Universidad Miguel Hernández de Elche

Programa de Doctorado en Recursos y Tecnologías Agrarias, Agroambientales y Alimentarias

Mejora de la calidad de la cereza en la cosecha y durante el almacenamiento mediante el uso de elicitores

Tesis Doctoral

Alberto Carrión Antolí

2025

Director: Dr. Daniel Valero Garrido

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La presente Tesis Doctoral, titulada "**Mejora de la calidad de la cereza en la cosecha y durante el almacenamiento mediante el uso de elicitores**", se presenta bajo la modalidad de **tesis por compendio** de las siguientes **publicaciones**:

PUBLICACIÓN 1

Título: Effects of Melatonin Treatment on Sweet Cherry Tree Yield and Fruit Quality
Autores: Carrión-Antolí, A., Lorente-Mento, J.M., Valverde, J.M., Castillo, S., Valero, D., & Serrano, M.
Fecha de publicación: 22 de diciembre de 2021
Revista: Agronomy
Editorial: Multidisciplinary Digital Publishing Institute (MDPI)
ISSN: 2073-4395
Factor de impacto (2023): 3.3
Factor de impacto (5 años): 3.7
Categoría JCR®: Agronomy
Rango: 20/126
Cuartil: Q1
CiteScore: 6.2
DOI: https://doi.org/10.3390/agronomy12010003

PUBLICACIÓN 2

Título: Melatonin Pre-harvest Treatments Leads to Maintenance of Sweet Cherry Quality During Storage by Increasing Antioxidant Systems
Autores: Carrión-Antolí, A., Martínez-Romero, D., Guillén, F., Zapata, P.J., Serrano, M., & Valero, D.
Fecha de publicación: 11 de abril de 2022
Revista: Frontiers in Plant Science
Editorial: Frontiers Media S.A.
ISSN: 1664-462X
Factor de impacto (2023): 4.1
Factor de impacto (5 años): 5.3
Categoría JCR®: Plant Sciences
Rango: 44/265
Cuartil: Q1
CiteScore: 7.3
DOI: https://doi.org/10.3389/fpls.2022.863467

PUBLICACIÓN 3

Título: Antioxidant Systems and Quality in Sweet Cherries Are Improved by Preharvest GABA Treatments Leading to Delay Postharvest Senescence

Autores: Carrión-Antolí, A., Badiche-El Hilali, F., Lorente-Mento, J.M., Díaz-Mula, H.M., Serrano, M., & Valero, D.

Fecha de publicación: 23 de diciembre 2023

Revista: International Journal of Molecular Sciences

Editorial: Multidisciplinary Digital Publishing Institute (MDPI)

ISSN: 1661-6596

Factor de impacto (2023): 4.9

Factor de impacto (5 años): 5.6

Categoría JCR®: Biochemistry & Molecular Biology

Rango: 66/313

Cuartil: Q1

CiteScore: 8.1

DOI: https://doi.org/10.3390/ijms25010260

PUBLICACIÓN 4

Título: Preharvest Gibberellic Acid Treatment Increases Both Modulus of Elasticity and Resistance in Sweet Cherry Fruit (cv. 'Bing' and 'Lapins') at Harvest and Postharvest During Storage at 0 °C

Autores: Carrión-Antolí, A., Zoffoli, J.P., Serrano, M., Valero, D., & Naranjo, P.

Fecha de publicación: 20 de noviembre de 2024

Revista: Agronomy

Editorial: Multidisciplinary Digital Publishing Institute (MDPI)

ISSN: 2073-4395

Factor de impacto (2023): 3.3

Factor de impacto (5 años): 3.7

Categoría JCR®: Agronomy

Rango: 20/126

Cuartil: Q1

CiteScore: 6.2

DOI: https://doi.org/10.3390/agronomy14112738

El **Dr. D. Daniel Valero Garrido**, director, y la **Dra. Dña. María Serrano Mula**, codirectora de la tesis doctoral titulada "**Mejora de la calidad de la cereza en la cosecha y durante el almacenamiento mediante el uso de elicitores**".

INFORMA/N:

Que **D. Alberto Carrión Antolí** ha realizado bajo nuestra supervisión el trabajo titulado "**Mejora de la calidad de la cereza en la cosecha y durante el almacenamiento mediante el uso de elicitores**" 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.

Lo que firmo/firmamos para los efectos oportunos, en Orihuela a 17 de marzo de 2025

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INFORMA:

Que **D. Alberto Carrión Antolí** ha realizado bajo la supervisión de nuestro Programa de Doctorado el trabajo titulado "**Mejora de la calidad de la cereza en la cosecha y durante el almacenamiento mediante el uso de elicitores**" 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.

Lo que firmo para los efectos oportunos, en Orihuela a 17 de marzo de 2025

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Esta Tesis Doctoral ha sido financiada por el Ministerio de Ciencia, Innovación y Universidades y por la Comisión Europea con los fondos FEDER, mediante el proyecto RTI2018-099664-B-I00 y también a través del proyecto PROMETEO/2021/089 de la Direcció General de Ciència i Investigació de la Generalitat Valenciana. Alberto Carrión Antolí ha sido beneficiario de una beca FPU (Formación Profesorado Universitario) con referencia FPU18/04112, que ha sido financiada por el Ministerio de Ciencia, Innovación y Universidades. Durante la estancia de investigación internacional en la Pontificia Universidad Católica de Chile, el Laboratorio de Fisiología y Tecnología Postcosecha participó en la financiación del trabajo de investigación.

Agradecimientos

Escribir estas líneas significa para mí que se aproxima la finalización de una etapa y el comienzo de otras, por ello quiero recordar a los que me ha acompañado y apoyado durante este periodo.

A mis directores el Dr. Daniel Valero y la Dra. María Serrano, por darme la oportunidad de realizar esta Tesis Doctoral, además de gestionar y dirigir el trabajo. Agradecer a la Dra. María Serrano su implicación y supervisión del trabajo realizado.

Al Dr. Domingo Martínez y al Dr. Juan Miguel Valverde, por su colaboración y compartir sus conocimientos en los distintos ámbitos. Al Dr. Fabián Guillén por su apoyo y buenos ratos que hemos compartido. Al Dr. Salvador Castillo, por inmortalizar las largas jornadas de trabajo.

A José Manuel, que más que un compañero de laboratorio es un gran amigo, gracias por colaborar en este trabajo y apoyarme durante su desarrollo, no olvidaré los momentos que compartimos.

A Fátima, Emma, Celeste, Alba, Fernando, Alberto, Ander y todos los compañeros de laboratorio con los que he compartido estos años, les agradezco su compañía y apoyo.

A los miembros de la Pontificia Universidad Católica de Chile, en especial al Dr. Juan Pablo Zoffoli y a Paulina Naranjo, por darme la oportunidad de realizar la estancia de investigación y orientarme en el trabajo realizado. A Román Toro, por su colaboración en las largas jornadas de campo y sus consejos, los cuales no olvidaré. A mis compañeros de laboratorio Angeli, Ángela, Ignacio, Javi y María Emilia, por su colaboración y por hacerme sentir como si estuviera en casa.

A las empresas Finca Toli y Garcés Fruit, por permitirnos utilizar sus instalaciones y cultivos.

A mis amigos, cuya amistad es un pilar fundamental, expreso mi más sincero agradecimiento por su compañía y respaldo en este proyecto.

A mi familia, por su apoyo y motivación. A mis padres, por todo su esfuerzo y buenos consejos para que pudiera llegar hasta aquí. A mi hermana, por su constante apoyo. A mis abuelos, gracias por todo.

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LISTADO DE ABREVIATURAS

1-MCP → 1-Metilciclopropeno

ASMT → Acetilserotonina metiltransferasa

CE → Comunidad Europea

EDTA → Ácido etilendiaminotetraacético

EFSA → Autoridad Europea de Seguridad Alimentaria

ES → Error estándar

GA3 → Ácido giberélico

GABA → Ácido gamma-aminobutírico

GAD → Glutamato descarboxilasa

HPLC → Cromatografía líquida de alta resolución

LMR → Límite máximo de residuos

MAP → Atmósfera modificada

PAL → Fenilalanina amonio liasa

PDH → Δ^1-pirrolina deshidrogenasa

PF → Peso fresco

PVDF → Fluoruro de polivinilideno

SC → Suspensión concentrada

SG → Gránulos solubles

SNAT → Serotonina N-acetiltransferasa

T5H → Triptamina hidrolasa

TDC → Triptófano descarboxilasa

UE → Unión Europea

RESUMEN

La cereza (*Prunus avium* L.) es un fruto muy apreciado por los consumidores por sus atributos internos, como el sabor, la relación sólidos solubles/acidez, la textura y el contenido de compuestos bioactivos, aunque también es muy valorada por sus características externas, como el tamaño, el color rojo intenso, la ausencia de defectos y pedicelos verdes y turgentes. Pero es un fruto muy sensible, en el que la maduración y senescencia evolucionan rápidamente tras la cosecha, reduciendo los atributos de calidad del fruto incluso en condiciones adecuadas de almacenamiento.

En los últimos años, se han investigado distintas estrategias precosecha enfocadas a incrementar la calidad del fruto en el momento de la recolección y mantenerla durante su almacenamiento. Por ello, la aplicación de elicitores en los cultivos es una estrategia interesante en la agricultura actual, ya que son compuestos que se encuentran naturalmente en las plantas, que inducen cambios fisiológicos y morfológicos que mejoran los parámetros de calidad de los frutos, de manera sostenible y respetuosa con el medio ambiente. En esta Tesis Doctoral se ha estudiado el efecto de la aplicación precosecha de tratamientos con melatonina, ácido gamma-aminobutírico (GABA) y ácido giberélico (GA3), con la finalidad de mejorar los parámetros de calidad de la cereza, tanto en el momento de la recolección como durante su almacenamiento postcosecha.

Los elicitores se aplicaron mediante pulverización foliar durante el desarrollo del fruto en el árbol, en las variedades 'Prime Giant', 'Lapins' y 'Sweet Heart', para melatonina se utilizaron dosis 0,1 mM, 0,3 mM y 0,5 mM, para GABA se emplearon dosis de 10 mM, 50 mM y 100 mM, en las disoluciones se añadió 0,1 % Tween-20 como surfactante. Para ambos elicitores se realizaron tres aplicaciones, coincidiendo con los estados fenológicos de endurecimiento de hueso, cambio de color y tres días antes de recolección, los ensayos se llevaron a cabo en la campaña de cultivo 2019 y 2020 en España. En la campaña de cultivo 2022 se realizó otro ensayo en Chile, en el que se realizaron tratamientos con GA3 en la variedad 'Bing' y 'Lapins', con 30 ppm fraccionadas en 15 ppm y 15 ppm, 45 ppm fraccionadas en 25 ppm y 20 ppm, y 60 ppm fraccionadas en 30 ppm y 30 ppm, la primera aplicación se realizó en endurecimiento de hueso y la segunda en color pajizo. En todos los ensayos se disponían de controles que fueron pulverizados con agua.

Los resultados muestran que durante dos años consecutivos, los tratamientos precosecha con melatonina o GABA mejoraron los atributos de calidad de la cereza. Los sólidos solubles, la acidez y firmeza fueron superior en los frutos tratados que en los

controles, tanto en el momento de la cosecha como durante el almacenamiento, estos elicitores también redujeron las pérdidas de peso durante el periodo postcosecha. Los compuestos bioactivos como fenoles y antocianinas, incrementaron su concentración cuando se aplicó GABA o melatonina y el color se incrementó correlacionándose con el contenido de antocianinas. La actividad de las enzimas antioxidantes como catalasa, ascorbato peroxidasa y peroxidasa aumentó en los frutos tratados, tanto en el momento de la cosecha como durante el almacenamiento. El incremento de los sistemas antioxidantes enzimáticos y no enzimáticos como consecuencia de la aplicación de GABA o melatonina, mejoraron la eficiencia de los tejidos para neutralizar las especies reactivas del oxígeno, retrasando el proceso de senescencia y manteniendo los parámetros de calidad durante el almacenamiento.

Respecto a los tratamientos con GA3, los resultados mostraron que la cosecha se retrasó entre dos y cuatro días en función de la dosis. Independientemente de la concentración utilizada, los frutos tratados presentaron una mayor resistencia en el momento de la cosecha, debido al incremento del módulo de elasticidad y de la tensión en el punto máximo, manteniéndose constante la deformación del tejido. Sin embargo, después de 35 días de almacenamiento a 0°C en bolsa de atmósfera modificada (MAP), los frutos mantuvieron una alta tensión en el punto máximo y un elevado módulo de elasticidad, pero disminuyó la deformación del tejido, lo que generó un fruto más rígido. Por otro lado, se observó que a medida que avanza la madurez del fruto, disminuye el daño mecánico inducido debido al aumento de la capacidad de deformación del tejido, por lo tanto, en la cosecha se debe de evaluar adecuadamente el grado de madurez del fruto para minimizar este problema.

En conclusión las aplicaciones precosecha con melatonina, GABA y GA3, han conseguido incrementar la calidad de la cereza en el momento de la cosecha y durante el almacenamiento, alargando la vida útil del fruto con unos parámetros de calidad aceptables. Teniendo en cuenta todas las dosis evaluadas y los resultados obtenidos, la dosis más efectiva fue 50 mM para GABA y 0,3 mM para melatonina, los resultados del GABA destacaron ligeramente respecto a la melatonina, además este elicitor tiene un coste inferior por unidad de superficie tratada, pero aun así su aplicación no es viable económicamente. Por otro lado, el GA3 es una herramienta que deben seguir utilizando los productores para incrementar la calidad de la cereza con un reducido coste. La dosis a emplear dependerá del objetivo perseguido, se ha observado que dos aplicaciones de 15 ppm han dado buenos resultados con un retraso mínimo en la cosecha.

ABSTRAC

Sweet cherry (*Prunus avium* L.) is a fruit highly appreciated by consumers, due to its internal attributes, such as flavour, acid/sugar balance, texture and content of bioactive compounds, although it is also highly valued for its external characteristics, such as size, deep red colour, absence of defects and turgid green pedicels. However, it is a very sensitive fruit, in which ripening and senescence advance quickly after harvest, reducing the quality attributes of the fruit even under proper storage conditions.

In recent years, different preharvest strategies have been investigated, focused on increasing the quality traits of the fruit at the time of harvest and maintaining it during storage. Therefore, the application of elicitors in crops is an interesting strategy in current agriculture, since elicitors are compounds that are naturally found in plants, which induce physiological and morphological changes that improve the quality traits of the fruits, in a sustainable and environmentally friendly way. In this Doctoral Thesis, the effect of the preharvest application of treatments with melatonin, gamma-aminobutyric acid (GABA) and gibberellic acid (GA3) has been studied, with the aim of improving the quality parameters of the sweet cherry at the time of harvest and during postharvest storage.

The elicitors were applied by foliar spraying during fruit development on the tree, in the varieties 'Prime Giant', 'Lapins' and 'Sweet Heart', for melatonin were used doses of 0.1 mM, 0.3 mM and 0.5 mM, for GABA were used doses of 10 mM, 50 mM and 100 mM, in the solutions 0.1% Tween-20 was added as a surfactant. For both elicitors, three treatments were applied, coinciding with the phenological states of pit hardening, colour change and three days before harvest, the trials were carried out in the 2019 and 2020 crop season in Spain. In the 2022 crop season, another trial was carried out in Chile, in which GA3 treatments were applied on the 'Bing' and 'Lapins' varieties, with 30 ppm divided into 15 ppm and 15 ppm, 45 ppm divided into 25 ppm and 20 ppm, and 60 ppm divided into 30 ppm and 30 ppm, the first application was carried out in pit hardening and the second in straw colour development. In all the trials there were controls that were sprayed with water.

The results show that for two consecutive years, preharvest treatments with melatonin or GABA improved sweet cherry quality attributes. The soluble solids, acidity and firmness were higher in treated fruits than in controls at harvest and during storage, these elicitors also reduced weight losses during the postharvest period. Bioactive compounds such as phenols and anthocyanins increased their concentration

when GABA or melatonin was applied and colour increased correlating with anthocyanin content. The activity of antioxidant enzymes such as catalase, ascorbate peroxidase and peroxidase increased in treated fruits, both at harvest and during storage. The increase in enzymatic and non-enzymatic antioxidant systems as a result of the application of GABA or melatonin improved the efficiency of the tissues to neutralize reactive oxygen species, delaying the senescence process and maintaining quality parameters during storage.

Regarding GA3 treatments, the results showed that harvest was delayed by two to four days depending on the dose. Regardless of the concentration used, the treated fruits showed greater resistance at harvest, due to the increase in the modulus of elasticity and the stress at maximum point, while maintaining constant tissue strain. However, after 35 days of storage at 0°C in a modified atmosphere packaging (MAP) bag, the fruits maintained a high stress at the maximum point and a high modulus of elasticity, but tissue strain decreased, which generated a more rigid fruit. On the other hand, it was observed that as the fruit matures the induced mechanical damage decreases due to the increase in the tissue's deformability, therefore, during harvest, the degree of fruit maturity must be adequately evaluated to minimize this problem.

In conclusion, preharvest applications of melatonin, GABA and GA3 have managed to increase the sweet cherry quality at harvest and during storage, extending the shelf life of the fruit with acceptable quality parameters. Taking into account all the doses evaluated and the results obtained, the most effective dose was 50 mM for GABA and 0.3 mM for melatonin, the results for GABA stood out slightly compared to melatonin, also this elicitor has a lower cost per unit of treated surface, but even so its application is not economically viable. On the other hand, GA3 is a tool that producers should continue to use to increase sweet cherry quality at a low cost. The dose to be used will depend on the objective pursued, it has been observed that two applications of 15 ppm have given good results with a minimum delay in the harvest.

1. Introducción

1 INTRODUCCIÓN

1.1 Botánica

Las especies de cerezos cultivadas más importantes son *Prunus avium* L. y P*runus cerasus* L., cuyos frutos se denominan cereza y guinda respectivamente, estas especies pertenecen a la familia de las rosáceas. Las cerezas son originarias de la zona de Asia Occidental, pero actualmente se cultivan en todo el mundo (Lezzoni, 2008).

La cereza es una drupa, la cual está formada por el epicarpio que es la capa más externa, a continuación, se encuentra el mesocarpio carnoso y en último lugar está el endocarpio leñoso, en cuyo interior se aloja la semilla (Figura 1). La drupa está unida al árbol a través de un pedicelo de color verde, el cual acompaña al fruto la mayoría de las veces durante el periodo de comercialización (Linke et al., 2010).

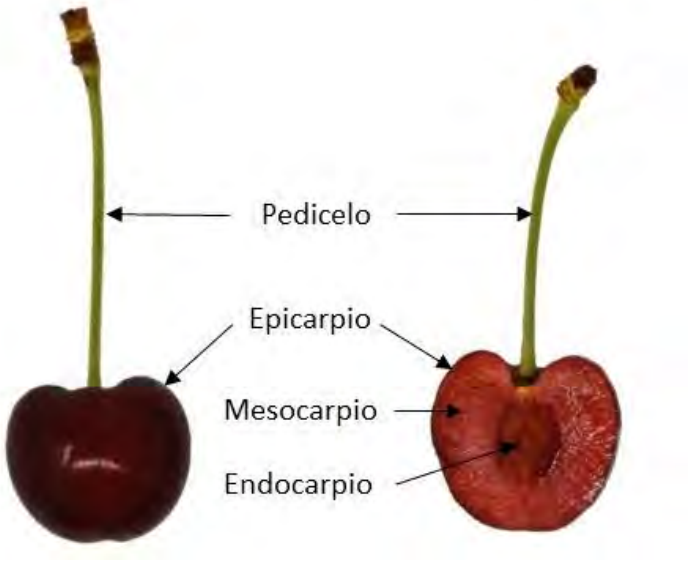


Figura 1. Partes de una cereza.

Fuente: Elaboración propia

1.2 Producción y superficie cultivada

1.2.1 En el mundo

La serie histórica de la producción y la superficie mundial de los últimos 25 años, muestra que el cerezo es un cultivo que está en expansión, ya que ha aumentado tanto la superficie como la producción, alcanzando valores de 451.064 ha y 2.732.413 t en el año 2021 (Figura 2).

Si se considera el año 1997 de referencia, se ha producido un incremento del 37,5 % de la superficie y un aumento del 67 % en la producción hasta el año 2021. El incremento de ambas variables no es proporcional, esto se debe a que con el paso del tiempo se ha mejorado la productividad de las parcelas, con el uso de variedades autocompatibles y con la incorporación de nuevas técnicas de cultivo (Figura 2).

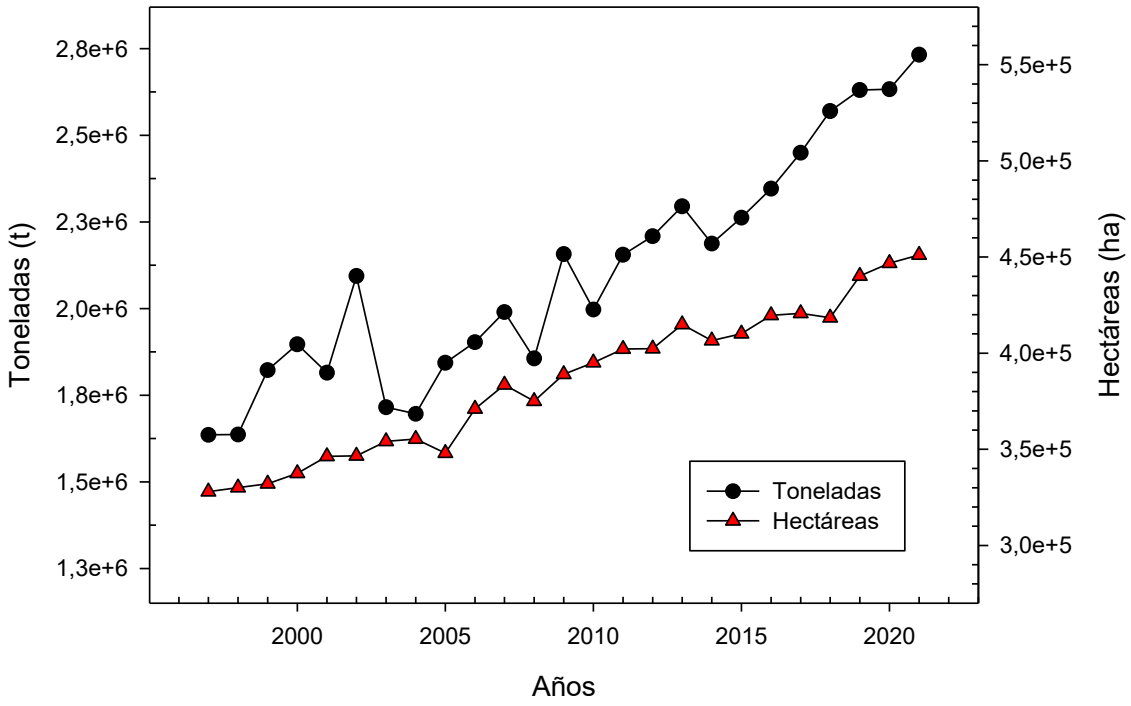


Figura 2. Evolución mundial de la superficie en hectáreas y de la producción en toneladas para el cultivo del cerezo en los últimos 25 años (1997-2021).

Fuente: FAOSTAT, 2021

Analizando la media de producciones en los últimos 25 años, el principal productor mundial es Turquía (414.000 t), seguido de Estados Unidos (274.000 t), Irán (217.000 t), Italia (112.000 t) y España que se encontraría en quinto lugar con una producción media de 96.000 t. Sin embargo, si se analiza la tendencia actual estudiando los últimos cinco años, se observa como Chile y Uzbekistán, entran en el ranking de los 5 países más productores desplazando a Italia y España (FAOSTAT, 2021).

1.2.2 En España

Actualmente en España se cultivan unas 29.500 ha de cerezos y la producción total se sitúa en torno a las 100.000 t. Si se analiza la evolución de este cultivo en los últimos 25 años, sobre el año 2000 se cultivaban unas 28.500 ha, pero el cultivo sufrió

una fuerte caída a partir del año 2004, situándose en años sucesivos en 24.000 ha, en los que también la producción se vio reducida y no fue hasta el año 2011 cuando el cultivo comenzó a recuperar superficie hasta situarse en los valores actuales (Figura 3).

Este cultivo se encuentra distribuido en las siguientes zonas productoras a nivel nacional: en primer lugar, estaría la zona de Aragón con una superficie de 10.500 ha, seguida de Extremadura con 7.500 ha, Cataluña con 2.650 ha y la Comunidad Valenciana con 2.600 ha (MAPA, 2022).

Hay que destacar que Aragón en los últimos años ha superado a Extremadura en producción, que es la zona tradicional del cultivo de la cereza, la cual dispone además de la Denominación de Origen Protegida Cereza del Jerte. Otra zona con tradición en el cultivo del cerezo se encuentra en la Comunidad Valenciana, donde disponen de la Indicación Geográfica Protegida Cerezas Montaña de Alicante.

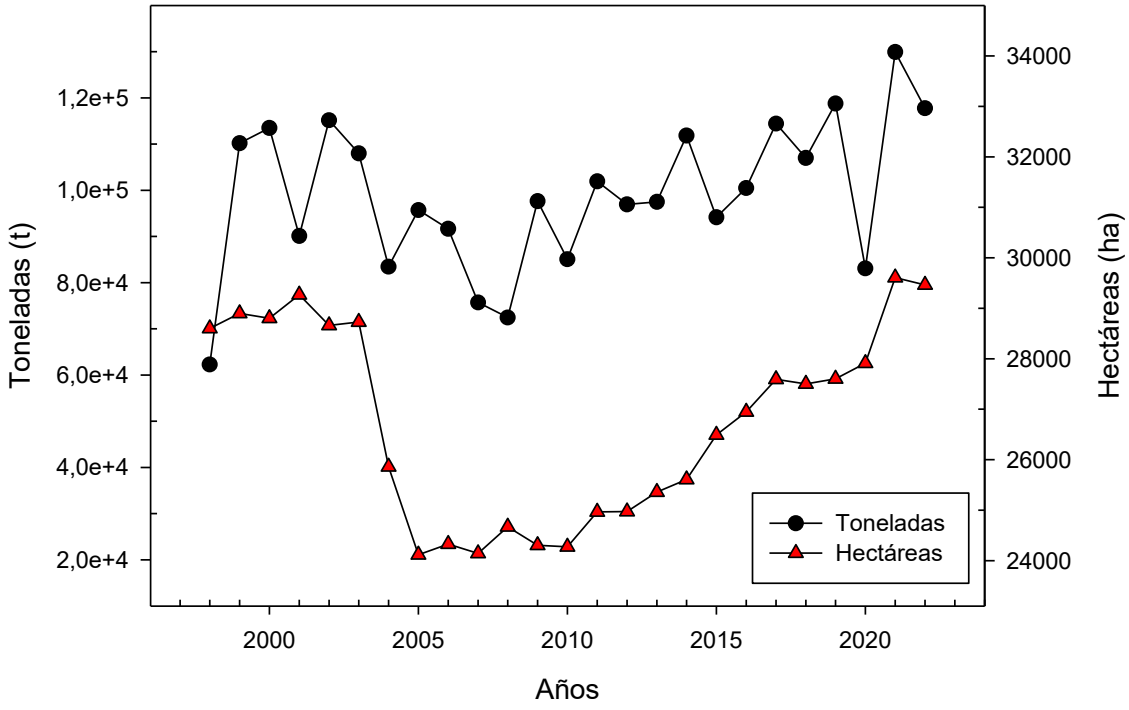


Figura 3. Evolución en España de la superficie en hectáreas y de la producción en toneladas para el cultivo del cerezo en los últimos 25 años (1998-2022).

Fuente: MAPA, 2021, 2022

1.3 Destino de la producción

La mayor parte de la producción española de cereza se destina al mercado en fresco, con una cuota aproximada del 50 % en el mercado nacional. Por otro lado, un 30 % se destina a la exportación, la cual genera un valor económico de unos 100

millones de euros (FEPEX, 2022a). El 20 % restante se utiliza en la industria agroalimentaria para realizar cereza confitada, en almíbar o para la elaboración de licores (MAPA, 2023).

Las comunidades autónomas que tienen un mayor volumen de exportación por orden de importancia son Extremadura, Cataluña y Aragón. Los principales destinos a los que se exporta la cereza son los países de la Unión Europea (UE), entre los que destacan Alemania, Francia, Italia, Portugal y Países Bajos, aunque en los últimos años está retomando cierta importancia la exportación a los países de fuera de la UE (FEPEX, 2022b).

1.4 Principales variedades

En el mundo hay muchos programas de mejora genética entorno al cerezo, de los que se han obtenido numerosas variedades. Normalmente todas estas variedades se clasifican en España en función de la fecha de recolección respecto a la variedad 'Burlat', la cual es una variedad temprana tradicional, aunque su cultivo se encuentra en descenso, ya que hay otras variedades modernas que tienen mejores características (MAPA, 2023).

Por lo tanto, las variedades se clasifican en función de la fecha de maduración en:

- Extra tempranas: son aquellas que se recolectan antes que 'Burlat', dentro de esta categoría se encuentran las variedades 'Early Bigi', 'Royal Tioga', 'Nimba' y 'Early Lory' (Rodrigo, 2018; Rodrigo y Negueroles, 2019).

- Tempranas: son las variedades que se recolectan entre 0 y 11 días respecto a 'Burlat', en este grupo se encuentran variedades como 'Red Pacific', 'Frisco', 'Brook', 'Sweet Ariana', 'Prime Giant', 'Santina', 'Sandon Rose', 'Celeste' y '13S 3 13' (Manzano et al., 2011).

- Media estación: son las variedades que maduran entre 12 y 19 días después de 'Burlat', dentro de esta clase se encuentran las variedades como 'New Star', 'Starking', 'Summit', 'Satin', 'Van', 'Samba' y 'Bing'.

- Tardías: se consideran las variedades cuya fecha de recolección se retrasa entre 20 y 27 días respecto a la variedad de referencia, en este grupo se encuentran 'Sunburst', 'Sonata', 'Lapins', 'Skeena' y 'Somerset'.

- Muy tardías: son las variedades que están retrasadas 28 días o más respecto a 'Burlat', en esta categoría se encuentran 'Ambrunesa', 'Pico Negro', 'Pico Colorado', 'Sweet Heart', 'Regina' y 'Staccato' (Rodrigo et al., 2016).

La incorporación de nuevas variedades ha permitido mejorar la calidad de la cereza, y también ampliar el calendario de recolección en las distintas zonas productoras al utilizar variedades más tempranas, respecto a las variedades más tardías el desarrollo ha sido menor (Rodrigo y Negueroles, 2019).

La variedad más cultivada en España es 'Lapins' con una importancia del 9,8 %, le siguen las variedades 'Prime Giant', 'Early Lory', 'Burlat' y 'Sweet Heart' que cada una tiene una importancia en torno al 5,5 %, después le seguirían variedades como 'Santina', 'Skeena' y 'Sonata' con un valor del 3 % para cada una de ellas, el resto de variedades tienen una importancia menor (Rodrigo, 2020) (Figura 4).

Figura 4. Cerezas de la variedad 'Lapins' a la izquierda y de 'Sweet Heart' a la derecha.

Fuente: Elaboración propia

1.5 Crecimiento y maduración de la cereza

La cereza tiene un patrón de crecimiento doble sigmoidal tanto en diámetro como en peso, que consta de tres etapas bien diferenciadas. La primera etapa es la fase I en la que predomina la división celular, le sigue la fase II en la que ocurre la lignificación del endocarpio y finaliza el desarrollo del embrión, en esta fase el crecimiento se reduce significativamente y no hay división celular. La última fase es la III en la que hay un rápido crecimiento del fruto, debido a la elongación celular

ocasionada por la incorporación de agua y fotoasimilados (Azarenko et al., 2008; Bastías et al., 2014) (Figura 5).

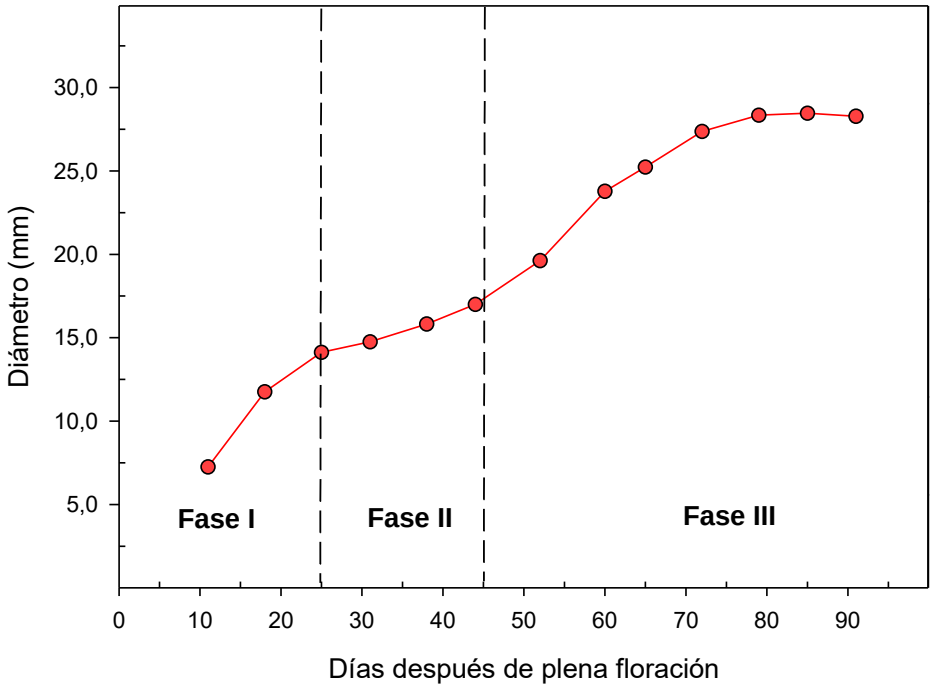


Figura 5. Patrón de crecimiento del diámetro del fruto para la variedad 'Lapins'.

Fuente: Elaboración propia

La maduración de la cereza engloba una serie de cambios morfológicos, fisiológicos y bioquímicos, que dan lugar a un fruto maduro con unos parámetros de calidad deseables, todos estos cambios están regulados por varias familias de genes que se expresan en mayor o menor medida en función del estado fenológico (Alkio et al., 2014; Qi et al., 2020).

Al final de la fase III del desarrollo de la cereza, es el momento en el que se produce la maduración, este proceso se caracteriza por la acumulación de pigmentos. En función del tipo de maduración los frutos se han clasificado tradicionalmente en climatéricos y no climatéricos, los primeros se caracterizan por tener un aumento en la producción de etileno y de la actividad respiratoria al inicio de la maduración (Paul et al., 2012). Sin embargo, la cereza es un fruto no climatérico cuyo proceso de maduración está relacionado con el ácido abscísico, cuyos niveles aumentan antes de la maduración y disminuyen cerca de la cosecha (Ren et al., 2011; Luo et al., 2014; Teribia et al., 2016). Por otro lado, se ha comprobado que la aplicación postcosecha de etileno en cereza temprana acelera los procesos de senescencia del fruto, e inhibidores como el 1-Metilciclopropeno (1-MCP) los ralentizan (Serradilla et al., 2019).

La cereza es un fruto no climatérico que debe ser cosechado en el punto óptimo de maduración, para que llegue al consumidor con la mejor calidad organoléptica, nutritiva y funcional. La cosecha temprana implicaría una reducción de los parámetros de calidad, ya que al ser un fruto no climatérico en la postcosecha no tendrá cambios positivos, por otro lado, las recolecciones tardías reducen la vida útil del fruto (Serrano et al., 2005; Serradilla et al., 2011; Ricardo-Rodrigues et al., 2021).

1.6 Parámetros de calidad de la cereza

El termino de calidad en los productos hortofrutícolas ha ido evolucionando a lo largo del tiempo y es un concepto variable en función de los distintos actores que participan en la cadena alimentaria, desde que se produce el fruto hasta que llega al consumidor final, pero en los últimos años estos criterios se centran principalmente en satisfacer las exigencias del consumidor final. Por lo tanto, la calidad se puede definir como el conjunto de propiedades que presenta el fruto, este concepto engloba las características organolépticas, nutritivas y funcionales. Dentro de las propiedades organolépticas se encuentra los parámetros que se puede percibir por los sentidos como el aroma, el sabor, el aspecto y la textura. La calidad nutritiva hace referencia al valor nutricional que proporciona el fruto y la calidad funcional a los componentes biológicamente activos que aportan un efecto beneficioso para la salud.

El dulzor de la cereza es consecuencia de los azúcares naturales presentes en el fruto, los mayoritarios son la glucosa y la fructosa, seguido de sorbitol y en menor medida de sacarosa. La acidez de la cereza se debe principalmente a la presencia de ácido málico, aunque en el fruto también se han determinado otros ácidos minoritarios como el ácido cítrico y succínico. Durante el proceso de maduración de la cereza incrementa la concentración total de azúcares, pero también incrementa el contenido de ácido málico, el cual disminuye en otras especies conforme se aproxima la fecha de recolección (Serrano et al., 2005). En función de la variedad de cereza, los sólidos solubles totales en el momento de la cosecha tienen valores comprendidos entre 12 y 24 °Brix, respecto a acidez total, estos valores oscilan entre los 0,50 y 1,25 g de ácido málico equivalente por cada 100 g de peso fresco (PF) (Díaz-Mula et al., 2009; Gonçalves et al., 2021). Teniendo en cuenta la aceptación del fruto por parte de los consumidores, es más importante hablar del índice de madurez, que es la relación entre los sólidos solubles totales y la acidez total, los valores altos se corresponden con frutos de poca acidez y por lo tanto menos atractivos para los consumidores, siendo la acidez un parámetro muy valorado (Crisosto et al., 2003; López et al., 2023).

El color de la piel de la cereza, es un indicador de calidad y del estado de madurez del fruto (Díaz-Mula et al., 2009), además también es un parámetro muy importante que valoran los consumidores a la hora de tomar la decisión de compra, siendo los colores más oscuros los mejor valorados (Crisosto et al., 2003). El color rojo característico de la cereza se adquiere en la última fase del desarrollo del fruto, cuando se produce la acumulación de antocianinas en la vacuola de las células, proceso que está directamente relacionado con el color del fruto (Díaz-Mula et al., 2009).

El calibre del fruto es un parámetro de calidad muy importante y valorado por los consumidores, pero en menor medida que el sabor y el color, en general los consumidores prefieren los frutos de mayor tamaño, pero hay cierta variación entre los consumidores de distintos países (Bujdosó et al., 2020). Turner et al. (2008) realizaron una evaluación sensorial y comprobaron que los consumidores tenían una preferencia significativa por los calibres más grandes de 30 mm o más.

El aspecto del pedicelo también es un buen indicador de la calidad del fruto, su aspecto debe de ser verde y turgente, la pigmentación del pedicelo se debe a su contenido en clorofila (Linke et al., 2010).

La firmeza es un atributo de calidad que disminuye conforme se desarrolla el fruto en el árbol, esta variable es muy dependiente de la variedad y por lo tanto hay grandes variaciones de ablandamiento entre cultivares. Es un parámetro muy valorado por los consumidores, y el ablandamiento excesivo es uno de los principales cambios asociados con el deterioro de la cereza (Gonçalves et al., 2021; Choi et al., 2002a).

Respecto a la calidad nutritiva, la cereza tiene un aporte por cada 100 g de 63 Kcal, 1,06 g de proteínas, 0,2 g de grasa, 16 gramos de carbohidratos y 2,1 g de fibra, además también aporta pequeñas cantidades de vitaminas (McCune et al., 2011). Al igual que todas las frutas, la cereza también tiene un importante contenido en agua entorno al 84 % (Serradilla et al., 2016).

La calidad funcional de la cereza se debe al contenido de compuestos bioactivos que presenta, los cuales tienen actividad biológica promoviendo mejores condiciones de salud. La cereza contiene β-carotenos y compuestos fenólicos entre los que se encuentran las antocianinas, la quercetina y los ácidos hidroxicinámicos (McCune et al., 2011; Fonseca et al., 2021). Los polifenoles mayoritarios en las cerezas son las antocianinas, las cuales aumentan considerablemente al final de la fase de maduración, este compuesto es el que le aporta la coloración roja al fruto, las antocianinas mayoritarias en la cereza son la cianidina 3-rutinósido, la pelargonidina 3-rutinósido y la cianidina 3-glucósido. La cereza también contiene enzimas antioxidantes, entre las que se encuentran, la superóxido dismutasa, la catalasa, la

peroxidasa y la ascorbato peroxidasa, todas estas enzimas junto con los compuestos antioxidantes, se encargan de neutralizar los radicales libres y las especies reactivas del oxígeno (Díaz-Mula et al., 2009; Martínez-Esplá et al., 2014).

1.7 Pérdidas de calidad de la cereza

1.7.1 Pérdidas precosecha

A la hora de hablar de pérdidas de calidad, normalmente el enfoque suele ser desde el punto de vista postcosecha, pero no hay que olvidar también la precosecha del fruto, porque es una fase importante en la que si no se hace un manejo adecuado, las pérdidas pueden ser significativas y además se condiciona la postcosecha del fruto.

La cereza durante el desarrollo en el árbol sufre unas pérdidas de calidad, ocasionada por factores abióticos y bióticos.

Dentro de los factores abióticos se encuentran las componentes climáticas como la temperatura, la humedad relativa, la radiación solar, la evapotranspiración y la velocidad del viento, entre otros. Todas estas variables cuando se salen de los niveles óptimos, ocasionan un estrés en la planta, causando una reducción de la producción tanto en cantidad como en calidad del fruto (Campoy et al., 2011; Wang et al., 2016; Chmielewski et al., 2018). Por otro lado, es importante también destacar la presencia de lluvia en la última fase de maduración, debido a la importancia que tiene sobre la incidencia del cracking, fenómeno que causa pérdidas cuantiosas debido a la rotura de la epidermis del fruto, lo que ocasiona su depreciación comercial (Correia et al., 2018; Winkler et al., 2020). El granizo sería otro factor meteorológico que tiene gran importancia en función del grado de afección, ya que aparte de dañar los frutos, puede afectar a la estructura del árbol condicionando las futuras cosechas.

La disponibilidad y calidad de agua y suelo, son factores muy importantes que están relacionados directamente con la productividad del árbol y la calidad de los frutos que se puede obtener (Asrey et al., 2018; Blanco et al., 2019; Aras et al., 2023). Además, estas variables son las que condicionan la elección del patrón, el cual tiene una gran influencia sobre la calidad final de la cereza (Cantín et al., 2010; López-Ortega et al., 2016).

Algunas de las plagas que ocasionan más daño en la cereza son *Drosophila suzukii*, *Rhagoletis cerasi*, *Myzus cerasi* y los insectos de la familia Thripidae, todos ellos producen la depreciación comercial de los frutos, ocasionando grandes pérdidas si no se realiza un manejo adecuado (Rodríguez et al., 2012; Papadopoulos et al., 2017).

Las principales enfermedades que afectan a la cereza en precosecha son *Monilia laxa*, *Monilia fructicola*, *Monilia fructigena*, *Botrytis cinerea*, *Alternaria alternata* y *Geotrichum candidum*, aunque estas enfermedades también se desarrollan en postcosecha (Feliziani et al., 2013; Serradilla et al., 2021)

1.7.2 Pérdidas postcosecha

Una vez recolectado el fruto del árbol comienza la postcosecha, periodo en el que también ocurren pérdidas de calidad, por lo tanto, para minimizarlas hay que realizar un manejo adecuado.

En el momento de la cosecha es cuando el fruto tiene un mayor contenido de agua, una vez recolectado se interrumpe su suministro, pero continua la traspiración por lo que hay una pérdida de agua. Debido a esto, durante el periodo de almacenamiento frigorífico y a temperatura ambiente se incrementa la pérdida de peso de las cerezas (Martínez-Romero et al., 2006).

