52	-	-	-	-
Daños por el frío	-	↓ 22,31	-	↓ 42,40	-

** Los valores de cada parámetro se han calculado con respecto a los obtenidos en los frutos control o sin tratar de las Publicaciones 1 y 2 y han sido expresados como valores medios de los dos distintos ciclos productivos de cada cultivar estudiado en esta Tesis Doctoral.*

La producción de etileno en las granadas fue basal y por tanto no mostró diferencias entre los frutos (datos no mostrados). De igual forma, los albaricoques recién recolectados en estado preclimatérico tampoco mostraron diferencias en el momento de la cosecha, pero sí se observaron durante el posterior almacenamiento a las distintas temperaturas estudiadas (**Tabla 3** y **Publicación 2**). De hecho, la producción de etileno de los albaricoques fue retrasada durante el almacenamiento en los frutos procedentes de los árboles tratados con melatonina en ambas variedades. Este retraso fue mayor en los albaricoques almacenados a 1 ºC. No existen estudios precosecha con melatonina donde se haya observado este comportamiento. Sin embargo, en estudios realizados con peras, se ha confirmado la menor expresión de genes como son el PcACS y el PcACO implicados en la codificación de enzimas que estimulan la producción de etileno, como el ácido 1-aminociclopropano-1-carboxílico (ACC sintasa) y la enzima reguladora ACC oxidasa respectivamente (Liu et al., 2019). De la misma forma, en bananas y manzanas los tratamientos poscosecha con melatonina (1 mM y 0.1 mM respectivamente) redujeron la producción de etileno mediante la menor expresión de los genes MdACO1, MdACO2 en ambas especies (Hu et al., 2017; Onik et al., 2021).

El efecto de las aplicaciones precosecha con melatonina tuvo un efecto claro en la respiración que fue reducida significativamente en ambas especies vegetales tanto en la cosecha como durante la conservación (**Tabla 3, Publicación 1** y **2**). Este efecto se observó especialmente con la menor concentración aplicada de melatonina (0,1 mM) en ambos ciclos productivos de granadas y albaricoques, independientemente de la temperatura de almacenamiento. Posteriormente a la Publicación 1 y 2, Michaelidis et al., (2021) observaron que las cerezas tratadas en precosecha con melatonina mostraban una menor respiración en el momento de la cosecha en coincidencia con nuestros resultados. En otros estudios, la reducción de la respiración tras aplicar melatonina en poscosecha se ha observado en diferentes frutos climatéricos como peras, mangos, melocotones, nectarinas, peras y mangos (Gao et al., 2016; Zhai et al., 2018; Liu et al., 2020b; Bal, 2021) y también en frutos no climatéricos como cerezas (Wang et al., 2019). Este efecto positivo observado sobre la respiración en los frutos tratados con melatonina, con respecto a los frutos control, se mantuvo durante el almacenamiento poscosecha posterior en las dos especies vegetales estudiadas independientemente de la temperatura de almacenamiento en el caso de los albaricoques (**Tabla 3** y **Publicaciones 1** y **2**).

Al igual que en la respiración, las aplicaciones precosecha de melatonina tuvieron efectos muy positivos sobre la firmeza de los frutos (**Tabla 3** y **Publicaciones 1** y **2)**. Los datos indicaron que estas aplicaciones dieron lugar a granadas y albaricoques más firmes, tanto en la cosecha como durante el almacenamiento a distintas temperaturas. Un estudio reciente (Carrión-Antolí et

al., 2022) describe el efecto del tratamiento precosecha con melatonina sobre la firmeza de cerezas en la cosecha mostrando resultados en consonancia con los obtenidos en esta Tesis Doctoral. La mayor firmeza de estos frutos podría estar relacionada con una disminución de la degradación de la pared celular a través del efecto de la melatonina sobre la inhibición de las enzimas relacionadas como la poligalacturonasa, la pectin metil esterasa y la β-galactosidasa (Fan et al., 2019; Lorente-Mento et al., 2023).

Un efecto similar se observó cuando se estudiaron las pérdidas de peso que sufrieron tanto granadas como albaricoques durante el almacenamiento poscosecha. En este sentido, las granadas tratadas con melatonina mostraron unas pérdidas de peso inferiores a los frutos controles (22,22 %) tras 60 días de almacenamiento a 10 ºC (**Tabla 3** y **Publicación 1**). Por otro lado, las pérdidas de peso observadas durante el almacenamiento de los albaricoques tras 21 días de almacenamiento fueron mayores en los frutos control que los frutos tratados con melatonina tanto a 1ºC (un 26,90 y 14,07 % más bajas que los controles para las variedades Colorado y Mikado respectivamente), como a 8 ºC (26,65 y 32,94 % más bajas que los frutos control respectivamente) (**Tabla 3 y Publicación 2**). Las pérdidas de peso son principalmente debidas a la transpiración a través de la superficie del fruto. La reducción de las pérdidas de peso en los frutos procedentes de los árboles tratados con melatonina podría ser atribuida al efecto de la melatonina incrementando el grosor de la cutícula que se ha descrito previamente en diferentes estudios poscosecha realizados en mangos (Rastegar et al., 2020) y nectarinas (Bal, 2021), así como a la menor respiración observada anteriormente.

El índice de madurez en el momento de la cosecha (**Tabla 3 y Publicaciones 1** y **2**) estuvo principalmente afectado por la acidez. Este índice fue menor en las granadas y los albaricoques cv. Colorado tratados en precosecha con melatonina cuando se evaluaron el día de la recolección. En las granadas tratadas con melatonina, los mayores niveles de acidez pudieron deberse al mayor contenido en los ácidos orgánicos málico, succínico y ascórbico de los frutos tratados en el momento de la cosecha (**Publicación 1**). Estos resultados fueron coincidentes con los encontrados en cerezas por Carrión-Antolí et al., (2022) en el momento de la cosecha. Pese a que los albaricoques cv. Colorado mostraron una menor acumulación de SST que los frutos control en la cosecha, el resto de cultivares de las distintas especies vegetales estudiadas mostraron una mayor acumulación de SST en dicho momento. Durante el almacenamiento poscosecha, tanto las granadas como los albaricoques tratados con melatonina mostraron un retraso en la acumulación de SST con respecto a la observada en los frutos control. De hecho, se observó como el elevado contenido en acidez de las especies vegetales tratadas con melatonina dio lugar a que tanto las granadas como los albaricoques mostrasen un menor índice de madurez que el determinado en los frutos control de cada especie y cultivar durante el almacenamiento (**Tabla 3** y

Publicaciones 1 y **2**). Los SST y la acidez total (AT) son factores claves en la aceptación de granadas y albaricoques por los consumidores (Pareek et al., 2015; Fan et al., 2018a). En este sentido, los mayores niveles observados durante el almacenamiento poscosecha mostraron que estos atributos fueron mejorados con respecto a los frutos control.