La pérdida de textura durante la conservación postcosecha es un problema importante, ya que reduce la vida útil de la cereza, este fenómeno está asociado tanto a la pérdida de agua que sufre el fruto por transpiración, como al desmantelamiento de la pared celular, el cual se debe a la solubilización de las pectinas y a la despolimerización de pectinas y hemicelulosas (Salato et al., 2013). Este ablandamiento se debe fundamentalmente a la actividad de la β-glucosidasa (Gerardi et al., 2001) y de la β-galactosidasa (Gerardi et al., 2012) durante el proceso de maduración de la cereza.

El color es un parámetro que aumenta durante la conservación de la cereza, este cambio está asociado al proceso natural de maduración del fruto, el cual continua tras la recolección y se observa como una disminución en el ángulo Hue, lo que implica que las cerezas almacenadas adquieren una tonalidad más oscura (Martínez-Romero et al., 2006; Díaz-Mula et al., 2012; Giménez et al., 2016).

La acidez total también es un parámetro que disminuye durante la conservación, esto se debe al metabolismo del fruto, el cual utiliza los ácidos orgánicos como sustrato primario en la respiración aeróbica de las células (Díaz-Mula et al., 2012). Este fenómeno ocasiona que el ratio sólidos solubles totales/acidez total, adquiera valores más altos que se asocian con un fruto menos atractivo para los consumidores (Crisosto et al., 2003).

Durante el almacenamiento se incrementa la concentración de compuestos bioactivos en la cereza, se ha observado que aumentan tanto fenoles como antocianinas, ambas variables están correlacionadas positivamente, lo que indica que las antocianinas son los principales compuestos fenólicos de la cereza. En este aspecto habría un aumento de la calidad durante las primeras semanas de conservación, pero estos valores disminuyen al final de la vida útil del fruto, ocasionando una pérdida de calidad funcional (Serrano et al., 2009; Valero et al., 2011).

Respecto a las podredumbres que se manifiestan en postcosecha, parte de estas enfermedades también ocasionan síntomas en precosecha, pero las más representativas en postcosecha son *Monilinia* spp., *Botrytis cinerea*, *Rhizopus stolonifer*, *Alternaria alternata*, *Penicillum expansum* y *Cladosporium spp.* (Romanazzi et al., 2008; Serradilla et al., 2021).

El pardeamiento y la deshidratación del pedicelo suponen una depreciación visual del fruto y es un aspecto de calidad muy valorado por los consumidores. El pardeamiento del pedicelo está relacionado con la degradación de las clorofilas, como consecuencia de la pérdida de agua, la temperatura y el daño mecánico. El tejido del pedicelo es más sensible que el fruto a la deshidratación, debido a que presenta una menor resistencia a la trasferencia de vapor de agua, por lo que las variaciones de humedad relativa le afectan en mayor medida (Linke et al., 2010; Golding et al., 2017) (Figura 6A).

El pitting es un problema postcosecha importante que se manifiesta durante el almacenamiento, se caracteriza por unas depresiones que aparecen en la superficie del fruto tras sufrir un daño mecánico (Toivonen et al., 2004). Este síntoma no se manifiesta rápidamente tras el daño, por lo que al principio es difícil detectarlo visualmente y las temperaturas bajas acentúan este desorden. A nivel celular, si se estudian las zonas en las que hay depresiones, se observa bajo la epidermis daño en las células del parénquima (Porritt et al., 1971). La recolección es el primer punto en el que se induce este tipo de daño mecánico, aunque suele ser bajo si se realizan unas buenas prácticas. Posteriormente, en las centrales de envasado, este porcentaje se incrementa a medida que el fruto pasa por las distintas fases. Además, la sensibilidad al pitting dependen principalmente de la variedad utilizada, de la temperatura de la pulpa en el momento del impacto y de la altura de la caída (Candan et al., 2014). Se distinguen dos tipos de daños, cuando se trata de pequeñas zonas hundidas se denomina pitting y cuando son áreas más extensas y aplanadas se denomina bruising. El primero se ocasiona por el impacto con los pedicelos o superficies duras en una pequeña área del fruto y el segundo se produce cuando la fuerza de impacto se distribuye en un área mayor (Grant y Thompson, 1997) (Figura 6B).

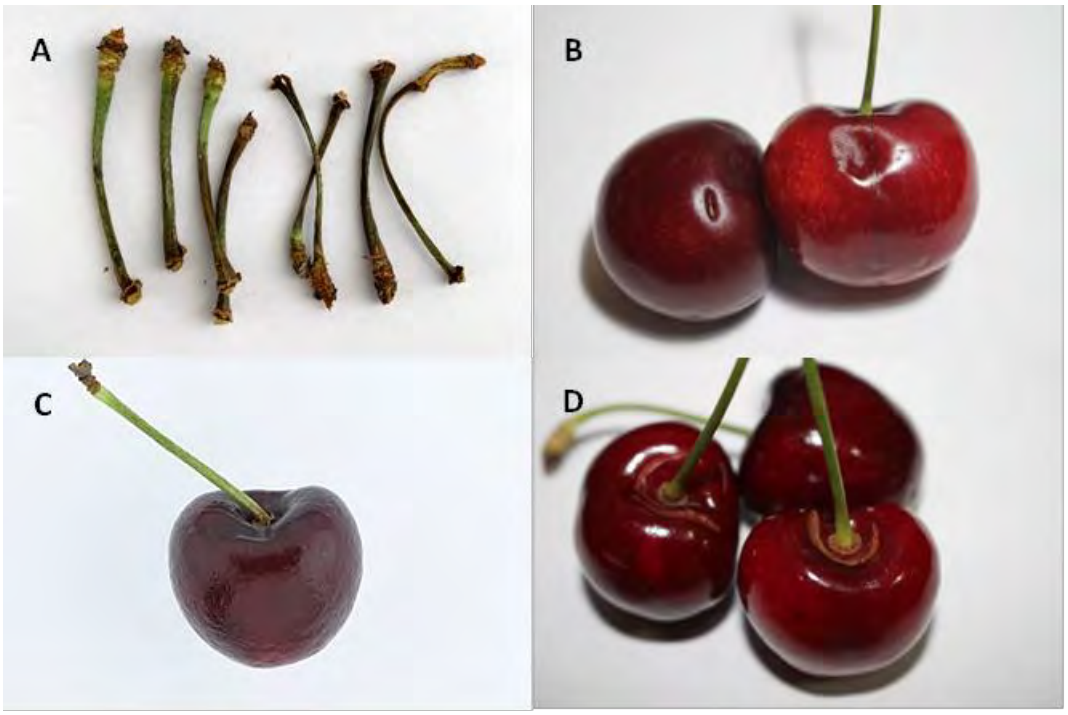


Figura 6. (A): Pedicelos deshidratados y pardeados; (B): Izquierda pitting y derecha bruising; (C): Piel de lagarto; y (D): Cracking postcosecha.

Fuente: Elaboración propia

Otro desorden que se puede manifestar en postcosecha en la cereza es el pebbling, también conocido como “piel de naranja”, “piel de lagarto” o “piel de cocodrilo”, este fenómeno se caracteriza por la formación de una superficie rugosa en la cereza, la cual está formada por áreas alternas de depresión y elevación que le dan ese aspecto heterogéneo. Este desorden es debido a la deshidratación de la piel y el factor varietal es importante ya que hay variedades más susceptibles (Schlegel et al., 2018) (Figura 6C).

El cracking, además de ser un problema que aparece en precosecha y ocasiona grandes pérdidas, también se manifiesta en postcosecha, sobre todo cuando la cereza entra en contacto con agua líquida o en un entorno con humedad relativa muy alta, lo que ocasiona la formación de microfracturas en la membrana cuticular y si persisten las condiciones se generará fracturas macroscópicas (Knoche y Peschel, 2006) (Figura 6D).

Cuando se prolongan los tiempos de almacenaje, algunas variedades son sensibles a desarrollar pardeamiento interno de la pulpa, esto se debe a la senescencia del tejido que ocasiona un cambio de coloración roja a tonalidades pardas, originando

además un cambio de sabor, la variedad 'Regina' es una de las más sensibles (Zoffoli et al., 2020).

1.8 Tecnologías precosecha para incrementar la calidad de la cereza

Muchos factores precosecha influyen en la calidad postcosecha de los frutos, afectando a parámetros como el calibre, el color, el sabor, la textura, la susceptibilidad a enfermedades, la composición de ácidos, azúcares y compuestos bioactivos, entre otras variables. Los factores más importantes que afectan a estos cambios son los genéticos, las condiciones ambientales y las prácticas agronómicas (Yahia et al., 2019).

1.8.1 Factores genéticos

Respecto a los factores genéticos, se puede influir en la elección del portainjerto y de la variedad comercial que se injertará sobre este. Esta combinación influye en gran medida en la calidad del fruto, ya que la expresión diferencial de genes en cada una de ellas inducirá las características típicas del fruto final. Se ha observado que el patrón 'Pikú 3', es el que mejores resultados de calidad ha obtenido en suelos pesados y calcáreos durante veranos cálidos y secos, frente a 'Mariana 2624', 'Adara', 'Mayor', 'Santa Lucia 64', 'MaxMa 14', 'Gisela 5', 'Gisela 6', 'Pikú 1' y 'Pikú 4' (López-Ortega et al., 2016). Los principales ácidos fenólicos y flavonoides también se ven influenciados por el portainjerto utilizado, presentando los valores más altos 'Weiroot 13' y 'Piku 1' (Jakobek et al., 2009). Respecto a las variedades que hay disponibles de cerezo, la gran variabilidad genética permite tener frutos con distintos parámetros de calidad y con un calendario más o menos extenso de producción (Díaz-Mula et al., 2009; Manzano et al., 2011; Rodrigo et al., 2016; Rodrigo y Negueroles, 2019).

1.8.2 Factores ambientales y prácticas agronómicas

Los factores ambientales son las variables más difíciles de controlar, pero se pueden emplear sistemas como son el uso de mallas, cubiertas e invernaderos, con la finalidad de reducir el impacto que tienen estos factores sobre la calidad final de los frutos. Los túneles altos han conseguido incrementar la temperatura del aire y la humedad relativa respecto a las condiciones de cultivo exterior, consiguiendo un adelanto en la fecha de recolección de 11 días (Blanco et al., 2021). También se ha

observado, que el uso de cubiertas para proteger a los cerezos de la lluvia, reduce las pérdidas por cracking y las podredumbres (Usenik et al., 2009).

Las prácticas agronómicas implican todas las operaciones y labores que se llevan a cabo durante el ciclo de cultivo, algunas de las más importantes y que tienen gran influencia sobre la calidad final del fruto son, el manejo del riego, la fertilización, el manejo de plagas y enfermedades, la poda y la aplicación de reguladores de crecimiento (Yahia et al., 2019).

El manejo del riego es un factor importante, ya que un déficit acusado puede ocasionar pérdidas en la producción, aunque estrategias de riego deficitario controlado mejoran algunos parámetros de calidad de la cereza, consiguiendo frutos más oscuros y dulces en cosecha y pedicelos más verdes en postcosecha, además de conseguir un ahorro significativo de agua (Blanco et al., 2019). Por otro lado, en otros estudios en los que se han realizado estrategias de riego deficitario en postcosecha, no se ha visto afectado el rendimiento y la calidad de la cereza en las campañas posteriores (Houghton et al., 2023).

Respecto al manejo de la fertilización, dosis altas de potasio mejoran el tamaño del fruto, los sólidos solubles y la acidez total, sin embargo, ocasionan una disminución de la actividad antioxidante y de los fenoles totales (Yener y Altuntaş, 2020). En el caso del nitrógeno, se ha observado que una alta fertilización nitrogenada incrementa este elemento en la cereza, causando una reducción de la firmeza del fruto, por lo tanto, hay que hacer una fertilización nitrogenada adecuada durante la precosecha para no afectar a la calidad de la cereza (Swarts et al., 2017).

1.8.3 Aplicaciones foliares con minerales y bioestimulantes

Una práctica muy utilizada en precosecha para mejorar los parámetros de calidad del fruto, es la realización de pulverizaciones foliares con abonos, bioestimulantes y minerales. A continuación, se muestra en la Tabla 1 un resumen de las principales estrategias utilizadas y los resultados obtenidos en cereza para mejorar la calidad.

Tabla 1. Tratamientos precosecha realizados mediante pulverización foliar con compuestos minerales y bioestimulantes en el cultivo del cerezo y efecto observado sobre la calidad del fruto.

Tratamiento	Variedad	Efecto	Referencia
Potasio	'Regina'	Reducción del cracking en cosecha y del pitting en postcosecha.	Bustamante et al., 2021
Ácido bórico	'Buttner's Red'	Aumento de sólidos solubles y antocianinas.	Wojcik y Wojcik, 2006
Hidróxido de calcio	'0900 Ziraat' 'Lambert' 'Van'	Reducción del cracking, disminución del peso del fruto e incremento de la firmeza.	Demirsoy y Bilgener, 1998
Cloruro de calcio	'Burlat'	Incremento del contenido de calcio en el fruto y reducción del cracking.	Wójcik et al., 2013
Silicato de sodio	'Van' 'Emperor Francis' 'New Star'	Reducción del cracking.	Rombolà et al., 2023
Ácido oxálico (OA)	'Sweet Heart' 'Sweet Late'	Aumento del tamaño del fruto, de la firmeza, del color, de las antocianinas y de los fenoles.	Martínez-Esplá et al., 2014
Glicina betaína	'Staccato'	Incremento de los sólidos solubles, los polifenoles, la vitamina C y la actividad antioxidante.	Gonçalves et al., 2020
	'Skeena'	Incremento de las antocianinas totales, pero descenso de los carotenoides y del ácido ascórbico.	Correia et al., 2020

Proteína harpin β	'Lapins' 'Regina'	Aumento de la fuerza de tracción del pedicelo y reducción de los desórdenes en postcosecha.	Li et al., 2020
Extracto de algas (*Ascophyllum nodosum*)	'Staccato'	Aumento de los sólidos solubles, la vitamina C y la actividad antioxidante.	Gonçalves et al., 2020
Extracto de algas (*Ecklonia maxima*)	'Bing'	Incremento del cuajado y de la producción.	Ureta Ovalle et al., 2019
Quitosano	'Sweet Heart' 'Blaze Star'	Reducción de las podredumbres en postcosecha.	Feliziani et al., 2013
Arginina	'Tak Danehe Mashhad'	Aumento de la actividad de las enzimas antioxidantes, de la firmeza, de la vitamina C y reducción de las pérdidas de peso.	Pakkish et al., 2022
Rizobacterias promotoras del crecimiento vegetal (PGPR)	'0900 Ziraat'	Incremento de la producción, del peso del fruto, de la longitud del brote y del contenido mineral de las hojas.	Esitken et al., 2006

1.8.4 Aplicaciones foliares con hormonas

En la tabla anterior no se han incluido las hormonas naturales y sintéticas utilizadas en el cultivo del cerezo, las cuales actúan en la planta a bajas concentraciones y se encargan de regular la actividad fisiológica. Estas moléculas orgánicas intervienen en el crecimiento, en el desarrollo, en la relación fuente sumidero y en la respuesta a los distintos tipos de estrés (Wani et al., 2016). Dentro de este grupo se encuentras las auxinas, giberelinas, citoquininas, ácido abscísico, ácido salicílico, poliaminas, ácido jasmónico, brasinoesteroides y etileno. En la Tabla 2 se muestra información más detallada sobre su efecto en la calidad de la cereza.

Tabla 2. Tratamientos precosecha hormonales aplicados mediante pulverización foliar en el cultivo del cerezo y efecto observado sobre la calidad del fruto.

Hormona	Variedad	Efecto	Referencia
Ácido 2-(2,4-diclorofenoxi) propiónico (2,4-DP)	'Satohnishiki'	Aumento de firmeza y disminución de antocianinas.	Kondo et al., 2000
Ácido 3,5,6-tricloro-2-piridiloxiacético (3,5,6-TPA) Ácido 2-(2,4-diclorofenoxi) propiónico (2,4-DP) Ácido 2,4-diclorofenoxiacético (2,4-D) + ácido naftalenacético (ANA)	'Bing'	Incremento del rendimiento y tamaño del fruto, como consecuencia del aumento de tamaño de las células parenquimáticas.	Stern et al., 2007
Ácido 4-clorofenoxiacético (4-CPA)	'Bing'	Adelanto de la coloración roja del exocarpio.	Zhang y Whiting, 2011
Tidiazurón (TDZ)	'Bing'	Incremento de los sólidos solubles y reducción de la firmeza	Sabir et al., 2021
Forclorfenurón (CPPU)	'Bing'	Reducción de los sólidos solubles y retraso del desarrollo del color.	Sabir et al., 2021
Meta-topolina [6-(3-hidroxibencilamino)purina]	'Bing'	Aumento del peso del fruto y retraso de la coloración del exocarpio.	Zhang y Whiting, 2011
Giberelina A1 (GA_1)	'Bing'	Incremento del tamaño del fruto	Zhang y Whiting, 2011
Giberelina A3 (GA_3)	'0900 Ziraat' 'Sweet Heart' 'Regina'	Aumento de la firmeza de la pulpa, retraso de la maduración de los frutos, disminución de los sólidos solubles, los fenoles totales, las antocianinas y la capacidad antioxidante.	Ozkan et al., 2016

Etefón	'Bing'	Reducción de la fuerza de retención del fruto, reducción de la firmeza y aumento del color.	Smith y Whiting, 2010
Ácido abscísico (ABA)	'Lapins' 'Bing'	Aumento de la coloración y disminución del diámetro, del peso y de la firmeza.	Time et al., 2021
Ácido salicílico (SA) Ácido acetilsalicílico (ASA)	'Sweet Heart' 'Sweet Late' 'Lapins'	Incremento del color, de la firmeza, de los sólidos solubles, de los fenoles, de las antocianinas y de la actividad de las enzimas antioxidantes.	Giménez et al., 2017
Jasmonato de metilo (MeJA)	'Early Lory' 'Prime Giant' 'Sweet Heart' 'Staccato'	Retraso del color, incremento de la firmeza y reducción del cracking.	Ruiz-Aracil et al., 2023a
Salicilato de metilo (MeSa)	'Sweet Heart' 'Sweet Late' 'Lapins'	Incremento de las antocianinas, los fenoles y las enzimas antioxidantes.	Valverde et al., 2015

1.8.4.1 Ácido giberélico (GA3)

De todas las hormonas que se han enumerado en la Tabla 2, hay que destacar que la más empleada a nivel comercial en el cultivo del cerezo es la giberelina A3 conocida como ácido giberélico (GA3) (Figura 7). Esta hormona se aisló a partir del hongo *Gibberella fujikuroi*, el cual ataca a las plantas de arroz y ocasiona la elongación de los tallos. En el año 1950 a partir del aislado del hongo se caracterizaron las giberelinas A1, A2 y A3, en los años posteriores se comenzó a estudiar el efecto de estos compuestos en los cultivos (Pradeepkumar et al., 2020). El GA3 es una hormona frecuentemente empleada, debido a que sus efectos están corroborados sobre un amplio abanico de variedades, patrones y distintas zonas edafoclimáticas. El efecto más significativo que buscan los productores de cereza a la hora de utilizar esta hormona es el incremento de firmeza (Kappel y MacDonald, 2002; Canli et al., 2009; Ozkan et al., 2016), pero el GA3 también tiene inconvenientes asociados, ya que ocasiona un

pequeño retraso en la maduración y por lo tanto en la recolección (Kappel y MacDonald, 2002; Cline y Trought, 2007). Además, en los momentos de lluvia los frutos tratados son más sensibles al cracking (Cline y Trought, 2007), aunque en otros estudios se ha observado que la aplicación de GA3 disminuye su incidencia (Usenik et al., 2005). Es importante definir adecuadamente la dosis a emplear para cada variedad, ya que esta hormona inhibe la inducción floral, ocasionando una reducción de la producción en el año posterior a la aplicación, efecto que se ve más acusado sobre todo cuando se emplean dosis muy altas (Lenahan et al., 2006).

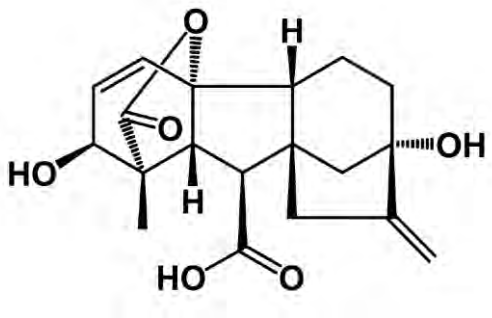


Figura 7. Estructura del ácido giberélico (GA3).

Fuente: Han et al. (2018)

1.8.5 Tratamientos innovadores

Tras analizar los últimos avances de los tratamientos empleados en el cultivo del cerezo, se decidió profundizar en el conocimiento de la melatonina y del ácido gamma-aminobutírico (GABA), ya que había muy poca información disponible sobre el uso de estos elicitores en el cultivo, y era necesario una mayor investigación para determinar qué efectos podría tener sobre la calidad de los frutos.

1.8.5.1 Melatonina

La melatonina (N-acetil-5-metoxitriptamina) se descubrió en el año 1958 en la glándula pineal bovina (Lerner et al., 1958) (Figura 8). Esta molécula se encuentra presente en casi todos los organismos de los cinco reinos de la naturaleza, que engloba moneras, hongos, protistas, animales y vegetales (Zhao et al., 2019a). En plantas este descubrimiento es relativamente reciente, ya que fue en el año 1995 cuando se describió esta molécula en *Pharbitis nil* (Vantassel et al., 1995). En humanos esta hormona se sintetiza en la glándula pineal, pero en los últimos años se ha observado que también se produce en otro tipo de órganos. La secreción de melatonina se ajusta

a los ciclos de luz y oscuridad, sincronizando los ritmos circadianos (Claustrat y Leston, 2015).

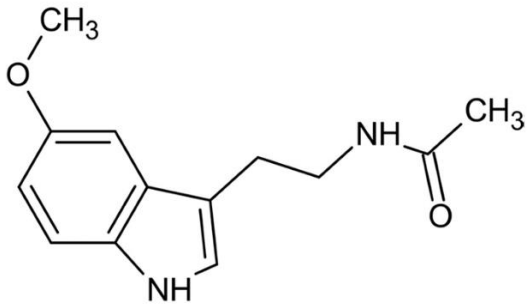


Figura 8. Estructura de la melatonina.

Fuente: Mannino et al. (2021)

Las plantas sintetizan la melatonina en los cloroplastos, ya que se ha demostrado que la enzima serotonina N-acetiltransferasa (SNAT) se encuentra únicamente en estos orgánulos, y esta enzima es necesaria como intermediaria para la obtención de la melatonina (Wang et al., 2020). Se ha observado que en condiciones normales la biosíntesis de melatonina se produce en los cloroplastos, pero si esta vía se bloquea, la síntesis pasa a realizarse en las mitocondrias para mantener los niveles (Tan y Reiter, 2020). La melatonina se sintetiza a partir de triptófano, en primer lugar actúa la enzima triptófano descarboxilasa (TDC) que da lugar a triptamina, la cual se transforma en 5-hidroxitriptamina (serotonina) por acción de la enzima triptamina hidrolasa (T5H). La SNAT convierte la serotonina en N-acetilserotonina, la cual es transformada en N-acetil-5-metoxitriptamina (melatonina) por la acción de la acetilserotonina metiltransferasa (ASMT) (Bhowal et al., 2021; Mannino et al., 2021) (Figura 9).

La melatonina tiene una gran capacidad antioxidante, siendo muy eficaz en la eliminación de radicales libres como las especies reactivas del nitrógeno y las especies reactivas del oxígeno, además de otros compuestos como toxinas. Por otra parte, la melatonina también es capaz de regular la actividad de las enzimas antioxidantes como la catalasa, la ascorbato peroxidasa, la peroxidasa y la superóxido dismutasa (Corpas et al., 2022; Zhang et al., 2022). La melatonina también tiene un papel importante en la regulación del estrés biótico, ya que es capaz de desencadenar una respuesta inmune e incrementar la expresión de los genes de defensa frente a los ataques de virus, bacterias y hongos (Singh y Gupta, 2023). Bajo factores de estrés abiótico como salinidad, sequía, frío, calor, radiación o exposición a compuestos químicos, la

melatonina ejerce una función reguladora para minimizar el estrés oxidativo ocasionado por todos estos agentes (Ahmad et al., 2023).

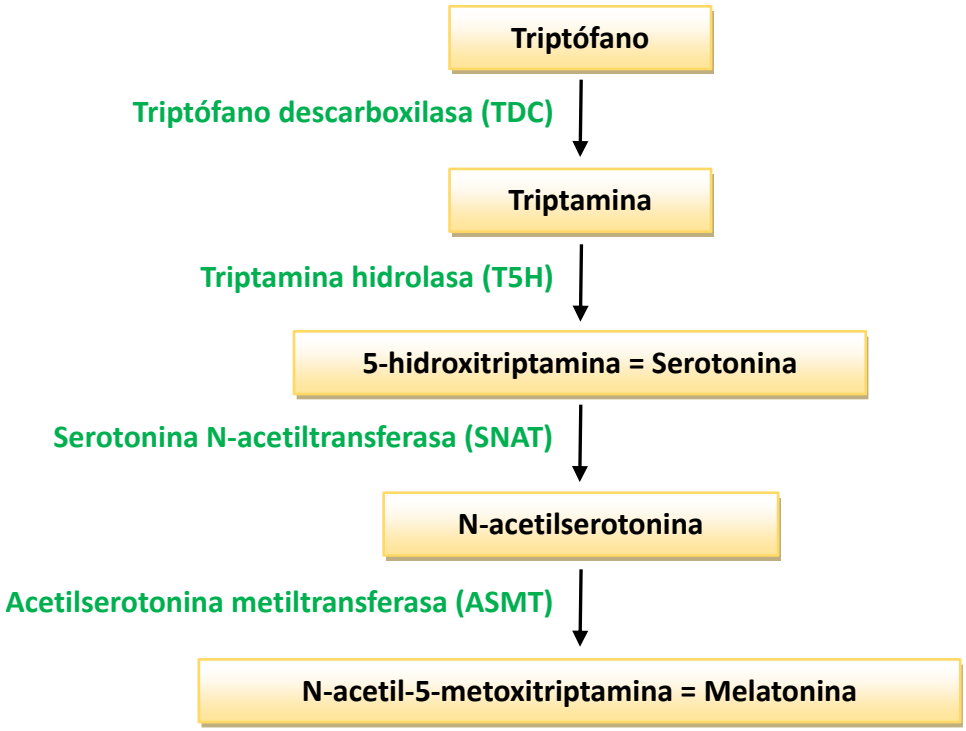


Figura 9. Ruta principal de biosíntesis de melatonina en plantas.

La melatonina también tiene un papel importante en el desarrollo de los frutos, ya que influye en la producción, en el proceso de maduración y en la calidad. Así por ejemplo, en granada la aplicación de melatonina aumentó la firmeza, el color, los fenoles, las antocianinas y la actividad antioxidante (Lorente-Mento et al., 2021), en ciruela se ha observado un retraso en el oscurecimiento del exocarpo y una reducción del ablandamiento, lo que se traduce en un incremento de la vida útil del fruto (Cortés-Montaña et al., 2023). Cuando se comenzó la investigación que ha dado como resultado esta Tesis Doctoral, no había información sobre la aplicación precosecha de melatonina en cereza, pero en los últimos años se han realizado estudios que muestran que la aplicación de este elicitor ha retrasado la maduración del fruto (Tijero et al., 2019), aunque también se ha observado una reducción de la respiración y un aumento de compuestos fenólicos (Michailidis et al., 2021). En el fruto de la cereza se ha cuantificado el nivel endógeno de melatonina, y se ha observado que el contenido disminuye conforme avanza la maduración (Xia et al., 2020). Además, la melatonina también tiene un importante efecto en la postcosecha de los frutos, ya que mantiene la

calidad, reduce los daños por frío, retrasa la senescencia y prolonga la vida útil (Ze et al., 2021).

La cantidad de melatonina es muy variable para las distintas especies vegetales y órganos analizados, en el caso de la cereza también hay una gran variabilidad entre variedades, con valores no detectados en 'Ambrunés', 0,01 ng/g PF en 'Van', 0,06 ng/g PF en 'Sweet Heart', 0,22 ng/g PF en 'Burlat' (González-Gómez et al., 2009), ~10 ng/g PF en 'Rainier' (Zhao et al., 2013) y 1,43 ng/g PF en 'Prime Giant' (Tijero et al., 2019).

1.8.5.2 Ácido gamma-aminobutírico (GABA)

El ácido gamma-aminobutírico también conocido como GABA, es un aminoácido no proteico de cuatro carbonos y fue descubierto por primera vez en el año 1949 en tubérculos de patata (Steward et al., 1949) (Figura 10). Este aminoácido se encuentra ampliamente distribuido en la naturaleza en microorganismos, plantas y animales (Ueno, 2000). En humanos se descubrió esta molécula en el cerebro en el año 1950, y se observó que el GABA se producía y acumulaba principalmente en este órgano (Awapara et al., 1950; Roberts y Frankel, 1950). Se ha comprobado que el GABA, actúa en el cerebro maduro como un neurotransmisor inhibidor reduciendo la actividad neuronal, aunque en las neuronas en desarrollo actúa como neurotransmisor excitador. Además, esta molécula está implicada como factor en el desarrollo del sistema nervioso (Owens y Kriegstein, 2002).

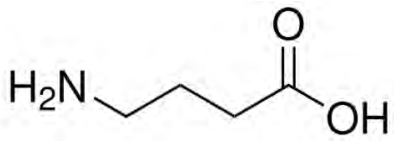


Figura 10. Estructura del ácido gamma-aminobutírico.

Fuente: Diana et al. (2014)

La síntesis de GABA en las plantas ocurre en el citosol y se obtiene a través de tres vías, a partir de la degradación de poliaminas, mediante la ruta de la derivación del GABA y a partir de prolina. La ruta a partir de poliaminas consiste en una serie de pasos enzimáticos que transforman estos compuestos en Δ^1-pirrolina, la cual es transformada en GABA mediante la acción de la enzima Δ^1-pirrolina deshidrogenasa (PDH) que se encuentra en los peroxisomas. En la ruta de derivación del GABA, este compuesto se sintetiza a partir del glutamato, el cual sufre una reacción de descarboxilación irreversible en el citosol, debido a la acción de la enzima glutamato descarboxilasa

(GAD). Una ruta alternativa para la biosíntesis de GABA es a partir de prolina, que en condiciones de estrés oxidativo da lugar a Δ^1-pirrolina, sustrato que utiliza la enzima PDH dando lugar a GABA (Podlešáková et al., 2019; Khan et al., 2021) (Figura 11).

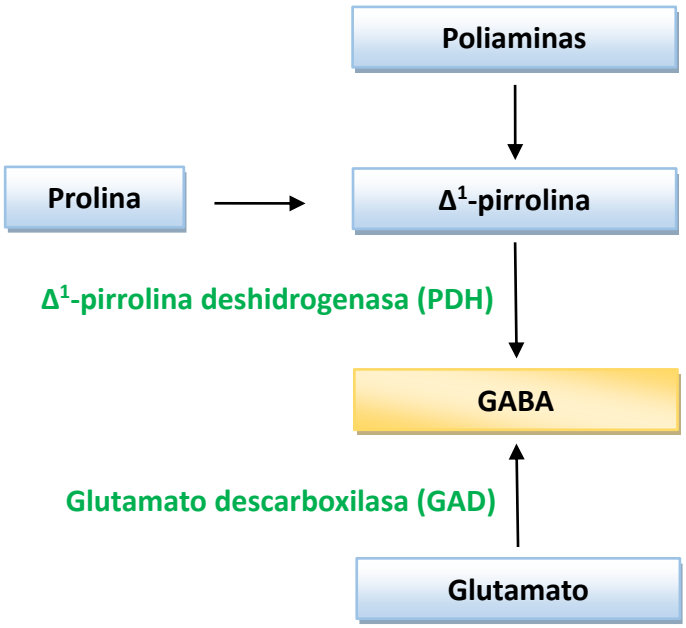


Figura 11. Resumen de las tres rutas de biosíntesis de GABA en plantas.

En las plantas el GABA se acumula principalmente en el citosol y posteriormente se transporta a las mitocondrias donde se cataboliza. El GABA es utilizado como sustrato en el ciclo de Krebs, ya que aporta energía y carbono bajo condiciones de estrés (Li et al., 2021). Esta molécula también es muy importante en el crecimiento y desarrollo de las plantas, ya que cuando se utilizan plantas mutantes en las que no funciona bien la ruta del GABA, se observan alteraciones en la floración, en el desarrollo de las hojas, en los frutos y en el tamaño (Jalil et al., 2019). El GABA tiene un papel importante en el balance de las reservas carbono-nitrógeno en las células vegetales y además, participa en el metabolismo del nitrógeno y en la senescencia de la planta (Khan et al., 2021). La ruta de derivación del GABA tiene un papel fundamental en la eliminación de las especies reactivas del oxígeno, y en la protección del sistema vegetal frente al daño oxidativo causado por las situaciones de estrés, las cuales producen un aumento de los niveles de GABA en las células (Ansari et al., 2021).

Se ha observado que los niveles de GABA son más altos en los frutos inmaduros, pero a medida que comienza la maduración disminuyen estos niveles (Takayama y Ezura, 2015). Respecto a la aplicación precosecha de GABA en frutos, en el momento que se decidió realizar esta investigación no había información disponible,

pero en los últimos años se han publicado varios trabajos. La aplicación precosecha de GABA en tomate, redujo los daños por frío durante el almacenamiento, mantuvo la calidad del fruto y aumento el contenido de licopeno (Zarei et al., 2020). En manzana la aplicación precosecha de GABA no afectó a las características externas del fruto, pero incrementó la firmeza de la pulpa, redujo la acidez, aumentó la relación azúcar-acidez e incrementó los niveles endógenos de glutamato y GABA (Cheng et al., 2023). En limón, la aplicación de este elicitor aumentó la producción y el número de frutos cosechados, en dos de las tres recolecciones que se realizaron, además, durante el almacenamiento postcosecha se redujeron las pérdidas de peso, la firmeza presentó valores superiores que los frutos control y se incrementaron los fenoles totales (Badiche-El Hilali et al., 2023).

El contenido de GABA es muy variable en las distintas especies vegetales, las que presentan los valores más altos son las patatas con 44,9 mg/100 g PF y los tomates amarillos con 36,8 mg/100 g PF, sin embargo, las frutas contienen niveles más bajos presentando la grosella europea el valor más alto con 13,2 mg/100 g PF, seguido del sauco menor con 11,1 mg/100 g PF y el arándano con 8,6 mg/100 g PF, mientras que en los frutos secos y los cereales los niveles son mínimos (Pencheva et al., 2023). En las variedades de cereza se han medido contenidos de 2,73 mg/100 g PF en ‘Della Recca’ y de 0,88 mg/100 g PF en ‘Del Monte’ (Pacifico et al., 2014).

1.8.6 Tecnología de las aplicaciones foliares y propiedades de las superficies vegetales

Una de la técnicas precosecha más utilizada para mejorar la calidad del fruto, son las pulverizaciones foliares con distintos compuestos, los cuales se han enumerado en los apartados anteriores (Tabla 1 y 2). Sin embargo, un factor muy importante para que estos tratamientos foliares sean eficientes, es que los compuestos aplicados sean absorbidos por las hojas y los frutos (Fernández et al., 2021).

Las superficies de las hojas, tallos, frutos y flores, están cubiertas con una cutícula que delimita los órganos y el medio que los rodea, tiene funciones protectoras actuando como barrera frente a los factores de estrés bióticos y abióticos (Fernández et al., 2016). La cutícula es una parte de la pared celular epidérmica compuesta por lípidos, entre los que se encuentran las ceras epicuticulares, las ceras intracuticulares, los polímeros de cutina y algunas veces de cután, también se encuentran en menor medida fenoles y elementos minerales (Guzmán-Delgado et al., 2016; Segado et al., 2016). Las superficies de las plantas tienen una gran heterogeneidad a nivel topográfico, presentando por un lado irregularidades a escala microscópica,

ocasionadas por elementos como los tricomas, papilas o estomas, y por otro lado, irregularidades a escala nanoscópica debido a las ceras epicuticulares y a los pliegues cuticulares (Almonte et al., 2021). El relieve de la superficie vegetal, junto con la composición química de la misma, determinará el grado de interacción de la disolución aplicada (Fernández et al., 2021).

Los compuestos aplicados mediante pulverización foliar, pueden ser absorbidos por la superficie de las plantas a través de la cutícula, de los estomas, las grietas o irregularidades cuticulares, o a través de estructuras epidérmicas tales como tricomas, lenticelas o venas (Fernández et al., 2021). Independientemente de la polaridad de la molécula, se ha observado que la penetración cuticular presenta restricción en cuanto al tamaño de partícula, el cual oscila entre los 0,3 y 5 nm de diámetro (Luque et al., 1995; Popp et al., 2005; Eichert y Goldbach, 2008).

En función de la topografía y composición de las superficies de las plantas, varia el grado de mojabilidad cuando las gotas de agua entran en contacto con las mismas. La mojabilidad es la capacidad que tiene la superficie vegetal para retener el agua, esta variable se cuantifica mediante el ángulo de contacto, que es el ángulo formado entre la tangente de la superficie del líquido y la superficie de la hoja (Papierowska et al., 2018). En las hojas de cerezos cultivados en el exterior se han medido ángulos de contacto promedios de 75°, 95° y 99°, para las variedades 'Souvenir', 'Samba' y 'Prime Giant' respectivamente (Hunsche y Noga, 2011). En otros ensayos, se ha medido el ángulo de contacto del fruto en varias variedades de cereza, siendo el valor promedio de 94,2° (Peschel et al., 2003). Utilizando la clasificación propuesta por Aryal y Neuner (2010) se considera que una superficie es super-hidrófila si el ángulo de contacto es inferior a 40°, es altamente mojable cuando el ángulo de contacto está comprendido entre 40° y 90°, es mojable si el ángulo está comprendido entre 90° y 110° y si es superior a 110º son superficies no mojables. En la mayoría de los casos las superficies vegetales del cerezo serían mojables, pero esta categoría no implica que las pulverizaciones foliares tengan un recubrimiento perfecto. Por este motivo, en las aplicaciones foliares, además de la materia activa que se pretende aplicar, se añaden adyuvantes para mejorar la tasa de mojado, retrasar el tiempo de secado y aumentar la penetración foliar (Fernández y Eichert, 2009).

1.9 Tecnologías postcosecha para mantener la calidad de la cereza

En el momento de la recolección cuando se desprende el fruto de la planta, comienza el periodo postcosecha, hasta este instante se han realizado una serie de manejos precosecha que se han detallado en los puntos anteriores, cuya finalidad era obtener un fruto con los máximos atributos de calidad, pero a partir de este momento hay que aplicar una serie de tecnologías postcosecha para mantener la calidad, hasta que el fruto llega al consumidor final.

La cosecha se realiza cuando la cereza alcanza el punto óptimo de madurez. La recolección puede ser mecánica, cuando el fruto va destinado a la industria agroalimentaria para elaborar productos procesados, o puede ser manual, cuando el destino es el mercado en fresco. La recolección manual es una labor que hay que realizar con cuidado para evitar dañar los frutos, además es muy importante tener en cuenta el diseño de los envases, para mantener la eficiencia durante la cosecha y reducir el daño mecánico (Sekse y Lyngstad, 1996). En algunas explotaciones en el momento de la cosecha, se suelen utilizar unas lonas cuya función es cubrir los recipientes con los frutos recién recolectados hasta que llegan al almacén, este sistema evita que se calienten, y además mantiene la humedad relativa en el entorno del fruto reduciendo la deshidratación (Schick y Toivonen, 2002).

La cereza es un fruto que se deteriora rápidamente tras la cosecha, debido a que tiene una alta tasa de respiración, la cual aumenta considerablemente con la temperatura de la pulpa (Crisosto et al., 1993). Por lo tanto, es importante realizar un enfriamiento rápido de los frutos tras la recolección, para ello se emplean sistemas de hydrocooling en los cuales los frutos reciben una ducha de agua fría aproximadamente a 1°C, con la finalidad de reducir la temperatura de la pulpa. La temperatura final alcanzada, dependerá principalmente de la temperatura de entrada del fruto y del tiempo de permanencia en el sistema (Alique et al., 2006). Se ha demostrado que el sistema de hydrocooling retrasa el deterioro y la senescencia de la cereza, reduce la deshidratación y mantiene los pedicelos más verdes y turgentes (Manganaris et al., 2007; Muñoz et al., 2017).

Durante la manipulación de la cereza en la línea es muy importante controlar la temperatura de la pulpa, ya que temperaturas bajas en torno a los 3°C ocasionan más lesiones que temperaturas entorno a los 6°C, por lo tanto se debe de controlar la temperatura del agua en las zonas en las que el fruto puede recibir más golpes. Además, los daños ocasionados durante la manipulación tanto por impacto como por

compresión no se ven instantáneamente, ya que se manifiestan tras una semana en almacenamiento frigorífico a 0°C (Zoffoli y Rodriguez, 2014a).

Una vez que la cereza se ha manipulado, es importante bajarle rápidamente la temperatura, esto se consigue mediante el empleo de túneles de aire forzado o mediante cámaras frigoríficas, el objetivo es bajar la temperatura a valores de conservación comprendidos entre -0,5°C y 0°C, de esta manera se retrasa la senescencia y se reduce la incidencia de podredumbres (Kupferman y Sanderson, 2005; Alonso y Alique, 2006). Por otro lado, se ha observado que si se almacenan las cerezas a temperaturas cercanas al punto de congelación biológico, de -1,5°C para la variedad 'Hongdeng' y -1,9°C para 'Lapins', se mejora la vida postcosecha, se retrasan los cambios de color, se reduce la respiración y se mantiene la calidad sensorial (Zhao et al., 2019b).

Junto con la temperatura, la humedad es otra variable fundamental que se debe de controlar durante el almacenamiento. Se recomiendan humedades relativas altas comprendidas entre 90-95 % para evitar la deshidratación, tanto del fruto como del pedicelo (Alonso y Alique, 2006; Golding et al., 2017).

Durante el procesado de los frutos es importante realizar una buena desinfección, debido a que contienen una gran cantidad de microorganismos que pueden ocasionar su deterioro. En postcosecha se suele manifestar *Monilinia* spp., *Botrytis cinerea*, *Rhizopus stolonifer*, *Alternaria alternata*, *Penicillum expansum* y *Cladosporium spp.* (Romanazzi et al., 2008; Serradilla et al., 2021). El cloro es el agente de desinfección más empleado en la industria agroalimentaria y se utiliza en un amplio rango de concentraciones de 50-200 ppm, en función de la cantidad de materia orgánica del agua, del pH, del tiempo y la temperatura de exposición (Goodburn y Wallace, 2013). En cereza se han realizado ensayos con desinfectantes alternativos al hipoclorito de sodio, ya que su uso ocasiona la formación de trihalometanos, cloroformo y bromodiclorometano, que son compuestos cancerígenos. Algunos de estos desinfectantes alternativos que se han propuesto son el ácido peracético, el peróxido de hidrogeno, desinfectantes a base de extractos naturales y ozono, de los cuales el ácido peracético mostró los mejores resultados, cuando se utilizaba en el agua de lavado del hydrocooling (Sehirli et al., 2020).

El control de los hongos es un desafío para la industria frutícola, ya que estos ocasionan pérdidas postcosecha significativas, y es muy importante controlarlos para que los frutos tengan una vida útil larga, para ello una vez procesada la cereza, en el último circuito de lavado se suelen añadir fungicidas. El único fungicida autorizado a nivel europeo en cereza para aplicar en las centrales de manipulación es el fludioxonil,

cuyo límite máximo de residuo (LMR) está establecido en 5 mg/kg (EC, 2024a). Se trata de un fungicida de bajo riesgo muy efectivo, ya que es capaz de inhibir el crecimiento del micelio y la germinación de las esporas (Leroux, 1996; Förster et al., 2007). En los últimos años se han buscado alternativas ecológicas para el control de las podredumbres en cerezas, las cuales se basan en el control biológico mediante el uso de levaduras y han dado resultados similares a los fungicidas químicos (Cabañas et al., 2023).

Una tecnología muy utilizada por la industria para conservar las cerezas por un largo periodo de tiempo, es el uso de bolsas de atmósfera modificada (MAP), las cuales están compuestas por una lámina plástica que tiene cierta permeabilidad a los gases, de manera que en el interior se genera una atmósfera propicia para mantener la calidad de los frutos durante semanas (Meheriuk et al., 1995). Es muy importante controlar la temperatura cuando la cereza esta envasada en bolsa MAP, debido a que las altas temperaturas aumentan la respiración y como consecuencia la cantidad de dióxido de carbono y se ha observado que concentraciones iguales o superiores al 20 % de CO_2 generan fermentaciones y ocasionan que los frutos adquieran un sabor desagradable (Remón et al., 2000). La atmósfera en el interior de la bolsa se puede alcanzar de manera activa, realizando el vacío e inyectando la concentración de gases adecuada, o de manera pasiva, por la propia respiración del fruto que consume el O_2 y genera CO_2 hasta que se alcanza el equilibrio. Se ha evaluado la efectividad de estos dos sistemas tras el almacenamiento frigorífico durante 42 días a 0°C y 4 días a 5°C, observándose que tras dos días se alcanzan concentraciones similares en ambas bolsas, y no hay diferencia de calidad entre los tratamientos, pero si una reducción de las podredumbres con respecto a los frutos control, los cuales están en una bolsa sin sellar (Zoffoli y Rodriguez, 2014b). Las bolsas MAP permiten reducir las pérdidas de peso del fruto, la respiración, retrasan los cambios fisicoquímicos relacionados con la pérdida de calidad, mantienen el color verde del pedicelo y reducen el crecimiento de bacterias y hongos (Wani et al., 2014). Son numerosas las concentraciones de gases que se han evaluado para mantener la calidad de las cerezas, pero se recomienda que sean de 3-10 kPa O_2 y de 10-12 kPa CO_2 (Artés et al., 2006), aunque estas pueden variar en función de los cultivares y del objetivo perseguido.

La aplicación de compuestos naturales presentes en las plantas como el ácido salicílico, el ácido acetilsalicílico y el ácido oxálico, se han evaluado realizando inmersiones de cerezas en soluciones con estos compuestos y tras 20 días de almacenamiento, en los frutos tratados se observó un retraso en el proceso de maduración, un aumento de los fenoles totales, de las antocianinas totales y de la actividad antioxidante (Valero et al., 2011). La aplicación de salicilato de metilo en

cerezas, redujo la respiración, la pérdida de peso, el ablandamiento y la pérdida de acidez, además mantuvo el contenido de compuestos bioactivos y la actividad antioxidante en valores más altos que los frutos control al final del almacenamiento (Giménez et al., 2016). La aplicación de jasmonato de metilo en cereza, redujo las podredumbres y mejoró la actividad de las enzimas antioxidantes y las enzimas relacionadas con la resistencia a enfermedades (Pan et al., 2022).

Los recubrimientos comestibles se utilizan para mejorar la conservación y aspecto de los frutos y además actúan como una barrera de protección. En cereza se ha evaluado el uso de quitosano, que es un biopolímero obtenido de los crustáceos y la aplicación postcosecha de este compuesto redujo el crecimiento microbiano, mientras que en el resto de propiedades fisicoquímicas los resultados fueron variables en función del tipo de quitosano empleado (Feliziani et al., 2013; Tokatlı y Demirdöven, 2020). También se han utilizado recubrimientos de alginato, que es un polisacárido natural que se extrae de algas marinas pardas, en cereza ha mostrado buenos resultados ya que retrasa el proceso de maduración del fruto, y tiene un efecto positivo en el mantenimiento de los fenoles totales y la actividad antioxidante (Díaz-Mula et al., 2012). También se han desarrollado recubrimientos comestibles a base de *Aloe vera*, que ofrecen una barrera física frente al deterioro, además de retrasar el proceso de maduración de la cereza, reducir la pérdida de peso y la respiración, presentando los frutos tratados una valoración sensorial superior que los frutos control (Martínez-Romero et al., 2006).

Respecto a los compuestos innovadores como la melatonina y el GABA, que se han explicado en el apartado anterior como estrategia precosecha para mantener la calidad, también han sido ensayados en postcosecha para observar su efecto en cereza, aunque la información disponible es limitada. Tratamientos postcosecha con melatonina realizados mediante inmersión, retrasaron la senescencia del fruto, redujeron las pérdidas de peso, incrementaron la firmeza, los sólidos solubles, la acidez y además mejoraron la actividad de las enzimas antioxidantes (Wang et al., 2019). En otro ensayo postcosecha con melatonina en cereza, también se observó una reducción de las pérdidas de peso, de la respiración y del pardeamiento del pedicelo, además parámetros como la firmeza, los sólidos solubles y las antocianinas se mantuvieron en valores superiores al control (Miranda et al., 2020). Respecto a los trabajos de GABA en postcosecha, solo hay un ensayo en cereza, en el cual este compuesto se aplica mediante inmersión de los frutos, obteniendo tras 30 días de almacenamiento un incremento de la actividad antioxidante, de los fenoles totales y de las enzimas antioxidantes respecto al control (Hassanpour et al., 2018).

2. Objetivos

2 OBJETIVOS

Teniendo en cuenta la problemática asociada al fruto de la cereza y la demanda de altos estándares de calidad por parte de los consumidores, se plantea la necesidad de investigar nuevas herramientas que den soluciones al cultivo del cerezo para minimizar los problemas asociados, utilizando alternativas sostenibles y respetuosas con el medio ambiente como es el uso de elicitores naturales.

El **objetivo principal** de esta Tesis Doctoral es incrementar la calidad de la cereza en el momento de la cosecha y mantenerla durante el almacenamiento postcosecha hasta que el fruto llega al consumidor final, aplicando tratamientos precosecha con melatonina, GABA y GA3, que son elicitores naturales que se encuentran presentes en las plantas. Para alcanzar el objetivo principal se plantean los siguientes **objetivos específicos**:

I. Evaluar la eficacia de los tratamientos en el rendimiento del cultivo.

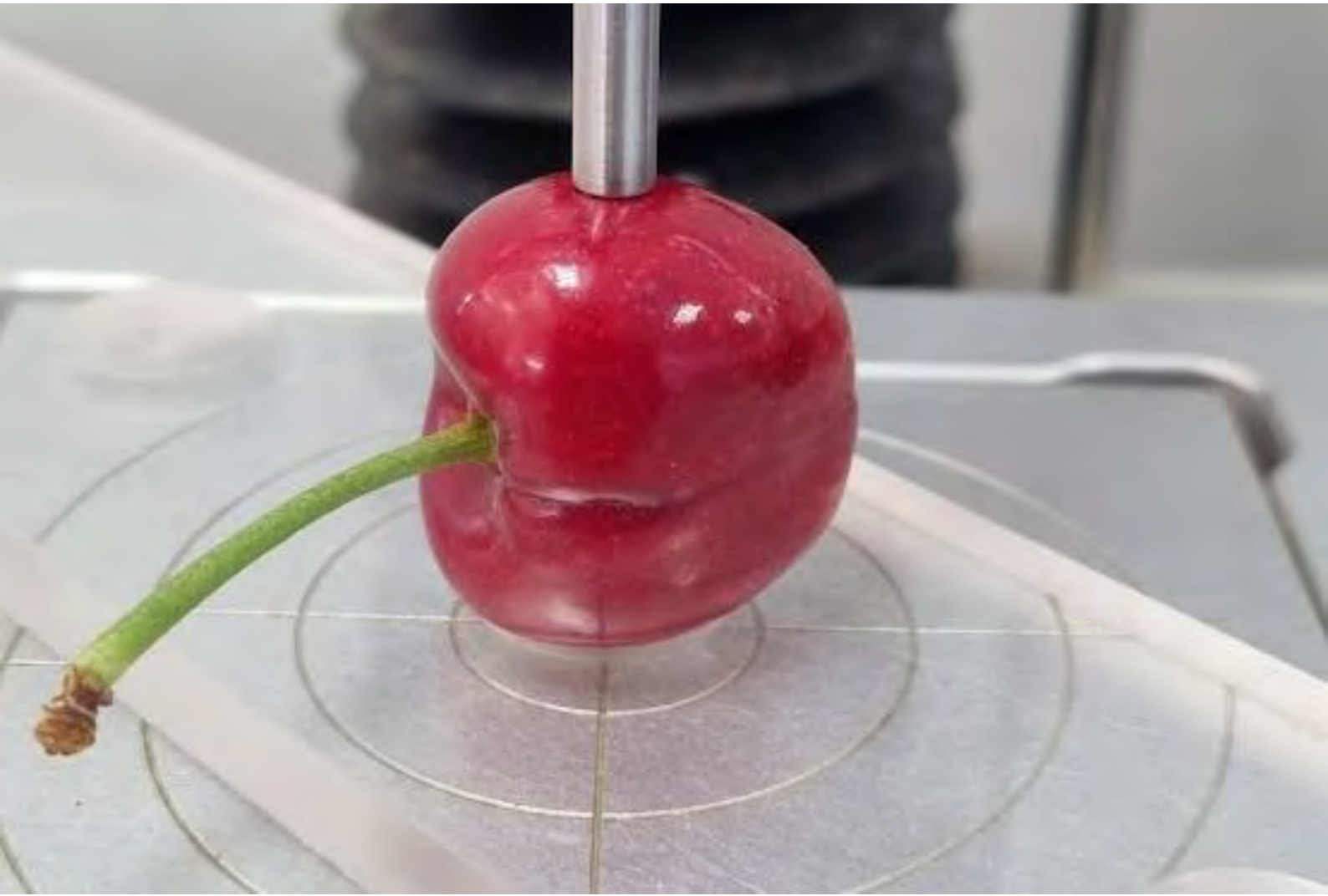
II. Determinar el efecto de los elicitores sobre los parámetros de calidad de la cereza, en el momento de la cosecha y durante su almacenamiento postcosecha.

III. Cuantificar el contenido de compuestos bioactivos totales e individuales.

IV. Determinar la actividad de las enzimas antioxidantes desde la cosecha hasta el final del almacenamiento.

V. Evaluar las variables reológicas del fruto y su relación con el daño mecánico.

3. Materiales y Métodos



3 MATERIALES Y MÉTODOS

3.1 Material vegetal y diseño experimental

Los cerezos empleados para realizar los distintos ensayos pertenecen a la especie *Prunus avium* y se han utilizado distintas variedades para obtener unos resultados más robustos. A continuación, se detallan las variedades utilizadas y sus principales características:

- 'Prime Giant': Variedad obtenida por Marvin Nies en California, presenta una fecha de recolección temprana, es autoincompatible, muestra un vigor medio-alto, tiene una productividad buena, la cereza tiene un gran tamaño y un color rojo oscuro (MAPA, 2023).

- 'Bing': Variedad obtenida en la Estación Lewelling en Oregón, presenta una fecha de recolección de media estación, es autoincompatible, presenta un vigor alto, tiene una productividad elevada, el fruto tiene un tamaño medio y un color caoba.

- 'Lapins': Variedad obtenida en la Estación Summerland en Canadá, presenta una fecha de recolección tardía, es autocompatible, muestra un vigor alto, tiene una productividad muy elevada, el fruto es de tamaño medio-alto y tiene un color rojo oscuro.

- 'Sweet Heart': Variedad obtenida en la Estación Summerland en Canadá, presenta una fecha de recolección muy tardía, es autocompatible, muestra un vigor alto, tiene una productividad muy elevada, el fruto es de tamaño medio-alto y tiene un color rojo.

3.1.1 Ensayo con melatonina y GABA

En el ensayo realizado en España se utilizó la melatonina y el GABA durante dos campañas de cultivo, en la finca comercial 'Finca Toli' situada en Jumilla (Murcia). En el año 2019 se utilizaron las variedades 'Prime Giant' y 'Lapins', en el año 2020 se emplearon las mismas variedades y además se añadió otra más que fue 'Sweet Heart' (Figura 12). Los árboles de la variedad 'Prime Giant' y 'Lapins' se plantaron en enero del año 2012 y los de la variedad 'Sweet Heart' en enero del año 2015, todos ellos están injertados sobre el patrón SL-64. La climatología en la zona de cultivo fue similar durante los dos años del experimento, presentando una temperatura media anual de 15,24 y 15,30°C, y una precipitación anual de 357 y 352 mm para el año 2019 y 2020, respectivamente. El sistema de formación del árbol es en vaso, el marco de plantación

es de 5 metros en las calles y 3 metros entre árboles, el mantenimiento del suelo es mediante cubierta espontánea sobre la que se realizan siegas y las labores culturales realizadas en las distintas variedades han sido similares todos los años.

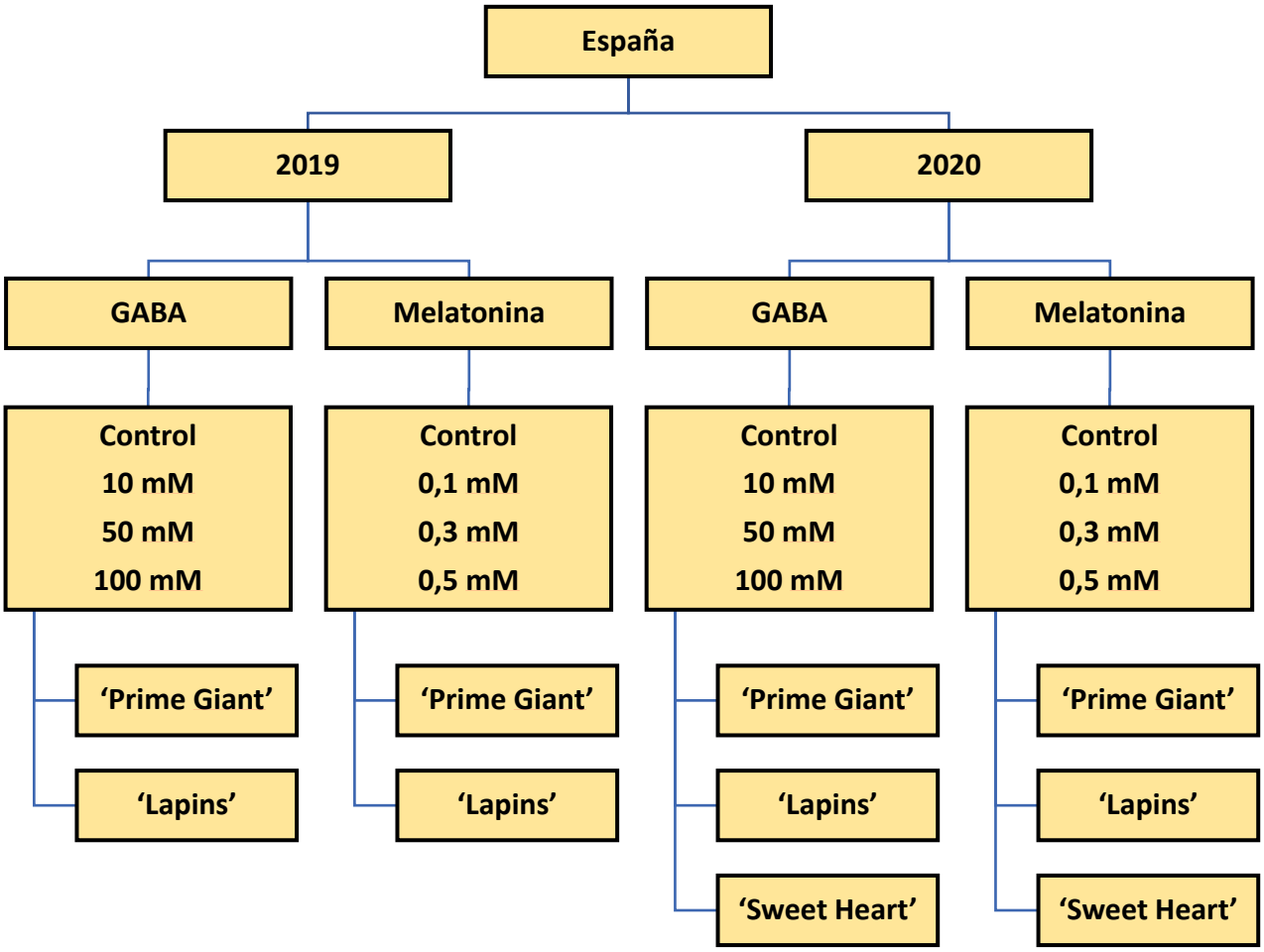


Figura 12. Esquema del diseño experimental de los tratamientos realizados en España.

Las dosis utilizadas fueron 10, 50 y 100 mM para el GABA y 0,1, 0,3 y 0,5 mM para la melatonina. Para cada elicitor y variedad se realizó un diseño experimental en bloques aleatorios y tanto para cada una de las dosis como para el control, se utilizaron 3 bloques, compuesto cada uno por tres árboles. Para evitar que la deriva de un bloque llegara al contiguo se dejó un árbol de separación, además se dejó una separación de 6 árboles respecto a los caminos perimetrales para evitar el efecto borde. Los elicitores empleados para preparar las soluciones tenían una pureza mayor o igual al 98 % y se adquirieron de la empresa Sigma-Aldrich (Madrid, España). Para mejorar el recubrimiento de las superficies vegetales se añadió Tween-20 como surfactante a una concentración de 0,1 %, estas disoluciones se prepararon directamente en el campo. Las aplicaciones se realizaron con un equipo de pulverización manual y se aplicó un

volumen de 3 litros por árbol, quedando las hojas y los frutos completamente mojados tras la aplicación. Estos tratamientos se realizaron por la tarde-noche cuando no había radicación solar directa, la temperatura era inferior a 25°C, en ausencia de viento y sin pronóstico de lluvia en las 24 horas posteriores a la aplicación. Cada uno de los tratamientos se aplicó tres veces, coincidiendo con los momentos claves del desarrollo del fruto, el primero (T1) se realizó en el endurecimiento de hueso, el segundo (T2) en cambio de color y el tercero (T3) 3 días antes de recolección. Para determinar estos estados fenológicos se tuvieron en cuenta los estudios previos de Giménez et al. (2017). Cuando la cereza alcanzó el punto óptimo de maduración para cada variedad, en base a criterios de color y sólidos solubles, se realizó la recolección de todos los tratamientos. Todos los árboles se recolectaron individualmente y de cada uno de ellos se cogió 1 kg de cerezas, obteniendo un total de 3 kg por réplica y 9 kg por tratamiento, que se transportaron al laboratorio en el menor tiempo posible. Cuando los frutos llegaron al laboratorio, se seleccionaron aleatoriamente para cada réplica lotes de 20 cerezas homogéneas en color, tamaño y sin defectos visuales. Estos lotes se conservaron a 2°C y 90 % de humedad relativa durante 7, 14, 21 y 28 días, pasado el tiempo de almacenamiento se sacaban los lotes correspondientes para realizar las determinaciones analíticas.

3.1.2 Ensayo con ácido giberélico (GA3)

Este ensayo se llevó a cabo en Chile y se realizaron aplicaciones con GA3 durante el año 2022 en las fincas comerciales de la empresa 'Garcés Fruit'. La variedad 'Bing' se encontraba en la comuna de Graneros y la variedad 'Lapins' en la comuna de Mostazal, ambas situadas en la Sexta Región. Los árboles de la variedad 'Bing' se plantaron en el año 2013 sobre un patrón 'Gisela 12' y los de la variedad 'Lapins' en el año 2016 sobre un patrón 'Colt'. La climatología en la zona de cultivo presentó una temperatura media anual de 14,12°C y una precipitación anual de 271 mm. El sistema de formación del árbol es en forma de 'Y' aprovechando una estructura auxiliar, en esta formación del tronco principal salen dos ejes, de manera que hay dos paredes frutales sobre la fila de cultivo, el marco de plantación empleado es 4 metros en las calles y 2 metros entre árboles. Las labores culturales, las condiciones climáticas y el tipo de suelo fueron similares en ambas fincas.

Las dosis empleadas de GA3 para cada variedad y tratamiento fueron de 15, 25 y 30 ppm en la primera aplicación, que se realizó en la fase de inicio de endurecimiento de hueso, en la segunda aplicación se utilizaron 15, 20 y 30 ppm en el estado fenológico de color pajizo. Los tratamientos se nombraron como la suma de ambas

aplicaciones denominándose T0 (control), T30 (15 ppm + 15 ppm), T45 (25 ppm + 20 ppm) y T60 (30 ppm + 30 ppm) (Figura 13).

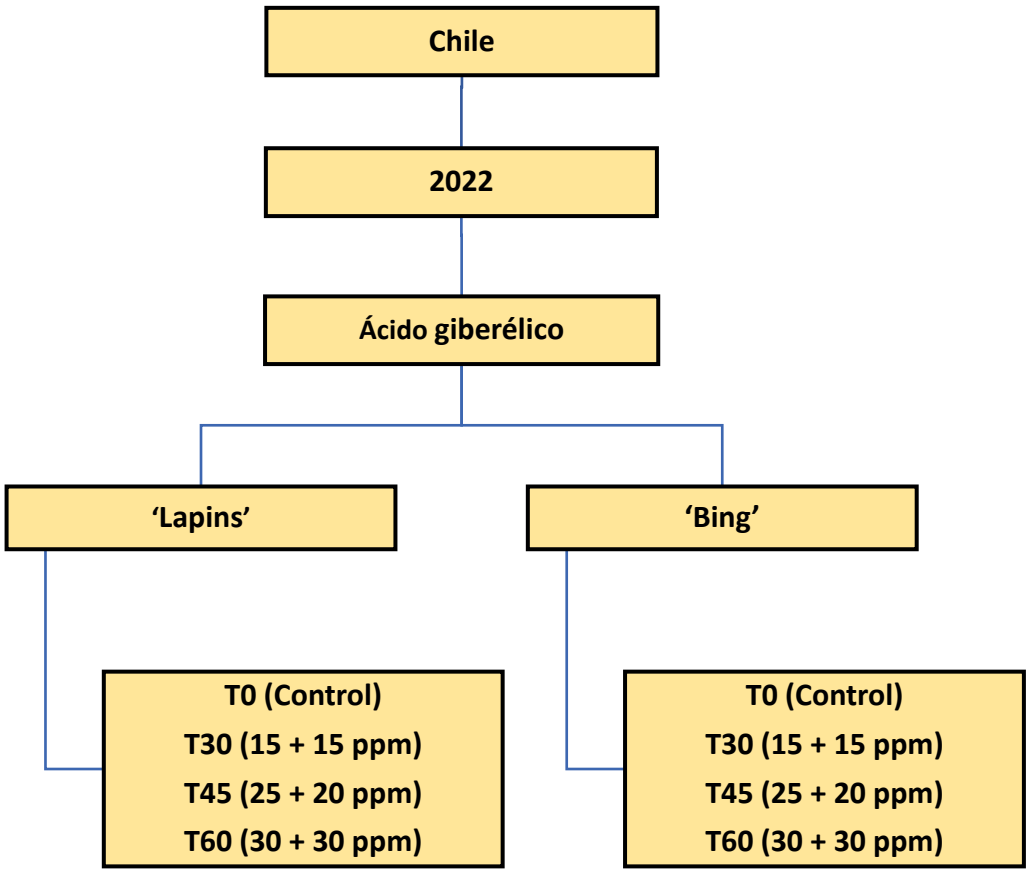


Figura 13. Esquema del diseño experimental de los tratamientos realizados en Chile.

Se realizó un diseño de bloques aleatorios para distribuir los tratamientos en la parcela, posteriormente se seleccionó una fila central en cada tratamiento de manera que no hubiera deriva de los adyacentes, y sobre esta fila se seleccionaron aleatoriamente 4 repeticiones de tres árboles que fueran representativos, además se dejó un mínimo de 6 árboles respecto a los caminos para evitar el efecto borde. El GA3 utilizado tenía una pureza del 40 % (ProGibb® 40 % SG; Valent BioSciences, Libertyville, Estados Unidos). Para realizar los tratamientos se emplearon los equipos de pulverización hidroneumáticos que se usan normalmente en la finca (NT-2000, Lerpain, Isla de Maipo, Chile). Se preparó una cantidad de 1000 litros para cada tratamiento y se aplicó a razón de 1500 l/ha (1,2 l/árbol). Las aplicaciones se realizaron a primera hora de la mañana con la vegetación seca, sin aire y con una temperatura inferior a 25°C, tras el tratamiento la vegetación quedó completamente mojada.

Se realizó un seguimiento durante el desarrollo de la cereza en el árbol y se realizaron dos recolecciones, una en color rojo caoba 3 y otra en caoba 3,5 (Tabla de color de cerezas 2022, Pontificia Universidad Católica de Chile). En cada uno de estos estados se recolectaron aleatoriamente 10 cerezas por réplica, dando lugar a 40 frutos por tratamiento, que se analizaron posteriormente en el laboratorio. En la recolección final en color 3,5 se seleccionaron aleatoriamente 100 frutos por réplica y se determinó la distribución de color y calibres mediante una máquina de visión óptica (Cherry roller, PT&I Chile, Santiago, Chile).

Para realizar las determinaciones postcosecha se recolectó 3 kg de cerezas en color 3,5 de cada réplica y los frutos se pasaron por hidrocooling a 0°C con una concentración de cloro libre de 80-100 ppm. Posteriormente la cereza se sumergió en una solución fungicida con una concentración 0,1 % de fludioxonil (SCHOLAR® 230 SC, 23 % p/v de fludioxonil; Syngenta Crop Protection Inc., Omaha, NE, Estados Unidos) y se seleccionaron 2,5 kg de frutos libres de defectos y con un calibre medio (26-28 mm), que se envasaron tras un día a 0°C en bolsa MAP (Crystal Cherry 826, San Jorge Packaging, Santiago, Chile) y se almacenaron durante 35 días a 0°C. La cereza se almacenó durante 35 días a 0°C para simular el tiempo que tarda en llegar al mercado asiático y posteriormente se dejó 3 días a 15°C para simular el tiempo hasta que llega al consumidor final. A la salida de frío se evaluó la incidencia en porcentaje para pudrición, piel de lagarto, bruising y pitting. La severidad se evaluó con una escala arbitraria valorando el daño como 1 leve, 2 moderado y 3 severo. La severidad se calculó como la suma de los frutos de cada categoría multiplicado por el valor del daño asignado y dividido por el total de frutos dañados.

3.2 Procesado y almacenamiento de muestras

En los muestreos las primeras determinaciones que se realizaron fueron las no destructivas, para posteriormente utilizar esos frutos en los ensayos destructivos. Una vez realizado los ensayos no destructivos, los 20 frutos de cada réplica se partieron en trozos eliminando el hueso y el pedicelo, alrededor de 50 gramos de esta muestra se congelaron con nitrógeno líquido. Posteriormente estas muestras se almacenaron en congeladores a -20°C, para realizar más adelante las determinaciones de antocianinas, fenoles y enzimas antioxidantes. Aproximadamente otros 50 gramos de frutos troceados se homogeneizaron manualmente en un mortero y se les extrajo el zumo mediante un prensado manual a través de dos capas de tela de algodón y este zumo se utilizó para determinar los sólidos solubles y la acidez total.

3.3 Variables productivas

En el momento de la cosecha se cuantificó la producción total por árbol, para ello se realizó la recolección individual de cada uno de ellos y se pesaron los frutos con una balanza expresando el resultado final en kg/árbol.

De cada árbol se cogieron 100 frutos al azar y se realizó un conteo para determinar el porcentaje de cereza comercial, los frutos que presentaban podredumbres, defectos visuales o cracking se consideraron frutos no comerciales, expresando este valor en %.

El peso medio del fruto se calculó para cada árbol teniendo en cuenta el peso de 100 frutos comerciales y expresando su valor en g/fruto.

3.4 Pérdida de peso

La pérdida de peso se determinó con una balanza digital KERN 440-35N (Balingen, Alemania) con dos cifras decimales de precisión en gramos. El día de la cosecha se pesaron los frutos de todos los lotes y tratamientos, posteriormente en las sucesivas semanas de muestreo a la salida de frío se pesaban nuevamente. La pérdida de peso se expresó en porcentaje (%) con respecto al peso inicial de cada lote, el resultado se indicó como la media ± error estándar (ES).

3.5 Color

El color externo de los frutos se midió con un colorímetro Minolta (CRC200, Minolta Camera Co., Osaka, Japón), en tres puntos equidistantes a lo largo del perímetro ecuatorial de cada fruto y el color se expresó como la relación a*/b* usando las coordenadas CIELab, ya que es un buen indicador de la evolución del color de la cereza (Díaz-Mula et al., 2009). Los resultados se expresaron como la media ± ES.

3.6 Propiedades reológicas del fruto

La firmeza de cada cereza se midió independientemente con un analizador de textura TX-XT2i (Stable Mycrosystems, Godalming, Reino Unido), utilizando un disco plano de acero como sonda. Se realizó un ensayo de compresión sobre el eje mayor del diámetro ecuatorial. En primer lugar, el equipo mide el diámetro del fruto y

posteriormente aplica una deformación sobre el mismo del 5 %, registrando la fuerza necesaria (N) y la distancia recorrida (mm), los resultados se expresan como la relación de ambas variables en el punto máximo en N/mm, como la media ± ES.

Las propiedades reológicas del tejido correspondientes al módulo de elasticidad, tensión y deformación, se midieron con un texturómetro TA.XT Plus (Stable Mycrosystems, Godalming, Reino Unido), equipado con una sonda cilíndrica de acero de 5 mm de diámetro, acabada en forma semiesférica y con una superficie de contacto de 19,6 mm^2. El ensayo de punción se realizó sobre el eje mayor del diámetro ecuatorial del fruto, a una velocidad de 0,3 mm s^{-1} para una penetración máxima de 5 mm, según la metodología propuesta por Param y Zoffoli (2016). Previamente la temperatura de la pulpa de los frutos fue homogeneizada a 15°C. Las variables reológicas se determinaron a partir de la curva fuerza/distancia, para el punto de inflexión, para el punto máximo (5 mm) y para el punto de bioyield, el cual ocurre cuando hay un incremento de la deformación sin cambio o con un ligero descenso en la fuerza, momento en el que comienza la ruptura de las células del tejido sin mostrar daño visible (Polat et al., 2012).

El módulo de elasticidad (E) (MPA) es la relación entre la tensión y la deformación en el punto de inflexión, este valor indica como de resistente es el fruto en el tramo elástico. Se calculó como:

$$E = \frac{FL}{A\Delta L}$$

Donde F es la fuerza (N), A el área de la sonda (mm^2), L la longitud inicial del fruto (mm) y ΔL cambio de longitud (mm) tras realizar el ensayo. La tensión (σ) (kPa) se calculó como la relación entre la fuerza aplicada y el área de la sonda (σ = F/A) y la deformación (ε) (%) como la relación entre ΔL y L (ε = ΔL/ L).

3.7 Daño mecánico inducido

Se realizaron dos tipos de ensayos para inducir el daño mecánico en la cereza, lo que permitió evaluar tanto el daño por compresión como por impacto en el momento de la cosecha. Se utilizaron cuatro repeticiones de 10 frutos, que se homogeneizaron previamente a una temperatura de pulpa de 15°C. El daño por compresión fue evaluado en los mismos frutos que se realizó el ensayo de punción, ya que esas condiciones son las que inducen este tipo de daño. Por otro lado, el daño por impacto también fue evaluado el día de la cosecha, para ello se utilizó una varilla de acero de 10 g con un diámetro de 5,4 mm y acabada en forma semiesférica, la cual se dejó caer

desde una altura de 10 cm sobre el eje mayor del diámetro ecuatorial, el dispositivo utilizado fue el descrito por Zoffoli et al. (2008). Una vez inducido el daño los frutos se colocaron en el interior de una bolsa polietileno y fueron almacenados durante 10 días a 0°C y un 100 % de humedad relativa. Para calcular el índice de daño se utilizó una escala de 5 puntos donde 0 no presentaba pitting y 4 era pitting muy severo, según la metodología propuesta por Param y Zoffoli (2016).

3.8 Sólidos solubles

Los sólidos solubles se midieron por duplicado para cada una de las réplicas a partir del zumo, con un refractómetro digital (Atago PR-101, Atago Co. Ltd., Tokio, Japón). En el ensayo con GA3 se utilizó otro modelo de refractómetro (PAL-1, Atago Co. Ltd, Tokio, Japón). En primer lugar, el refractómetro se calibró con agua destilada y posteriormente se realizaron las medidas a 20°C, expresando el resultado en g 100 g^{-1} PF como la media ± ES.

3.9 Acidez total

La acidez total se determinó por duplicado, a partir de 1 mL de zumo de cada réplica que se diluyó en 25 mL de agua destilada, posteriormente se realizó una valoración con una disolución de NaOH 0,1 N hasta pH 8,1 con un valorador automático (785 DMP Titrino, Metrohm, Herisau, Suiza). En el ensayo de GA3 se realizó la valoración manual de las muestras (Edge HI2002, Hanna Instruments, Woonsocket, RI, USA). Los resultados son la media ± ES expresados como g de ácido málico equivalente en 100 g^{-1} PF.

3.10 Fenoles totales

Los fenoles totales se extrajeron según Díaz-Mula et al. (2009) pero con ligeras modificaciones. Se partió con 5 g de la muestra que se congeló en N_2 líquido, a la que se le añadió 10 mL de una disolución de agua:metanol (2:8, v:v) que contenía 2 mM de NaF, para inactivar la actividad de la polifenol oxidasa y prevenir la degradación de los fenoles. Esta mezcla se homogeneizó durante 30 segundos en un Ultraturrax (T18 basic, IKA, Staufen, Alemania). A continuación, las muestras se dejaron en una bandeja con hielo durante 1 hora sobre un agitador orbital, a una velocidad media para favorecer la extracción de los compuestos. Posteriormente, los tubos se centrifugaron a

10000 x g durante 10 minutos a una temperatura de 4°C, a continuación se midió el volumen del sobrenadante y se cuantificaron los fenoles por duplicado para cada una de las muestras iniciales. La determinación de los fenoles se realizó mezclando 50 µL del extracto con 450 µL agua:metanol (2:8, v:v) para diluir la muestra, a continuación se añadió 2,5 mL de reactivo Folin Ciocalteau diluido (1:10), se agitó durante 5 segundos en un vortex (IKA, Staufen, Alemania) y se dejó reposar durante 2 minutos a temperatura ambiente. Luego se añadieron 2 mL de carbonato de sodio con una concentración de 75 g L^{-1} y se volvió a agitar con el vortex, a continuación los tubos de ensayo se pusieron en un baño de agua caliente a 50°C durante 15 minutos y se dejaron enfriar a temperatura ambiente. Por último, se midió la absorbancia a 760 nm con un espectrofotómetro (UV-1700 PharmaSpec, Shimadzu, Kioto, Japón), la curva de calibración se realizó con ácido gálico y los resultados fueron expresados como la media ± ES en mg ácido gálico equivalentes en 100 g^{-1} PF.

3.11 Antocianinas totales

Las antocianinas totales se extrajeron a partir de 2 g de la muestra que se congeló en N_2 líquido, a la que se le añadió 10 mL de metanol:agua:HCl (80:19:1, v/v/v) que contenía 2 mM de NaF para evitar la degradación de los compuestos fenólicos, esta mezcla se homogenizó durante 30 segundos en un Ultraturrax (T18 basic, IKA, Staufen, Alemania). Después las muestras se centrifugaron a 10000 x g durante 10 minutos a una temperatura de 4°C, a continuación se midió el volumen del sobrenadante y se cuantificaron las antocianinas por duplicado para cada una de las repeticiones, para ello se midió la longitud de onda a 530 nm con un espectrofotómetro (UV-1700 PharmaSpec, Shimadzu, Kioto, Japón). Esta longitud de onda se estableció en función de los estudios previos, realizados en las diferentes variedades para determinar el pico máximo de absorción. Las antocianinas totales se calcularon utilizando el coeficiente de extinción molar de la cianidina 3-glucósido que tiene un valor de 23900 L cm^{-1} mol^{-1} y un peso molecular de 449,2 g mol^{-1}, los resultados se expresaron como la media ± ES en mg de cianidina 3-glucósido equivalentes 100 g^{-1} PF.

3.12 Antocianinas individuales

Las antocianinas individuales se midieron por duplicado a partir del extracto obtenido para cuantificar las antocianinas totales, cuyo protocolo se ha definido anteriormente. Este extracto se filtró a través de un filtro de 0,45 µm de fluoruro de

polivinilideno (PVDF) (Millex HV13, Millipore, Bedford, MA, Estados Unidos) y se cuantificó mediante cromatografía líquida de alta resolución (HPLC) en las condiciones que describe Martínez-Esplá et al. (2014). Se inyectó un volumen de 20 µL en un sistema HPLC (Agilent HPLC 1200 Infinity series, Santa Clara, CA, Estados Unidos) y los cromatogramas se registraron a 530 nm, las antocianinas individuales se cuantificaron mediante la comparación con las curvas de calibración estándar, realizadas con cianidina 3-rutinósido, pelargonidina 3-rutinósido y cianidina 3-glucósido. Los resultados se expresaron como la media ± ES para cada antocianina individual en mg 100 g^{-1} PF.

3.13 Actividad de las enzimas antioxidantes

Las enzimas antioxidantes catalasa, ascorbato peroxidasa y peroxidasa se extrajeron a partir de 5 g de la muestra que se congeló en N_2 líquido, a la que se le añadió 10 mL de tampón fosfato 50 mM a pH 7, el cual contiene 1 mM de ácido etilendiaminotetraacético (EDTA) que actúa de agente quelante de iones, de manera que se inhibe la acción de las enzimas que necesitan la intervención de un ion metálico y un 1 % (p/v) de polivinilpirrolidona que es un agente clarificador que permite extraer la mayor parte de las impurezas. La mezcla anterior se homogenizó durante 30 segundos en un Ultraturrax (T18 basic, IKA, Staufen, Alemania), posteriormente se centrifugó a 15000 x g durante 30 minutos a una temperatura de 4°C y a continuación se midió el volumen del sobrenadante y se cuantificó la actividad de los enzimas antioxidantes según Giménez et al. (2017).

3.13.1 Catalasa

Para determinar la actividad de la enzima catalasa se añadió 100 µL del extracto descrito anteriormente más 2,9 mL de tampón fosfato 50 mM a pH 7 que contenía 15 mM H_2O_2, ocurriendo la reacción final en un volumen total de 3 mL. Se midió por duplicado el descenso de absorbancia debido a la degradación del H_2O_2 a una longitud de 240 nm durante 1 minuto. Se definió una unidad enzimática (U) como el descenso de 0,01 unidades de absorbancia por minuto y los valores de la actividad de la enzima catalasa se expresaron como la media ± ES en U min^{-1} g^{-1}.

3.13.2 Ascorbato peroxidasa

La actividad de la enzima ascorbato peroxidasa se determinó a partir de 100 µL del extracto inicial, al que se le añadió una disolución que contenía tampón fosfato 50 mM a pH 7 con 0,5 mM de ácido ascórbico y 1 mM H_2O_2, dando lugar a un volumen final de 3 mL. El descenso de absorbancia se midió por duplicado a 290 nm durante 1 minuto y la actividad de la enzima ascorbato peroxidasa se expresó como la media ± ES en U min^{-1} g^{-1}, considerando una unidad enzimática (U) como el descenso de 0,01 unidades de absorbancia por minuto.

3.13.3 Peroxidasa

La actividad de la enzima peroxidasa se cuantificó mediante la mezcla de tampón fosfato 50 mM a pH 7, 14 mM de guayacol, 12 mM H_2O_2 y 100 µL del extracto enzimático, dándose la reacción en un volumen final de 3 mL. Se midió el incremento de absorbancia debido a la oxidación del guayacol a tetraguayacol a 470 nm desde el tiempo 0 a 1 minuto. Se consideró como una unidad enzimática (U) el ascenso de 0,01 unidades de absorbancia por minuto y los resultados se expresaron como la media ± ES en U min^{-1} g^{-1}.

3.14 Residuos insolubles en alcohol

El contenido de pared celular fue cuantificado en color 3 y 3,5 mediante los residuos insolubles en alcohol, a partir la metodología propuesta por Choi et al. (2002a) y adaptado según Param y Zoffoli (2016). Esta determinación se realizó a partir de una muestra de 25 gramos de pulpa por repetición, la cual se congeló en nitrógeno líquido y se pulverizó. A continuación, se mezcló con etanol al 95 % y se hirvió a 84°C durante 30 minutos. Posteriormente los residuos se filtraron con un papel de filtro y se lavaron dos veces, primero con 50 mL de acetona al 80 % y después con 50 mL de acetona al 100 % quedando el residuo incoloro. Después se dejó secar en estufa a 35°C y se pesó, el resultado se expresó como g 100 g^{-1} PF y en mg/fruto.

3.15 Análisis estadístico

El diseño en campo se realizó aleatoriamente y para cada tratamiento había tres o cuatro réplicas en función del ensayo, compuesta cada una por 3 árboles. Los resultados se corresponden a la media ± ES de las repeticiones de cada uno de los

ensayos. Las cerezas que se conservaron y analizaron en cada repetición, provenían de una muestra representativa de los tres árboles que la formaban. Se realizó un análisis de varianza (ANOVA) mediante el programa SPSS versión 22 para Windows (SPSS Inc., Chicago, IL, Estados Unidos) o con InfoStat v 2020 (InfoStat Group, Universidad Nacional de Córdoba, Córdoba, Argentina). Las medias fueron comparadas mediante el test de Tukey o mediante la diferencia mínima significativa (LSD), considerando que hay diferencias estadísticamente significativas para *p*-valor < 0,05. También se utilizó un análisis de regresión lineal para ver la relación entre dos variables y se indicó el coeficiente de determinación para mostrar la variabilidad explicada por el modelo. Las figuras se realizaron con SigmaPlot v 11.0 (Systat Software Inc., San Jose, CA, Estados Unidos) y los datos se representaron como la media ± ES.

4. Publicaciones

4 PUBLICACIONES

4.1 Publicación 1

PUBLICACIÓN 1 (Acceso abierto)

Título: Effects of Melatonin Treatment on Sweet Cherry Tree Yield and Fruit Quality

Autores: Carrión-Antolí, A., Lorente-Mento, J.M., Valverde, J.M., Castillo, S., Valero, D., & Serrano, M.

Fecha de publicación: 22 de diciembre de 2021

Revista: Agronomy

Editorial: Multidisciplinary Digital Publishing Institute (MDPI)

ISSN: 2073-4395

Factor de impacto (2023): 3.3

Factor de impacto (5 años): 3.7

Categoría JCR®: Agronomy

Rango: 20/126

Cuartil: Q1

CiteScore: 6.2

DOI: https://doi.org/10.3390/agronomy12010003

agronomy

MDPI

Article

Effects of Melatonin Treatment on Sweet Cherry Tree Yield and Fruit Quality

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Abstract: The effects of preharvest melatonin treatment, applied as foliar spray at 0.1, 0.3 and 0.5 mM concentration at three key points of fruit development (pit hardening, initial colour changes and 3 days before harvesting), on crop yield and fruit quality properties at harvest was evaluated in three sweet cherry cultivars, 'Prime Giant', 'Lapins' and 'Sweet Heart', and two years, 2019 and 2020. The results showed that melatonin treatment had no effect on crop yield, except for the 'Lapins' cultivar, in which increases were found. However, decayed and cracked fruit percentage was decreased in all cultivars in 2020 when adverse weather conditions occurred and commercial crop yield was increased, especially for 0.3 mM dose. Fruit quality traits at harvest, such as fruit weight, colour, firmness, total soluble solids and titratable acidity, were enhanced by melatonin treatments in all sweet cherry cultivars and in both years. Moreover, bioactive compounds, such as total phenolics and total and individual anthocyanins, were also found at higher levels in fruit from melatonin-treated trees with respect to controls. Thus, taking into account all these effects, 0.3 mM melatonin foliar spray, at three key points of fruit developmental stages, could be a useful tool to improve crop yield and quality traits of sweet cherries, especially their content on bioactive compounds with antioxidant properties and health beneficial effects.