Uno de los objetivos de esta Tesis Doctoral ha sido evaluar si la melatonina podría ser una herramienta precosecha útil a la hora de incrementar la coloración de los frutos en el momento de la cosecha especialmente en las granadas "Mollar de Elche" depreciadas por la baja coloración externa e interna. En este sentido, la aplicación de melatonina en los árboles mejoró la coloración externa de las granadas y la coloración de los arilos desde el momento de la cosecha. Además, se mantuvo durante el almacenamiento ya que el mayor descenso del ángulo CIE *hue** fue observado en las granadas tratadas con melatonina. Los valores bajos de ángulo CIE *hue** se corresponden a coloraciones más oscuras y rojizas en estos frutos. Sin embargo, este efecto fue menos significativo en los albaricoques (**Tabla 3** y **Publicación 1** y **2**). Así los albaricoques cv. Colorado incrementaron ligeramente su coloración mientras que los albaricoques cv. Mikado retrasaron este parámetro. Aunque en los albaricoques no se observó un efecto tan claro, el color rojizo en los frutos viene dado por compuestos polifenólicos como las antocianinas (Gómez-Martínez et al., 2021) y carotenoides (Batool et al., 2022). En este sentido, en general en ambas variedades, se mantuvieron mayores niveles de polifenoles totales que no afectaron a los observados en los polifenoles individuales evaluados (**Publicación 2**). El perfil polifenólico y la concentración de estos compuestos tanto en granada como albaricoque están influenciados por varios factores, incluido el estado de maduración, las condiciones agronómicas y ambientales, así como el tejido vegetal analizado (Drogoudi et al., 2008; Campbell et al., 2013; Mphahlele et al., 2014). Los cultivares de albaricoque de la presente investigación tenían una concentración fenólica total en la cosecha de aproximadamente 20 mg 100 g^{-1} de peso fresco, lo que demuestra que el contenido fenólico en los cultivares 'Colorado' y 'Mikado' es bajo en comparación con otros cultivares de albaricoque. La catequina, el ácido clorogénico, el ácido neoclorogénico, la epigalocatequina y la rutina han sido descritos como los principales compuestos fenólicos en el albaricoque, aunque sus concentraciones relativas dependen del cultivar y la etapa de maduración (Kan et al., 2014). En el caso de las granadas los compuestos fenólicos más representativos son las antocianinas, las cuales son responsables de la coloración rojiza tanto de la piel como de los arilos, cuyo contenido además aumenta durante la maduración del fruto (Kulkarni et al., 2005). En el caso de las granadas las antocianinas más importantes son la cianidin 3-glucósido y la delfinidin 3-glucósido (García-Pastor et al., 2020b). Aunque en las granadas estas antocianinas no fueron determinadas, la mayor coloración observada tanto en la piel como en los arilos estaría relacionada con un aumento general de estos compuestos. Los mayores niveles de

antocianinas han sido relacionados con el incremento de la tolerancia a los daños por frío en melocotones, facilitando el mantenimiento de la fluidez de las membranas celulares (Gao et al., 2016; 2018).

La aplicación de bajas temperaturas suele ser una alternativa eficaz para retrasar los parámetros de maduración y de senescencia en los frutos. Sin embargo, las temperaturas subóptimas pueden provocar daños por frío tanto en las granadas como en los albaricoques. No obstante, y aunque durante el almacenamiento de las granadas no se observaron daños por frío al no aplicar temperaturas subóptimas en este estudio, estos daños sí aparecieron durante el almacenamiento a 1 ºC en ambas variedades de albaricoque (**Tabla 3** y **Publicación 2**). En este sentido el efecto protector del tratamiento con melatonina fue significativo reduciendo estos daños tanto en la cv Colorado como en la Mikado, con respecto a los frutos control (22,31 y 42,40 % menos afectados respectivamente). De esta forma también se pudo observar que la cv. Mikado mostró mayor sensibilidad a esta fisiopatía con respecto a la cv. Colorado. Atendiendo a nuestros resultados, la reducción del daño por frío en los albaricoques tras los tratamientos precosecha con melatonina, podría estar relacionada con la mayor concentración de compuestos polifenólicos como previamente se describe en la Publicación 2. En relación a nuestros resultados, se ha observado una menor actividad en la enzima polifenol oxidasa (PPO) en albaricoques tratados con melatonina en poscosecha (Koushesh et al., 2012). Por tanto, el mayor contenido en compuestos fenólicos y la menor incidencia del daño por frío en albaricoques tratados en precosecha con melatonina podrían estar relacionados con una menor actividad de esta enzima. Jannatizadeh et al. (2019), observaron esta relación también en granadas tratadas en poscosecha. Además, tanto en frutos climatéricos, como kiwi y tomate (Yu et al., 2019; Jiao et al., 2020), como en no climatéricos como las granadas (Kashash et al., 2019), se ha observado que la reducción en la producción de etileno provocada por los tratamientos poscosecha con melatonina también está relacionada con el daño por frío. En este sentido, la menor producción de etileno observada en los albaricoques de la presente Tesis Doctoral sugiere que la inhibición de la biosíntesis de etileno con tratamientos de melatonina en precosecha podría estar contribuyendo a la reducción del daño por frío que sufren los albaricoques durante el almacenamiento a temperaturas subóptimas en poscosecha.

5.3. Efecto de las aplicaciones poscosecha con melatonina sobre la calidad y los daños por frío de piñas y chirimoyas

Son diversos los estudios que han evaluado las propiedades de la melatonina como tecnología poscosecha. En ellos se describe que cada especie necesita ser estudiada de forma independiente, ya que las condiciones de tratamiento para una especie vegetal no se corresponden con las

observadas en otras especies, especialmente con respecto a la concentración de melatonina óptima para conseguir un efecto favorable.