Keywords: *Prunus avium*; yield; firmness; acidity; soluble solids; phenolics; anthocyanins

Citation: Carrión-Antolí, A.; Lorente-Mento, J.M.; Valverde, J.M.; Castillo, S.; Valero, D.; Serrano, M. Effects of Melatonin Treatment on Sweet Cherry Tree Yield and Fruit Quality. *Agronomy* **2022**, *12*, 3. https://doi.org/10.3390/agronomy12010003

Academic Editors: Vasileios Ziogas and Konstantinos Papoutsis

Received: 29 November 2021
Accepted: 16 December 2021
Published: 22 December 2021

1. Introduction

Sweet cherry fruit (*Prunus avium* L.) is highly valued by consumers worldwide due to its excellent quality properties, mainly its colour, sugar and organic acid content, flavour, texture and juiciness [1–3]. In addition, sweet cherry fruit are rich in bioactive compounds, namely phenolics (including anthocyanins) and ascorbic acid, which are responsible of the health benefit effects attributed to sweet cherry consumption, the major ones being antimicrobial, antidiabetic, anticancer and anti-inflammatory effects, as well as neuroprotection and cardiovascular protection activity [4–6]. According to FAOSTAT [7], Spain is the sixth largest sweet cherry producing country in the world, with 118,380 tons, after Turkey, the USA, Chile, Uzbekistan and Iran.

Melatonin was identified in 1995 in plants from mono- and dicotyledonous species [8], and since then, its effect on regulating a wide range of plant physiological processes, from seed germination to fruit maturation and senescence, as well as on inducing plant resistance to biotic and abiotic stresses, has been reported in a wide range of plant species [9,10]. In addition, recent reports have shown a role of melatonin on regulating fruit ripening, although most of them have been focused on postharvest treatments [11,12]. However, the effect of pre-harvest melatonin treatments on on-tree fruit ripening and quality traits at harvest has been evaluated in very few papers, and different effects have been reported

depending on fruit species, concentration or application time. Thus, the application of 0.1 mM melatonin to tomato plants in the irrigation system increased lycopene and sugar content in fruits showing an acceleration of fruit ripening [13]. However, melatonin foliar spray treatment of apricot trees increased crop yield and fruit weight, but no effect on fruit on-tree ripening was observed [14]. In addition, enhanced apricot quality parameters at harvest and maintenance during storage, at chilling and not-chilling temperatures, have recently been reported in apricot fruits from melatonin-treated trees [15]. Accordingly, melatonin treatment of 'Mollar de Elche' pomegranate trees increased fruit quality traits at harvest, including anthocyanin and phenolic content, and overall fruit quality was maintained during storage at higher levels as compared with fruit from control trees [16].

Specifically, in sweet cherry, it has been recently reported that postharvest melatonin treatments, by dipping in 0.05–1.0 mM melatonin solutions for 5 min, delayed senescence and maintained fruit quality during storage in some sweet cherry cultivars, such as 'Siah Mashhad' [17], 'Sunburst' [18], 'Santina' and Royal Rainier' [19]. On the other hand, melatonin (0.01 and 0.1 mM) applied directly to fruit surface at stage II of development (green and large in size fruit) delayed fruit ripening, manifested by delayed anthocyanin accumulation, in the 'Prime Giant' cultivar [20]. On the contrary, foliar spray of sweet cherry 'Ferrovia' with 0.5 mM melatonin 2 and 1 weeks prior to harvest led to fruit with a higher content of the individual anthocyanins cyaniding 3-rutinoside, cyaniding 3-galactoside and cyaniding 3-glucoside at harvest, showing an acceleration of the fruit ripening process [21]. However, as far as we know, no literature is available regarding the effect of pre-harvest melatonin treatment on sweet cherry tree yield or fruit quality properties at harvest. Thus, the aim of the present experiment was to evaluate the effects of foliar spray of sweet cherry trees with melatonin on yield and on fruit quality and nutritional and functional properties at harvest on three cultivars and two experimental years.

2. Materials and Methods

2.1. Plant Material and Experimental Design

Experiments were performed in a commercial field plot located at Jumilla (Murcia, Spain, UTMX: 463.700 UTMY: 4.268.900) with sweet cherry trees (*Prunus avium* L.) of cultivars 'Prime Giant' and 'Lapins' in 2019 and 2020. In addition, the 'Sweet Heart' cultivar was also assayed in 2020. 'Prime Giant' and 'Lapins' cultivars were planted in January 2012 and 'Sweet Heart' in January 2015 and all of them grafted onto SL-64 rootstock. Climatic conditions in the crop field were: mean annual temperatures 15.24 and 15.30 °C for 2019 and 2020, respectively, and an accumulated rainfall of 357 and 352 mm for 2019 and 2020, respectively. Sweet cherry trees were under similar agronomic practices for both years with 60:30:100 kg ha^{-1} N:P:K fertilisation and open-centre pruning. Melatonin treatments were performed by applying 3 L per tree (with a manual sprayer machine) of freshly prepared melatonin solutions at 0.1, 0.3 and 0.5 mM containing 1 mL L^{-1} Tween. Similarly, 3 L of distilled water with 1 mL L^{-1} Tween were applied to control trees. For each treatment and cultivar, three replicates of three trees were used. Each treatment was repeated three times, at pit hardening, at the beginning of colour changes and 3 days before harvest (Table 1). Sweet cherries were harvested at commercial ripening stage, according to commercial practices, based on the characteristic skin colour of each cultivar. Total yield per tree was recorded as kg $tree^{-1}$ and a sample of 100 fruit per tree was taken at random and weighed to obtain data of fruit weight average. Lots for each replicate were mixed and transported to laboratory in 3 h. Then, 3 lots of 20 fruits, homogenous in size and colour and without visual defects, were taken at random from each field replicate and treatment and used for the following analytical measures.

Table 1. Dates for treatments (T1, T2 and T3) and harvest date in the 2019 and 2020 experiments.

Year	Cultivar	T1	T2	T3	Harvest
2019	'Prime Giant'	4 May	22 May	8 June	11 June
	'Lapins'	5 May	1 June	17 June	21 June
2020	'Prime Giant'	23 April	15 May	30 May	4 June
	'Lapins'	24 April	26 May	13 June	17 June
	'Sweet heart'	28 April	1 June	27 June	2 July

2.2. Quality Parameters

Colour was measured independently in each fruit with a Minolta colorimeter (CRC200, Minolta Camera Co., Tokyo, Japan), and the CIELab coordinates. Three readers were taken for each fruit at three equidistant points along the equatorial perimeter and colour was expressed as a*/b* ratio, which is a good index for sweet cherry colour [1]. The results are the mean ± SE. To measure fruit firmness, a TX-XT2i Texture Analyser (Stable Mycrosystems, Godalming, UK) equipped with a flat probe was used. The machine measured fruit diameter and applied a force to achieve a 3% fruit diameter deformation. Fruit firmness was expressed as the relation between the applied force and the travelled distance (N mm^{-1}) and results are the mean ± SE. After that, the flesh of the 20 fruit of each replicate was cut in small pieces to obtain a homogeneous sample. About 50 g were squeezed through two layers of cotton cloth and the juice was used to measure, in duplicate, the total soluble solids (TSS) and titratable acidity (TA). Other 50 g of sample was frozen under liquid N_2, ground and stored at −20 °C to measure total phenolics and anthocyanins. To measure TA, 1 mL of juice was diluted in 25 mL of distilled H_2O and titrated with 0.1 N NaOH up to pH 8.1 by using an automatic titration system (785 DMP Titrino, Metrohm, Herisau, Switzerland). The results (mean ± SE) were expressed as g malic acid equivalent 100 g^{-1} in fresh weight basis. TSS were measured in the juice of each sample by using a digital refractometer (Atago PR-101, Atago Co. Ltd., Tokyo, Japan) at 20 °C and results (mean ± SE) were expressed as 100 g^{-1} on a fresh weight basis.

2.3. Total Phenolic and Anthocyanin Quantification

To extract the total phenolis compounds, 5 g of pulp were homogenised with 15 mL of water:methanol (2:8, v/v) containing 2 mM NaF for 30 s, by using an Ultraturrax (T18 basic, IKA, Berlin, Germany). The extracts were centrifuged at 10,000× g for 10 min at 4 °C and the supernatant was used to quantify total phenolics in duplicate according to Díaz-Mula et al. [1]. Briefly, 50 µL of appropriately diluted extracts were mixed with 2.5 mL of water-diluted Folin-Ciocalteau reagent and incubated for 2 min at room temperature. Then, 2 mL of sodium carbonate (75 g L^{-1}) was added and shaken vigorously. Thereafter, the mixture was incubated in a water bath at 50 °C for 15 min, and finally, the absorbance was measured at 760 nm. A calibration curve was performed with gallic acid and results (mean ± SE) were expressed as mg gallic acid equivalent 100 g^{-1} on a fresh weight basis. Total anthocyanins were extracted by homogenising 5 g of flesh with 15 mL of methanol/formic acid/water (25:1:24, $v/v/v$). The extracts were centrifuged at 10,000× g for 10 min at 4 °C and the supernatant was used to quantify total anthocyanins by reading absorbance at 520 nm according to previous report [16]. The results were expressed as mg 100 g^{-1} of cyaniding 3-glucoside equivalent (cyn 3-glu, molar absorption coefficient of 26,900 L cm^{-1} mol^{-1} and molecular weight of 449.2 g mol^{-1}). Individual anthocyanins were quantified, in duplicate, in the previous extracts after filtration through 0.45 µm PVDF filter (Millex HV13, Millipore, Bedford, MA, USA) by using HPLC analysis, as previously described by Martínez-Esplá et al. [3]. In brief, 20 µL of the extracts were injected into a HPLC system (Agilent HPLC 1200 Infinity series, Santa Clara, CA, United States) and chromatograms were recorded at 520 nm. Individual anthocyanins were quantified by comparison with standard calibration curves performed with cyanidin 3-rutinoside (Cyn 3-rut), pelargonidin 3-rutinoside (Pelg 3-rut) and cyanidin 3-glucoside Cyn 3-gluc) and

results were expressed as mg 100 g^{-1} (mean ± SE of measures in duplicate in each of the three replicates).

2.4. Statistical Analysis

A factorial design with melatonin treatments (0, 0.1, 0.3 and 0.5 mM) with three triplicates (n = 3) of three trees per replicate was performed for each sweet cherry cultivar and year (2019 and 2020). For all the measured parameters, year and cultivar, data are the mean ± SE of three replicates (n = 3). An analysis of variance (ANOVA) was performed by using the SPSS software version 20 (SPSS Inc., Chicago, IL, USA) and means were compared by Tukey's test to find significant differences among treatments at $p < 0.05$. In addition, a t-test was performed by comparison between control and melatonin-treated fruit, for each cultivar and year. Finally, linear regressions were performed between colour a*/b* index and anthocyanin content and between total anthocyanin and phenolic concentration separately for each cultivar and each year.

3. Results

3.1. Crop Yield and Fruit Weight

Sweet cherry fruit were harvested when fruit reached their commercial ripening stage, according to commercial practices. Preharvest melatonin treatments at 0.3 and 0.5 mM concentrations significantly ($p < 0.05$) increased the total yield of the 'Lapins' cultivar in 2019, while in the 2020 experiment, significant increases were found for 0.3 mM (Figure 1). However, no significant effects of melatonin treatments on yield were observed for 'Prime Giant' or 'Sweet Heart' cultivars. In addition, important differences in total yield were observed for both years. Thus, for the 'Lapins' cultivar, the total yield of control trees was 19.02 ± 2.31 kg $tree^{-1}$ in 2019 and 35.33 ± 1.92 kg $tree^{-1}$ in 2020. On the contrary, for the 'Prime Giant' cultivar, the yield of control trees was 2.5-fold reduced from 2019 to 2020 (Figure 1). Moreover, a great proportion (50.50 ± 3.19%) of the 'Prime Giant' harvested fruit from control trees in the 2020 experiment were decayed or cracked fruit (unmarketable fruit), while this proportion was significantly ($p < 0.05$) reduced in melatonin-treated trees, in a concentration-dependent way, up to 32.66 ± 2.53% in 0.5 mM treated trees, leading to significant increases in commercial yield (Figure 2). The percentage of unmarketable fruit (decayed and cracked fruit) from control trees in the 2020 experiment was very low for 'Lapins' and 'Sweet Heart' cultivars (less than 5%), although they were also significantly ($p < 0.05$) reduced by melatonin treatments and commercial yield was increased, the higher effect being observed with 0.3 mM concentration (Figure 2). Fruit weight of 'Prime Giant' cherries from control trees was ca. 11 and 13.5 g in 2019 and 2020, respectively, and significantly higher ($p < 0.05$) in fruit from melatonin-treated trees (except for 0.5 mM dose in 2019), the major increases (9–13%) being found for 0.1 and 0.3 mM doses in both years. For the 'Sweet Heart' cultivar, fruit weight was also increased by melatonin treatments, from 4 to 8% for 0.1 to 0.5 mM doses, while for the 'Lapins' cultivar, the effect of melatonin treatments on increasing fruit weight was significant only for the 0.5 mM dose in the 2020 experiment (Figure 3).

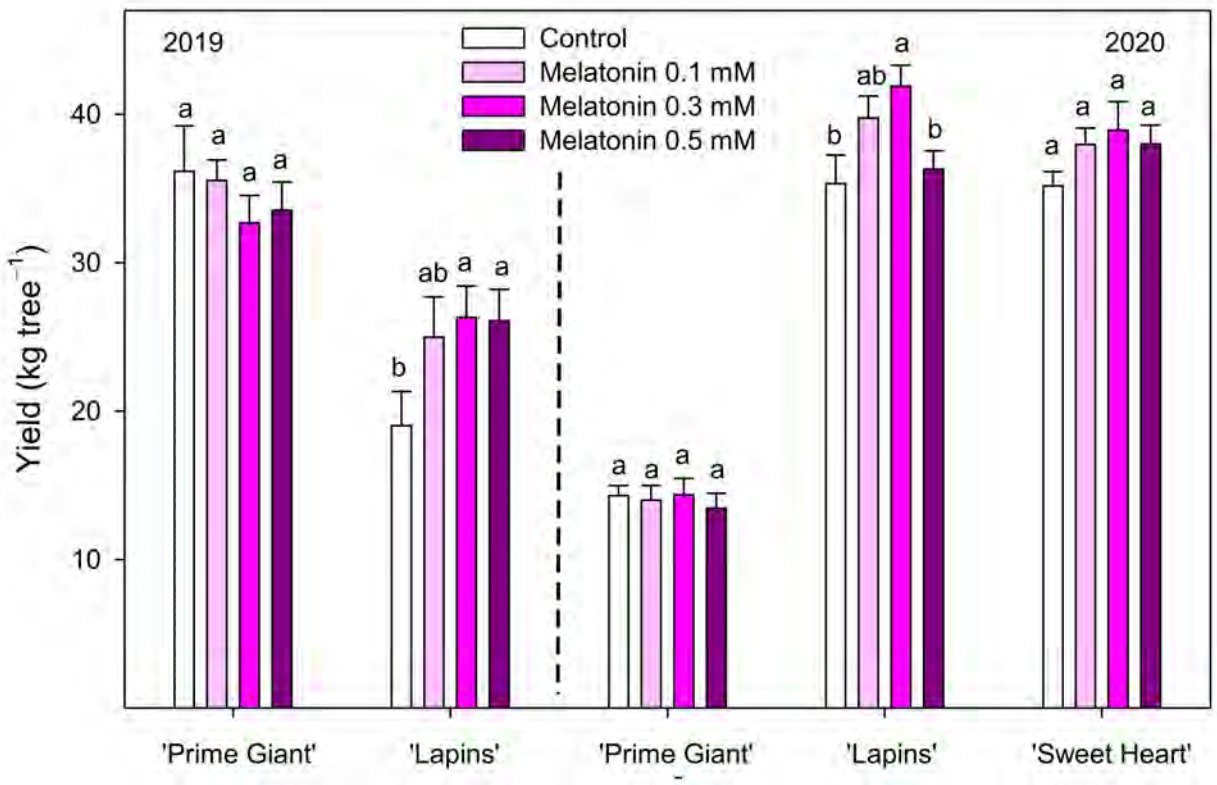

Figure 1. Yield (kg tree^{-1}) in control and melatonin-treated trees in the 2019 and 2020 experiments. Data are the mean ± SE of three replicates of three trees. Different letters show significant differences ($p < 0.05$) between treatments for each cultivar and year.

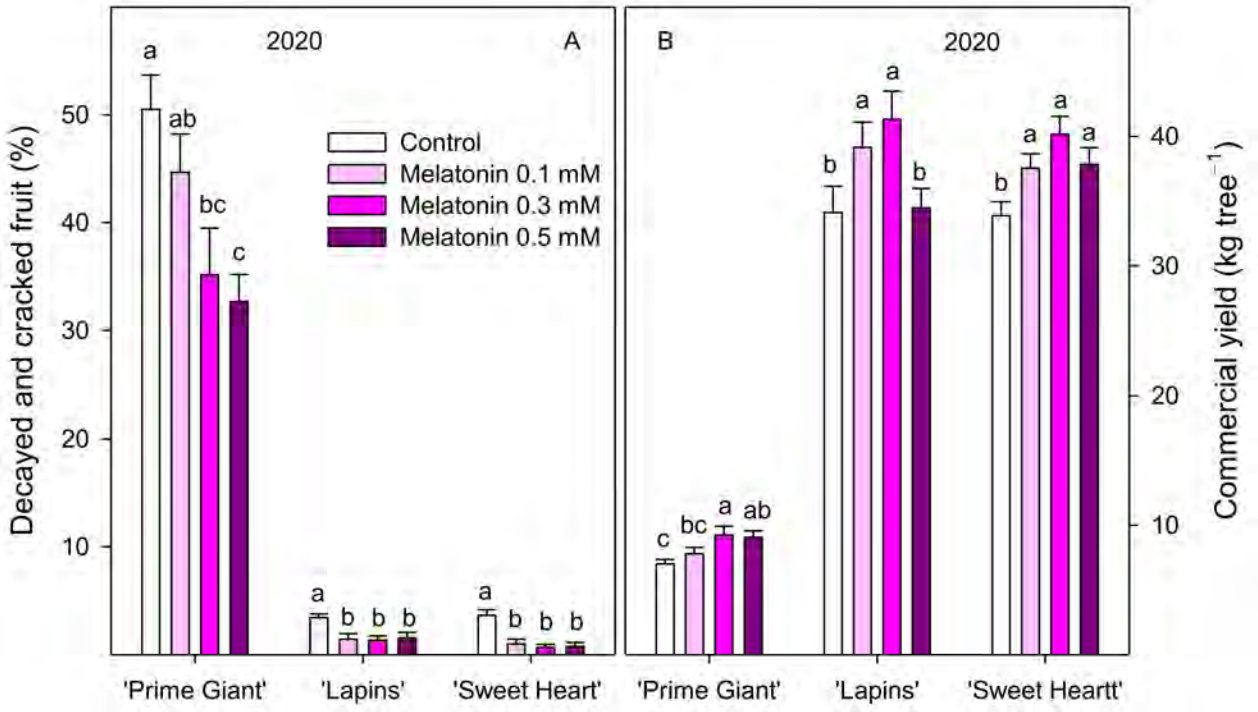

Figure 2. Decayed and cracked fruit (**A**) and commercial yield (**B**) in control and melatonin-treated trees in the 2020 experiments. Data are the mean ± SE of three replicates of three trees. Different letters show significant differences ($p < 0.05$) between treatments for each cultivar and year.

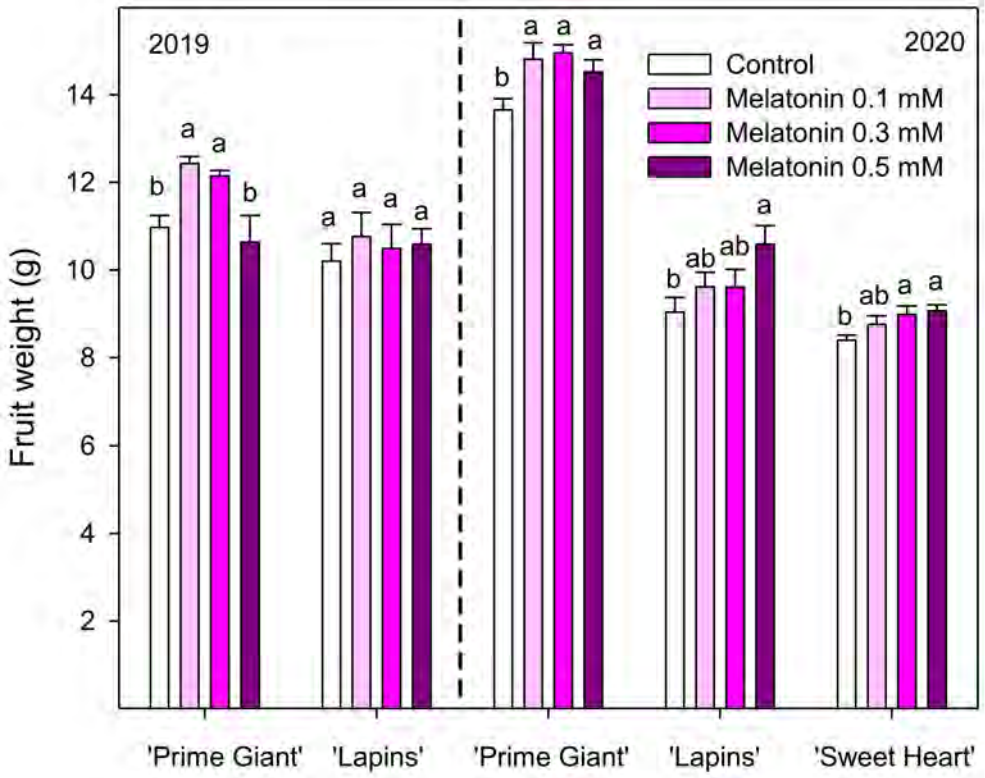

Figure 3. Effects of sweet cherry trees' melatonin treatments on fruit weight (g) in the 2019 and 2020 experiments. Data are the mean ± SE of 100 fruit taken at random from commercial fruit of control or treated trees. Different letters show significant differences ($p < 0.05$) between treatments for each cultivar and year.

3.2. Fruit Quality Parameters

The surface skin colour, expressed as the a*/b* index, was 3.1–3.2 in 'Prime Giant' and 'Sweet Heart' cultivars and ca. 4.3 in 'Lapins' for control fruit, showing a deeper red colour in the last cultivar (Table 2). However, sweet cherries from melatonin-treated trees showed significantly ($p < 0.05$) higher a*/b* colour index for all assayed cultivars and, in general, the highest values were found for 0.5 mM dose. Significantly ($p < 0.05$) higher values of fruit firmness were also observed in fruit from melatonin-treated trees with respect to those from control trees. However, for this quality parameter, no significant differences were observed among the applied melatonin doses, except for 'Prime Giant' in 2019, in which the highest firmness values were found in fruit from 0.3 and 0.5 mM melatonin-treated trees (Table 2). With respect to TSS content, values of ~20.4 and 22.3 g 100 g^{-1} were found in control fruit of the 'Prime Giant' cultivar for 2019 and 2020, respectively, and ~20.4 and 19.0 g 100 g^{-1} for 'Lapins', showing differences on this quality parameter between different growing years and cultivars. Nevertheless, a similar effect of preharvest melatonin treatments on TSS was observed for all cultivar and years, since TSS values were significantly ($p < 0.05$) enhanced by these treatments (Table 2). In general, the highest increases were found with 0.3 and 0.5 mM doses, ranging from 15% in 'Prime Giant' in the 2019 experiment to 5% in 'Sweet Heart' in the 2020 experiment. Finally, TA values were also significantly ($p < 0.05$) increased by preharvest melatonin treatments, ranging from 10 to 20%, depending on cultivar and year, without significant differences attributed to melatonin concentration (Table 2).

Table 2. Effects of preharvest melatonin treatments at 0.1, 0.3 and 0.5 mM concentration on fruit colour, firmness, total soluble solids and titratable acidity.

		Melatonin Concentration			
Cultivar	**Year**	**Control**	**0.1 mM**	**0.3 mM**	**0.5 mM**
		Fruit Colour (a*/b*)			
'Prime Giant'	2019	3.20 ± 0.07c	3.47 ± 0.07b	3.47 ± 0.06b	3.92 ± 0.06a
'Prime Giant'	2020	3.12 ± 0.09c	3.37 ± 0.11b	3.50 ± 0.07b	3.72 ± 0.11a
'Lapins'	2019	4.25 ± 0.07c	4.55 ± 0.07b	4.50 ± 0.07b	4.76 ± 0.07a
'Lapins'	2020	4.33 ± 0.11b	4.77 ± 0.14a	4.73 ± 0.09a	4.68 ± 0.09a
'Sweet Heart'	2020	3.13 ± 0.17c	3.43 ± 0.11b	3.59 ± 0.03b	3.79 ± 0.07a
		Fruit firmness (N mm^{-1})			
'Prime Giant'	2019	1.59 ± 0.04c	1.83 ± 0.04b	2.02 ± 0.04a	1.91 ± 0.03a
'Prime Giant'	2020	1.49 ± 0.08b	1.84 ± 0.09a	1.99 ± 0.06a	1.78 ± 0.10a
'Lapins'	2019	1.56 ± 0.05b	1.73 ± 0.04a	1.80 ± 0.04a	1.77 ± 0.04a
'Lapins'	2020	1.60 ± 0.05a	1.83 ± 0.06a	1.81 ± 0.04a	1.80 ± 0.04a
'Sweet Heart'	2020	1.54 ± 0.07b	1.87 ± 0.06a	2.05 ± 0.09a	1.85 ± 0.05a
		Total soluble solids (g 100 g^{-1})			
'Prime Giant'	2019	20.43 ± 0.24c	22.45 ± 0.12b	23.00 ± 0.12ab	23.47 ± 0.41a
'Prime Giant'	2020	22.28 ± 0.22b	23.93 ± 0.45a	23.77 ± 0.23a	24.27 ± 0.17a
'Lapins'	2019	20.36 ± 0.14b	21.40 ± 0.26a	22.10 ± 0.47a	22.17 ± 0.16a
'Lapins'	2020	18.98 ± 0.12c	20.07 ± 0.06b	20.52 ± 0.22ab	21.05 ± 0.08a
'Sweet Heart'	2020	19.70 ± 0.21b	20.92 ± 0.07a	20.75 ± 0.16a	20.72 ± 0.38a
		Titratable acidity (g 100 g^{-1})			
'Prime Giant'	2019	1.10 ± 0.01b	1.31 ± 0.02a	1.24 ± 0.03a	1.22 ± 0.01a
'Prime Giant'	2020	1.25 ± 0.01b	1.40 ± 0.02a	1.39 ± 0.02a	1.36 ± 0.03a
'Lapins'	2019	0.95 ± 0.01b	1.15 ± 0.03a	1.12 ± 0.01a	1.09 ± 0.02a
'Lapins'	2020	1.08 ± 0.02b	1.21 ± 0.01a	1.18 ± 0.01a	1.17 ± 0.01a
'Sweet Heart'	2020	1.22 ± 0.01b	1.37 ± 0.04a	1.35 ± 0.02a	1.36 ± 0.03a

Data are the mean ± SE of fruits harvested from three replicates of three trees for the 2019 and 2020 experiments. For each cultivar and year, different lowercase letters show significant differences ($p < 0.05$) between treatments.

3.3. Total Phenolics and Total and Individual Anthocyanins

Total phenolic concentration was different depending on cultivar, year and melatonin dose applied. In control fruit of the 'Prime Giant' cultivar, the total phenolic content at harvest was 80.05 ± 3.84 and 73.67 ± 2.24 mg 100 g^{-1} for 2019 and 2020, respectively, while for 'Lapins', cultivar important differences were found for both years, 64.45 ± 1.22 and 107.48 ± 3.96 mg 100 g^{-1}, respectively. However, in general, for all cultivars and years, preharvest treatments with melatonin led to significant increases ($p < 0.05$) in phenolic concentration, the major effects being observed for 0.3 and 0.5 mM doses, with increases up to 45% in the 'Lapins' cultivar in the 2019 experiment compared to the controls (Figure 4). With respect to the anthocyanin concentration, it was significantly increased ($p < 0.05$) by all applied melatonin doses in both experimental years and cultivars. The highest effects were found in the 2019 experiment for the 0.5 mM dose, which led to 82 and 57% increases in 'Prime Giant' and 'Lapins' cultivars, respectively. In the 2020 experiment, all melatonin treatments increased anthocyanin concentration, these increases ranging from 14 to 25%, although no significant differences ($p < 0.05$) among melatonin concentrations were observed for any of the three assayed cultivars (Figure 5).

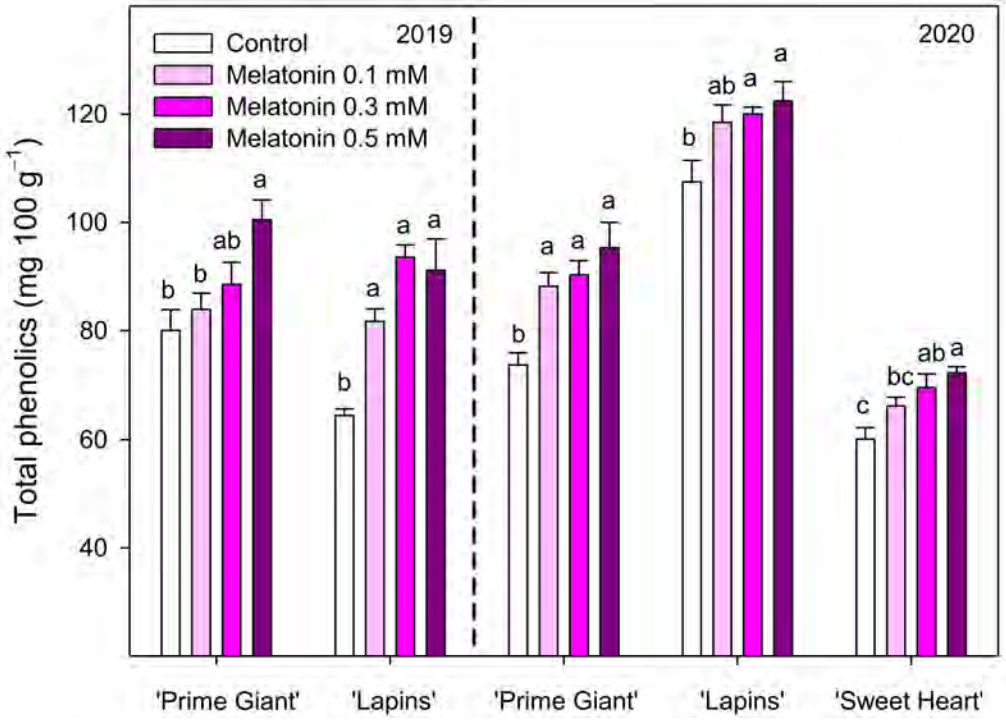

Figure 4. Total phenolic concentration in sweet cherries from control and melatonin-treated trees in the 2019 and 2020 experiments. Data are the mean ± SE of determinations made in duplicate in three replicates. Different letters show the significant differences ($p < 0.05$) between treatments for each cultivar and year.

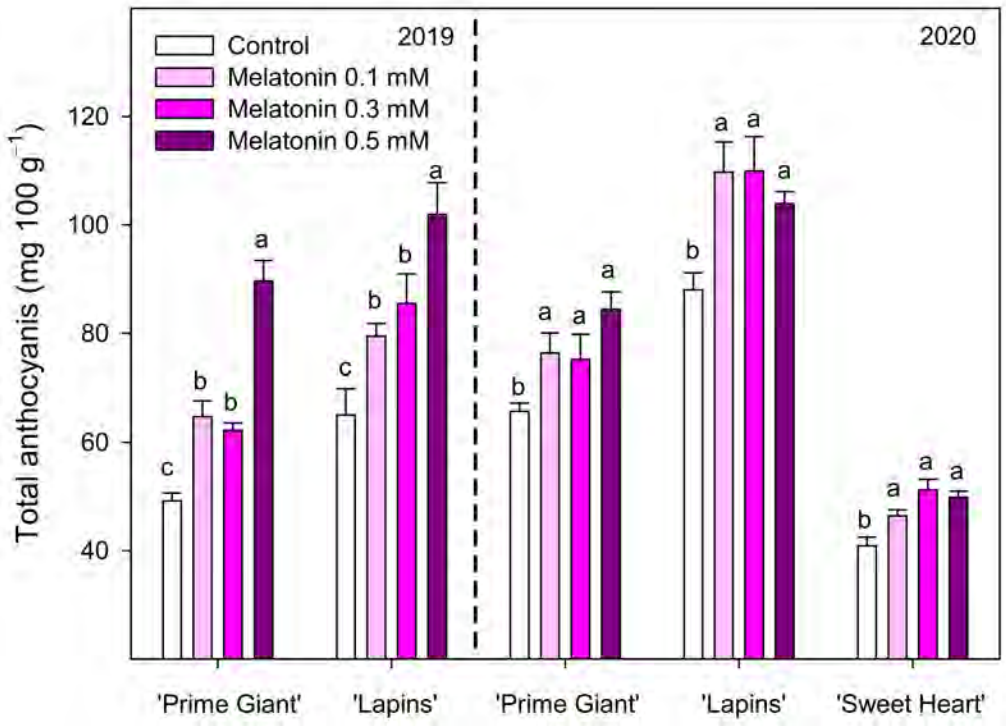

Figure 5. Total anthocyanin concentration in sweet cherries from control and melatonin-treated trees in the 2019 and 2020 experiments. Data are the mean ± SE of determinations made in duplicate in three replicates. Different letters show significant differences ($p < 0.05$) between treatments for each cultivar and year.

The concentration of individual anthocyanins were measured in the 2020 experiment and the result showed that cyaniding 3-*O*-rutinoside (Cyn 3-rut) was the major anthocyanin in all sweet cherry cultivars, with concentrations of 76.17 ± 1.30, 88.65 ± 2.27 and 52.11 ± 0.62 mg 100 g^{-1} in control fruit of 'Prime Giant', 'Lapins' and 'Sweet Heart' cultivars, respectively (Figure 6). Pelargonidin 3-*O*-rutinoside (Pelg 3-rut) was found at a much lower concentration, from 7.5 to 9.1 mg 100 g^{-1} for 'Lapins' and 'Prime Giant', respectively, in control fruit. Cyaniding 3-*O*-glucoside (Cyn 3-gluc) was only found in control fruit of the 'Sweet Heart' cultivar and at very low concentration, ca. 0.5 mg 100 g^{-1}. However, it is worth noting that concentrations of all individual anthocyanins were significantly ($p < 0.05$) enhanced as a consequence of melatonin pre-harvest treatments. These increments ranged from 20 to 40% for Cyn 3-rut and from 10 to 50% for Pelg 3-rut depending on the cultivar, although no significant differences were observed among the applied melatonin doses (Figure 6).

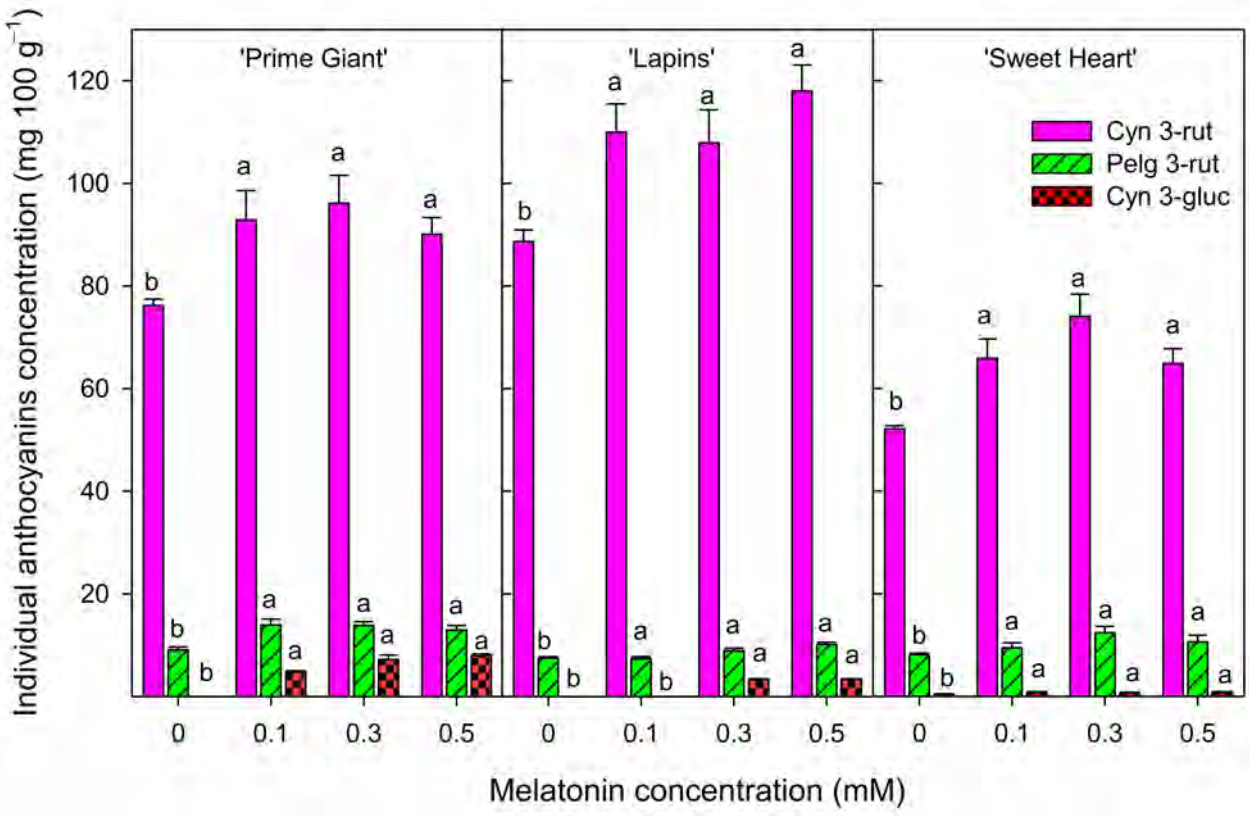

Figure 6. Individual anthocyanins concentration (cyaniding 3-rutinoside, cyn 3-rut, pelargonidin 3-rutinoside, pelg 3-rut, and cyanidin 3-glucoside, cyn 3-gluc) in sweet cherries from control and melatonin-treated trees in the 2020 experiment. Data are the mean ± SE of determinations made in duplicate in three replicates. Different letters show significant differences ($p < 0.05$) between treatments for each cultivar.

4. Discussion

Melatonin treatments of cherry trees increased crop yield in the 'Lapins' cultivar in both experimental years, especially with 0.3 mM dose, but no significant effects were observed for 'Prime Giant' or 'Sweet Heart' cultivars (Figure 1). No previously published papers are available in the literature regarding the effect of melatonin treatment of sweet cherry trees on crop yield for comparative purposes, and only a few reports are available in other fruit species. Thus, increases in crop yield as a consequence of melatonin tree treatment during fruit development have been reported in 'Colorado', 'Mikado' and 'Canino' apricots [14,15], as well as in tomato plants when seeds were soaked with melatonin or melatonin was applied in the irrigation system [13]. These effects were attributed to an increase in plant photosynthetic rate and leaf chlorophyll content. The yield of pomegranate tree was also

increased by 0.1 mM melatonin preharvest treatment due to increases in fruit size and number of fruit harvested by tree [22]. Similarly, a 14% of crop yield was obtained in well-irrigated tomato plants when melatonin was applied at 30 and 50 days after transplanting, while this increase was 37% under water deficit stress [23]. However, in another previous paper, increases in tomato crop yield in melatonin-treated plants were only found when plants were exposed to rain acid stress but not in tomato plants under optimal growth conditions [24]. Then, the effect of melatonin treatment during fruit development on plant on increasing crop yield is clear when plants are under abiotic or biotic stresses. Nevertheless, under optimal conditions this effect depends on the plant species or cultivars, the concentration applied or the plant development stage and deserves further research [25].

On the other hand, the total yield was quite different in both experimental years. Thus, for the 'Lapins' cultivar, the yield of control trees was ca. 19 and 35 kg tree^{-1} in 2019 and 2020, respectively, which could be attributed to the normal variability occurring in cherry tree between the years. In this sense, a three-fold higher yield was observed in 'Skeena' cherry trees grown in Portugal in 2016 with respect to 2015, which was attributed to a higher crop load rather than larger fruit size [26]. However, for the 'Prime Giant' cultivar, the yield of 2020 experiment was 2.5-fold reduced with respect to 2019, either in control as in treated trees (Figure 1). This reduction was due to a heavy rainfall (15 L m^2) that occurred on May 16, causing dropping of many fruits that were at the initial colour change stage. On the contrary, 'Lapins' and 'Sweet Heart' cultivars were at the pit hardening stage when the heavy rainfall occurred and were not as highly affected as the 'Prime Giant' cultivar, reaching high yield values, which for 'Lapins' were even higher than in the 2019 experiment. In addition, in 'Prime Giant', ca. 50% of the harvested fruit from control trees in 2020 were unmarketable fruit, due to the appearance of cracking and/or fungal decay as a consequence of the heavy rain that occurred. However, the percentage of unmarketable fruit was reduced in melatonin-treated trees, leading to increases in commercial yield, especially with a 0.3 mM dose (Figure 2). For 'Lapins' and 'Sweet Heart' cultivars, in spite of being much less affected by these high rains and rendering a much lower percentage of unmarketable fruit in 2020, these percentages were also reduced in melatonin-treated trees.