Tanto piñas como chirimoyas incrementaron las pérdidas de peso a lo largo del almacenamiento independientemente de la concentración de melatonina aplicada (**Publicaciones 3** y **4**). El tratamiento con melatonina en las piñas no afectó a las pérdidas de peso con ninguna de las concentraciones y tiempos de inmersión estudiados. Tampoco se vio afectado este parámetro en las chirimoyas que fueron tratadas con las concentraciones de melatonina mayores y menores (0,01 y 0,1 mM). Sin embargo, la concentración intermedia (0,05 mM) sí llegó a retrasar las pérdidas de peso alrededor de un 12 % (**Tabla 4**).

Tabla 4: *Análisis comparativo porcentual (%) entre los valores medios determinados en piñas y chirimoyas tratadas en poscosecha con melatonina a distintas concentraciones y los frutos control durante su almacenamiento tras 14 días (piñas) y 21 días (chirimoyas) respectivamente a temperaturas subóptimas.*

	PIÑA	CHIRIMOYA		
PARAMETROS	**MT 0,1 mM**	**MT 0,01 mM**	**MT 0,05 mM**	**MT 0,1 mM**
Pérdida de peso (%)	-	-	↓ 12,07	-
Respiración (mg kg^{-1} h^{-1})	↓ 12,70	-	↓ 13,93	-
Etileno (nL g^{-1} h^{-1})	-	↓ 50,12	↓ 27,28	-
Color CIE *L**	↑ 3,45	↑ 1,58	↑ 7,58	↑ 2,72
Color CIE *a**	↓ 22,24	-	↓ 9,44	↓ 2,46
Clorofilas (mg $100g^{-1}$)	↑ 17,69	↑ 10,29	↑ 34,25	-
Firmeza (N)	↑ 7,28	↑ 20,97	↑ 4,12	↑ 4,18
Daños por frío	↓ 52,71	↓ 50,01	↓ 67,52	↓ 11,22
Fuga de electrolitos (%)	-	-	↓ 15,19	↓ 11,22

** Los valores de cada parámetro se han calculado con respecto a los obtenidos en los frutos control o sin tratar de las Publicaciones 3 y 4 descritos en esta Tesis Doctoral.*

En las pérdidas de peso son factores clave la transpiración y la respiración tanto en las chirimoyas (Alique et al., 1994) como en las piñas (Paull, 1993). En estas últimas, la transpiración de las hojas que conforman la corona contribuye de forma adicional a las pérdidas de peso y particularmente

a la pérdida de la calidad estética de forma más acelerada (Chen y Paull, 2001). Los efectos positivos en la reducción de las pérdidas de peso producidos por las aplicaciones exógenas de melatonina han sido descritos previamente en una gran variedad de frutos como melocotones, fresas y ciruelas (Gao et al., 2016; Liu et al., 2018; Bal, 2019). Sin embargo, al igual que en el caso de las piñas estudiadas en esta Tesis Doctoral, no se han observado efectos provocados por la melatonina sobre las pérdidas de peso de frutos climatéricos como el tomate (Sun et al., 2015) y en no climatéricos como es el caso de las granadas (Molla et al., 2022). El efecto en la reducción del metabolismo del fruto también se observó en las piñas y en las chirimoyas durante el almacenamiento (**Publicación 3** y **4**). Tras evaluar las distintas concentraciones de melatonina, se pudo comprobar que, pese a las diferencias existentes entre las especies, en ambos casos las mayores concentraciones de melatonina ensayadas condujeron a producciones de CO_2 retrasadas o reducidas frente a los frutos control de cada especie (**Tabla 1** en **Publicación 3** y **Figura 3** en **Publicación 4**). En consonancia con la respiración, las mayores concentraciones aplicadas de melatonina en las chirimoyas (0,05 y 0,1 mM), fueron las que al comienzo del almacenamiento redujeron significativamente la producción de etileno (**Publicación 4**). Sin embargo, la concentración más baja (0,01 mM) retuvo la producción de etileno con menores niveles que los mostrados por el resto de lotes observados hacia el final del experimento (**Tabla 4** y **Publicación 4**). Tanto la respiración como el etileno son parámetros que nos permiten evaluar la intensidad metabólica de los productos y tejidos vegetales (Valero y Serrano, 2010). Procesos tales como la maduración y la propia senescencia del fruto incrementan el coste energético al que hacen frente los tejidos vegetales a costa de sus reservas energéticas. En los estudios que se abordan en este epígrafe, las piñas y las chirimoyas son sometidas a un estrés adicional como es el almacenamiento a temperaturas subóptimas. En este sentido, la reducción tanto de la respiración como del etileno se ha relacionado recientemente con una mayor tolerancia a este tipo de estrés tanto en frutos climatéricos como no climatéricos tratados con melatonina (Madebo et al., 2022) y mediante otras tecnologías (Hakim et al., 1999; Park et al., 2021; Hasan et al., 2021). En los últimos años, ha sido demostrada la relación entre la reducción del metabolismo celular en los frutos tratados con melatonina y su acción estimulando la ruta de derivación del GABA. Esta ruta va a aportar sustratos energéticos adicionales como el propio GABA que son claves en situaciones de estrés con mayor demanda metabólica (Aghdam et al., 2018). Así, la melatonina a través de la estimulación de esta ruta estaría estabilizando el balance energético y con ello asegurando el correcto funcionamiento celular de forma más eficiente durante más tiempo. De hecho, cuando evaluamos la tolerancia al estrés provocado por las bajas temperaturas evaluando los daños por frío encontramos las diferencias porcentuales (**Tabla 4**) y visuales (**Publicaciones 3** y **4**) más importantes entre los frutos tratados con melatonina y controles en ambas especies. La concentración de sólidos solubles totales y acidez total tanto en las piñas como

en las chirimoyas fue similar al inicio del experimento en los frutos tratados con melatonina y controles (**Publicaciones 3** y **4**). Las aplicaciones con melatonina no alteraron el contenido en sólidos solubles durante el almacenamiento de las piñas, aunque sí retrasaron este parámetro en las chirimoyas. Además, en ambas especies los tratamientos con melatonina retrasaron la evolución de la acidez. Mientras que en la mayoría de los distintos frutos durante la maduración posrecolección o senescencia se da una pérdida de acidez (Valero y Serrano, 2010), la chirimoya se caracteriza por mostrar un incremento de la acidez total durante su maduración poscosecha (Martínez et al., 1993; Dattola et al., 2019). En este fruto tropical el retraso tanto en la acumulación de azúcares como en la evolución de la acidez total tuvo un comportamiento dosis-dependiente, ya que conforme se incrementó la concentración de melatonina aplicada a las chirimoyas el retraso en la evolución de estos parámetros fue superior (**Publicación 4**). Por esta razón, se puede constatar que el enlentecimiento en la maduración de las chirimoyas durante el almacenamiento podría ser debido a un retraso en la producción de etileno como se ha confirmado en chirimoyas tratadas mediante otras tecnologías (Yonemoto et al., 2002; Maldonado et al., 2004; Li et al., 2009). Asimismo, el retraso en la senescencia de las piñas y con ello, los mayores niveles de acidez encontrados en estos frutos tratados con melatonina podrían incrementar la aceptación del consumidor de esta variedad, caracterizada por bajos niveles de acidez al afectar a la relación azúcares-acidez de estos frutos (Bal, 2019). Un efecto similar al encontrado en las piñas se ha observado en otros frutos tratados con melatonina en los que, pese a que el contenido en sólidos solubles totales no fue alterado, sí se mantuvieron los niveles de acidez por más tiempo, tanto en fresas como en ciruelas tratadas con melatonina (Liu et al., 2018; Bal, 2019). En diferentes especies vegetales, se ha observado como las aplicaciones con melatonina estimulan la regulación de genes relacionados con la estructura celular, mejorando la estructura y la fluidez de la membrana (Sun et al., 2016; Zhai et al., 2018), al incrementar la concentración de ácidos grasos insaturados (Wang et al., 2020; Kong et al., 2020). Además, también se mantiene la firmeza en valores más elevados en los frutos tratados con melatonina con respecto a los frutos controles. Este efecto lo hemos observado también en las piñas y las chirimoyas donde las concentraciones estudiadas mantuvieron los niveles de firmeza desde el comienzo de los diferentes estudios (**Tabla 4** y **Publicaciones 3** y **4**). Es de destacar, que en el caso de las chirimoyas las mayores concentraciones de melatonina fueron las que mayores niveles de firmeza mantuvieron (**Tabla 4** y **Publicación 4**). Este hecho pudo deberse a la menor producción de etileno que observamos en las chirimoyas tratadas en poscosecha con melatonina, que al igual que en las piñas se observó un metabolismo reducido. La mayor firmeza junto con el mantenimiento del balance energético que permite el correcto funcionamiento celular, podrían estar evitando la muerte celular, además de los daños estructurales. A nivel celular, pudimos comprobar en la piel de las chirimoyas, como la compartimentalización celular se mantuvo de forma superior en los

frutos tratados con melatonina mediante la evaluación de la fuga de electrolitos (**Tabla 4** y **Publicación 4**). Este colapso celular conlleva oxidaciones de compuestos fenólicos y la aparición de los pardeamientos en los distintos frutos (Wang et al., 2020).

La concentración de melatonina que menos afectó a la tolerancia de las chirimoyas a las bajas temperaturas fue la más baja (0.01 mM) (**Tabla 4** y **publicación 4**). Esta dosis mostró un efecto escaso en el mantenimiento de la firmeza y de la integridad celular como se observó al evaluar la fuga de electrolitos. Sin embargo, es la que redujo por más tiempo la producción de etileno. De forma contraria, las concentraciones mayores, aunque no retrasaron tanto la producción de etileno, mantuvieron mayores niveles de firmeza y de integridad de las membranas. En este sentido fue la dosis intermedia la que mejor controló el mantenimiento de firmeza, y la reducción de la fuga de electrolitos por lo que finalmente los daños por frío también fueron menores a esta concentración. Esto nos llevaría a la hipótesis de que probablemente la mejora del estatus energético celular que permite el mantenimiento de las funciones celulares estimulado por la melatonina podría tener una repercusión mayor en la vida útil del fruto que la que la reducción de etileno "*per se*" provoca en la vida útil de los frutos climatéricos.

Los pardeamientos internos en el caso de las piñas (Paull y Rohrbach,1985) y los externos en la chirimoya (Pareek et al., 2011) disminuyen la calidad y vida útil de los frutos siendo esta fisiopatía agravada por el almacenamiento a bajas temperaturas. De hecho, el color CIE *L** externo de las chirimoyas mostró valores más bajos en los frutos controles que en los tratados con melatonina donde la incidencia del daño por frío fue inferior (**Tabla 4** y **publicación 4**). Este mantenimiento del color CIE *L** provocado por los tratamientos con melatonina también fue observado en la corteza externa de la piña. Este retraso se observó tanto durante el almacenamiento en frío como en el posterior almacenamiento a temperatura ambiente lo que denota un retraso en la senescencia de los frutos. Este efecto ha sido observado en un amplio número de productos vegetales como en fresas, lichi y brócoli tratados con melatonina en poscosecha (Liu et al., 2018; Zhang et al., 2018; Wu et al., 2020). Tanto en las piñas como en las chirimoyas este comportamiento está ligado a la pérdida de clorofilas. En el caso de la chirimoya, la pérdida de clorofilas además da paso a la formación de pardeamientos externos, fruto de la pérdida de la estabilidad de las membranas que facilitan la acción de la enzima polifenol oxidasa (PPO) (Alique et al., 1994). Esta enzima es también la responsable del pardeamiento interno que sufren las piñas (Murai et al., 2021). Además, en estudios anteriores se ha demostrado que el daño por frío en estos frutos es consecuencia de esa desestructuración de membranas, que causa la disfuncionalidad de las mitocondrias en los tejidos de los frutos almacenados a temperaturas subóptimas (Gutiérrez et al., 1992; Nukuntornprakit et al., 2020). De esta forma se provocaría la muerte celular debido a una producción

insuficiente de ATP para contrarrestar el estrés producido por las temperaturas subóptimas (Aghdam et al., 2018). La melatonina es capaz de reducir estos daños de membrana en varias frutas y hortalizas como el melocotón, la fresa, yuca y la sapota, a través de un mejor balance energético y una reducción de la peroxidación lipídica, aumentando la relación entre los ácidos grasos insaturados y saturados que conduce a una mayor integridad de la membrana. Además, se ha descrito la melatonina como un compuesto directamente relacionado con el mantenimiento de la funcionalidad de las mitocondrias a través de su actividad antioxidante (Reiter et al., 2016) y no sólo por el aporte extra de ATP mencionado anteriormente (Aghdam et al., 2018). De esta forma, el tratamiento con melatonina en las piñas y chirimoyas podría estar retrasando la aparición de los daños por frío mediante un mantenimiento de la homeostasis celular y el correcto funcionamiento de la célula.