Sweet cherry size is a quality parameter highly valued by consumers and large-sized fruit reached higher prices on the market than small ones. The results of the present experiments showed, in general, an effect of 0.3 and 0.5 mM melatonin treatments on increasing fruit weight, although in the 'Lapins' cultivar, this effect was significant only for 0.5 mM dose in 2020 (Figure 3). Increases in fruit size have been recently reported in pomegranate fruit from melatonin-treated trees [22] as well as on grape berries [27], which were attributed to an increase of the fruit sink strength, leading the fruit to uptake more sugars and reach larger-size at harvest. Fruit colour, firmness and TSS and TA contents are also quality parameters highly valued by consumers in sweet cherry fruit and it is worth noting that all of them were found at higher levels in fruit from melatonin-treated trees than in controls (Table 2). It has been reported in a wide range of sweet cherry cultivars that fruit firmness decreased during the on-tree ripening process, while increases occurred in colour, TSS and TA contents [1,28,29]. Thus, as melatonin treatment led to fruit with higher firmness at harvest, it could be inferred a delay of fruit ripening as a consequence of melatonin treatment. On the contrary, colour, TA and TSS were enhanced as a consequence of melatonin treatments, which would show an accelerate ripening. In fact, ripening in sweet cherry, as well as in other fruit species, is a complex and coordinated process and each single parameter evolves at a particular pace [30]. In the 'Ferrovia' cultivar, foliar spray with 0.5 mM melatonin 2 and 1 weeks prior to harvest did not affect the ripening process, leading to fruit with similar TSS, TA and colour values than controls [21]. Accordingly, no significant effects of 0.1 or 0.01 mM melatonin treatment (applied 19 days before harvesting) on fruit firmness, TA or TSS were reported for the 'Prime Giant' cultivar by Tijero et al. [20], although anthocyanin content was significantly decreased. These authors proposed that sweet cherry ripening is modulated by a delicate hormonal balance. Thus, melatonin, in combination with jasmonic and salicylic acids, would have inhibitory roles in fruit ripening,

since these three plant hormones decreased as ripening started, while abscisic acid would have a ripening stimulatory effect since it increased with ripening. On the contrary, three-fold application of 0.05 and 0.1 mM melatonin (at week intervals, starting just before the fruit turned red) on 'Hongdeng' sweet cherry trees led to higher contents on TSS and total anthocyanins, although TA content was decreased [31]. These findings suggest that the effects on melatonin preharvest treatments on sweet cherry ripening and quality traits are notably influenced by the applied concentration, developmental stage and cultivar, among other possible factors. However, when melatonin was applied as postharvest dipping general effects on delaying postharvest fruit ripening, maintaining fruit quality traits and extending shelf-life have been reported in several fruit species, such as banana, peach, pear, kiwi, strawberry and pomegranate, as recently revised by Ze et al. [32], although the applied dose and time dipping was different for each fruit species. These effects were attributed to a reduced accumulation of reactive oxygen species (ROS) due to increases in the enzymatic activities and gene expressions of antioxidant enzymes. Even in sweet cherry, postharvest melatonin treatments, especially with 0.1 mM dose, led to a delay of the postharvest ripening process of 'Sunbust' [18] and 'Siah Mashhad' [17] cultivars, through increasing the activity of antioxidant enzymes and concentration of antioxidant compounds, such as ascorbic acid, reduced glutathione and phenolics.

Sweet cherries are fruit with high content of phenolic compounds, mainly anthocyanins, which are primarily responsible for the beneficial health effects attributed to cherry consumption [6,33–35]. In fact, phenolic compounds and especially anthocyanins have strong antioxidant activity and preventive effects on a wide range of age-related and chronic diseases, such as neurodegenerative, cardiovascular and oncologic diseases, hypertension, obesity and diabetes, among others [36–38]. The results of the present research show enhanced levels of phenolic and total and individual anthocyanin concentrations in fruit from melatonin-treated trees in the three sweet cherry cultivars tested. In addition, high correlation was found between a*/b* colour index and total anthocyanin concentration ($y = 0.02x + 2.24$; $r^2 = 0.672$), as well as between total phenolic and total anthocyanin concentrations ($y = 0.76x + 31$; $r^2 = 0.801$), taking into account data from all cultivar and years. Thus, fruit surface colour, measured by the a*/b* index, is a good indicator of anthocyanin concentration, the pigments responsible for sweet cherry colour. Moreover, these pigments are the major phenolic compounds in these sweet cherry cultivars contributing to their antioxidant potential and health beneficial effects [33,35,39]. The predominant anthocyanin in the three sweet cherry cultivars was Cyn 3-rut followed by Pelg 3-rut and Cyn 3-gluc, in agreement with previous reports on other cherry cultivars [3,40–43]. Accordingly, Cyn 3-rut was the major anthocyanin in the 'Ferrovia' cultivar, although cyanidin 3-*O*-galactoside and Cyn 3-gluc were the minor ones [21]. Postharvest melatonin treatments have shown a general trend on increasing phenolic content, including anthocyanins, and antioxidant activity in a wide range of fruit species, such as 'Santa Rosa' plum [44], strawberry [45], nectarine [46], tomato [17], litchi [47] and pomegranate [48]. Accordingly, preharvest treatment of pomegranate trees led to enhanced concentrations of total phenolics and total and individual anthocyanins as well as of total antioxidant activity in arils at harvest, showing that melatonin treatment stimulated the anthocyanin biosynthesis pathway [16].

Specifically, in sweet cherry, 0.1 mM melatonin dipping treatment increased anthocyanin concentration in 'Santina' and 'Royal Rainier' cultivar during storage due to an induced overexpression of two key genes coding for dihydroflavonol 4-reductase (DFR) and anthocyanidin 3-*O*-glucosyltransferase (UFGT), two enzymes involved in the last steps of anthocyanin biosynthesis partway [19]. Similarly, 0.1 mM melatonin dipping treatment led to enhanced total phenolics, flavonoids and anthocyanins during storage in 'Siah Mashhad' cultivar, these effects being accompanied by an increased radical scavenging potential and attributed to higher phenylalanine ammonia lyase (PAL) and chalcone synthase (CHS) activities, along with decreased PPO activity [17]. However, the effect of melatonin preharvest treatment on phenolic and anthocyanin contents of sweet cherry are not as clear as those of the postharvest ones previously mentioned. Thus, Tijero et al. [20] reported

two-fold increases in anthocyanin concentration at harvest in 'Prime Giant' after preharvest 0.1 mM melatonin treatment at stage II, while two-fold decreases occurred with 0.01 mM dose. In 'Hongdeng' cultivar; moreover, an increase in the total phenolic content at harvest was found after preharvest treatment with 0.05, 0.01 and 0.1 mM melatonin (applied three times at weekly intervals from initial colour changes), while for the anthocyanin content, increases were found for 0.05 and 0.1 mM—no effect was observed for 1 mM [31]. Finally, in the 'Ferrovia' cultivar, 0.5 mM melatonin foliar spray treatment, 2 and 1 weeks before harvest did not affect individual phenolic or anthocyanin contents at harvest, although the expression of PAL codifying gene was significantly increased [21]. Taking into account these previous results and those obtained in the present experiments, it is clear that to obtain an increase in the phenolic and anthocyanin concentration by melatonin treatments, 0.3 and 0.5 mM doses would be effective, provided the treatments were applied at key points of fruit development on the tree, namely pit hardening, initial colour changes and three days before harvest, as was performed in the present experiments.

5. Conclusions

The results show that melatonin treatment of sweet cherry trees at key points of fruit development had little effect on crop yield, except for the 'Lapins' cultivar, although the decayed and cracked fruit percentage was decreased under adverse weather conditions and commercial crop yield was increased, especially for the 0.3 mM dose. Quality parameters at harvest, such as fruit weight, colour, firmness, TSS and TA, were enhanced by melatonin treatments in all sweet cherry cultivars and 2019 and 2020. Moreover, antioxidant compounds, such as total phenolics and total and individual anthocyanins, were also found at higher levels in fruit from melatonin-treated trees with respect to controls. Thus, considering all these effects, 0.3 mM melatonin treatments at three key points of fruit developmental stages could be a useful tool to improve quality traits of sweet cherries and specially their content on bioactive compounds with antioxidant properties and health beneficial effects, with additional effects increasing crop yield under unfavourable climatic conditions during fruit ripening.

Author Contributions: A.C.-A., M.S., D.V. and S.C. conceived and designed the work in association with other authors. A.C.-A., J.M.V. and J.M.L.-M. performed the field treatments. A.C.-A. and J.M.L.-M. performed most of the analytical determination in collaboration with J.M.V. Finally, M.S. and D.V. analysed the data and wrote the manuscript. Funding acquisition, D.V. and M.S. All authors have read and agreed to the published version of the manuscript.

Funding: This work is included in the Project RTI2018-099664-B-I00 founded by the Spanish Ministry of Science, Innovation and Universities and the European Commission with FEDER funds.

Acknowledgments: The authors thank Spanish Ministry of Science, Innovation and Universities and Miguel Hernández University for PhD-scholarships of A.C.-A. and J.M.L.-M, respectively and to FincaToli Frutas S.L. for providing field experiments.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study, collection, analyses or interpretation of data, in the writing of the manuscript or in the decision to publish the results.

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4.2 Publicación 2

PUBLICACIÓN 2 (Acceso abierto)

Título: Melatonin Pre-harvest Treatments Leads to Maintenance of Sweet Cherry Quality During Storage by Increasing Antioxidant Systems

Autores: Carrión-Antolí, A., Martínez-Romero, D., Guillén, F., Zapata, P.J., Serrano, M., & Valero, D.

Fecha de publicación: 11 de abril de 2022

Revista: Frontiers in Plant Science

Editorial: Frontiers Media S.A.

ISSN: 1664-462X

Factor de impacto (2023): 4.1

Factor de impacto (5 años): 5.3

Categoría JCR®: Plant Sciences

Rango: 44/265

Cuartil: Q1

CiteScore: 7.3

DOI: https://doi.org/10.3389/fpls.2022.863467

frontiers | Frontiers in Plant Science

ORIGINAL RESEARCH
published: 11 April 2022
doi: 10.3389/fpls.2022.863467

Melatonin Pre-harvest Treatments Leads to Maintenance of Sweet Cherry Quality During Storage by Increasing Antioxidant Systems

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Melatonin has been reported to have an important role in fruit ripening, although the effect of pre-harvest melatonin treatment on sweet cherry quality properties during storage is still unknown. In the present experiments, the effects of melatonin (0.1, 0.3, and 0.5 Mm) by foliar spray treatments of 'Prime Giant' and 'Sweet Heart' sweet cherry trees on fruit quality traits and antioxidants systems during storage was evaluated. Results showed that these treatments reduced weight losses during storage, as well as losses in firmness and titratable acidity. In addition, changes in fruit colour and total soluble solid content were also delayed in fruit from melatonin treated trees with respect to controls. Moreover, in general, total phenolic and anthocyanin concentrations were higher in fruit from treated trees than in those from control ones, either at harvest or during the whole storage period. Finally, the activity of the antioxidant enzymes catalase, ascorbate peroxidase and peroxidase was also enhanced as a consequence of melatonin treatment. Overall results show that pre-harvest melatonin treatment delayed the post-harvest ripening process of sweet cherry fruit, leading to maintenance of their quality properties in optimum levels for consumption 2 weeks more with respect to fruit from control trees. Antioxidant systems, both enzymatic and non-enzymatic ones, were also enhanced by melatonin treatments, which would account for the delay on fruit post-harvest ripening process and fruit quality maintenance during storage.

Keywords: ***Prunus avium*, phenolics, anthocyanins, firmness, colour, soluble sugars, acidity, antioxidant enzymes**

OPEN ACCESS

Edited by:
Leo Marcelis, Wageningen University and Research, Netherlands

Reviewed by:
Kaituo Wang, Chongqing Three Gorges University, China
Qingguo Wang, Shandong Agricultural University, China

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Specialty section:
This article was submitted to Crop and Product Physiology, a section of the journal Frontiers in Plant Science

Received: *27 January 2022*
Accepted: *17 March 2022*
Published: *11 April 2022*

Citation:
Carrión-Antolí A, Martínez-Romero D, Guillén F, Zapata PJ, Serrano M and Valero D (2022) Melatonin Pre-harvest Treatments Leads to Maintenance of Sweet Cherry Quality During Storage by Increasing Antioxidant Systems. Front. Plant Sci. 13:863467. doi: 10.3389/fpls.2022.863467

INTRODUCTION

Sweet cherry fruit (*Prunus avium* L.) have excellent organoleptic and nutritional properties, such as appearance, colour, texture, flavour, juiciness, and sugar and organic acid content (Usenik et al., 2008; Díaz-Mula et al., 2009; Martínez-Esplá et al., 2014). In addition, sweet cherries are rich in bioactive compounds with antioxidant properties, mainly phenolics, and ascorbic acid, which are responsible for their health beneficial properties, namely, anti-inflammatory, antidiabetic, antimicrobial, and anticancer effects as well as cardiovascular and neuroprotection activities (McCune et al., 2011; Blando and Oomah, 2019; Faienza et al., 2020). However, they are very perishable fruit suffering from quickly quality losses after harvest even under storage in cold

conditions. Thus, different post-harvest treatments combined with cold storage have been reported to be useful to maintain sweet cherry fruit quality for longer time, such as alginate coating (Díaz-Mula et al., 2012), *Aloe vera* gel containing rosehip oil (Paladines et al., 2014), nano-silica coating (Meng et al., 2022), 1-methylcyclopropene and chlorine dioxide treatments, alone or in combination (Serradilla et al., 2019; Zhao et al., 2021) or salicylic (SA), acetylsalicylic (ASA), and oxalic (OA) acids treatments (Valero et al., 2011), as well as storage under modified atmosphere conditions (Cozzolino et al., 2019), among others (Correia et al., 2017).

In addition, different pre-harvest treatments, such as gibberellic acid (Einhorn et al., 2013), oxalic acid (Martínez-Esplá et al., 2014), salicylic acid (SA), acetyl salicylic acid (ASA), and methyl salicylate (MeSa) (Giménez et al., 2014, 2017; Valverde et al., 2015) have been performed aimed to increase sweet cherry fruit quality attributes at harvest. These treatments led to enhanced fruit size, firmness and total anthocyanin and phenolic contents at harvest and these quality parameters were maintained during storage, leading to fruit with increased shelf life. In addition, the activity of antioxidant enzymes, such as peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX), and superoxide dismutase (SOD), was also enhanced by these salicylate treatments (Valverde et al., 2015; Giménez et al., 2017). These antioxidant enzymes are involved on scavenging reactive oxygen species (ROS) species, such as hydrogen peroxide (H_2O_2), superoxide radical ($O2^{\bullet-}$), hydroxyl radical ($OH^{\bullet}$), or $^1O^2$, which are inevitably generated in normal metabolism of plant cells but accumulated during fruit ripening and senescence, contributing to peroxidation of membrane lipids, damage to DNA and proteins, and acceleration of senescence processes. Thus, treatments aimed to increase the ability of fruit tissues to decrease ROS levels, by enhancing antioxidant enzyme activities and/or antioxidant compounds, such as phenolics or anthocyanins, have been reported to delay ripening and senescence process and, in turn, to maintain fruit quality in a wide range of fruit species including sweet cherry. In this sense, post-harvest treatments of sweet cherry with hexanal or 1-methylcyclopropene led to higher levels of SOD and APX activities during storage as compared with control cherries (Sharma et al., 2010). Similarly, higher antioxidant enzyme activities during storage were found in sweet cherry fruit coated with chitosan (Dang et al., 2010) or after vacuum cooling treatment (He et al., 2013), as well as in sweet cherry fruit from SA, ASA, or MeSa treated trees (Giménez et al., 2015; Valverde et al., 2015).

Recently, melatonin, which was identified in plants in 1995 (Dubbels et al., 1995), is gaining a broad interest as a universal plant signalling molecule having pivotal roles on regulating a wide range of plant physiological processes, with great potential for its application in the horticultural industry (Aghdam et al., 2020; Arnao and Hernández-Ruiz, 2020a; Tiwari et al., 2020). In particular, post-harvest melatonin treatments have been shown to delay fruit ripening in a wide range of fruit species (Xu et al., 2019; Arnao and Hernández-Ruiz, 2020b). For instance, dipping treatment with 0.5 mM melatonin delayed ripening in mangoes, due to inhibition of ABA and ethylene biosynthesis (Liu et al., 2020), as well as in banana fruit (Hu et al., 2017), which were dose-dependent in the range of 0.05–0.5 mM. Accordingly, a delay of ripening has been reported in peaches and nectarines after post-harvest melatonin treatment (Gao et al., 2016; Bal, 2021). However, the effects of melatonin applied as pre-harvest treatment on on-tree fruit ripening and quality traits have been evaluated in a very few papers and different effects have been found depending on concentration, application time and fruit species. Thus, tomato plant treatment with melatonin, applied in the irrigation system led to increases in lycopene and sugar contents, showing acceleration of the fruit ripening process (Liu et al., 2016). However, melatonin treatment by foliar spray of apricot trees did not affect the on-tree ripening process although positive effects were observed on crop yield and fruit quality parameters at harvest, which were maintained during storage, either at chilling or non-chilling temperatures, as compared with apricots from control trees (Abd El-Naby et al., 2019; Medina-Santamarina et al., 2021). Similar effects of melatonin pre-harvest treatments have been reported recently for 'Mollar de Elche' pomegranate (Lorente-Mento et al., 2021).

In sweet cherry fruit, post-harvest dipping melatonin treatments have been recently reported to maintain fruit quality during storage throughout a delay of the senescence process in 'Sunburst' (Wang et al., 2019), 'Siah Mashhad' (Sharafi et al., 2021), 'Santina,' and 'Royal Rainier' (Miranda et al., 2020) cultivars. On the other hand, melatonin treatment, applied directly to fruit surface during on-tree fruit development delayed fruit ripening in 'Prime Giant' cultivar (Tijero et al., 2019). On the contrary, foliar spray treatment with 0.5 mM melatonin 2 and 1 weeks prior to harvest accelerated fruit ripening of sweet cherry 'Ferrovia' (Michailidis et al., 2021). In our previous paper, pre-harvest foliar spray with 0.1, 0.3, and 0.5 mM melatonin led to fruit with enhanced quality traits at harvest, such as fruit weight, colour, firmness, total soluble solid content and titratable acidity (Carrión-Antolí et al., 2022). However, as far as we know, there is not available literature regarding the effect of pre-harvest melatonin treatment on the maintenance of sweet cherry fruit quality properties during storage. Thus, the aim of the present experiment was to evaluate the effects of melatonin foliar spray of sweet cherry trees of 'Prime Giant' and 'Sweet Heart' cultivars on fruit quality parameters, with especial interest on bioactive compounds and the activity of antioxidant enzymes.

MATERIALS AND METHODS

Plant Material and Experimental Design

The experiments were carried out in a commercial field located at Jumilla (Murcia, Spain, UTMX: 463.700 UTMY: 4.268.900) with 'Prime Giant' and 'Sweet Heart' sweet cherry (*P. avium* L.) cultivars, in 2019 and 2020 years, respectively. 'Prime Giant' was planted in January 2012 and 'Sweet Heart' in January 2015 and both were grafted onto SL-64 rootstock. Climatic conditions in the crop field were similar for 2019 and 2020 years: mean annual temperatures 15.24 and 15.30°C for 2019 and 2020, respectively, and accumulated rainfall of 357 and 352 mm for 2019 and 2020, respectively. Agronomic practices were similar for both

cultivars with fertilisation of 60:30:100 kg ha^{-1} N:P:K and base type open centre pruning. For each cultivar, three blocks of three trees were selected at random for 0 (control), 0.1, 0.3, and 0.5 mM melatonin treatments. Treatments were applied with a manual sprayer machine (3 L per tree) by using freshly prepared melatonin solutions (containing 1 mL L^{-1} Tween as surfactant) at three key points of fruit development (pit hardening, starting of colour changes and 3 days before harvest), according to previous reports (Giménez et al., 2017; Carrión-Antolí et al., 2022). Sweet cherries were harvested according to commercial practices, when reached their commercial ripening stage, based on the soluble solid content and skin colour of each cultivar. About 3 kg of fruit from each treatment and replicate were taken and transported to laboratory in 3 h. Then, lots of 20 fruits, homogenous in colour and size and without visual defects, were performed at random and stored at 2°C and 90% RH. After 0, 7, 14, 21, and 28 days of storage one lot of each replicate and treatment was taken to perform the following analytical determinations.

Quality Parameter

Fresh weight of each fruit lot was measured at harvest and at each sampling date during storage by using a digital balance KERN 440-35N (Balingen, Germany) and weight losses were expressed as percentage with respect to weight at day 0. Colour was measured with a Minolta colorimeter (CRC200, Minolta Camera Co., Osaka, Japan), at three equidistant points along the equatorial perimeter of each fruit and was expressed as a*/b* ratio by using the CIELab coordinates. Results are the mean ± SE. Fruit firmness was measured independently in each fruit by using a TX-XT2i Texture Analyzer (Stable Mycrosys-tems, Godalming, United Kingdom) equipped with a flat probe. A force to achieve a 5% fruit diameter deformation was applied and fruit firmness was expressed as the relation between the applied force and the travelled distance (N mm^{-1}). Results are the mean ± SE. Then, flesh of the 20 fruit of each replicate was cut in small pieces to obtain a homogeneous sample. A ≈50 g sample was used for total soluble solids (TSS) and titratable acidity (TA) measures, in duplicate, after being squeezed through two layers of cotton cloth. TSS in fruit juice were measured by using a digital refractometer (Atago PR-101, Atago Co. Ltd., Tokyo, Japan) and TA by titration of 1 mL of juice, diluted in 25 mL of distilled H_2O, with 0.1 N NaOH up to pH 8.1 by using an automatic titration system (785 DMP Titrino, Metrohm, Herisau, Switzerland). TSS and TA results are expressed as g 100 g^{-1} and are the mean ± SE. Other 50 g fruit sample was ground under liquid N_2, and stored at −20°C until total phenolic and anthocyanin concentrations and antioxidant enzyme activities were measured.

Total Phenolic and Anthocyanin Quantification

Phenolics were extracted by homogenising 5 g of frozen tissue with 10 mL of water:methanol (2:8) containing 2 mM NaF (to inactivate polyphenol oxidase activity and prevent phenolic degradation) in a Ultraturrax homogeniser (T18 basic, IKA, Berlin, Germany). Then, the extracts were centrifuged at 10,000 × *g* for 10 min at 4°C and total phenolics were quantified in duplicate in the supernatant by using the Folin-Ciocalteu reagent as previously described (Díaz-Mula et al., 2009). Results were expressed as mg gallic acid equivalent 100 g^{-1} and are the mean ± SE. Anthocyanins were extracted by homogenising 2 g of fruit sample with 10 mL of methanol/HCl/water (80:1:19, v/v/v) as addressed above. After centrifugation, anthocyanins were quantified in duplicate in the supernatant by reading absorbance at 530 in a spectrophotometer (UNICAM Helios-α, Artisan Technology Group, Champaign, IL, United States). Total anthocyanins were calculated by using cyanidin-3-glucoside molar absorption coefficient of 23,900 L cm^{-1} mol^{-1} and molecular weight of 449.2 g mol^{-1}. Results were expressed as mg cyanidin 3-glucoside equivalent 100 g^{-1} and were the mean ± SE.

Measure of Antioxidant Enzyme Activities

To obtain crude extract of POD, CAT and APX, 5 g of sweet cherry samples were homogenised with 10 mL of phosphate buffer 50 mM, pH 7.0, containing 1 mM ethylen-diamine-tetraacetic acid (EDTA) and 1% (w/v) polyvinylpyrrolidone. Then, the homogenate was centrifuged at 15,000 × *g* for 30 min at 4°C and antioxidant enzyme activities were measured in the supernatant as previously described (Giménez et al., 2017). Briefly, for POD determination, the reaction mixture contained 50 mM phosphate buffer pH 7.0, 14 mM guaiacol, 12 mM H_2O_2 and 100 μL of enzymatic extract in a total volume of 3 mL. The increase of absorbance at 470 nm from time 0 to 1 min, due to guaiacol oxidation, was measured and POD activity was expressed as U min^{-1} g^{-1}, one enzymatic unit (U) being defined as 0.01 absorbance increase per min. The reaction mixture for CAT activity contained 100 μL of the above extract and 2.9 mL 50 mM phosphate buffer pH 7.0, containing 15 mM H_2O_2 and the decrease of absorbance at 240 nm for 1 min due to H_2O_2 degradation was measured and CAT activity expressed as U min^{-1} g^{-1}, one enzymatic unit (U) being defined as 0.01 absorbance decrease per minute. Finally, the assay mixture for APX quantification contained 50 mM potassium phosphate pH 7.0, 0.5 mM ascorbic acid, 1 mM H_2O_2 and 100 μL of crude extract in a final volume of 3 mL. The decrease of absorbance at 290 nm during 1 min was measured and one enzymatic unit of APX (U) was defined as the amount of enzyme that oxidises 1 mmol of ascorbate per minute, and APX was expressed as U min^{-1} g^{-1}.

Statistical Analysis

The field experiments were performed by using three replicates of three trees per treatment for each cultivar in a completely randomised design. Fruit samples from each replicate were taken and used for storage experiment. Experimental data from each cultivar were independently subjected to ANOVA analysis. For each cultivar, sources of variation were treatment and storage time. All analyses were performed with SPSS software package v. 22.0 for Windows (SPSS, 2011). Least significant differences (LSD) at $p < 0.05$ were calculated and values shown in each figure.

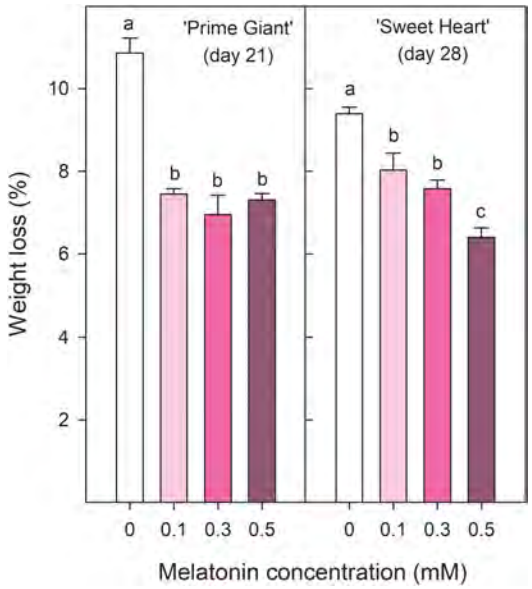

FIGURE 1 | Weight losses for 'Prime Giant' and 'Sweet Heart' cultivars after 21 and 28 days of storage at 2°C, respectively, as affected by melatonin pre-harvest treatments. Data are the mean ± SE of three replicates of 20 fruits. Different letters show significant differences ($p < 0.05$) among treatments for each cultivar.

RESULTS

Fruit Quality Parameters

'Prime Giant' and 'Sweet Heart' cultivars were stored for 21 and 28 days, respectively, until control fruit reached an over-ripening and senescence stage in which quality attributes were considered as not optimum for consumption. Weight loss increased during storage in both cherry cultivars, either in fruit from control trees as from treated trees, although they were delayed in the last ones. Thus, for 'Prime Giant' weight losses in control fruit reached values of 10.86 ± 0.36% after 21 days of storage while significantly ($p < 0.05$) lower values, ≈7% were reached in fruit from melatonin control trees independently of the applied dose. For 'Sweet Heart' cultivar, weight losses were also significantly lower ($p < 0.05$) and dose dependent in fruit from melatonin treated trees than in controls, the lowest weight losses being found for 0.5 mM dose, with values of 6.40 ± 0.23% after 28 days as compared to 9.39 ± 0.16% in controls (**Figure 1**). Colour index (a*/b*) at harvest was significantly increased ($p < 0.05$) as a consequence of melatonin treatments with respect to controls in both cultivars and a similar trend was observed during storage, with increases during the first 1–2 weeks and decreases thereafter, except for 'Prime Giant' from 0.5 mM melatonin treated fruit, in which no changes occurred from day 0 to day 14 of storage (**Figures 2A,C**). However, colour index showed higher values, 9.21, 7.77, and 11.63%, in fruit from 0.1, 0.3, and 0.5 mM treated trees, respectively, for 'Prime Giant' and 2.46, 7.86, and 8.48% for 'Sweet Heart' than in controls taking into account data from all sampling dates. Fruit firmness was also found at significantly higher levels ($p < 0.05$) in fruit from melatonin treated trees than in controls at harvest and these differences were maintained during the whole storage period, in spite of the firmness decreases observed in all fruit for both cultivars (**Figures 2B,D**). At harvest, the highest effect on fruit firmness was observed for 0.3 and 0.1 mM melatonin doses in 'Prime Giant' and 'Sweet Heart,' respectively. However, during storage, no significant differences were observed between melatonin doses, with 25–30 and 15–20% higher firmness levels in treated fruit than in controls for 'Prime Giant' and 'Sweet Heart' cultivars, respectively, taking into account data of all sampling dates.

Total soluble solids in control fruit at harvest were 20.43 ± 0.24 and 19.70 ± 0.21 g 100 g^{-1} for 'Prime Giant' and 'Sweet Heart,' respectively, and significant increases ($p < 0.05$) occurred during storage (**Figures 3A,C**). Melatonin pre-harvest treatments led to significant ($p < 0.05$) enhanced TSS concentrations at harvest, the highest effects being observed for 0.1 mM in 'Prime Giant' (24.45 ± 0.12 g 100 g^{-1}), while no significant differences among doses were observed for 'Sweet Heart' cultivar (≈21 g 100 g^{-1}). Nevertheless, it is worth noting that TSS was higher in fruit from melatonin treated trees than in controls during the whole storage period. TA at harvest was 1.05 ± 0.01 and 1.31 ± 0.02 g 100 g^{-1} in control fruit of 'Prime Giant' and 'Sweet Heart' cultivars, respectively, and significant decreases ($p < 0.05$) occurred during storage. However, in fruit from melatonin treated trees, TA losses were delayed with respect to controls in both cultivars, and significantly ($p < 0.05$) higher values, ca. 15 and 10%, were observed as a consequence of melatonin treatments, either at harvest or during storage, in 'Prime Giant' and 'Sweet Heart', respectively (**Figures 3B,D**).

Antioxidant Compounds and Antioxidant Enzymes

Total phenolic concentrations at harvest was significantly increased by melatonin treatments in a dose-dependent way, from 74.84 ± 4.23 mg 100 g^{-1} in fruit from control trees to 100.48 ± 3.67 mg 100 g^{-1} in those from 0.5 mM treated ones, in 'Prime Giant' and from 60.08 ± 2.14 to 72.36 ± 0.96 mg 100 g^{-1} in 'Sweet Heart.' During storage, total phenolics were maintained at higher levels in fruit from melatonin treated trees than in controls, although no significant differences among melatonin doses were observed (**Figures 4A,B**). With respect to anthocyanin concentration, significant enhanced ($p < 0.05$) values were also found, in general, as a consequence of melatonin treatments, either at harvest or during storage, for both cultivars (**Figures 4C,D**). Taking into account data of all sampling date, total phenolic and anthocyanin concentration was ca. 25% lower in 'Sweet Heart' than in 'Prime Giant,' while the increase in concentrations of these bioactive compounds by melatonin treatments was higher in 'Sweet Heart' than in 'Prime Giant.'

In general, the activity of antioxidant enzymes CAT, APX, and POD was significantly higher ($p < 0.05$) in fruit from 0.3 mM melatonin treated trees than in controls, either at harvest or during storage, except POD activity in 'Prime Giant' cultivar (**Figure 5**). The highest effects were found for CAT activity, which was 30 and 20% higher in treated fruits for 'Prime Giant' and 'Sweet Heart,' respectively, during the whole storage period, while ≈15% increases were observed for APX activity in both cultivars.

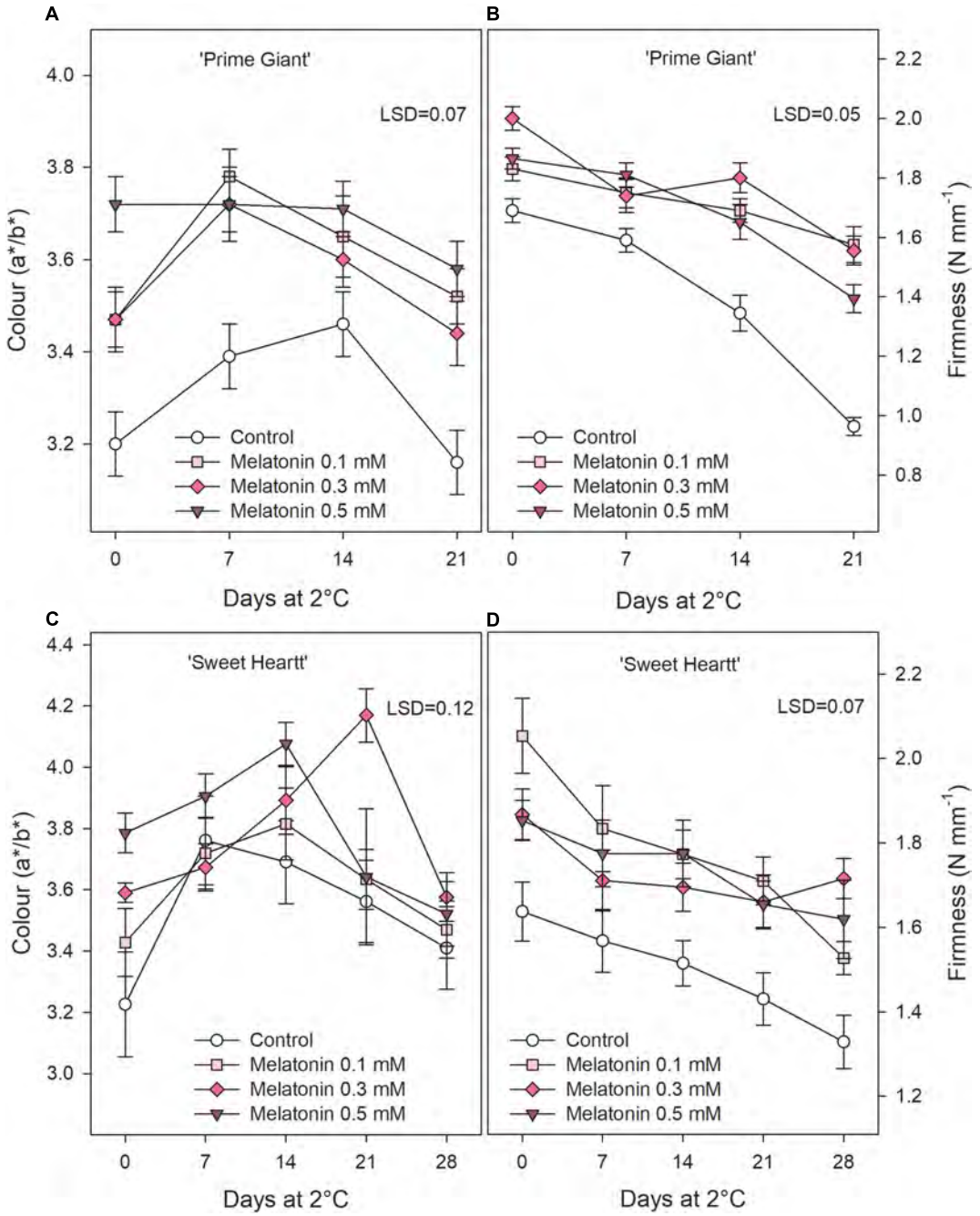

FIGURE 2 | Fruit colour **(A)** and firmness **(B)** for 'Prime Giant' and 'Sweet Heart' **(C,D)** cultivars during storage at 2°C as affected by melatonin pre-harvest treatments. Data are the mean ± SE of three replicates of 20 fruits. LSD values at ($p < 0.05$) are shown in each figure.

DISCUSSION

Sweet cherry fruit quality traits, such as absence of visual defects, fruit size, colour, stem freshness, and length, firmness, aroma, flavour, sweetness, and sourness are the major responsible for consumer purchase decisions, although important differences have been reported among cultivars (Díaz-Mula et al., 2009; Serradilla et al., 2012; Correia et al., 2017). However, these quality parameters evolved quickly during fruit storage, even if storage is performed at appropriate temperature, leading to fruit with no optimal quality for consumption (Serrano et al., 2009; Chockchaisawasdee et al., 2016; Giménez et al., 2017; Zhang et al., 2021). These changes are mainly related to softening, losses of fruit weight and TA, and increases in TSS and colour as well as to fruit decay and browning and desiccation of the pedicel. Accordingly, the present results show increases in weight loss,

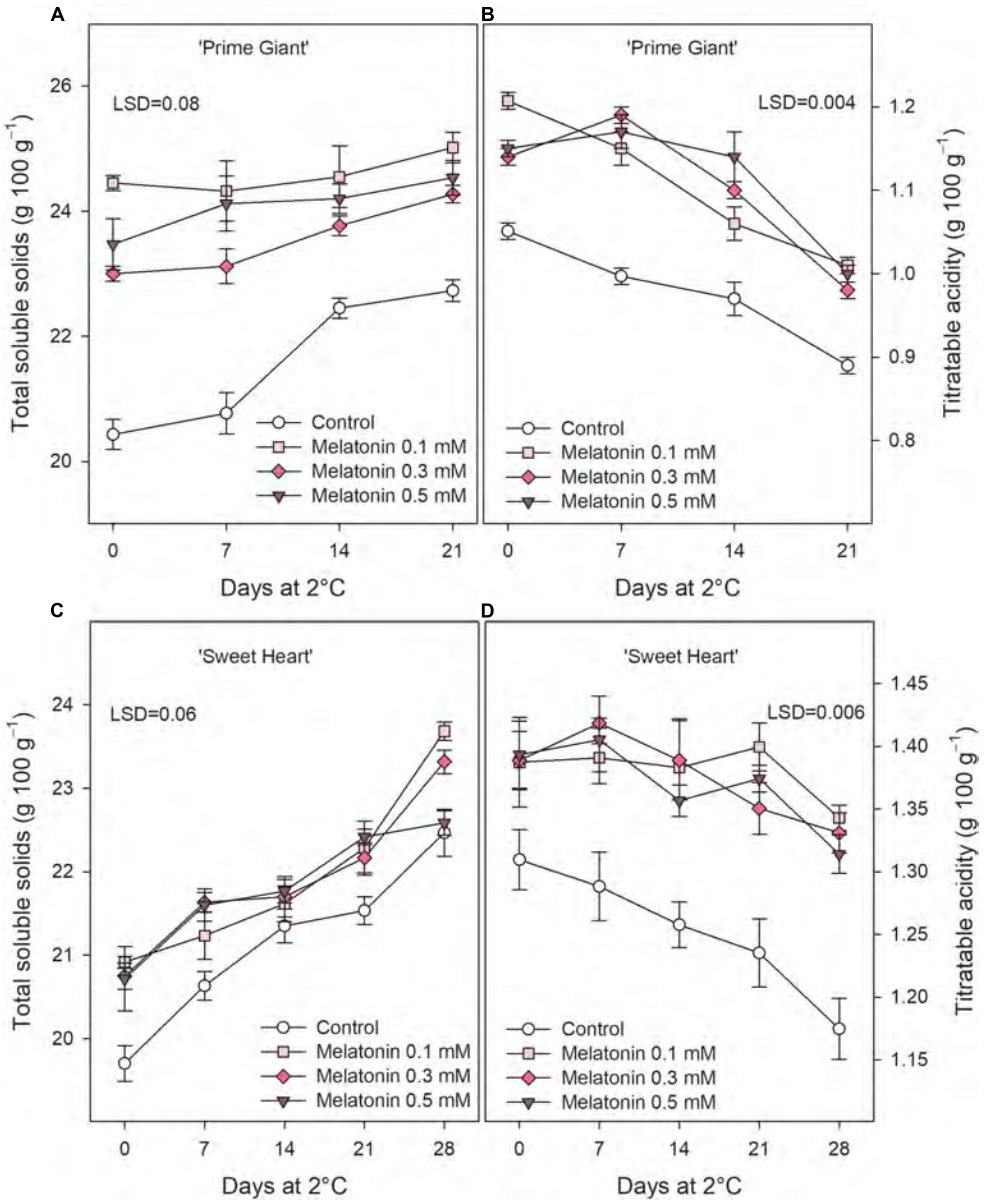

FIGURE 3 | Total soluble solids **(A)** and titratable acidity **(B)** for 'Prime Giant' and 'Sweet Heart' **(C,D)** cultivars during storage at 2°C as affected by melatonin pre-harvest treatments. Data are the mean ± SE of three replicates. LSD values at ($p < 0.05$) are shown in each figure.

TSS and colour and decreases in fruit firmness and TA, although these changes were significantly delayed in fruit from melatonin treated trees with respect to controls (**Figures 1–3**). Firmness and TA maintenance as a consequence of pre-harvest melatonin treatments are major factor contributing to preserve fruit during storage, since cherries with higher firmness are much appreciated by consumers and TA retention during storage led to cherries with the aroma and taste of recently harvested cherries (Valero et al., 2011; Díaz-Mula et al., 2012; Serradilla et al., 2012).

Thus, taking into account all these quality parameters, storage time with optimal fruit quality properties for consumption in control cherries for both cultivars was 14 days, while it could be extended up to 21 and 28 days in fruit from melatonin treated trees for 'Pime Giant' and 'Sweet Heart' cultivars, respectively. Accordingly, post-harvest ripening was delayed in 'Guifei' mangoes by 0.5 mM melatonin dipping treatment for 1 h (Liu et al., 2020) and in banana in a concentration dependent manner in the range of 0.05–0.5 mM (Hu et al., 2017), leading to extension of fruit shelf life. Similar results have been reported in peaches (Gao et al., 2016), and nectarines (Bal, 2021), and these effects were attributed to inhibition of ethylene production in those climacteric fruit species. Maintenance of fruit quality traits and extension of shelf-life seem to be general fruit responses to melatonin post-harvest dipping treatments since they have been

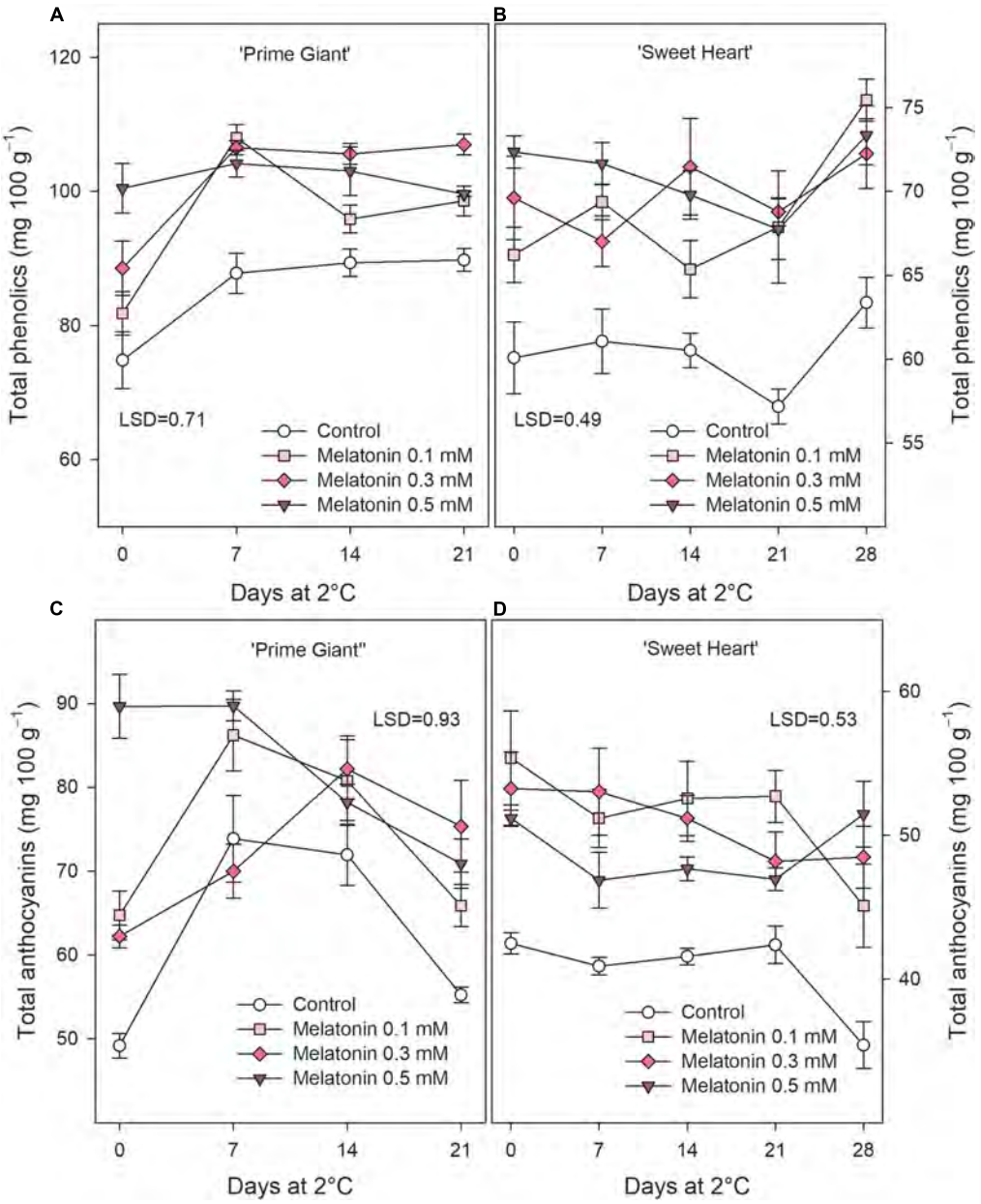

FIGURE 4 | Total phenolics **(A,B)** and total anthocyanins **(C,D)** for 'Prime Giant' and 'Sweet Heart' cultivars during storage at 2°C as affected by melatonin pre-harvest treatments. Data are the mean ± SE of three replicates. LSD values at ($p < 0.05$) are shown in each figure.

also reported in non-climacteric fruit, such as pomegranate and strawberry as recently revised by Ze et al. (2021). Specifically, in 'Sunburst' sweet cherries, post-harvest 0.05, 0.1, and 0.15 Mm melatonin treatments led to delay the post-harvest ripening process (Wang et al., 2019) and in 'Siah Mashhad' cultivar dipping with 0.001, 0.01, 0.1, and 1 mM melatonin reduced flesh browing and decay after 45 days of storage, the highest effect being found with 0.1 mM dose (Sharafi et al., 2021). However, post-harvest fruit treatments have consumers' concerns and legal restrictions and then, there is a need of research regarding pre-harvest treatments with effect on fruit quality properties at harvest and during storage. In this sense, pre-harvest treatments of apricot tree with melatonin increased fruit quality parameters at harvest and these quality traits were maintained during storage (Medina-Santamarina et al., 2021). Higher values of quality parameters, either at harvest or during storage, were observed on pomegranate fruit as a consequence of melatonin tree treatments during on-tree fruit development (Lorente-Mento et al., 2021). In sweet cherry, 0.05, 0.1, and 0.2 mM melatonin applied on tree canopy (3, 2, and 1 weeks before harvest) resulted in fruit with higher TSS and lower TA in the 'Hongdeng' cultivar (Xia et al., 2020), but no storage experiment was performed in this research.