La medida del color CIE *a** externo de los frutos también mostró una evolución más retrasada, tanto en las piñas como en las chirimoyas que fueron tratadas con melatonina, lo que pudo ser debido a los mayores niveles de clorofilas observados en la piel de las chirimoyas o en la corteza de las piñas (**Tabla 4** y **Publicación 3** y **4**). Esta pérdida de clorofila, tanto en los frutos como en la corona de la piña, induce a que otros parámetros de color como el ángulo CIE *hue** se reduzcan. La pérdida de clorofilas también está relacionada con la pérdida de la integridad celular además de ser una de las principales dianas de las especies reactivas de oxígeno (ROS) producidas por el metabolismo celular en los distintos tejidos vegetales (Khanna-Chopra, 2012), ya que los sistemas de detoxificación de los ROS se reducen a lo largo de la senescencia de las plantas. Son varios los estudios que demuestran el efecto de las aplicaciones exógenas con melatonina a la hora de mantener los niveles de clorofilas. En ellos se observa que la melatonina a través del mantenimiento de la turgencia celular, además de una reducción en la actividad clorofilasa, producen una estimulación de la actividad fotosintética y un adecuado balance del sistema antioxidante, lo que reduce la oxidación de las clorofilas (Arnao y Hernández-Ruiz, 2019; Sun et al., 2021).

Aunque en las piñas la acción de la melatonina sobre la actividad antioxidante no se evaluó, este efecto se estudió en la pulpa de las chirimoyas sometidas a diferentes concentraciones de melatonina (**Tabla 5**).

Tabla 5: *Análisis comparativo porcentual (%) entre los valores medios determinados en chirimoyas tratadas en poscosecha con melatonina a distintas concentraciones y los frutos control durante su almacenamiento tras 21 días de almacenamiento a temperaturas subóptima.*

	CHIRIMOYA		
PARAMETROS	**MT 0,01 mM**	**MT 0,05 mM**	**MT 0,1 mM**
AAT (Hidrosoluble) (mg 100 g^{-1})	↓ 36,93	↓ 47,01	↓ 30,54
AAT (Liposoluble) (mg 100 g^{-1})	↓ 41,27	↓ 62,87	↓ 47,86
Polifenoles totales (mg 100 g^{-1})	↓ 28,24	↓ 50,04	↓ 32,39

** Los valores de cada parámetro se han calculado con respecto a los obtenidos en los frutos control o sin tratar de la Publicaciones 4 descrita en esta Tesis Doctoral.*

A través de los resultados obtenidos, pudimos comprobar que tanto los niveles de actividad antioxidante como los del contenido en compuestos fenólicos totales estuvieron muy afectados por el tratamiento con melatonina. En general, observamos en todos los frutos un retraso en la acumulación de compuestos fenólicos, así como en la actividad antioxidante de la fracción hidrosoluble y liposoluble. Además, el mayor retraso en estos parámetros fue el observado para la concentración de melatonina que mejor controló los distintos parámetros de calidad poscosecha descritos anteriormente (0,05 mM). Las chirimoyas, al igual que otros frutos climatéricos y no climatéricos, incrementan sus propiedades antioxidantes y su contenido en polifenoles totales a lo largo de su maduración (Campos-Vargas et al., 2008). Son muchos los estudios en los que se observa una mayor actividad antioxidante y una mayor estimulación en la acumulación de compuestos fenólicos en los frutos tratados con melatonina (Bhardwaj et al., 2022; Mirshekari y Madani, 2022). Pero también, son varios los estudios en los que se observa una menor acumulación de compuestos bioactivos en los frutos tratados con melatonina. En este sentido, los tratamientos con melatonina incrementaron en menor medida el contenido fenólico en la piel del plátano, lo que además condujo a un menor daño por frío (Wang et al., 2022). Este efecto de la melatonina retrasando la acumulación en compuestos fenólicos también se ha observado en otros frutos como las ciruelas (Xu et al., 2022). Por tanto, el retraso producido por la melatonina sobre la maduración de los frutos sería la causa por la que los niveles antioxidantes fueron menores que los observados en los frutos controles. Este hecho indicaría que los tratamientos con melatonina no redujeron los daños por frío debido a un mantenimiento o incremento del estatus antioxidante, sino por una mayor homeostasis celular que mejoraría la firmeza y por un menor

metabolismo celular como se ha observado en parámetros anteriormente estudiados indicando un importante retraso en la senescencia del fruto.

5.4. Efecto sinérgico de la melatonina y del 1-metilciclopropeno como tecnología combinada sobre la calidad y vida útil del calabacín.

Tras aplicar los distintos tratamientos basados en aplicaciones de melatonina y 1-MCP por separado en los frutos o bien mediante una combinación de ambos tratamientos pudimos comprobar que no todos los tratamientos aplicados fueron capaces de retrasar las pérdidas de peso (**Tabla 6** y **Publicación 5**).

Tabla 6: *Análisis comparativo porcentual (%) entre los valores medios determinados en calabacines tratados en poscosecha con melatonina 1 mM y con 1-MCP de forma individual o combinada con respecto a los frutos control durante su almacenamiento tras 9 días de almacenamiento a temperaturas subóptimas.*

	CALABACÍN		
PARAMETROS	**MT**	**1-MCP**	**MT + 1-MCP**
Pérdida de peso (%)	-	-	↓ 21,11
Firmeza (N)	↑ 8,39	↑ 10,39	↑ 19,82
Daños por frío (escala 0-5)	-	-	↓ 23,8
Respiración (mg kg^{-1} h^{-1})	↓ 1,51	↓ 11,23	↓ 29,64
Etileno (nL g^{-1} h^{-1})	↓ 37,19	↓ 33,36	↓ 28,62
MDA (µmol kg^{-1})	↓ 24,49	↓ 41,75	↓ 38,96
Fuga de electrolitos (%)	↓ 16,70	↓ 35,56	↓ 53,46
Clorofilas (mg 100 g^{-1})	-	↑ 11,20	↑ 12,95
Color CIE *hue**	↑ 0,20	↑ 0,22	↑ 0,45
Sólidos solubles totales (g 100 g^{-1})	-	↑ 9,92	↑ 3,19
Acidez total (g 100 g^{-1})	-	-	↑ 12,05

** Los valores de cada parámetro se han calculado con respecto a los obtenidos en los frutos control o sin tratar de la Publicación 5 descrita en esta Tesis Doctoral.*

Las pérdidas de peso incrementaron durante el almacenamiento refrigerado en todos los lotes. Sin embargo, pese a que los tratamientos con melatonina y 1-MCP por sí solos no afectaron a este parámetro, la aplicación de los tratamientos de forma combinada redujo las pérdidas de peso hasta un 21 % las perdidas de peso tras 9 días de almacenamiento a 4 ºC más dos días a 20 ºC. Sin embargo, todos los tratamientos fueron eficaces a la hora de mantener la firmeza de los frutos. Los tratamientos con melatonina, así como los frutos tratados con 1-MCP, sólo o combinado con melatonina, mantuvieron mayores niveles de firmeza que los observados en los frutos controles. Los mayores niveles de firmeza se observaron de forma general en los frutos que fueron tratados mediante la combinación de los tratamientos (MT + 1-MCP), ya que mostraron valores de firmeza superiores a los observados en los frutos que se trataron con estas sustancias por separado (**Tabla 6** y **Publicación 5**).