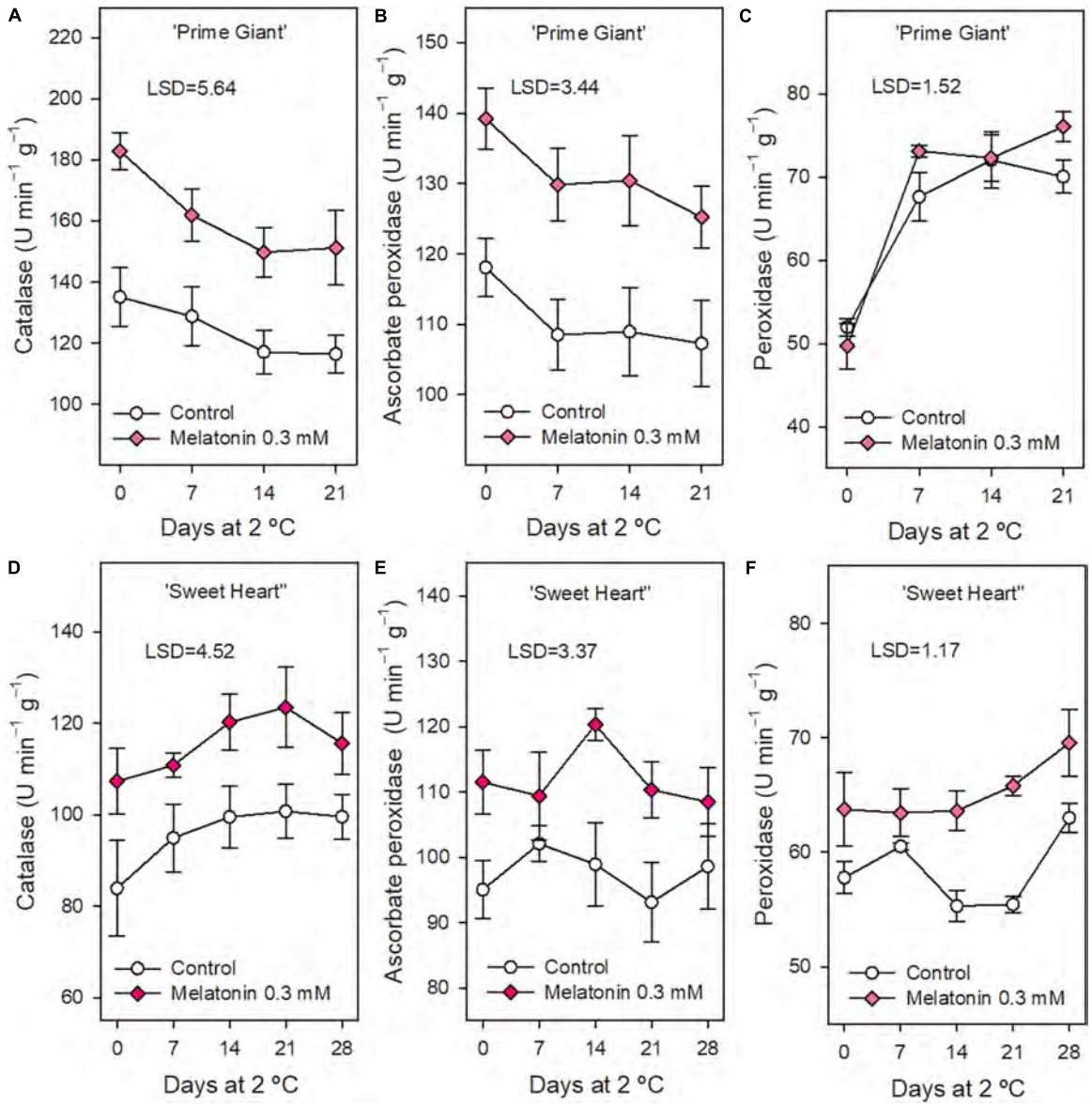

FIGURE 5 | Catalase, ascorbate peroxidase, and peroxidase activities in 'Prime Giant' (**A–C**, respectively) and 'Sweet Heart' (**D–F**, respectively) cultivars during storage at 2°C as affected by melatonin pre-harvest treatments. Data are the mean ± SE of three replicates. LSD values at ($p < 0.05$) are shown in each figure.

On the contrary, similar treatments with 0.5 mM melatonin did not show significant effect on 'Ferrovia' fruit quality parameters at harvest and softening was the only parameters related to fruit quality and senescence delayed after 14 days of cold storage (Michailidis et al., 2021). Thus, the effects of pre-harvest melatonin treatment on delaying fruit ripening and senescence will be different depending on cultivar, applied concentration or fruit developmental stage, among other factors.

In the last decade, special attention has been paid to the content on bioactive compounds with antioxidant activity, such as anthocyanins and other phenolic compounds, in sweet cherry due to their positive impact on human health, by reducing the risk of suffering from several degenerative diseases (Correia et al., 2017; Gonçalves et al., 2018, 2019; Antognoni et al., 2020; Faienza et al., 2020; Luo et al., 2021). In this fruit species, the red colour intensity is due to their content of anthocyanins and their profile, the major anthocyanin being cyanidin 3-*O*-rutinoside comprising around 90% of total anthocyanins, and 70% of total phenolic compounds, in most of the studied cultivars, including 'Prime Giant' and 'Sweet Heart' (Usenik et al., 2008; Serrano et al., 2009; Martínez-Esplá et al., 2014; Antognoni et al., 2020; Gonçalves et al., 2021; Carrión-Antolí et al., 2022). Total phenolic concentration showed an upward trend from day 0 until the end of storage in fruit from control and treated trees for both cultivars (**Figures 4A,B**), while total anthocyanins, generally, increased during the first

weeks of storage and decreased thereafter (**Figures 4C,D**). These results are in agreement with previous reports in other cherry cultivars, which have been related to the ongoing ripening process after harvesting (Serrano et al., 2009; Valero et al., 2011; Giménez et al., 2014; Sharafi et al., 2021). However, it is wort noting that phenolic and anthocyanin contents at harvest were enhanced as a result of melatonin treatments and maintained at higher levels in treated fruit than in controls during storage (**Figures 4A–D**). Accordingly, post-harvest melatonin dipping treatments have been reported to increase phenolic and anthocyanin concentrations during storage in some fruit species, such as strawberry (Liu et al., 2018), tomato (Sharafi et al., 2019), and pomegranate (Aghdam et al., 2020) and even in sweet cherry, as has been recently reported by Sharafi et al. (2021). These effects were attributed to melatonin stimulation of the phenylpropanoid pathway mainly by enhancing phenylalanine ammonialyase and chalcone synthase activities. However, literature regarding the impact of pre-harvest melatonin treatments on phenolic and anthocyanin evolution during storage is scarce.

In sweet cherry, the effects of pre-harvest melatonin treatments on anthocyanins and phenolic content have been reported only in three previous papers and contradictory results are observed. Thus, higher total phenolic and anthocyanin contents at harvest in 'Hongdeng' cultivar were found after tree treatment with 0.05 and 0.1 mM melatonin 3, 2, and 1 week before harvest (Xia et al., 2020). On the contrary, fruit treatment of 'Prime Giant' cultivar at stage II with 0.1 mM did not show impact on anthocyanin content at harvest while 0.01 mM dose led to twofold lower anthocyanin concentration as compared with control (Tijero et al., 2019). In 'Ferrovia' cultivar, 0.5 mM melatonin treatments, 2 and 1 week before harvest, had no effects on individual phenolic or anthocyanin compounds at harvest (Michailidis et al., 2021). However, as far as we know, only Michailidis et al. (2021) have reported the effects of cherry tree pre-harvest melatonin treatment on these bioactive compound evolution during storage and showed higher levels of neochlorogenic acid and cyaniding 3-*O*-rutinoside (the major phenolic and anthocyanin, respectively) after 12 days of cold storage in fruit from treated trees than in controls.

Fruit ripening and senescence are associated with ROS accumulation, such as H_2O_2, $O_2^{-\bullet}$ and $OH^{-\bullet}$, which are involved in DNA and proteins damage, peroxidation of membrane lipids and acceleration of senescence processes (Hodges et al., 2004). These ROS are generated in normal metabolism of plant cells and scavenged by antioxidant compounds (such as phenolics, tocopherols, carotenoids, and ascorbic acid) and by antioxidant enzymes, mainly superoxide dismutase (SOD), POD, CAT, and APX contributing to repair cell oxidative damage (Hodges et al., 2004; Kumar et al., 2014). Antioxidant enzymes were measured in fruit from control and 0.3 mM treated trees, since, in general, similar effects on maintaining cherry quality properties were observed for 0.3 and 0.5 mM concentrations, as well as on increasing crop yield (Carrión-Antolí et al., 2022). The results of the present study show higher activities of these antioxidant enzymes in melatonin treated trees than in controls during the whole storage period in both cultivars (**Figures 5A–F**). Thus, the occurrence of increased activity of antioxidant enzymes and enhanced content of the antioxidant compounds, phenolics and anthocyanins, could be responsible for the delay in the fruit post-harvest ripening process and maintenance of fruit quality attributes observed in sweet cherries from melatonin treated trees. Accordingly, different post-harvest treatments aimed to delay the sweet cherry post-harvest ripening and senescence processes also increased these antioxidant enzymes during storage. Thus, chitosan coating enhanced CAT and POD activities (Dang et al., 2010), as well as vacuum cooling treatment before storage (He et al., 2013) and 1-methylcyclopropene and hexanal increased SOD activity and reduced decreases in APX activity during storage compared to control cherries (Sharma et al., 2010). Nano-silica-chitosan solution and pressurised Argon treatment, and specially the combination of both treatments, led also to increased activities of CAT, APX, SOD, POD, and glutathione reductase (GR) and reduced accumulation of H_2O_2 and $O_2^{\bullet-}$ during sweet cherry storage as compared with controls, resulting in fruit with delayed senescence and extended shelf life (Meng et al., 2022). Pre-harvest sweet cherry treatments with SA, ASA, and SaMe led also to higher activities of CAT, POD, APX, and SOD and increased concentrations of phenolics and anthocyanins in treated fruit at harvest and during storage as compared with controls (Valverde et al., 2015; Giménez et al., 2017). Thus, treatments leading to increase sweet cherry ROS elimination systems, as observed in the present experiments for cherries from melatonin-treated trees, could contribute to delaying the post-harvest ripening and senescence processes and extending their shelf life. Accordingly, post-harvest melatonin treatment significantly induced enzymatic antioxidants and non-enzymatic antioxidants during storage in mango, kiwifruit, pomegranate, and peach fruit as reviewed by Xu et al. (2019) and Ze et al. (2021). The expression of genes encoding for antioxidant enzymes was upregulated by melatonin treatment, although the molecular mechanism underlying these effects needs further research. In sweet cherry, increased activity of antioxidant enzymes during storage due to post-harvest melatonin treatment has also been recently reported (Sharafi et al., 2021), although these effects due to melatonin applied as foliar spray treatment to sweet cherry trees have been reported for the first time in the present experiments.

CONCLUSION

Overall results showed that melatonin treatments during sweet cherry fruit on-tree development reduced weight and TA losses, softening and changes in fruit colour and TSS during cold storage. In addition, total phenolic and anthocyanin concentrations were higher in fruit from treated trees than in those from control ones, either at harvest or during the whole storage period. Finally, the activity of the antioxidant enzymes CAT, APX, and POD was also enhanced as a consequence of melatonin treatment. Thus, the storage period of fruit with quality properties in optimum levels for consumption was extended by one and 2 weeks for 'Prime Giant' and 'Sweet

Heart' cultivars, respectively, with respect to fruit from control trees. The increase of antioxidant systems, both enzymatic and non-enzymatic ones, as a consequence of melatonin treatments would lead to a more efficient ROS elimination accounting for delaying the post-harvest ripening process and maintaining fruit quality during storage.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

DV and MS conceived and designed the work in association with other authors. AC-A performed field treatments and most of the analytical determination, in collaboration with DM-R, PJZ, FG, MS, and DV. MS and DV analysed the data and wrote the manuscript. DV and MS were responsible for funding acquisition. All authors contributed to review the article and approved the submitted version.

FUNDING

This work was funded by Spanish Ministry of Science, Innovation and Universities and European Commission with FEDER funds through Project RTI2018-099664-B-100 Ph.D scholarship FPU18/04112 funded by Ministry of Science, Innovation and Universities.

ACKNOWLEDGMENTS

We thank Spanish Ministry of Science, Innovation and Universities for Ph.D.-scholarship of AC-A and Finca Toli Frutas S.L. for providing field experiments.

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4.3 Publicación 3

PUBLICACIÓN 3 (Acceso abierto)

Título: Antioxidant Systems and Quality in Sweet Cherries Are Improved by Preharvest GABA Treatments Leading to Delay Postharvest Senescence

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Fecha de publicación: 23 de diciembre 2023

Revista: International Journal of Molecular Sciences

Editorial: Multidisciplinary Digital Publishing Institute (MDPI)

ISSN: 1661-6596

Factor de impacto (2023): 4.9

Factor de impacto (5 años): 5.6

Categoría JCR®: Biochemistry & Molecular Biology

Rango: 66/313

Cuartil: Q1

CiteScore: 8.1

DOI: https://doi.org/10.3390/ijms25010260

International Journal of
Molecular Sciences

MDPI

Article

Antioxidant Systems and Quality in Sweet Cherries Are Improved by Preharvest GABA Treatments Leading to Delay Postharvest Senescence

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Abstract: γ-Aminobutyric acid (GABA) plays important roles in plant development, including the maintenance of fruit quality when applied as postharvest treatment. However, little information is available about the effects of preharvest GABA treatments. Thus, GABA (10, 50 and 100 mM) was applied as foliar spray at key points of fruit development in three sweet cherry cultivars and over two years. The results show that quality parameters, such as total soluble solid content, titratable acidity and firmness were higher in the fruit from GABA-treated trees than in the controls, either at harvest or during four weeks of cold storage. In addition, the total phenolic and total and individual anthocyanin concentrations were also enhanced by GABA treatments and the fruit color was improved. The activities of the antioxidant enzymes catalase, ascorbate peroxidase and peroxidase were also enhanced by the GABA treatments. The most effective concentration was 50 mM, which led to extending the storage period of sweet cherries with high quality traits to up to four weeks, while for the controls this was two weeks. Thus, GABA treatment had a clear effect on delaying the postharvest ripening and senescence processes in sweet cherries, with an additional effect on enhancing the content of bioactive compounds, such as phenolics and anthocyanins, with antioxidant properties and health benefits.

Keywords: anthocyanins; ascorbate peroxidase; catalase; firmness; peroxidase; phenolics; *Prunus avium* L.; storage

Citation: Carrión-Antolí, A.; Badiche-El Hilali, F.; Lorente-Mento, J.M.; Díaz-Mula, H.M.; Serrano, M.; Valero, D. Antioxidant Systems and Quality in Sweet Cherries Are Improved by Preharvest GABA Treatments Leading to Delay Postharvest Senescence. *Int. J. Mol. Sci.* **2024**, *25*, 260. https://doi.org/10.3390/ijms25010260

Academic Editor: Bo Sun

Received: 28 November 2023
Revised: 13 December 2023
Accepted: 19 December 2023
Published: 23 December 2023

1. Introduction

Sweet cherry (*Prunus avium* L.) fruit have high-quality properties, namely juiciness, texture, color, taste and flavor, making them highly appreciated by consumers around the world [1–4]. In addition, they have bioactive compounds, such as phenolic acids, anthocyanins, flavonoids and ascorbic acid, which have antioxidant properties and are the major responsible actors for the beneficial effects of sweet cherry consumption to human health, which include lower risk of suffering from degenerative illnesses, such as diabetes, cardiovascular, inflammatory and several kinds of cancer, among others [5–7]. However, the postharvest ripening and senescence processes evolve rapidly in sweet cherry fruit and their quality deteriorates in a short period of time. The most usual practice to maintain fruit quality is storage at cold temperature as soon as possible after harvest, but even in this case, the shelf life of cherries is no longer than 2–3 weeks, depending on cultivar and other preharvest factors [8,9]. In this sense, the combination of cold storage with other postharvest technologies, such as edible coatings based on alginate [10], *Aloe vera* gel, alone or combined with aromatic plant extracts [11], and chitosan [12] or nano-silica coating

combined with pressurized Ar [13], 1-methylcyclopropene [14,15] or salicylates and oxalic acid [16] treatments, led to the maintenance of the sweet cherry fruits' quality properties and the extension of their shelf life. On the other hand, preharvest treatments with salicylic acid, acetyl salicylic acid or methyl salicylate [17], as well as with oxalic acid [2], gibberellic acid [18], melatonin [19,20] or methyl jasmonate [21] proved to have important effects on increasing fruit quality traits (size, color, firmness and sugar content) at harvest and on their maintenance at higher levels, as compared with fruit from control trees during storage.

γ-Aminobutyric acid (GABA) is a four-carbon non-protein amino acid which plays important roles in plants and animals. In humans, GABA has many health-related effects, acting against inflammatory, diabetic, hypertensive and cancer illnesses [22]. In plants, GABA has been reported to regulate many plant physiological processes; the first ones discovered being the plant resistance induction to abiotic and biotic stresses [23–25]. Glutamate is the precursor for GABA synthesis by action of glutamate decarboxylase, and GABA could be metabolized through the GABA shunt pathway, rendering α-ketoglutarate and succinate in two consecutive reactions. In addition, the GABA shunt plays important roles in reducing reactive oxygen species (ROS) and acting as a signaling molecule regulating several stress-related mechanisms [25,26]. More recently, beneficial effects of postharvest fruit GABA treatments on maintaining quality properties and reducing chilling injury damage have been reported in a wide range of fruit species, such as table grape [27], cornelian [28], peaches [29], mango [30], loquat [31] and kiwifruit [32], among others.

However, the literature regarding the effects of GABA application as preharvest treatment in fruit quality attributes is scarce. Foliar spray with GABA solutions of pomegranate trees led to increased crop yield and fruit quality properties at harvest, which were also maintained at higher levels during storage as compared to fruit from control trees. In 'Fino-95' lemon, the GABA treatment of the trees increased crop yield (with ca. 15% kg per tree with respect to controls), without affecting fruit firmness, total soluble solids or titratable acidity [33]. Finally, GABA foliar spray application to apple trees, 1 or 2 weeks before harvest, decreased soft scald symptoms after cold storage, although no effects were observed in other fruit quality parameters [34].

Specifically, in sweet cherry fruit, no previous reports are available about GABA treatments either applied as post- or as preharvest treatments, although Wang et al. [35,36] assayed the effects of postharvest treatments with β-aminobutyric acid (BABA), a GABA isomer. In these papers, it was reported that BABA dipping treatment for 10 min reduced weight loss and softening and maintained high levels of sugars, organic acids, phenolics and antioxidant enzyme activities during storage at 20 °C, leading to maintaining overall sweet cherry fruit quality.

According to the previous literature, it was hypothesized that preharvest GABA treatments could have beneficial effects on sweet cherry fruit quality traits either at harvest or during storage, which was the main goal of the present experiments. In addition, it was important to know whether these effects could be dependent on cultivar or growing season. For this purpose, different GABA concentrations were assayed in three sweet cherry cultivars and for two years, in order to obtain more broad conclusions regarding the effects of GABA.

2. Results

2.1. Sweet Cherry Quality Parameters during Storage

Weight loss increased during storage in all cherry cultivars, reaching final values in control fruits of 9–10% after 28 days of storage, depending on the cultivar and growing cycle. However, weight losses were significantly lower ($p < 0.05$) in fruit from GABA-treated trees, the lowest weight losses being found for 50 and 100 mM concentrations (Figure 1). With respect to fruit firmness, significant effects of GABA treatments were also observed, since firmness values were higher in fruit from GABA-treated trees than in controls, either at harvest or during the whole storage time. In general, the highest effects ($p < 0.05$) on diminishing firmness losses during storage were observed for the 50 mM GABA treatment,

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although for "Lapins" in the 2019 experiment, no significant differences were observed among the GABA-applied doses (Figure 2). In addition, it is worth noting that the fruit firmness of "Lapins" from the 50 mM GABA treatment was 17% higher than for the controls, while for the remaining cultivars' fruit firmness values were 30–35% higher than in the controls during the whole storage periods. Thus, the effect of preharvest GABA treatments on maintaining this important quality trait was lower for the "Lapins" cultivar.

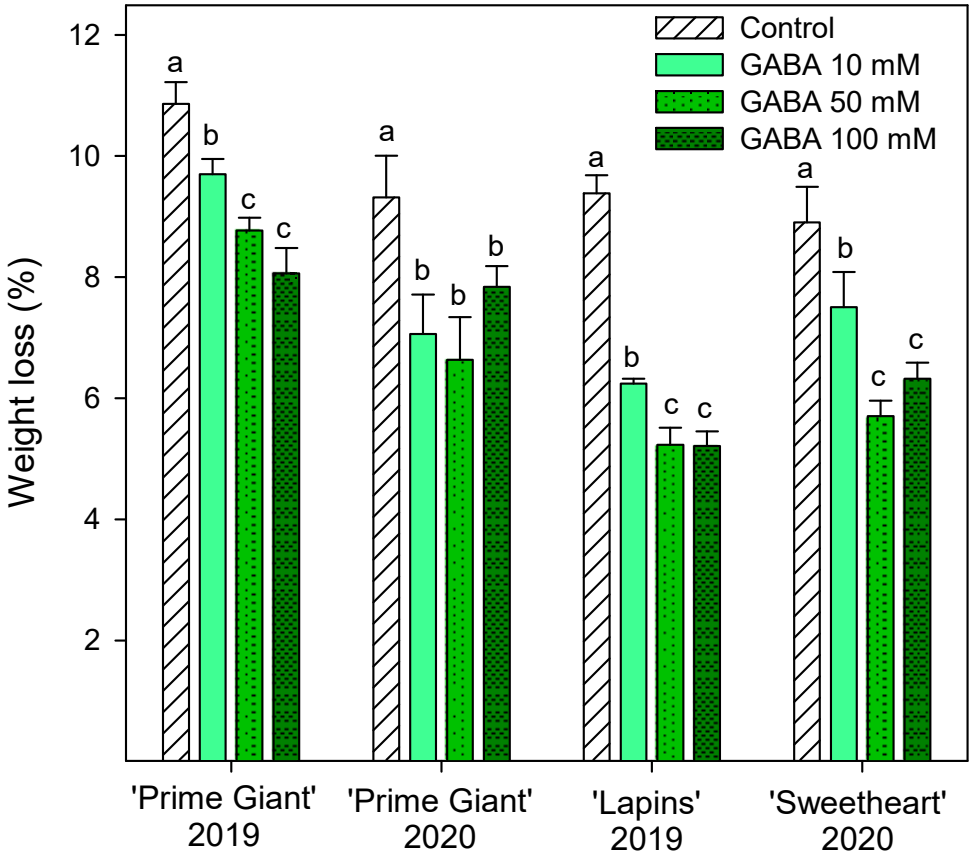

Figure 1. Weight loss of sweet cherries from control and γ-aminobutyric acid (GABA)-treated trees after 28 days of storage at 2 °C. Data are the mean ± SE of three replicates. Different letters show significant differences ($p < 0.05$) among treatments for each cultivar and growing cycle.

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Total soluble solids (TSSs) and titratable acidity (TA) were also significantly affected ($p < 0.05$) by GABA treatments, either at harvest or during storage (Table 1). Thus, TSS and TA values at harvest were increased as a consequence of the GABA treatments, the effect being dose-dependent for TSSs in "Prime Giant" and "Sweetheart", while for 'Lapins' similar values were obtained with all the GABA doses assayed as well as for TA for all cultivars and growing cycles (Table 1). During storage, a significant increase in TSSs occurred, although at the end of the storage time the TSS content was still higher in the fruit from the GABA-treated trees than in the controls (Table 1). On the contrary, TA significantly decreased during storage whether in the controls or in the treated fruit. However, the TA decreasing rate was delayed by the GABA treatments since, at the end of storage, the TA values were significantly higher in fruit from treated trees than in the controls (Table 1) for all cultivars and years.

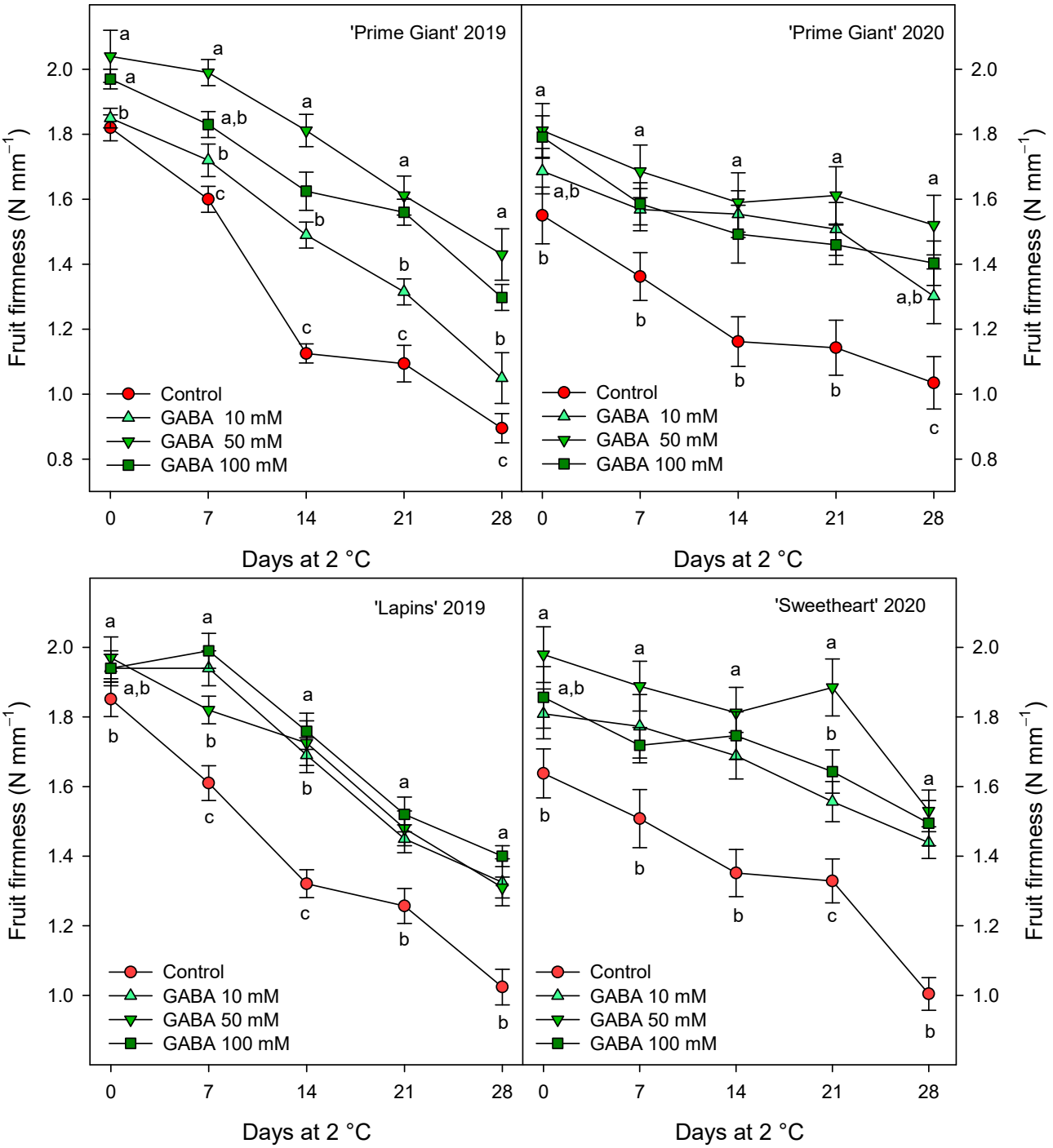

Figure 2. Fruit firmness of sweet cherries from control and γ-aminobutyric acid (GABA)-treated trees during 28 days of storage at 2 °C. Data are the mean ± SE of three replicates. Different letters show significant differences (at $p < 0.05$) among treatments for each sampling date, for each cultivar and growing cycle.

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Table 1. TSS (°Brix) and titratable acidity (TA) at harvest and after 28 days of storage at 2 °C of sweet cherry fruit from control and γ-aminobutyric acid (GABA)-treated trees.

Cultivar		TSS (Day 0)	TSS (Day 28)	TA (Day 0)	TA (Day 28)
"Prime Giant" 2019	Control	20.43 ± 0.24^{aA}	22.73 ± 0.17^{aB}	1.11 ± 0.02^{aA}	0.85 ± 0.02^{aB}
	GABA 10 mM	21.88 ± 0.23^{bA}	24.55 ± 0.35^{bB}	1.21 ± 0.02^{bA}	0.96 ± 0.01^{bB}
	GABA 50 mM	23.03 ± 0.08^{cA}	25.17 ± 0.41^{bcB}	1.23 ± 0.03^{bA}	0.98 ± 0.02^{bB}
	GABA 100 mM	23.88 ± 0.18^{dA}	26.32 ± 0.14^{cB}	1.17 ± 0.04^{abA}	0.99 ± 0.01^{bB}
"Prime Giant" 2020	Control	22.63 ± 0.11^{aA}	23.42 ± 0.15^{aB}	1.47 ± 0.03^{aA}	1.12 ± 0.03^{aB}
	GABA 10 mM	23.47 ± 0.17^{bA}	25.73 ± 0.45^{bB}	1.53 ± 0.03^{abA}	1.27 ± 0.02^{bB}
	GABA 50 mM	24.08 ± 0.08^{cA}	27.03 ± 0.23^{cB}	1.61 ± 0.02^{bA}	1.37 ± 0.01^{cB}
	GABA 100 mM	24.57 ± 0.09^{dA}	26.57 ± 0.33^{cB}	1.59 ± 0.01^{bA}	1.36 ± 0.01^{cB}
"Lapins" 2019	Control	20.90 ± 0.17^{aA}	21.83 ± 0.08^{aB}	1.11 ± 0.03^{aA}	0.86 ± 0.03^{aB}
	GABA 10 mM	21.85 ± 0.15^{bA}	23.10 ± 0.35^{bB}	1.15 ± 0.03^{abA}	0.98 ± 0.03^{bB}
	GABA 50 mM	21.92 ± 0.14^{bA}	22.95 ± 0.16^{bB}	1.20 ± 0.02^{bA}	1.03 ± 0.01^{bB}
	GABA 100 mM	22.02 ± 0.20^{bA}	23.32 ± 0.09^{bB}	1.21 ± 0.03^{bA}	0.99 ± 0.01^{bB}
"Sweetheart" 2020	Control	20.03 ± 0.21^{aA}	21.53 ± 0.17^{aB}	1.32 ± 0.01^{aA}	1.27 ± 0.02^{aB}
	GABA 10 mM	21.48 ± 0.23^{bA}	22.40 ± 0.09^{bB}	1.39 ± 0.04^{abA}	1.28 ± 0.01^{aB}
	GABA 50 mM	22.08 ± 0.04^{cA}	24.20 ± 0.14^{cB}	1.45 ± 0.02^{bA}	1.40 ± 0.02^{bB}
	GABA 100 mM	21.78 ± 0.20^{cA}	25.83 ± 0.17^{dB}	1.44 ± 0.01^{bA}	1.38 ± 0.01^{bB}

Different capital letters within a row show significant differences at $p < 0.05$ from day 0 to day 28 for each parameter and treatment. Different lowercase letters within a column show significant differences at $p < 0.05$ among treatments for each cultivar and growing cycle.

2.2. Phenolic and Anthocyanin Concentrations during Storage

The total phenolic concentration was significantly ($p < 0.05$) enhanced by preharvest GABA treatments, and in general, the highest effect was found for the 50 mM dose from day 0 to the end of storage for all cultivars and years (Figure 3). Nevertheless, this effect was more dependent on cultivar than on growing cycle, since increases ca. 25% were observed for "Prime Giant" in 2019 and 2020, and ca. 30 and 40% for "Sweetheart" in 2020 and "Lapins" in 2019, respectively. Similarly, significantly higher ($p < 0.05$) concentrations of total anthocyanins were observed in sweet cherries from GABA-treated trees as compared with those from the controls, during the whole storage period (Figure 4). The highest increases in this parameter were also found for the 50 mM dose, which were of H75 % for "Lapins" in 2019 and H50% for "Sweetheart" in 2020 and for "Prime Giant" in both years. In addition, a similar trend was found for total phenolic and anthocyanin concentrations during storage, with increases from day 0 to day 14–21 and decreases thereafter, independently of the treatments, cultivars or years (Figures 3 and 4). In fact, high correlations ($r^2 = 0.76 - 0.85$) were observed between phenolic and anthocyanin concentrations for all cultivars and years when data from all sampling dates were considered (Figure 5).

Individual anthocyanin concentration was measured at harvest (day 0, 2019 for "Prime Giant" and "Lapins" and 2020 for "Sweetheart") and the results show that cyanidin 3-*O*-rutinoside (Cyn 3-*O*-rut) was the major one, followed by pelargonidin 3-*O*-rutinoside (Pelg 3-*O*-rut) and cyanidin 3-*O*-glucoside (Cyn 3-*O*-gluc). These three individual anthocyanins were significantly ($p < 0.05$) enhanced by GABA treatments in all cultivars, and in general, the highest increases were observed for the 50 mM GABA dose (Figure 6).

2.3. Antioxidant Enzymes

Antioxidant enzymes, APX, CAT and POD were measured in sweet cherries from control and 50 mM GABA-treated trees since, generally, this dose was the more effective for maintaining higher values of quality parameters and antioxidant compounds during storage. The results show significantly higher values ($p < 0.05$) in cherries from treated trees than in controls during the whole storage period (Figures 7–9). For APX activity, a decrease trend in the control fruits was observed after 14–21 days of storage while, for fruit from GABA-treated trees, this activity remained at similar levels than at harvest during

the whole storage time (Figure 7). However, it is worth noting that the effects of GABA treatment were similar for all cultivars and years, with increases ranging from 20 to 28% when data for all sampling dates were taken into account.

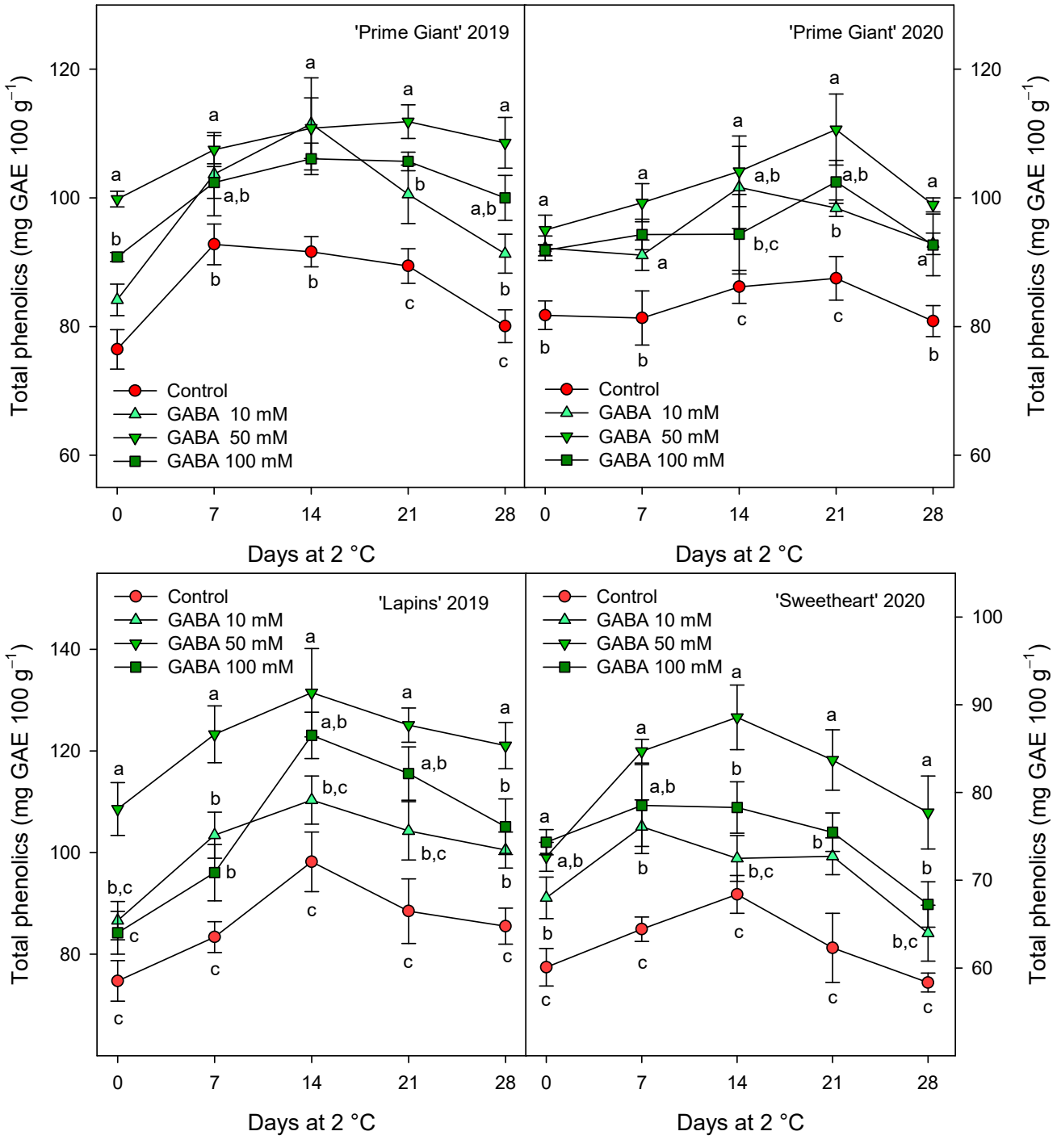

Figure 3. Total phenolic content (equivalent to gallic acid (GAE) 100 g^{-1}) in sweet cherries from control and γ-aminobutyric acid (GABA)-treated trees during 28 days of storage at 2 °C. Data are the mean ± SE of three replicates. Different letters show significant differences (at $p < 0.05$) among treatments for each sampling date, for each cultivar and growing cycle.

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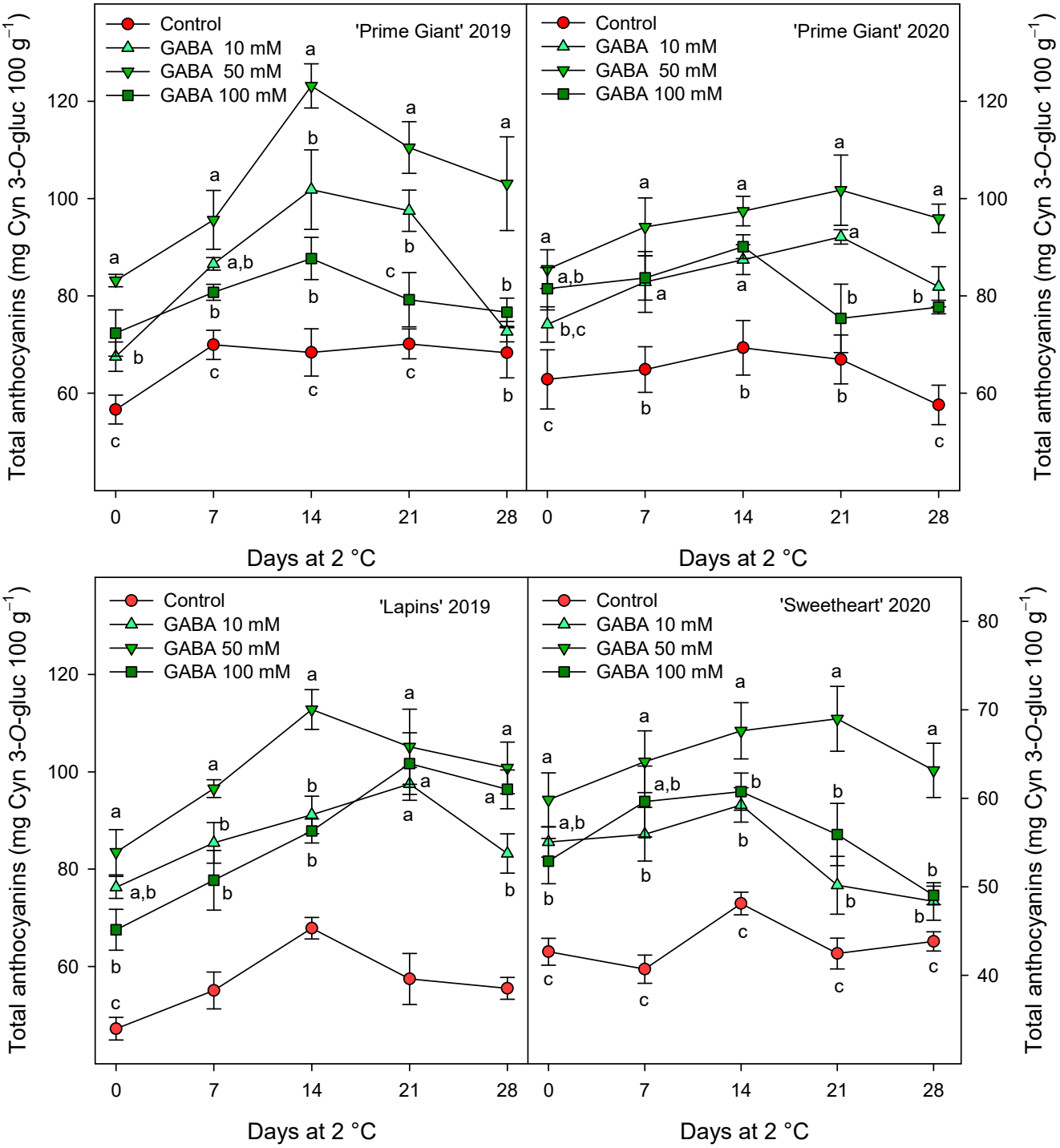

Figure 4. Total anthocyanin content (mg cyanidin 3-O-glucoside equivalent (Cyn 3-O-gluc) 100 g^{-1}) in sweet cherries from control and γ-aminobutyric acid (GABA)-treated trees during 28 days of storage at 2 °C. Data are the mean ± SE of three replicates. Different letters show significant differences (at $p \leq 0.05$) among treatments for each sampling date, for each cultivar and growing cycle.