Los calabacines son frutos muy sensibles a las bajas temperaturas. De hecho, todos los lotes mostraron esta sensibilidad a lo largo del almacenamiento. Aunque tanto la melatonina como el 1-MCP retrasaron la aparición de estos daños, la combinación de tratamientos fue capaz de retrasar hasta 6 días la incidencia de los daños por el frío con respecto a los frutos controles y hasta 3 días más con respecto a los frutos tratados únicamente con 1-MCP. De hecho, este efecto se puede observar claramente en la **Tabla 6** como tras 9 días de almacenamiento sólo los frutos que recibieron el tratamiento combinado continuaba mostrando tolerancia al almacenamiento refrigerado.

Durante el almacenamiento a temperaturas subóptimas, tanto el peso como la firmeza descienden principalmente debido a la transpiración que además se ve incrementada debido a la incidencia del pitting que sufre el calabacín en estas condiciones. Por esta razón, las pérdidas de peso afectan a la turgencia celular reduciendo la firmeza (Lin y Pitt, 1986; Hayes y Sealey, 1996), por lo que estos dos parámetros suelen estar correlacionados entre ellos y con el daño por frío. El efecto observado sobre las pérdidas de peso, la firmeza y los daños por frío al aplicar melatonina y 1-MCP como tratamiento combinado fue significativamente mayor que cuando se aplicaron estas sustancias por separado. Por esta razón podemos considerar que se establece un efecto sinérgico entre ambos tratamientos. Los tratamientos realizados únicamente con 1-MCP en los calabacines de este estudio (**Publicación 5**) y de estudios anteriores con esta misma variedad (Megías et al., 2016), no afectaron a las pérdidas de peso, aunque coincidiendo con nuestros resultados el 1-MCP por sí sólo fue capaz de retrasar ligeramente los daños por frío. De forma similar, los tratamientos realizados únicamente con melatonina no afectaron a las pérdidas de peso del calabacín como se ha observado en otros frutos como tomates y ciruelas (Sun et al., 2015; Bal, 2019) aunque ha mostrado efectos positivos controlando las pérdidas de peso en otras especies vegetales (Jayarajan y Sharma, 2021). Sin embargo, sí observamos un efecto

positivo aunque débil a la hora de retrasar tanto las pérdidas de firmeza como la evolución del daño por frío (**Publicación 5**). Estudios previos han confirmado estimulación o regulación positiva de la expresión de distintos genes relacionados con la estructura de la pared celular (Sun et al., 2015; Zhai et al., 2018). Además, no se puede descartar el efecto positivo adicional que podría estar ejerciendo la actividad antioxidante de la melatonina evitando la peroxidación de las membranas celulares que podría reducir la oxidación de compuestos fenólicos mediante una inhibición de enzimas tales como la peroxidasa o la PPO (Dan et al., 2015; Zhang et al., 2018). Por esta razón en esta Tesis Doctoral proponemos que la aplicación conjunta de 1-MCP con melatonina podría estar ejerciendo un efecto sinérgico mediante el retraso de la desintegración celular, y un menor estrés oxidativo como la razón por la que en los calabacines tratados con un tratamiento combinado el control sobre el daño por el frío fue más eficaz.

Tanto la respiración como la producción de etileno aumentaron a lo largo del almacenamiento hasta el final del estudio, en cuyo muestreo final ambos parámetros se redujeron. Estos dos parámetros fueron reducidos por los distintos tratamientos aplicados de forma individual o combinada (**Tabla 6** y **Publicación 5**). La respiración está relacionada con las pérdidas de peso por lo que estos parámetros suelen estar correlacionados. En la variedad 'Cronos' objeto de este estudio, trabajos anteriores han demostrado la capacidad del 1-MCP a la hora de reducir tanto la respiración como la producción de etileno siendo coincidente con nuestros resultados (Megías et al., 2016). Una menor respiración y una menor producción de etileno se han relacionado con un menor impacto de los daños por el frío en cucurbitáceas (Hakim et al., 1999; Megías et al., 2016). La melatonina estimula la ruta de derivación del GABA y su acumulación de este tanto en frutos climatéricos como en no climatéricos (Shelp et al., 1999; Aghdam y Fard, 2017; Sharafi et al., 2019). Esta estimulación proporciona a las células un inmediato sustrato energético que utilizan para reponerse del estrés causado por las bajas temperaturas y el proceso de senescencia, cubriendo las necesidades energéticas inmediatas de los tejidos vegetales al mejorar el balance energético (Aghdam y Fard, 2017). Aunque en este estudio la melatonina por sí sola no afectó de forma importante a la respiración, el 1-MCP sí fue capaz por sí sólo de retrasar este parámetro. Sin embargo, cuando los tratamientos se aplicaron de forma combinada se observó una acción sinérgica entre ambos ya que este tratamiento tuvo un importante impacto reduciendo la respiración de los frutos con respecto del resto de tratamientos aplicados. Este beneficio adicional también se observó en la producción de etileno, donde la aplicación conjunta tuvo un mayor efecto en la reducción del etileno que la aplicación de los tratamientos por separado (**Publicación 5**), pese a que estos también tuvieron un efecto positivo en el retraso de este parámetro (**Tabla 6** y **Publicación 5**). La melatonina ha sido descrita como un regulador de la expresión de distintos genes involucrados en la síntesis de etileno, siendo capaz de reducir esta expresión (Kou et al., 2021).