Fig. 4. Total anthocyanin content (mg cyanidin 3-O-glucoside equivalent (Cyn 3-O-gluc) 100 g−1) in sweet cherries from control and γ-aminobutyric acid (GABA)-treated trees during 28 days of storage at 2 °C. Data are the mean ± SE of three replicates. Different letters show significant differences (at p ≤ 0.05) among treatments for each sampling date, for each cultivar and growing cycle.

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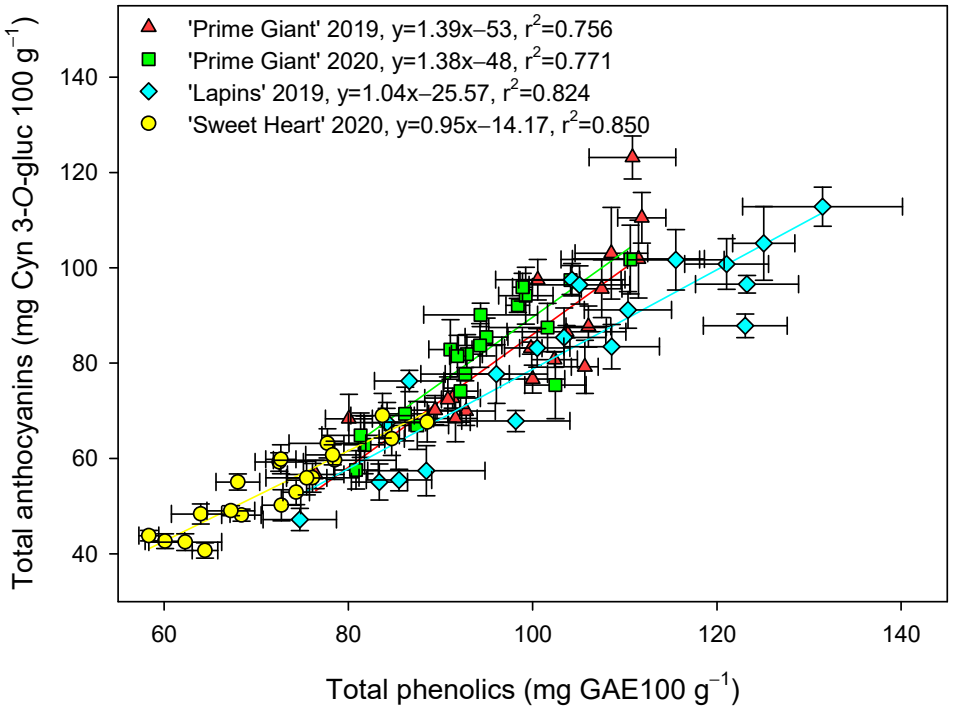

Figure 5. Correlation between total phenolic (mg gallic acid equivalent (GAE) 100 g^{-1}) and total anthocyanin (mg cyanidin 3-*O*-glucoside equivalent (Cyn 3-*O*-gluc) 100 g^{-1}) concentrations for each cultivar and year, taking into account data from control and treated fruit at each sampling date. Data are the mean ± SE of three replicates.

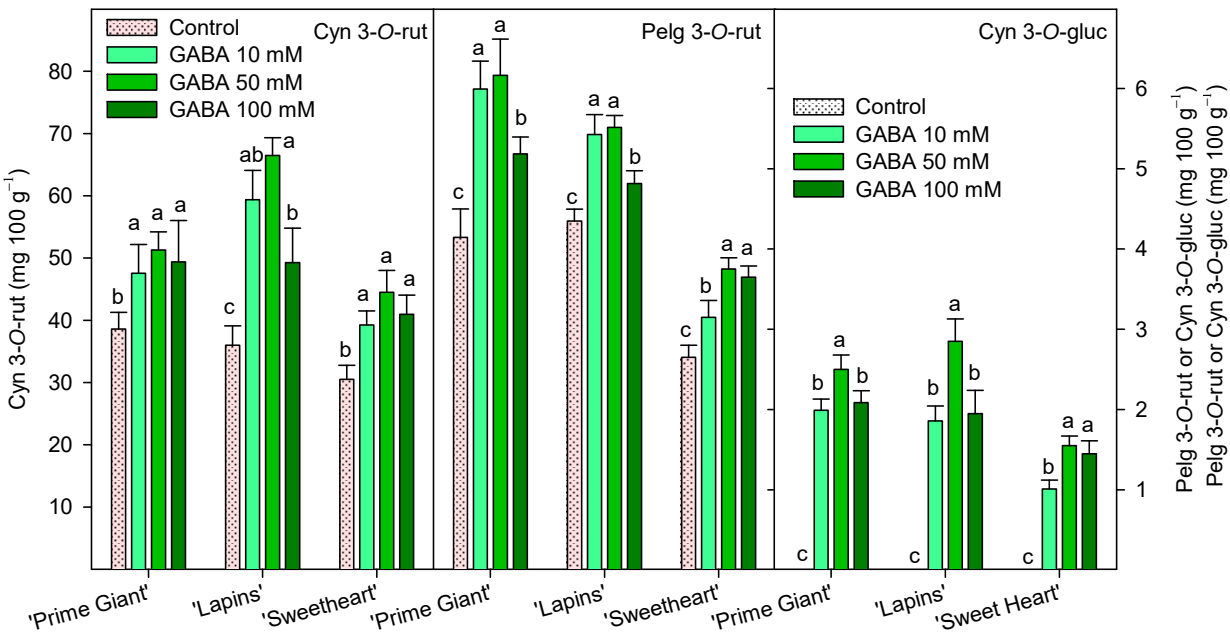

Figure 6. Individual anthocyanin concentration, cyanidin 3-*O*-rutinoside (Cyn 3-*O*-rut, left axis), pelargonidin 3-*O*-rutinoside (Pelg 3-*O*-rut, right axis) and cyanidin 3-*O*-glucoside (Cyn 3-*O*-gluc, right axis) at harvest (day 0, 2019 for "Prime Giant" and "Lapins" and 2020 for "Sweetheart") in sweet cherries from control and γ-aminobutyric acid (GABA)-treated trees. Data are the mean ± SE of three replicates. Different letters show significant differences at $p < 0.05$ for each cultivar and growing cycle.

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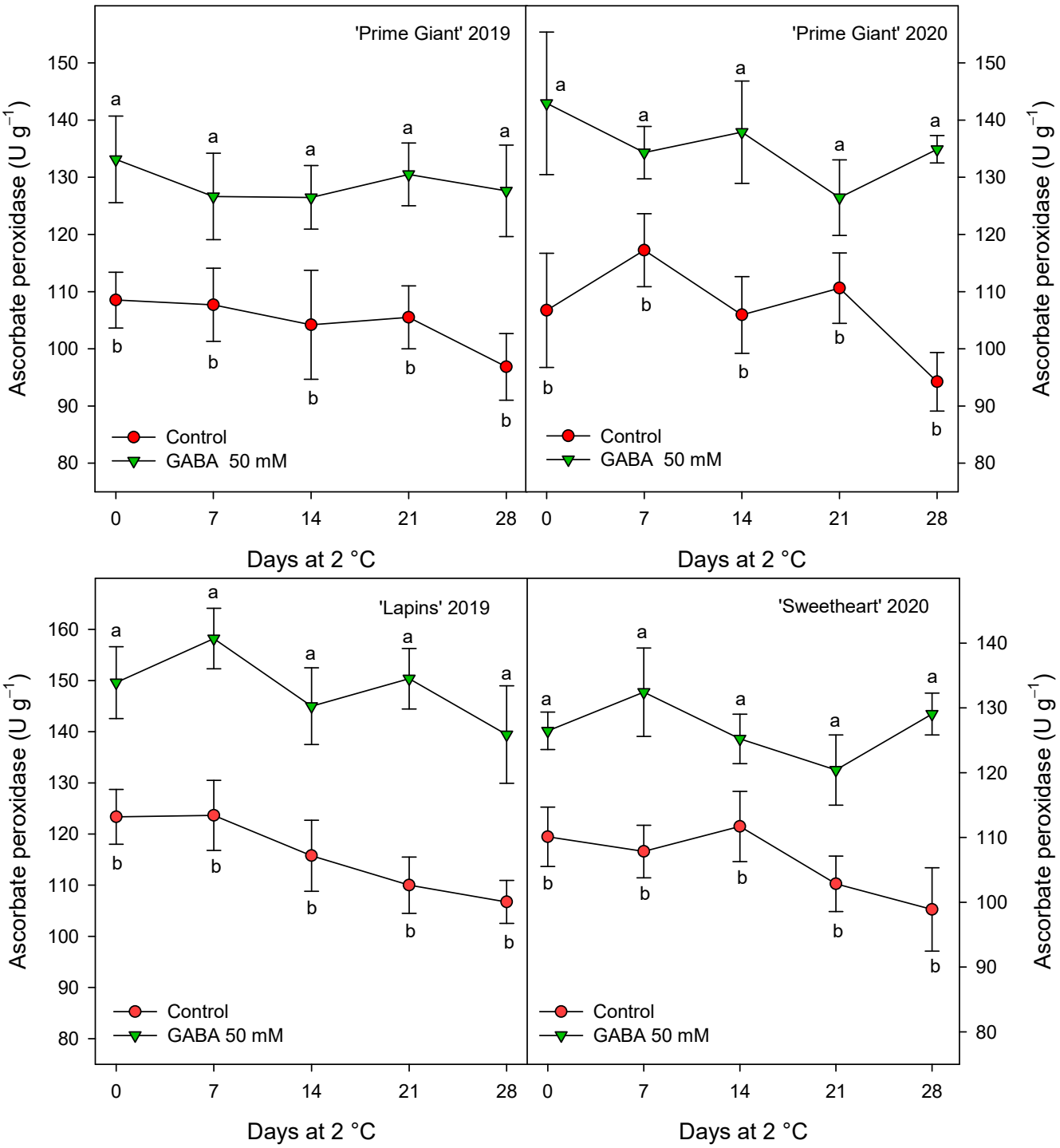

Figure 7. Ascorbate peroxidase activity in sweet cherries from control and γ-amino
(GABA)-treated trees during 28 days of storage at 2 °C. Data are the mean ± SE of three rep
Different letters show significant differences (at $p < 0.05$) between treatments for each sa
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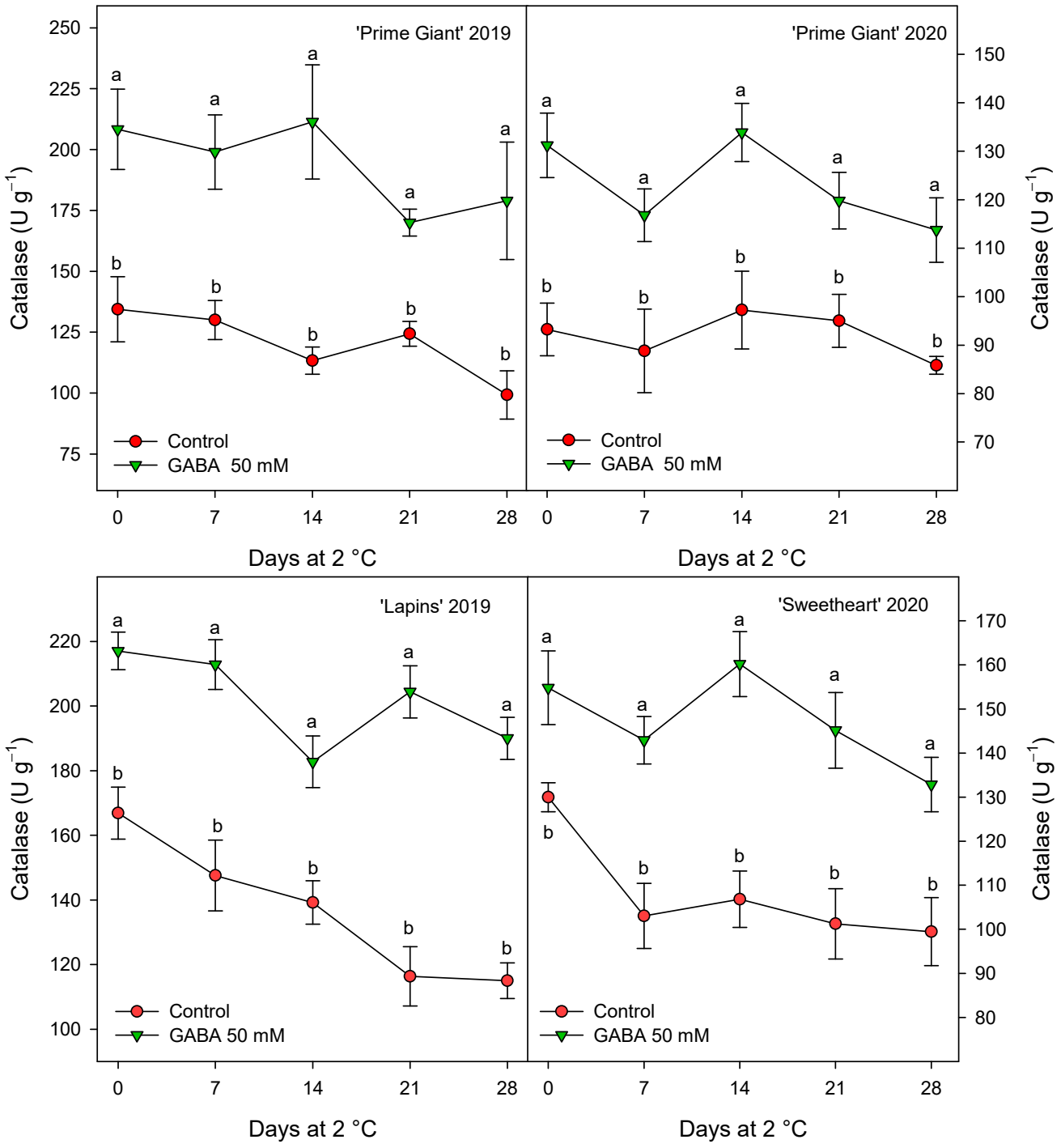

Figure 8. Catalase activity in sweet cherries from control- and γ-aminobutyric acid (GABA)-treated trees during 28 days of storage at 2 °C. Data are the mean ± SE of three replicates. Different letters show significant differences (at $p < 0.05$) between treatments for each sampling date, for each cultivar and growing cycle.

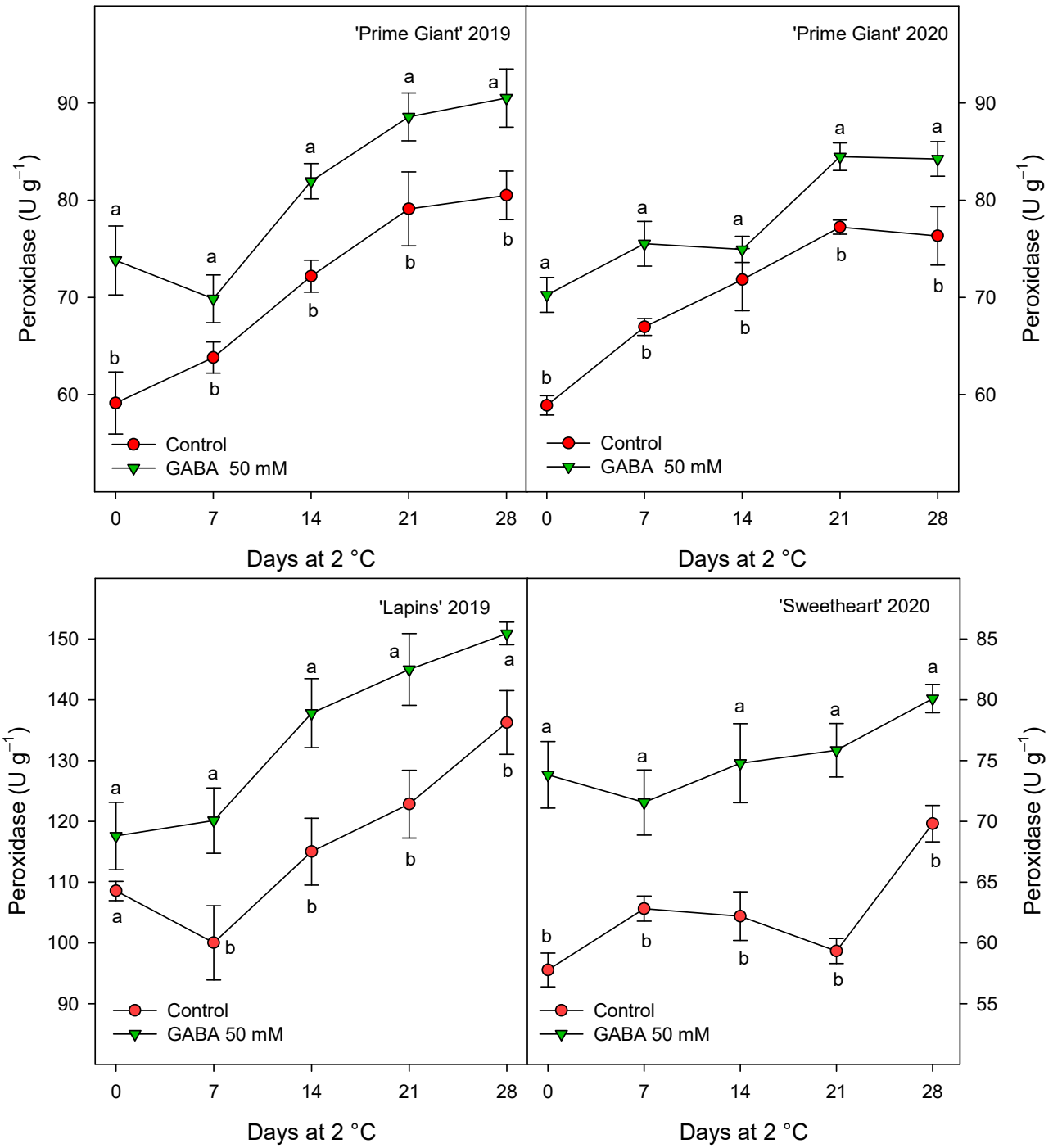

Figure 9. Peroxidase activity in sweet cherries from control and γ-aminobutyric acid (GABA)-treated trees during 28 days of storage at 2 °C. Data are the mean ± SE of three replicates. Different letters show significant differences (at $p < 0.05$) between treatments for each sampling date, for each cultivar and growing cycle.

Figure 9. Peroxidase activity in sweet cherries from control and γ-aminobutyric acid (GABA)-treated trees during 28 days of storage at 2 °C. Data are the mean ± SE of three replicates. Different letters show significant differences (at $p < 0.05$) between treatments for each sampling date, for each cultivar and growing cycle.

CAT activity was also found at higher levels in fruits from GABA-treated trees as compared with controls, although for this activity the highest increases, ca. 60%, were observed for "Prime Giant" in 2019, followed by "Lapins" in 2019, ca. 50%, while for "Prime Giant" and "Sweetheart" in 2020, the increases were ca. 35% (Figure 8). Finally, POD activity was significantly increased by GABA treatment although the increases were lower than those for APX and CAT activities, since they were of 15–20% with respect to the controls, independently of the cultivar or growing cycle (Figure 9).

3. Discussion

The main quality attributes of sweet cherry fruit are visual appearance, such as absence of defects, size and color, stem freshness and length, organoleptic properties including juiciness, firmness, sweetness, sourness, taste, aroma and flavor, sweetness and sourness which affect consumer purchase intentions, and vary depending on the cultivars [1,3,8]. However, these quality traits decrease rapidly after harvest, mainly due to stem browning and fruit weight, firmness and acidity losses, leading to decreased fruit juiciness and freshness, so the fruits lose their organoleptic properties and taste

3. Discussion

The main quality attributes of sweet cherry fruit are visual appearance, such as absence of defects, size and color; stem freshness and length; organoleptic properties including juiciness, firmness, sweetness, sourness, taste, aroma and flavor; sweetness and sourness which affect consumer purchase intentions, and vary depending on the cultivars [1,3,8]. However, these quality traits decrease rapidly after harvest, mainly due to stem browning and fruit weight, firmness and acidity losses, leading to decreased fruit juiciness and freshness, so the fruits lose their organoleptic properties and taste over-ripened [9,12,17,36,37]. The present results show that preharvest GABA treatments enhanced red color, fruit firmness and TSS and TA contents at harvest, leading to fruit with higher organoleptic properties. According to the previous reports commented above, increases in fruit weight loss (Figure 1), due to dehydration by transpiration process, and in TSSs (Table 1), as well as decreases in fruit firmness (Figure 2) and TA (Figure 3) were observed in sweet cherries during cold storage. However, these changes were significantly delayed in fruit from GABA-treated trees as compared with the controls in the "Prime Giant" and "Lapins" cultivars in the 2019 experiment, and these effects were confirmed for the "Prime Giant" cultivar and for the "Sweetheart" cultivar in the 2020 experiment and, in general, the highest effects were observed for the 50 mM concentration. Taking into account the results of all these quality parameters, control cherries could be stored at cold temperatures for two weeks with optimal properties for consumption, while this period was extended to up to four weeks for cherries from the 50 mM GABA-treated trees. Postharvest GABA dipping treatments have been proven to successfully maintain fruit quality properties in cornelian cherry [28], loquat [31], peach [29] and tomato [38], with additional effects on reducing chilling injury symptoms. Moreover, other postharvest treatments with effects on maintaining fruit quality, such as sodium nitroprusside (SNP) in peach [39], calcium in apple [40] or melatonin [41] and methyl jasmonate [42] in tomato, increased GABA content and the GABA-shunt pathway, proving the effects of GABA on delaying the postharvest ripening and senescence processes, which has been attributed to an enhanced mitochondrial energy status [29]. It is important to note that consumers have concerns about post-harvest fruit treatments, which have also more legal restrictions than preharvest ones; meanwhile, preharvest treatments with GABA, which is a natural amino acid, are considered to be safe and to have beneficial properties for human health [43], so this could be a suitable and environmentally friendly approach to increase sweet cherry quality and their storage time for longer periods.

The content of phenolic compounds and especially anthocyanins in sweet cherry fruit have attracted increasing interest in recent years due to their antioxidant properties responsible for their positive impact on human health, namely by reducing the risk of suffering from degenerative diseases [7,8,44–46]. In the present results, a significant increase in total phenolic and anthocyanin content was observed as a consequence of GABA treatment, either at harvest or during the whole storage period (Figures 3 and 4). In general, total phenolic and anthocyanin concentrations increased from day 0 to day 7–14 in control fruit and decreased thereafter, these changes being related to the evolution in the postharvest ripening process of sweet cherry [9,16,21], which was delayed in the fruit from GABA-treated trees. In addition, a higher content in individual anthocyanin concentration was found in treated cherries at harvest as compared with controls (Figure 6). The major anthocyanin in "Prime Giant", "Lapins" and "Sweetheart" was cyn 3-*O*-rut in the three cultivars, in agreement with previous reports for other cultivars [2,4,9,46–49]. No previous studies are available in the literature regarding the effects of preharvest GABA treatments on the biosynthesis of phenolic compounds during fruit on-tree development for comparative purposes, although some reports have been published for postharvest treatments. For instance, GABA dipping treatment increased phenolics and flavonoids in carambola [50,51], cornelian cherry fruits [28] and tomato [52], as well as in fresh pistachio fruit [53], although in the last fruit species higher effects were observed when GABA was combined with carboxymethyl cellulose coating and CaO. These effects, in aonla and

carambola fruits, have been attributed to a higher activity of phenylalanine ammonia lyase (PAL) and reduced activity of polyphenol oxidase (PPO), leading to increased total phenols accumulation [50,54]. However, preharvest GABA treatments seem to be more effective in increasing the content in these bioactive compounds, as has been observed in the present experiments and in previous ones with pomegranates [55]. Thus, GABA treatment may lead to increases in the health benefits of sweet cherries, since phenolics and especially anthocyanins exhibit protective roles against heart, vision and neurological diseases, among others [44–46,56]. These effects are attributed to their widely recognized antioxidant, anti-inflammatory and antiapoptotic properties, which also depend on the anthocyanins which significantly depend on the human being's gut microbiota activities [57].

Oxygen free radicals (ROS), mainly H_2O_2, $O_2^{-\bullet}$ and $OH^{-\bullet}$, accumulate during fruit ripening and senescence, leading to protein and DNA damage membrane lipid peroxidation and senescence process acceleration [58]. Vegetable cells have antioxidant systems able to scavenge these ROS and repair de oxidative damage, including antioxidant compounds (namely ascorbic acid, phenolic compounds, tocopherols and carotenoids) and antioxidant enzymes, such as POD, CAT, APX and superoxide dismutase (SOD), among others [58,59]. The activity of the antioxidant enzymes APX, CAT and POD was found to be higher in cherries from the 50 mM GABA-treated fruit than in the controls for the three cherry cultivars at harvest and for all sampling dates during storage (Figures 7–9). Thus, the higher antioxidant systems, enzymatic and non-enzymatic ones, found as a consequence of preharvest GABA treatments, could account for delaying post-harvest ripening and senescence processes and being responsible for the maintenance of fruit quality traits. In fact, the accumulation of ROS, such as H_2O_2, $O_2^{-\bullet}$ and $OH^{-\bullet}$, among others, is a general event occurring during fruit ripening and senescence processes [58,59]. Then, enhancing the fruit cell antioxidant system, both enzymatic and non-enzymatic ones would account for the observed delayed senescence and quality traits maintenance. Accordingly, the antioxidant enzymes SOD, CAT, POD, APX and glutathione reductase were increased in blueberry and carambola fruits by postharvest GABA dipping treatment, leading to delay in the senescence process [50,60]. In addition, different pre- and postharvest treatments with effects on delaying quality properties losses in sweet cherries also increased these antioxidant systems. For instance, vacuum cooling [61], chitosan coating [57,62] or the combination of chitosan with Argon [13] enhanced antioxidant enzyme activities and antioxidant compounds, resulting in extending the sweet cherry shelf life [13], as well as preharvest treatments with salicylates [17], oxalic acid [2] or melatonin [20].

4. Materials and Methods

4.1. Plant Material and GABA Treatments

Field experiments were performed in a commercial farm, located at Jumilla (Murcia, Spain, coordinates UTMX: 463.700 and UTMY: 4.268.900) by using three replicates of three trees for each cultivar and GABA treatment. In 2019, the assays were made with "Prime Giant" and "Lapins" cultivars, which were 7 years old, and for 2020, the experiment was repeated with "Prime Giant" and a new cultivar, "Sweetheart" (which was 5 years old), was added. Mean annual temperatures in the field during the trials were 15.24 and 15.30 °C for 2019 and 2020, respectively, the accumulated rainfalls were 357 and 352 mm for 2019 and 2020, respectively, and the relative humidity mean values were 60.5 and 65.1%, respectively. All cultivars were grafted onto SL-64 rootstock and were grown under normal agronomic conditions for both years, applying 60:30:100 kg ha^{-1} of N:P:K fertilizers and 5250 m^3 ha^{-1} of water along the growing cycle and performing an open-center pruning. GABA treatments were performed by applying 3 L of 10, 50 or 100 mM GABA freshly prepared solutions, containing 0.1% Tween 20 as surfactant, as foliar spray with a hand spray machine in order to wet the whole tree canopy. Control trees were treated with tap water containing 0.1% Tween 20. Control and treated trees were separated by other rows of trees to avoid treatment drift. Sweet cherry fruit were harvested at commercial ripening stage, according to characteristic fruit size, color and total soluble solids content of each

cultivar, and a sample of 3 kg (1 kg of each tree) was taken for each treatment, replicated and transported to the laboratory (at 15 °C and 70% RH) in 2 h for storage experiments. Once at the laboratory, five lots of 20 fruits, homogeneous in size and color, were selected for each replicate, weighted and stored at 2 °C and 90% RH for 0, 7, 14, 21 and 28 days.

4.2. Fruit Quality Parameter Measures

Fruit weight was measured at day 0 and after each storage period, by using a digital balance (KERN 440-35N, Balingen, Germany), and weight loss was expressed as percentage with respect to weight at harvest. Fruit firmness was determined in each individual fruit by using a Texture Analyzer (TX-XT2i model, Stable Mycrosys-tems, Godalming, UK) as previously reported [20], and the results are expressed as N mm^{-1}. Then, sweet cherry fruit were cut into small pieces to obtain a homogeneous sample for the 20 fruits of each replicate. One portion of each sample was used for total soluble solids (TSSs) and titratable acidity (TA) measures (immediately after cutting) and another one was frozen and ground under liquid N_2 and stored at −20 °C until phenolics, anthocyanins and antioxidant enzyme activities were measured. TSSs and TA were measured (in duplicate) in the juice obtained from 50 g of fruit sample, through squeezing and filtration with a double cotton fabric, by a hand refractometer (Atago PR-101, Atago Co. Ltd., Tokyo, Japan) and titration with NaOH 0.1 N until pH 8.1 with the 785 DMP Titrino automatic titration system (Metrohm, Herisau, Switzerland), respectively. TSSs were expressed as °Brix and TA as g of malic acid equivalent to 100 g^{-1}.

4.3. Measures of Total Phenolic Compounds and Total and Individual Anthocyanins

Phenolics were extracted and quantified according to Carrión-Antolí et al. [23]. Briefly, fruit samples (5 g) were homogenized with 10 mL of water:methanol (2:8, *v*:*v*) plus 2 mM NaF (to avoid phenolic degradation by suppressing the activity of polyphenol oxidase) in an Ultraturrax homogenizer (T18-basic model, IKA, Berlin, Germany). The supernatant was used to quantify total phenolic content (in duplicate in each extract) by addition of the Folin-Ciocalteu reagent as described by Díaz-Mula et al. [1]. The results (mean ± SE) are expressed in gallic acid equivalent, mg 100 g^{-1} on a fresh weight basis. For anthocyanin extraction, 2 g of sample and 10 mL of methanol/water/HCl (80:19:1) were homogenized and centrifuged as described above and anthocyanins were measured in the supernatant by reading absorbance at 530 nm by using an UNICAM Heliosα spectrophotometer (Artisan-Technology-Group, Champaign, IL, USA). The results (mean ± SE) are expressed as cyanidin 3-*O*-glucoside (cyn 3-*O*-gluc) equivalents, taking into account the coefficient of molar absorption of cyanidin 3-*O*-glucoside, 23,900 L cm^{-1} mol^{-1} and its molecular weight, 449.2 g mol^{-1}. Anthocyanin extracts were filtered through a 0.45 µm PVDF filter (Millex-HV13, Millipore, Bedford, MA, USA) and used to quantify individual anthocyanins (in duplicate in each extract) in an HPLC analysis system (Agilent HPLC-1200-Infinity series, Santa Clara, CA, USA), according to Martínez-Esplá et al. [2]. Cyn 3-*O*-rut, pelg 3-*O*-rut and cyn 3-*O*-gluc were used to perform standard calibration curves and each individual anthocyanin was expressed as mg 100 g^{-1}.

4.4. Determination of Antioxidant Enzyme Activities

The extracts for APX, CAT and POD quantification were obtained by homogenizing 5 g of fruit sample with 10 mL of 50 mM phosphate buffer, pH 7.0, with 1 mM EDTA (ethylen–diaminetetraacetic acid) and 1% PVP (polyvinylpyrrolidone) and centrifuging at 15,000× *g* for 30 min at 4 °C [17]. APX quantification was conducted by reading the absorbance at 290 nm for 1 min in a 3 mL volume reaction containing 50 mM potassium phosphate buffer, pH 7.0, 0.5 mM ascorbic acid, 1 mM H_2O_2 and 0.1 mL of crude extract. The results are expressed as U g^{-1} and one unit of enzyme activity (U) was defined as a 0.01 absorbance decrease per min. For measuring CAT activity, 0.1 mL of extract was added to 2.9 mL of phosphate buffer (50 mM, pH 7.0), containing 15 mM H_2O_2 and the absorbance decrease at 240 nm from 0 time to after 1 min was measured. CAT was expressed as U g^{-1},

one U being a 0.01 absorbance decrease per minute. Finally, the reaction mixture for POD measure contained 2.9 mL of phosphate buffer (50 mM, pH 7.0), 12 mM H_2O_2, 14 mM guaiacol and 0.1 mL of enzymatic extract. The absorbance was measured at 470 nm at time 0 and after 1 min, and the increase in absorbance due to guaiacol oxidation was calculated and the activity of POD was expressed as U g^{-1}. One U was defined as a 0.01 absorbance increase per min.

4.5. Statistical Analysis

Field experiments were conducted in a randomized design by using three replicates (of three trees) for each treatment and cultivar in both experimental years. Fruit samples of each replicate were used for storage experiments and, for all the analyzed parameters, sweet cherry cultivar and year data are the mean ± SE of three replicates (n = 3). The SPSS software version 20 (SPSS-Inc., Chicago, IL, USA) was used to perform an analysis of variance (ANOVA) and Tukey's test was used for mean comparisons to find significant differences among treatments at $p < 0.05$. In addition, linear regressions were performed between total phenolic and anthocyanin content for each cultivar and each year.

5. Conclusions

The overall results lead us to conclude that preharvest GABA treatments, especially at a 50 mM dose, make for increased sweet cherry organoleptic quality at harvest which is maintained during storage at higher levels than in control fruits, due to reduced weight, firmness and acidity losses. In addition, antioxidant compounds are enhanced, leading to improved health benefits for sweet cherry fruit consumption. Finally, the higher activity of antioxidant enzymes, together with the higher content in phenolics and anthocyanins, could contribute to reduce the oxidative stress in fruit and to delay the postharvest ripening and senescence process, and in turn, the storage period with proper quality could be extended.

Author Contributions: Conceptualization, D.V. and M.S.; methodology, A.C.-A., F.B.-E.H., J.M.L.-M. and H.M.D.-M.; validation, A.C.-A., D.V. and M.S.; formal analysis, A.C.-A., D.V. and M.S.; investigation, A.C.-A., H.M.D.-M., D.V. and M.S.; resources, D.V. and M.S.; data curation, D.V. and M.S.; writing—original draft preparation, A.C.-A.; writing—review and editing, D.V. and M.S.; project administration, D.V. and M.S.; funding acquisition, D.V. and M.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was founded by the Spanish Ministry of Science, Innovation and Universities and the European Commission with FEDER funds, Project RTI2018-099664-B-I00.

Data Availability Statement: Data are presented in the article and further inquiries could be directed to the corresponding author.

Acknowledgments: Authors thank to Spanish Ministry of Science, Innovation and Universities for the scholarships of A.C.-A. and F.B.-E.H., to Miguel Hernández University for the scholarship of J.M.L.-M., and to Finca Toli Frutas S.L. for providing field experiments.

Conflicts of Interest: The authors declare no conflict of interest. The founders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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4.4 Publicación 4

PUBLICACIÓN 4 (Acceso abierto)

Título: Preharvest Gibberellic Acid Treatment Increases Both Modulus of Elasticity and Resistance in Sweet Cherry Fruit (cv. 'Bing' and 'Lapins') at Harvest and Postharvest During Storage at 0 °C

Autores: Carrión-Antolí, A., Zoffoli, J.P., Serrano, M., Valero, D., & Naranjo, P.

Fecha de publicación: 20 de noviembre de 2024

Revista: Agronomy

Editorial: Multidisciplinary Digital Publishing Institute (MDPI)

ISSN: 2073-4395

Factor de impacto (2023): 3.3

Factor de impacto (5 años): 3.7

Categoría JCR®: Agronomy

Rango: 20/126

Cuartil: Q1

CiteScore: 6.2

DOI: https://doi.org/10.3390/agronomy14112738

agronomy

MDPI

Article

Preharvest Gibberellic Acid Treatment Increases Both Modulus of Elasticity and Resistance in Sweet Cherry Fruit (cv. 'Bing' and 'Lapins') at Harvest and Postharvest During Storage at 0 °C

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Abstract: Fruit firmness in sweet cherries (*Prunus avium* L.) is a critical quality parameter highly valued by consumers as it is associated with fruit freshness. In general, firm fruit also cope better with storage and handling. Gibberellic acid (GA) is commonly used by sweet cherry producers to increase firmness, soluble solids content and fruit size. This study evaluated the effects of GA on the rheological properties of sweet cherry fruit at harvest and postharvest storage. Specifically, GA's influence on susceptibility to mechanical damage during handling was evaluated. The following GA treatments were applied to two sweet cherry cultivars 'Bing' and 'Lapins': T0, control, T30—GA at 15 ppm applied at pit-hardening and straw-colour stages; T45—GA at 25 ppm at pit-hardening and GA at 20 ppm at straw-colour; and T60—GA at 30 ppm applied at pit-hardening and straw-colour. The results indicate that GA delayed harvest by two to four days in both cultivars, with 'Lapins' also showing a significant increase in fruit size. Regardless of spray concentration, GA increased the modulus of elasticity and fruit resistance evaluated as stress at the maximum point at harvest. These effects persisted after 35 days of storage at 0 °C and an additional three days of shelf-life at 15 °C. While the strain or deformation capacity of the fruit at bioyield at harvest was constant across treatments, it was, however, lower in the GA-treated fruit than in the controls during storage at 0 °C under the high-humidity conditions of modified atmosphere packaging. The less mature fruit harvested at colour 3.0 (red/mahogany) were stiffer (reduced deformation) and more sensitive to induced mechanical injury than the fruit harvested later at colour 3.5 (mahogany). The GA treatments increased fruit resistance to damage without increasing tissue deformability. Other questions associated with stiffer tissues and lower deformability during storage at 0 °C under high humidity should be further studied, specifically cultivars that are naturally high in box-cracking sensitivity during storage.

Keywords: *Prunus avium*; rheological properties; strain at bioyield; stress; firmness; bruising; postharvest; mechanical damage

Citation: Carrión-Antolí, A.; Zoffoli, J.P.; Serrano, M.; Valero, D.; Naranjo, P. Preharvest Gibberellic Acid Treatment Increases Both Modulus of Elasticity and Resistance in Sweet Cherry Fruit (cv. 'Bing' and 'Lapins') at Harvest and Postharvest During Storage at 0 °C. *Agronomy* **2024**, *14*, 2738. https://doi.org/10.3390/agronomy14112738

Academic Editors: Isaac Duah Boateng and Likai Wang

Received: 12 October 2024
Revised: 15 November 2024
Accepted: 18 November 2024
Published: 20 November 2024

1. Introduction

Sweet cherries (*Prunus avium* L.) are highly valued by consumers for their internal attributes of a distinctive flavour, a pleasing acid/sugar balance and a firm texture. They are also valued for their visual characteristics, such as a heart-like shape, a one-bite size and a deep red colour. Cherries are especially valued if they are without visual defects and with shiny skins and turgid green pedicels (stalks) [1–3]. Fruit firmness is also important, being associated with longer shelf-life (key for distributors) and with greater acceptance by consumers (who associate firmness with freshness and softness with old fruit) [4]. Hence,

it is important to be able to measure fruit firmness. This study deals with the rheological relationships between an external applied force acting on the fruit and the resistance and deformation that results. A viscoelastic behaviour is observed that can be quantified via measurements of the stress–strain relationship with subsequent time-dependent relaxations [5]. For small forces and deformations, the fruit exhibits elastic (reversible) behaviour, and then, when some critical deformation is exceeded, the deformation is plastic (irreversible). Beyond the plastic limit, greater forces and deformations cause tissue damage (cell rupture) [6]. A fruit may exhibit different behaviours depending on how the test is performed. Most methods measure uniaxial force deformations, but biaxial tests have also been performed that are closer to reality since they simulate the actual stresses and strains associated with the fruit's three-dimensional growth [7,8].

Rheological variables vary between cultivars, as has been demonstrated in sweet cherry, so genetic factors are clearly important [9], but the rheological properties also vary in the same fruit depending on temperature, ripeness, transpiration and water uptake [7]. For commercial producers and distributors, sweet cherry firmness is expressed in Durofel, a measurement made using a durometer. A number of other devices have been used to measure fruit firmness. Some of these employ their own measurement units or use special probes, making it difficult to compare results from different workers [10]. The study of the rheological variables of stress, strain, energy and modulus of elasticity in samples at the inflection point, bioyield point and maximum point provides more robust information when characterising the different plant tissues and their sensitivities to mechanical damage [11]; hence, high values of deformation (strain) characterise fruit that are more resistant to mechanical damage [9].

In sweet cherry, fruit firmness changes during development. It increases during growth Stage I, reaches a maximum in Stage II at pit-hardening, and then decreases during Stage III as ripening occurs, reaching a minimum at harvest [12,13]. During the postharvest period, two firmness behaviours have been reported, depending on storage conditions. If fruit are stored in a modified atmosphere packaging (MAP) bag at 100% relative humidity, firmness is maintained or increases slightly [14], but if not, then firmness decreases [15,16], especially when the fruit is removed at high temperature and low moisture conditions. Susceptibility to mechanical damage is related to the rheological properties of the fruit at the time of harvest [17]. Thus, there is a positive relationship between firmness and pitting resistance [18], with higher values of stress and strain at the bioyield point being associated with higher resistance to pitting [9].

The rheological properties of sweet cherry fruit can be modified through the use of various agronomic managements. Thus, firmness is increased by fruit thinning [19,20], by foliar applications of calcium [21,22], and by the application of elicitor compounds such as methyl jasmonate, salicylic acid or melatonin [23–25]. The phytohormone gibberellic acid (GA) is widely used by cherry producers, which increases fruit firmness [26–28]. In a recent study with numerous cultivars and GA application rates, it was found that GA increased firmness, soluble solids and titratable acidity and reduced stem browning and surface pitting. However, the genotype did not have a strong influence on the response [29]. GA inhibits floral bud induction in sweet cherry, leading to a reduction in flower number, with a consequent decrease in yield in the season following application. It has been observed that a double application of 50 ppm or a single application of 100 ppm significantly reduces yield; however, commercial applications do not exceed these thresholds, with rates around 30 ppm [30].

Sweet cherries are classified as non-climacteric fruit, meaning they must complete ripening on the tree and do not experience a significant peak of ethylene during the process. Additionally, other hormones, such as abscisic acid, are involved, increasing prior to maturation and decreasing as harvest approaches [31,32]. The rise in abscisic acid in sweet cherries has been linked to the activation of metabolic pathways associated with ripening, including anthocyanin biosynthesis, decreased firmness and increased sugar content [32–34]. Moreover, treatments with GA have been shown to delay the accumulation

of abscisic acid at the onset of ripening, effectively delaying the natural ripening process of the fruit [35]. The objective of this study is to understand how GA modifies the rheological properties of a sweet cherry at harvest and during storage and how these modifications affect the sensitivity of the fruit tissues to mechanical damage.

2. Materials and Methods

2.1. Plant Material and Experimental Design

The experiment was conducted in the 2021–2022 season with the sweet cherry cultivars 'Bing' and 'Lapins' in commercial sweet cherry orchards (*Prunus avium* L.) located in Graneros (lat. 34°03′36.7″ S, long. 70°44′01.3″ W) and Mostazal (lat. 34°00′32.0″ S, long. 70°42′25.7″ W) in the central valley of Chile, Sixth Region, respectively. The cultivar (rootstock) combinations 'Bing' (Gisela 12) and 'Lapins' (Colt) were planted in 2013 and 2016, respectively. In both orchards, trees were trained to a Y-shape trellis, and spacing was at 4 × 2 m, with 1250 trees ha^{-1}. Similar soil, climate conditions and agronomics cultural practices were employed with each cultivar in each orchard.