Por otro lado, el 1-MCP es un potente inhibidor de la acción del etileno lo que explicaría la reducción en la producción de esta fitohormona pese a que la combinación de tratamientos no redujo el etileno en mayor proporción que cuando se aplicaron de forma individual (**Tabla 6** y **Publicación 5**). Por esta razón, y dado que los valores de firmeza se mantuvieron mejor en los calabacines tratados de forma conjunta con ambas sustancias, la mayor tolerancia a los daños por frío podría estar apuntando a un mejor balance antioxidante provocado por la aplicación de melatonina. Este hecho se confirmó al evaluar el contenido en MDA que es un producto de la peroxidación lipídica de las membranas plasmáticas (Bi et al., 2022). Así, el contenido en MDA fue inferior a lo largo de todo el estudio en los frutos tratados con melatonina o 1-MCP individualmente o mediante un tratamiento combinado con respecto al observado en los frutos control. De forma análoga, esta efectividad fue observada también a la hora de reducir la fuga de electrolitos consecuencia de la desintegración de las membranas celulares. Los tratamientos aplicados individualmente retrasaron la fuga de electrolitos con respecto a los frutos control, pero cuando se aplicaron de forma conjunta la reducción fue mayor (**Tabla 6** y **Publicación 5**). En estudios previos se ha observado cómo tanto la melatonina (Arnao y Hernández-Ruiz, 2019; Wang et al., 2020) como el 1-MCP (Cao et al., 2012; Xu y Liu, 2017) son capaces de estimular la actividad de los sistemas antioxidantes enzimáticos implicados en la detoxificación celular de radicales libres oxidativos, reduciendo los niveles de MDA y de fuga de electrolitos en consonancia con nuestros resultados. Por tanto, esta podría ser la causa del efecto positivo observado en ambos parámetros, así como del mayor impacto en estos parámetros cuando ambos tratamientos se aplicaron de forma conjunta, que podría estar dotando a los frutos de una mayor tolerancia a las bajas temperaturas.

Con respecto al contenido en clorofilas de la piel y las connotaciones derivadas de este contenido sobre la tonalidad del fruto, pudimos comprobar que, pese a que las aplicaciones con melatonina de forma individual no afectaron a estos parámetros, el 1-MCP y su combinación con melatonina tuvieron un efecto marcadamente positivo en ambas determinaciones (**Tabla 6** y **Publicación 5**). De hecho, estos dos tratamientos mantuvieron mayores niveles de clorofilas y retrasaron la evolución del ángulo CIE *hue**. En este sentido los mejores resultados se obtuvieron cuando se aplicaron los tratamientos de forma combinada. El color verde en el calabacín 'Cronos' así como en otras especies vegetales, viene determinado por el contenido en clorofila y su degradación está correlacionada con una bajada en el ángulo CIE *hue**. Esta degradación en la variedad de calabacín objeto de estudio es principalmente debida la pérdida de integridad celular, que reduce la firmeza y contribuye a la degradación de las clorofilas (Blanco-Díaz et al., 2016). En este sentido, el 1-MCP ha demostrado su capacidad en el mantenimiento de la firmeza de los tejidos y del contenido en clorofila retrasando la senescencia tanto en frutos climatéricos como no climatéricos (Watkins, 2006; Du et al.,

2021). Además, las clorofilas son las principales dianas de las ROS, y estos radicales libres no son detoxificados apropiadamente conforme avanza la senescencia de la planta o durante distintos tipos de estrés (Khanna-Chopra, 2012). Asimismo, en distintos estudios se ha puesto de manifiesto la capacidad de los tratamientos poscosecha con melatonina a la hora de retrasar el desverdizado de los frutos manteniendo los niveles de clorofilas en brócoli, mango y otras cucurbitáceas (Wei et al., 2020; Dong et al., 2021; Madebo et al., 2021). Sin embargo, aunque en nuestro estudio los tratamientos con melatonina no mostraron este efecto en los calabacines, el 1-MCP sí fue capaz de mantener ambos parámetros. Por esta razón, planteamos la hipótesis de que el efecto sinérgico observado cuando se aplican ambas sustancias de forma conjunta podría ser debido a una mejor actuación de la melatonina sobre las ROS producidas por una mejora en la homeostasis celular de la que sería responsable el 1-MCP. Es decir, el mantenimiento en la integridad celular provocado por el 1-MCP podría estar permitiendo de forma óptima la actividad de la melatonina en la detoxificación de las ROS. De hecho, cuando el 1-MCP se aplica de forma aislada, retrasa el incremento del MDA, la fuga de electrolitos y la evolución del color satisfactoriamente, mientras que la melatonina por sí sola tiene un impacto menor en estos parámetros (**Tabla 6** y **Publicación 5**).

Los SST en el calabacín, componen una importante característica de calidad que refleja la concentración de azúcar en las células que suele incrementar tras la hidrólisis del almidón a lo largo del almacenamiento. Sin embargo, en este estudio y en consonancia con otros estudios realizados en calabacín, parece que a 20 ºC el consumo de los azúcares se da a un ritmo más acelerado que su propia acumulación conforme avanza la senescencia de este fruto (Zhang et al., 2019; Jafari et al., 2022). Por otro lado, el contenido de acidez, al igual que en otros frutos, suele descender durante la conservación del calabacín debido a la movilización de los ácidos orgánicos como sustratos de la respiración (Valero y Serrano, 2010; Jafari et al., 2022). En nuestro estudio, tanto los niveles de SST como de AT descendieron a lo largo de la conservación del calabacín en todos los lotes aunque con diferencias dependiendo del tratamiento aplicado (**Publicación 5**). Así, los frutos tratados únicamente con melatonina fueron los que menores valores tanto de SST como de AT mostraron junto con los frutos control. Sin embargo, tanto los frutos tratados con 1-MCP como aquellos tratados con el tratamiento combinado (MT + 1-MCP), mantuvieron niveles superiores con respecto al control y los frutos tratados con melatonina en ambos parámetros. Asimismo, los frutos que recibieron la combinación de tratamientos fueron los únicos que mantuvieron mayores niveles de acidez con respecto a los frutos control tras 9 días de almacenamiento en frío más 2 días a 20 ºC (**Tabla 6** y **Publicación 5**).

Distintos estudios han revelado el importante efecto del 1-MCP en distintas variedades de calabacín retrasando los procesos de maduración y

reduciendo la respiración del fruto (Megías et al., 2016) y así manteniendo el contenido en SST y AT en diferentes especies vegetales (Watkins, 2006; Valero y Serrano, 2010). Aunque no existen estudios previos que evalúen el impacto de la melatonina sobre los calabacines, en una reciente revisión la melatonina ha sido descrita como una sustancia capaz de inducir tolerancia al almacenamiento en frío mediante la acumulación tanto de azúcares como de ácidos orgánicos en distintas especies (Zeng et al., 2022). Sin embargo, los tratamientos que contenían únicamente melatonina no incrementaron ni los SST ni la AT lo que probablemente fue debido al patrón de respiración similar al observado en los frutos control y tratados (**Publicación 5**). Por esta razón, y basándonos en nuestros resultados, el tratamiento combinado (MT + 1-MCP) aunque no incrementó el contenido en SST a mayor concentración que el observado en los frutos tratados con 1-MCP, sí que mostró un efecto sinérgico manteniendo el contenido en AT de los frutos tratados con melatonina y 1-MCP de forma conjunta. Este efecto podría ser debido a un retraso en el proceso de senescencia de los frutos y en la respiración provocado por el 1-MCP, así como al mayor contenido en ácidos orgánicos que son capaces de provocar los tratamientos con melatonina, como se ha observado en la mayor parte de especies vegetales tratadas en poscosecha con melatonina (Zeng et al., 2022). En este sentido, una mayor concentración de solutos en las células vegetales es un factor beneficioso a la hora de mantener la osmorregulación del protoplasma mejorando así la tolerancia al frío. De hecho, altos niveles tanto de SST como AT están directamente relacionados con el mantenimiento de la homeostasis celular reduciendo el impacto de los daños por frío a través de un mayor contenido en azúcares y ácidos orgánicos tales como el ácido ascórbico o el ácido cítrico (Boonyaritthongchai y Supapvanich, 2017; Sangsoy et al., 2022; Zeng et al., 2022).

6 Conclusiones

6. CONCLUSIONES

En esta Tesis Doctoral se ha estudiado la aplicación de melatonina como una estrategia precosecha y poscosecha de origen natural capaz de mejorar la calidad de frutos climatéricos y no climatéricos tanto en la cosecha como durante su almacenamiento poscosecha. El estudio de nuevas herramientas de origen natural que muestran un impacto positivo en la calidad de los frutos satisface las demandas tanto de consumidores como de productores. Por tanto, las aplicaciones con melatonina podrían constituir una novedosa alternativa de origen natural, capaz de mejorar importantes aspectos en la calidad de los frutos dadas las conclusiones generales que se detallan a continuación.

1. Las aplicaciones precosecha con melatonina 0,1 mM incrementaron la producción de granadas y albaricoques, principalmente debido a un mayor tamaño del fruto, entre otros factores, por lo que esta tecnología podría suponer un importante beneficio económico para los productores.

2. La melatonina 0,1 mM aplicada en precosecha incrementó la calidad en la cosecha de granadas y albaricoques, al aumentar la firmeza de los frutos y los niveles de sólidos solubles y acidez. Además, incrementaron la coloración de los frutos, hecho que es especialmente de interés en las granadas estudiadas, las cuales incrementaron la coloración tanto externa como interna de los frutos.

3. Las granadas y los albaricoques tratados con melatonina 0,1 mM en precosecha fueron capaces de mostrar una mayor vida útil tanto a temperaturas óptimas como a temperaturas subóptimas al retrasar la maduración y la senescencia de los distintos frutos estudiados.

4. Los frutos tratados con melatonina en poscosecha incrementaron su vida útil al reducir su actividad metabólica y la evolución de los parámetros de maduración y senescencia.

5. Los daños por frío fueron drásticamente reducidos durante la conservación poscosecha en albaricoques y piña tratados con melatonina 0,1 mM. Asimismo, un efecto similar se observó en calabacín y chirimoya tratados con melatonina 1 mM y 0,05 mM respectivamente. Esta reducción se debe probablemente a la mayor firmeza y mantenimiento de las membranas celulares que mostraron los frutos estudiados.

6. En esta Tesis Doctoral se demuestra por primera vez que los aspectos beneficiosos que aportan los tratamientos de los calabacines con melatonina son mejorados si esta estrategia natural es aplicada en combinación con tecnologías aplicadas comercialmente como el 1-

metilciclopropeno. Los efectos combinados sobre la firmeza de los frutos y el mantenimiento de distintos compuestos bioactivos podrían ser la razón por la que la vida útil del fruto es incrementada y la acción de los daños por frío reducida.

7

Futuras líneas de investigación

7. FUTURAS LÍNEAS DE INVESTIGACIÓN

Los resultados obtenidos en esta Tesis Doctoral han demostrado los beneficios que pueden proporcionar, tanto a nivel productivo como durante la conservación, permitiendo además la aplicación de temperaturas más bajas y por tanto alargando la vida útil de los frutos. Los hallazgos observados en este estudio podrían permitir la transferencia de esta tecnología a las empresas y cooperativas interesadas. Además, abre nuevas posibilidades de estudio para incrementar u optimizar los tratamientos con melatonina por lo que las futuras líneas de actuación serían las siguientes:

1. Desarrollar la transferencia de esta tecnología natural mediante la realización de proyectos CDTI que permitan el desarrollo comercial de las aplicaciones con melatonina en pre y poscosecha. La melatonina muestra un excelente potencial a nivel productivo y como agente conservador. Es eficiente a la hora de incrementar la firmeza de los frutos, y ese podría ser un factor crítico que pudiera mejorar la resistencia de los frutos a otras fisiopatías que no se han estudiado en esta Tesis Doctoral, como el agrietado de los frutos en precosecha, la incidencia de las plagas y la falta de coloración de los frutos. Estas fisiopatías están muy relacionadas con el cambio climático por lo que la melatonina podría ser una herramienta con la que trabajar en esta línea.

2. Pese a que la mayor firmeza en los frutos tratados con melatonina, tanto en precosecha como en poscosecha, podría ser la responsable principal en la reducción de los daños por frío de las distintas especies vegetales estudiadas en esta Tesis Doctoral, la evaluación de la actividad de las enzimas antioxidantes, el contenido en ácido gamma-aminobutírico, así como la expresión de distintos genes relacionados con la integridad de las membranas sería de gran interés y ayudaría a dilucidar los mecanismos de acción de la melatonina que podrían estar actuando de forma conjunta.

3. La aplicación de melatonina podría mejorar otras tecnologías de conservación ya existentes como el uso de atmósferas controladas o las aplicaciones con 1-metilciclopropeno. Esta última, combinada con melatonina ha mostrado efectos sinérgicos, por lo que abre la puerta a la experimentación con tecnologías combinadas que permitan la comercialización a mercados lejanos con los mayores estándares de calidad.

8 Referencias

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