Gibberellic acid (ProGibb ® 40% soluble granule, Valent BioSciences, Libertyville, IL, USA) treatments were named T30, T45 and T60. For T30, GA at 15 ppm was applied at pit-hardening (start of Stage II) and straw-colour (end of Stage II) stages; for T45, GA was applied at 25 ppm at pit-hardening and at 20 ppm at straw-colour; and for T60, GA at 30 ppm was applied at both fruit growth stages. Control trees (T0 treatment) were sprayed with water (Table 1). These rates were chosen based on commercial use by farmers and previous research conducted by Zoffoli et al. [36]. The decision was made to broaden the range to evaluate the effect of higher doses on the behaviour of the rheological variables. The beginning of pit-hardening occurred 28 days after full bloom (DAFB) in 'Lapins' and 'Bing', and straw-colour was reached at 44 DAFB in 'Bing' and 45 DAFB in 'Lapins'. Whole canopies were sprayed using hydropneumatics spraying equipment (NT-2000, Lerpain, Isla de Maipo, Chile) at a rate of 1500 L ha^{-1}, so a notional rate of 1.2 L $tree^{-1}$. The sprays were applied between 7:00 and 9:00 a.m. to avoid dew and at temperatures below 25 °C. Coverage was uniform and complete—i.e., to run-off.

Table 1. Description of gibberellic acid (GA) treatments and harvest dates (days after full bloom, DAFB) at colour 3.5 on 'Bing' and 'Lapins' sweet cherry cultivars.

Cultivar	Treatment	GA (ppm)	Time of Application (DAFB)	Harvest (DAFB)
Bing	T0	0	-	82
	T30	15 + 15	Pit-hardening (28) + Straw-colour (44)	84
	T45	25 + 20	Pit-hardening (28) + Straw-colour (44)	86
	T60	30 + 30	Pit-hardening (28) + Straw-colour (44)	86
Lapins	T0	0	-	87
	T30	15 + 15	Pit-hardening (28) + Straw-colour (45)	87
	T45	25 + 20	Pit-hardening (28) + Straw-colour (45)	91
	T60	30 + 30	Pit-hardening (28) + Straw-colour (45)	91

The experimental units were arranged in a randomised complete block design for each cultivar. Subsequently, three rows were selected, and the treatments were applied in the central row to avoid spray drift from adjacent rows. Four replicates of three trees of similar vigour, size and fruit load were selected and randomly assigned to each treatment. A minimum of six trees were left with respect to the roads to avoid edge effects. Fruit was harvested at two maturity stages when more than 80% of the cherry population achieved the skin colour 3 or colour 3.5 (cherry colour chart scale 2022, Pontificia Universidad Católica de Chile), resembling colour numbers 4 and 5 according to the CTIFL colour chart (Centre Technique Interprofessionnel des Fruits et Légumes, Paris, France), respectively. Two groups of 10 fruit per replicate for each colour stage were collected, one for the evaluation of maturity and the other for the determination of rheological properties.

One hundred fruit per replicate were randomly selected from the exterior of the canopy at the time of harvest with colour 3.5, and the size and colour distribution were determined using an optical vision machine (Cherry roller, PT&I Chile, Santiago, Chile). The size distribution was described in terms of percentage of fruit in each commercial category (Undersize < 22 mm; L 22.0–23.9 mm; XL 24.0–25.9 mm; J 26.0–27.9 mm; 2J 28.0–29.9 mm; 3J 30.0–31.9 mm; 4J 32.0–33.9 mm; and 5J > 34 mm) and the colour distribution by the proportion of fruit in each category (i.e., 3 and 3.5, red/mahogany and mahogany).

2.2. Storage and Fruit Quality Postharvest

One 3 kg group of fruit of colour 3.5 was harvested separately per replicate, placed in a plastic box and hydrocooled with 0 °C sanitised water. The water sanitisation was performed using a 70% calcium hypochlorite solution (Unión Química Spa, Lampa, Chile) and had 80–100 ppm of free chlorine. Then, fruit were immersed in a fungicide solution with a 0.1% fludioxonil (SCHOLAR ® 230 Suspension Concentrated (SC) formulation containing 23% p/v of fludioxonil; Syngenta Crop Protection Inc., Omaha, NE, USA), and 2.5 kg of fruit that were free of visible damage and of uniform diameter (26–28 mm) were selected and packaged in a modified atmosphere bag (Crystal Cherry 826, San Jorge Packaging, Santiago, Chile) after one day of storage at 0 °C. The fruit was then stored for 35 days at 0 °C to simulate the time it takes for Chilean sweet cherries to reach consumers in the Asian market, the most significant market for the Chilean cherry industry. Following this, a shelf-life period of 3 days at 15 °C was included to simulate the time until the fruit is consumed.

Fruit quality after 35 days at 0 °C was characterised in terms of decay, orange-skin disorder, bruising and pitting, and incidences were calculated and expressed as percentages from samples of 70 fruit. There was only one category of damage assessed following the method described by Zoffoli and Rodriguez [37]. The severity of damage was assessed using an arbitrary scale: mild = 1, moderate = 2 and severe = 3. The severity was calculated as the sum of the number of fruit in each category (n_1, n_2 and n_3) multiplied by each factor 1, 2 and 3, respectively, and the total was divided by the number of damaged fruit.

2.3. Fruit Growth and Evolution of Maturity Parameters

Samples of eight fruit of 'Bing' or 'Lapins' from the exterior of the canopy were identified on each of the four trees (replicate) 23 and 25 days after full bloom, and the increases in fruit diameter (mm) were determined weekly. At the same times, groups of 10 fruit per replicate were transported to the laboratory, where composite juice samples were created, and soluble solids (%) and titratable acidity (%) were determined using a digital thermo-compensated refractometer (PAL-1, Atago Co. Ltd., Tokyo, Japan) and by titration with NaOH 0.1 N until pH 8.1 (Edge HI2002, Hanna Instruments, Woonsocket, RI, USA), respectively.

The crude cell wall content was assessed in colours 3 and 3.5 by the alcohol insoluble residue (AIR) method as described by Choi et al. [38] and modified by Param and Zoffoli [9] from a sample of 25 g of ground fruit flesh.

2.4. Rheological Properties and Increased Sensitivity to Damage

The rheological properties of the fruit tissue were determined by a compression test using a Texturometer TA.XT plus analyser (Stable Micro Systems Ltd., Godalming, England) fitted with a 5 mm diameter cylindrical steel probe with a hemispherical end with a contact surface area of 19.6 mm^2. The fruit's mechanical parameters of modulus of elasticity, stress and strain were obtained using the protocol of Param and Zoffoli [9] at two harvest stages—colour 3 and 3.5, in storage after 35 days at 0 °C and after 35 days at 0 °C plus 3 days of shelf-life at 15 °C. The measurements were carried out on the cheek of each fruit (10 fruit per replicate) after previously homogenising the fruit at 15 °C. The compression force was applied to the major axis of the fruit's equatorial diameter. The loading rate was 0.3 mm s^{-1} for a maximum penetration depth set at 5 mm (maximum point) to avoid tissue

disruption. The modulus of elasticity (E) (MPa) is the ratio between stress and strain at the inflection point (just before the start of plastic deformation), and this value indicates how resistant the fruit is to elastic deformation. It was calculated as:

$$E = \frac{FL}{A\Delta L} \quad (1)$$

where F is the force (N), A is the probe area (mm^2), L is the initial length of the fruit (mm) and ΔL is the change length of the fruit (mm) after the test. The stress (σ) (kPa) was calculated as the ratio between the applied force and the area of the probe (σ = F/A), and the strain (ε) (%) was calculated as the ratio ΔL between L (ε = ΔL/L). These variables were calculated from the force/distance curve at the inflection point, at the maximum point (5 mm) and at the bioyield point, which occurs where there is an increase in deformation with a decrease or no change in the force, or the point at which flesh cells begin to rupture but without visible external damage [39].

The compression damage sensitivity was assessed in the same fruit as that in which the compression test was performed because the conditions of this test are those that induce compression damage. On the other hand, the impact damage was evaluated on the day of the harvest on 10 fruit per replicate at 15 °C by dropping a 10 g stainless steel rod of 5.4 mm hemispherical head diameter from a height of 10 cm onto one side of each fruit; the device used was described by Zoffoli et al. [40]. After performing the compression test and impact test, the fruit was placed on a tray inside a polyethylene bag and stored for 10 days at 0 °C and 100% relative humidity. To calculate the fruit damage index, a 5-point scale was used where 0 = no pitting and 4 = very severe pitting; the method and visual scale used were those proposed by Param and Zoffoli [9].

2.5. Statistical Analysis

Statistical analyses were performed using the InfoStat v 2020 software (InfoStat Group, National University of Córdoba, Córdoba, Argentine), the data were analysed using analysis of variance (ANOVA) and mean separations were performed using Fisher's least significant difference (LSD) test when the applicable p-value ≤ 0.05. The data in the figure were graphed with SigmaPlot v 11.0 (Systat Software Inc., San Jose, CA, USA) and are presented as the means and standard error.

3. Results

3.1. Crop Yield, Fruit Growth and Quality Parameters at Harvest

Gibberellic acid treatments delayed the harvest date for 'Bing' at colour 3.5 by two days for T30 and by four days for T45 and T60. Similar four-day harvest delays occurred for 'Lapins' with the higher GA rates. Furthermore, this delay in fruit pigmentation was observed during the fruit ripening period, 77 and 79 days after full bloom for 'Bing' and 'Lapins', respectively (Figure 1). The untreated fruit of 'Bing' was harvested at 82 DAFB and of 'Lapins' at 87 DAFB (Table 1). Data obtained from the optical vision machine for random sampling at harvest confirmed that more than 80% of the fruit on the tree attained the 3.5 colour at harvest. The average production of 'Bing' was between 22.5 and 23.9 kg $tree^{-1}$, and the average fruit weight was similar between GA and control fruit (in the range of 8.3 and 8.5 g $fruit^{-1}$). On the contrary, for 'Lapins', the average weight (8.6 g $fruit^{-1}$) of the control fruit was significantly lower (p-value = 0.0001) than the weight (10.3 g $fruit^{-1}$) of GA. In addition, fruit production per tree was also increased in 'Lapins', with values of 10 kg $tree^{-1}$ in controls and 14.5 kg $tree^{-1}$ in GA-treated trees (p-value = 0.0406).

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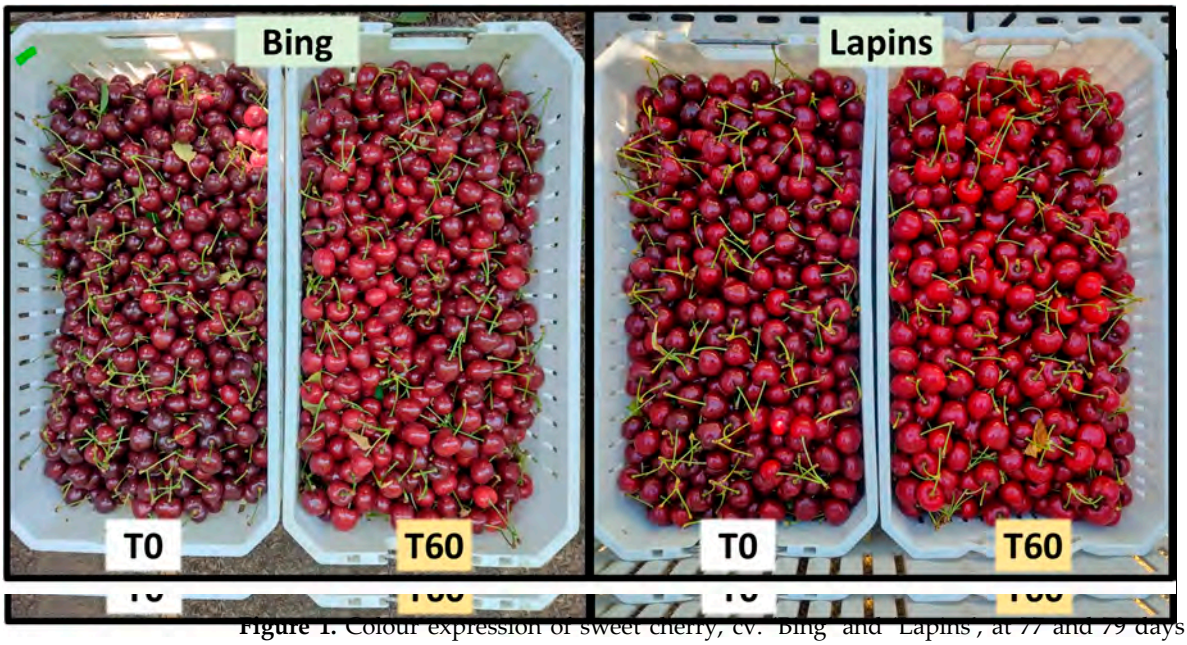

Figure 1. Colour expression of sweet cherry, cv. 'Bing' and 'Lapins', at 77 and 79 days after full bloom, respectively, for fruit treated with GA: T0: control and T60: GA at 30 ppm applied at pit hardening and straw colour stages.

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The GA treatments did not affect soluble solids accumulation at harvest (colour 3.5) in 'Bing', with average soluble solids of 24.4% or in 'Lapins', with an average of 22.1%. The average titratable acidity in 'Bing' across all treatments was 1.23%, and in 'Lapins' between 0.85% and 1.02%.

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Fruit diameter was similar among GA treatments and control in 'Bing', but in 'Lapins', control fruit were smaller than GA fruit. The main differences showed up in the last stages of development, where fruit diameter reached ca. 26 mm in controls compared with 28 mm in GA-treated ones (Figure 2).

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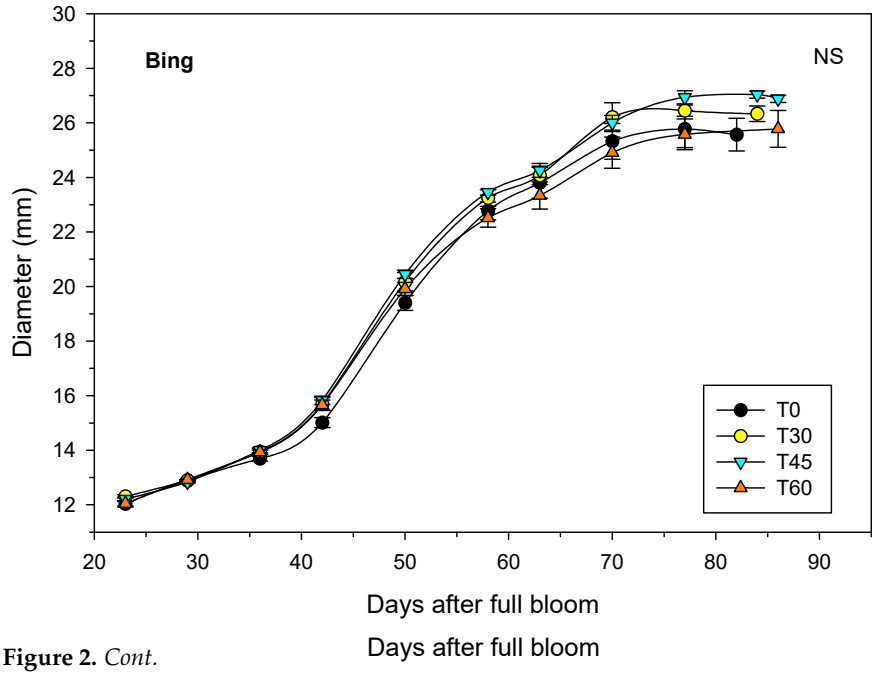

Days after full bloom

Figure 2. *Cont.*

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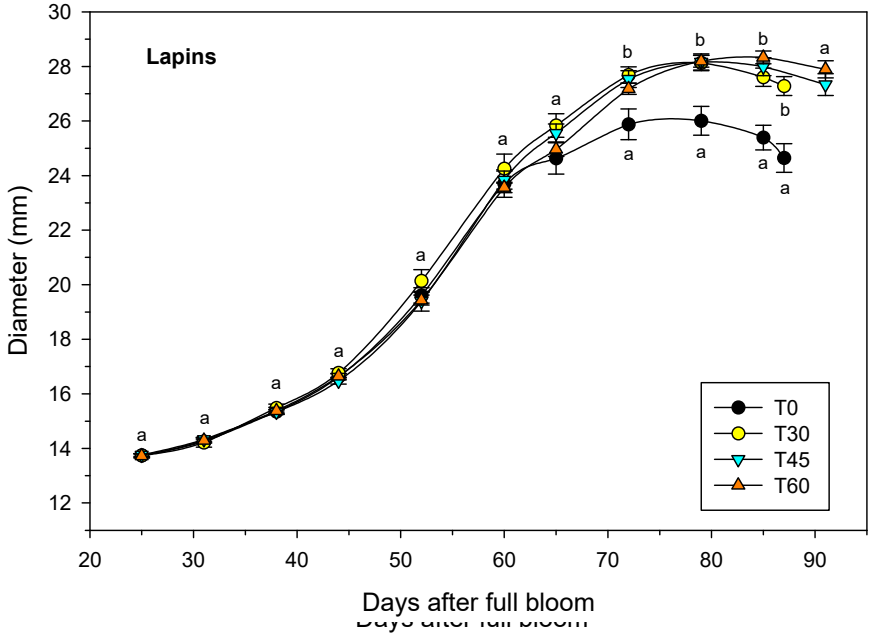

Figure 2. *Growth in fruit diameter during development for control and gibberellic acid (GA)-treated fruit of cv. 'Bing' and 'Lapins' sweet cherries. Treatments: T0 (control): 0 ppm GA; T30: 15 ± 15 ppm GA (pit-hardening + straw-colour); T45: 25 ± 20 ppm GA (pit-hardening + straw-colour); T60: 30 + 30 ppm GA (pit-hardening + straw-colour). Different letters for each day show significantly different mean values for Fisher's LSD test, with p-value < 0.05. NS: non-significant at p-value < 0.05.*

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As we expected from above, the size distribution of 'Bing' fruit was not affected by GA treatment, but 97% of the population of GA-treated 'Lapins' fruit had diameters greater than 26 mm, while only 76% of the population of control fruit had diameters greater than 26 mm (Figure 3).

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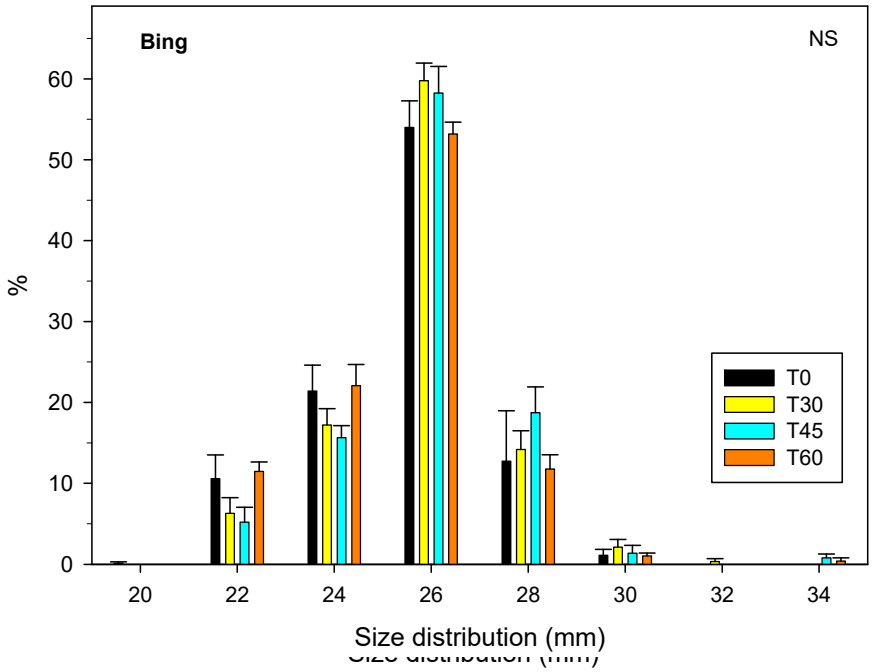

Figure 3. *Cont.*

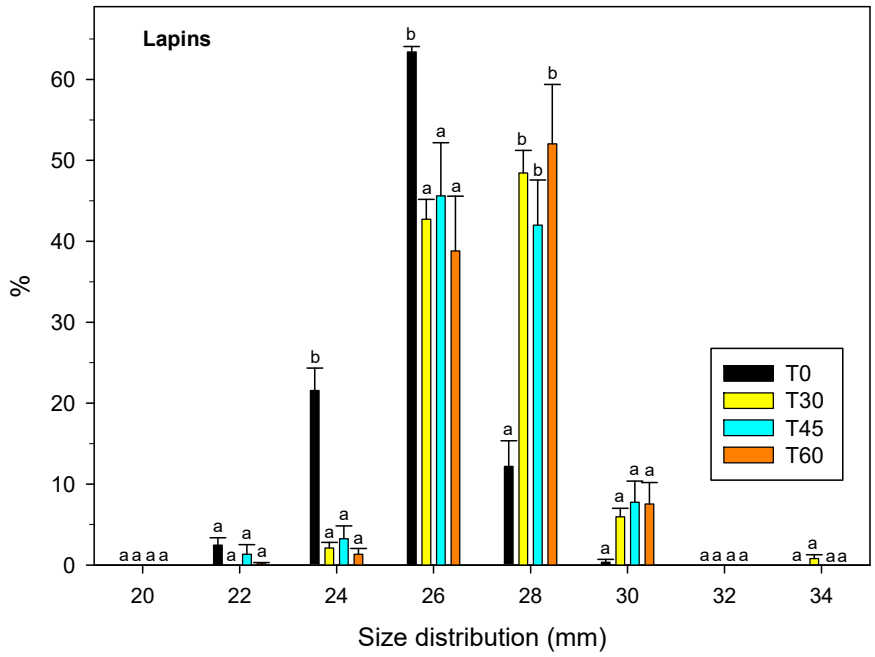

Figure 3. Fruit size distribution at harvest for cv. 'Bing' and 'Lapins' sweet cherries depends on the rate of gibberellic acid (GA) application. Treatments: T0 (control), 0 ppm GA; T30, 15 + 15 ppm GA (pit-hardening + straw-colour); T45, 25 + 20 ppm GA (pit-hardening + straw-colour); T60, 30 + 30 ppm GA (pit-hardening + straw-colour). Different letters for each size show significantly different mean values for Fisher's LSD test, with p-value < 0.05. NS: non-significant at p-value < 0.05.

3.2. Rheological Properties at Harvest and Postharvest

In 'Lapins', the application of GA increased the modulus of elasticity at harvest. Treatment T60 induced the highest modulus of 1.92 MPa compared to 1.23 MPa for the control. There were no significant differences among the different GA treatments. 'Bing' behaved similarly, with the modulus of elasticity of the control being 1.73 MPa and the GA treatments all increasing it, with T30 giving the highest modulus of elasticity at 2.19 MPa. The strain at bioyield had a mean value of 11.0% for 'Bing' and 10.4% for 'Lapins', without significant differences among treatments. In 'Bing', the maximum stress value was increased by GA treatment, with 262.4 kPa being reached with T30 compared to the 223 kPa in the control. In 'Lapins', GA increased the maximum stress by an average of 21% compared with 185.8 kPa in the control (Table 2).

Evaluations after 35 days of storage at 0 °C showed similar patterns in terms of the rheological properties of the fruit. The modulus of elasticity and maximum stress were significantly higher in the GA treatments than in the control. In the 'Bing' cultivar, the modulus of elasticity was 1.93 MPa and 2.42 MPa, respectively, in the control; they increased by about 12% and 16% in the GA-treated fruit. No significant differences were found among the different GA treatments. In 'Lapins', a dose-dependent effect was observed in which the highest rates induced the highest modulus of elasticity and stress. The modulus of elasticity of the control was 1.38 MPa, and the maximum stress was 194 kPa. The T60 treatment increased these values by about 97% and 38%, respectively. In both cultivars, the strain at bioyield was significantly higher in the controls than in the GA treatments. In 'Bing', the control was 10.37%, and T30 was 8.33%, distinct from the other GA treatments. In 'Lapins', the control was 10.09%, and the GA treatments averaged 8.74% (Table 3).

Table 2. Rheological properties of the fruit of 'Bing' and 'Lapins' sweet cherries at harvest as affected by GA treatments on the elasticity properties of the skin. Treatments: T0 (control), 0 ppm GA; T30, 15 + 15 ppm GA (pit-hardening + straw-colour); T45, 25 + 20 ppm GA (pit-hardening + straw-colour); T60, 30 + 30 ppm GA (pit-hardening + straw-colour).

Cultivar	Treatment	Modulus of Elasticity (MPa)	Strain at Bioyield (%)	Maximum Stress (kPa)
Bing	T0	1.73 a	10.65	223.0 a
	T30	2.19 b	11.01	262.4 b
	T45	1.92 ab	11.12	245.3 ab
	T60	1.92 ab	11.30	244.3 ab
p-value		0.0257	NS	0.0366
Lapins	T0	1.23 a	10.95	185.8 a
	T30	1.71 b	10.11	224 b
	T45	1.74 b	10.21	220.8 b

Table 2. Effect of gibberellic acid (GA) treatments on the rheological properties of 'Bing' and 'Lapins' sweet cherries at harvest in colour 3.5. Treatments: T0 (control), 0 ppm GA; T30, 15 + 15 ppm GA (pit-hardening + straw-colour); T45, 25 + 20 ppm GA (pit-hardening + straw-colour); T60, 30 + 30 ppm GA (pit-hardening + straw-colour).

Cultivar	Treatment	Modulus of Elasticity (MPa)	Strain at Bioyield (%)	Maximum Stress (kPa)
Bing	T0	1.73 a	10.65	223.0 a
	T30	2.19 b	11.01	262.4 b
	T45	1.92 ab	11.12	245.3 ab
	T60	1.92 ab	11.30	244.3 ab
p-value		0.0257	NS	0.0366
Lapins	T0	1.23 a	10.95	185.8 a
	T30	1.71 b	10.11	224 b
	T45	1.74 b	10.21	220.8 b
	T60	1.92 b	10.14	231.5 b
p-value		0.0016	NS	0.0021

Different letters for each cultivar and in each column show significantly different mean values for Fisher's LSD test, with p-value < 0.05. NS: non-significant at p-value < 0.05.

Table 3. Effect of gibberellic acid (GA) on the rheological properties of 'Bing' and 'Lapins' sweet cherries of colour 3.5 after 35 days of storage at 0 °C. Treatments: T0 (control), 0 ppm GA; T30, 15 + 15 ppm GA (pit-hardening + straw-colour); T45, 25 + 20 ppm GA (pit-hardening + straw-colour); T60, 30 + 30 ppm GA (pit-hardening + straw-colour).

Cultivar	Treatment	Modulus of Elasticity (MPa)	Strain at Bioyield (%)	Maximum Stress (kPa)
Bing	T0	1.93 a	10.37 c	242.9 a
	T30	2.51 b	8.33 a	275.1 b
	T45	2.51 b	8.80 b	287.6 b
	T60	2.64 b	8.86 b	281.8 b
p-value		0.0011	<0.0001	0.0403
Lapins	T0	1.38 a	10.09 b	194.0 a
	T30	2.29 b	8.83 a	241.7 b
	T45	2.45 bc	8.64 a	255.4 bc
	T60	2.72 c	8.75 a	267.7 c
p-value		<0.0001	0.0380	0.0001

Different letters for each cultivar and in each column show significantly different mean values for Fisher's LSD test, with p-value < 0.05.

Gibberellic acid treatments did not affect the incidence of postharvest decay; incidences were 0.28% in 'Bing' and 0.7% in 'Lapins', and average pitting values were 32% in 'Bing' and 23% in 'Lapins'. The incidence of bruising in 'Bing' was lower for the higher rates of GA (T45 and T60), and damage severity was significantly lower in the GA treatments than in the control. In 'Lapins', bruising incidence was lower in T30 and T60 than in the control, but the lowest damage severity was in T45 (Table 4). The incidence of orange-skin disorder was high in both the controls and the GA treatments, with an average value of 97% in 'Bing' and 99% in 'Lapins'.

Table 4. Effect of gibberellic acid (GA) treatment on bruising in 'Bing' and 'Lapins' sweet cherries in colour 3.5 after 35 days of storage at 0 °C. Treatments: T0 (control), 0 ppm GA; T30, 15 + 15 ppm GA (pit-hardening + straw-colour); T45, 25 + 20 ppm GA (pit-hardening + straw-colour); T60, 30 + 30 ppm GA (pit-hardening + straw-colour).

Cultivar	Treatment	Bruising	
		Inc. [1] (%)	Sev. [2] (1–3)
Bing	T0	32 b	2.27 b
	T30	30 b	1.78 a
	T45	16 a	1.69 a
	T60	19 a	1.71 a
p-value		0.0001	0.0193
Lapins	T0	27 b	2.54 c
	T30	8 a	2.43 bc
	T45	18 ab	1.76 a
	T60	13 a	2.01 ab
p-value		0.0185	0.0118

[1] Inc. refers to the incidence, that is, the proportion of fruit affected. [2] Sev. refers to the severity of the damage, which was evaluated with an arbitrary scale where 1 = mild, 2 = moderate and 3 = severe. Different letters for each cultivar and in each column show significantly different mean values for Fisher's LSD test, with *p*-value < 0.05.

After postharvest storage at 0 °C and three days of shelf-life at 15 °C, the modulus of elasticity and the maximum stress were significantly different, with the GA treatments being higher than the controls. In 'Bing', there were no differences in the modulus of elasticity and the maximum stress between the GA treatments, but in 'Lapins', the modulus of elasticity was highest for T60 (76%), while the maximum stress was higher (31%) than the control. The strain at bioyield in 'Bing' was significantly higher in T45 and T60, with an average value of 9.09% compared with the control of 8.12%. In 'Lapins', the control value was 10.75%, and the GA treatments averaged 9.25% (Table 5). The GA treatments did not affect soluble solids at postharvest in 'Bing', with average soluble solids of 23.7%, and the 'Lapins' average was 21.9%. The average titratable acidity in 'Bing' across all treatments was 1.08%, and in 'Lapins', it was 0.9%.

Table 5. Effect of gibberellic acid (GA) treatments on the rheological properties for 'Bing' and 'Lapins' sweet cherries at harvest in colour 3.5 after 35 days of postharvest storage at 0 °C and three days of shelf-life at 15 °C. Treatments: T0 (control), 0 ppm GA; T30, 15 + 15 ppm GA (pit-hardening + straw-colour); T45, 25 + 20 ppm GA (pit-hardening + straw-colour); T60, 30 + 30 ppm GA (pit-hardening + straw-colour).

Cultivar	Treatment	Modulus of Elasticity (MPa)	Strain at Bioyield (%)	Maximum Stress (kPa)
Bing	T0	1.84 a	8.12 a	236.7 a
	T30	2.35 b	8.67 ab	288.0 b
	T45	2.49 b	8.91 b	312.7 b
	T60	2.54 b	9.27 b	312.7 b
p-value		0.0276	0.0168	0.0031
Lapins	T0	1.40 a	10.75 b	211.3 a
	T30	1.97 b	9.06 a	241.4 b
	T45	2.26 bc	9.38 a	260.2 bc
	T60	2.46 c	9.31 a	276.4 c
p-value		0.001	0.0317	0.0011

Different letters for each cultivar and in each column show significantly different mean values for Fisher's LSD test, with *p*-value < 0.05.

3.3. Effect of Maturity on Rheological Properties and Induced Mechanical Damage

The incidences of impact and compression damage were higher in colour 3 than in colour 3.5 in both 'Bing' and 'Lapins', with significant differences except for with the impact on 'Lapins'. If 'Bing' is allowed to ripen to colour 3.5, the impact damage was 33% less. For compression damage, reductions of around 40% and 27% were achieved with GA for 'Bing' and 'Lapins', respectively. During fruit ripening from colour 3 to 3.5, the modulus of elasticity decreased by 17% and 18% for 'Bing' and 'Lapins', respectively, while the strain increased by 8% and 13%, respectively. Maximum stress decreased by 11% in 'Bing', but decreases were not significant in 'Lapins' (Table 6). Gibberellic acid treatments did not affect the fruit damage index (see Supplementary Material Table S1).

Table 6. Effect of maturity (colour indices 3 and 3.5) on rheological properties and induced mechanical damage (fruit damage index) by compression and impact tests on 'Bing' and 'Lapins' sweet cherry cultivars.

		Fruit Damage Index [1]		Rheological Properties		
Cultivar	**Colour**	**Compression Test**	**Impact Test**	**Modulus of Elasticity (MPa)**	**Strain at Bioyield (%)**	**Maximum Stress (kPa)**
Bing	Colour 3	3.54 b	1.44 b	2.34 b	10.24 a	275.08 b
	Colour 3.5	2.13 a	0.96 a	1.94 a	11.02 b	243.76 a
	p-value	<0.0001	0.0052	<0.0001	0.0108	0.002
Lapins	Colour 3	3.74 b	1.38	2.01 b	9.19 a	233.08
	Colour 3.5	2.73 a	1.24	1.65 a	10.35 b	215.55
	p-value	<0.0001	NS	0.0054	0.001	NS

[1] Each fruit was evaluated on an arbitrary 5-point scale where 0 = no pitting, 1 = mild pitting, 2 = moderate pitting, 3 = severe pitting and 4 = very severe pitting. Different letters for each cultivar and in each column show significantly different mean values for Fisher's LSD test, with p-value < 0.05. NS: non-significant at p-value < 0.05.

3.4. Alcohol Insoluble Residues (AIR)

In 'Bing', the concentration of AIR in colour 3 increased as the rate of GA increased: the controls had the lowest AIR value of 1.49 g 100g^{-1} FW, and T60 had the highest value of 2.06 g 100 g^{-1} FW. However, the AIR content per fruit was 178.03 mg/fruit, compared to 139.6 mg/fruit in the control, without significant differences among the GA treatments. In 'Lapins', the behaviour was similar at colour 3: the highest concentration of AIR was in T60 with 1.81 g 100 g^{-1} FW, compared with the control with 1.73 g 100 g^{-1} FW. The lowest fruit content was in the control, with 169.58 mg/fruit, and the highest was in the T60 treatment, with 204.87 mg/fruit. For colour 3.5, there were no significant differences in AIR between controls and treatments, which had average AIR concentrations for 'Bing' and 'Lapins' of 2.07 g 100 g^{-1} FW and 1.95 g 100 g^{-1} FW, respectively, with average AIR contents of 204.51 mg/fruit and 204.43 mg/fruit, respectively (Table 7).

Table 7. Effect of gibberellic acid (GA) treatments on cell wall concentrations of alcohol insoluble residues (AIR) in 'Bing' and 'Lapins' sweet cherries at colours 3 and 3.5. Treatments: T0 (control), 0 ppm GA; T30, 15 + 15 ppm GA (pit-hardening + straw-colour); T45, 25 + 20 ppm GA (pit-hardening + straw-colour); T60, 30 + 30 ppm GA (pit-hardening + straw-colour).

		Colour 3		Colour 3.5	
Cultivar	**Treatment**	**AIR (g 100 g^{-1} FW)**	**AIR (mg/fruit)**	**AIR (g 100 g^{-1} FW)**	**AIR (mg/fruit)**
Bing	T0	1.49 a	139.60 a	1.89	188.07
	T30	1.76 b	172.43 b	2.12	205.62
	T45	1.86 bc	174.63 b	1.96	193.96
	T60	2.06 c	187.03 b	2.32	230.40

Table 7. *Cont.*

Cultivar	Treatment	Colour 3		Colour 3.5	
		AIR (g 100 g^{-1} FW)	**AIR (mg/fruit)**	**AIR (g 100 g^{-1} FW)**	**AIR (mg/fruit)**
p-value		0.0004	0.0149	NS	NS
Lapins	T0	1.73 b	169.58 a	2.09	199.15
	T30	1.53 a	170.85 a	1.81	199.12
	T45	1.69 ab	187.22 ab	1.96	215.78
	T60	1.81 b	204.87 b	1.94	203.68
p-value		0.0385	0.0461	NS	NS

Different letters for each cultivar and in each column show significantly different mean values for Fisher's LSD test, with *p*-value < 0.05. NS: non-significant at *p*-value < 0.05.

4. Discussion

In this trial, the 'Bing' and 'Lapins' trees were 9 and 6 years old, respectively, and both varieties exhibited full production, judging by the yields recorded during the season. The average production from the previous season was 9400 kg/ha for 'Bing' and 12,300 kg/ha for 'Lapins'. Regardless of the trees' age, fruit load plays a much more critical role in influencing the size and quality of the fruit, as shown in other studies [20].

Regardless of the application rate, GA delayed harvest by between 2 and 4 days in 'Lapins' and 'Bing', as has been reported previously [12,41]. This harvest delay has significant implications: early-season producers may experience lower prices, while mid-season and late-season producers may find it advantageous for extending their harvest season. Additionally, for larger farms, this delay serves as a strategy to stagger the harvesting of different cultivars. This delay in anthocyanin synthesis is explained by the lower activity of the phenylalanine ammonia-lyase enzyme, which is hindered by GA [42,43]. Additionally, the delay in sweet cherry ripening and the modification of quality parameters may be attributed to the interaction between exogenous gibberellins (GA) and abscisic acid (ABA). This interaction leads to a reduction in ABA levels at the onset of sweet cherry ripening, thereby impacting the natural ripening process of the fruit [35]. GA also influences the transcript levels of certain genes involved in ABA homeostasis and signalling while also affecting various other pathways. The regulation of GA appears to differ based on whether the sweet cherry variety is early or mid-season. In this study, mid-season varieties were used, indicating that ripening control may occur through the regulation of PP2C gene expression [44,45]. Notably, in climacteric fruits, exogenous GA can also influence ripening and senescence by regulating ethylene-related pathways [46]. In terms of postharvest preservation, combining preharvest GA applications with modified atmosphere packaging (MAP) technology has proven effective in minimising storage losses and maintaining fruit quality during cold storage [47].

The increasing fruit size explains the main effect of GA on 'Lapins', where similar results have been reported for the cv. 'Skeena', 'Sweetheart' and 'Staccato' [29] and in 'Sweetheart' [48]. In 'Bing', however, GA did not increase fruit size, which may be explained by the naturally high crop load in the 'Bing'/'Gisela 12' combination. Similar results were found by Zhang and Whiting [49] but not by Facteau et al. [26], where there was a significant increase in weight. The GA treatments did not increase the already high soluble solids contents (23%) found in the controls.

Other changes, such as the content of crude cell wall extract, quantified here as AIR, achieved similar values among the GA treatments at colour 3; however, in 'Bing', the GA treatment T60 increased the concentration by 38%. This increase has also been observed in other trials, in which it has also been correlated with lower incidences of surface disorders [50,51]. An increase in firmness has been associated with high levels of AIR [52] in cv. 'Kordia', and a slight positive correlation has been observed between firmness and AIR content [53].

Rheological properties, such as the modulus of elasticity, the strain at bioyield and the maximum stress, were characterised at harvest during storage and ripening. Applications of GA increased the modulus of elasticity evaluated at the elastic mode of the tissue at harvest as a result of increased tissue stress since no effect was observed on strain. Hence, high values of the modulus of elasticity are related to a more rigid fruit. The main effect of GA treatment was found in tissue stress at the maximum point, where the GA-treated fruit were more resistant (higher stress values) than the control, without significant differences among the various GA treatments. 'Lapins' fruit treated with GA showed more uniform effects than 'Bing', where high variability was found among the treatments. A single application at the pit-hardening or straw-colour stages, as well as applications in both phenological states, also increased the modulus of elasticity and stress at the maximum point in cv. 'Bing' and 'Sweetheart' [36]. Applications with calcium in Stage I have increased the modulus of elasticity, as reported by Matteo et al. [22], rendering the fruit more resistant to mechanical damage.

The evaluation of these characteristics after 35 days of storage at 0 °C demonstrates that fruit of both cultivars, when treated with GA, maintain a higher resistance (stress at maximum point) and modulus of elasticity; however, fruit had lower values of deformability (strain at bioyield point) compared with control fruit. This reduction by GA treatment reinforces the rigidity of the tissue, so it remains firm under tension and thus should be more sensitive to skin fracturing. This postharvest behaviour can be explained by the conditions of the fruit inside the MAP bag since it was a water-saturated environment, and the fruit will have been under maximum stress. However, fruit softening in sweet cherry under saturated conditions is controversial, with the manipulation of water status not having demonstrated an effect on fruit pressure [54]. Tapia García et al. [14] observed that sweet cherry firmness increases during storage under MAP. On the other hand, it is known that fruit temperature also influences the rheological variables and the sensitivity to mechanical damage [7,37,55]; for this reason, in the development of this experiment, the tests were carried out at a constant pulp temperature of 15 °C.

After 3 days of shelf-life, the fruit modulus of elasticity was slightly lower and stress at the maximum point slightly higher than at the time of removal from cold storage at 0 °C, maintaining significant differences at all times between the GA treatments with higher values than the control. The strain at bioyield increased slightly compared with removal from cold storage at 0 °C, except in the case of the 'Bing' control, which decreased, resulting in a less deformable fruit than that of the GA treatments. In general terms, these changes in rheological variables may be related to increases in temperature in this phase, which increases fruit metabolism and, therefore, increases respiration [55]; also, transpiration due to a greater vapour pressure deficit causes fruit to lose water. Trials on sweet cherry have confirmed that increasing fruit temperature decreased the modulus of elasticity and the fracture pressure [7].

The application of GA reduced the incidence and severity of bruising in both the cultivars examined here. The effect of reduced severity has also been observed in sweet cherries with a single application of 10 or 20 ppm GA and with a double application of 10 ppm [56]. Param and Zoffoli [9] showed that the increase in stress and strain in sweet cherry tissue makes the fruit more resistant to mechanical damage. This trial shows how GA applications are able to increase the stress, making the fruit more resistant to bruising.

The GA treatments did not show significant differences from the controls for the compression and impact tests, but there were differences between the different degrees of maturity, with the more mature fruit, represented by colour 3.5, having the lowest damage index value. The greater resistance to tissue damage has mainly been related to the increase in strain at bioyield due to the natural ripening process, causing the fruit to be more deformable. Lidster et al. [50] observed that the ripest mahogany-coloured fruit appeared to have a maximum resistance to the different forms of impact damage compared with the earlier ripening states. Pitting rating decreased as fruit colour increased, so as maturity progressed, the fruit became less susceptible to pitting [18].

Sweet cherry tissue exhibits viscoelastic properties when deformed [5]. Consequently, when slow loading rates are applied to the fruit, the cherry matrix can deform and flow without rupturing cells, which is associated with its inherent capacity for deformation. In contrast, less mature tissue is stiffer; when subjected to a load, it compresses the parenchyma cells, leading to cell wall fractures and surface pitting.

During the earlier stages of ripening, the cell wall structure undergoes changes that affect both the mechanical strength of the cell walls and cell-to-cell adhesion [57]. This alteration results in different capacities to withstand external mechanical forces. Viscoelastic tissue should ideally strike a balance between deformation and cell wall resistance. As the fruit ripens, it transitions from a stage of high resistance with low deformation to one of weaker resistance, which increases sensitivity to mechanical damage. Therefore, identifying and prioritising the optimal stage of harvest that balances high resistance with sufficient deformation capacity is crucial for minimising damage and ensuring fruit quality.

5. Conclusions

Gibberellic acid is a phytohormone widely used by cherry producers. Determining the best application rate and timing is a difficult decision for farmers and agronomists, so this work contributes by providing more knowledge on this topic. In fact, it can be concluded that GA treatments delayed the harvest date for 2 to 4 days in both cultivars; increased crop yield in 'Lapins' due to enhanced fruit weight and size; and led to fruit being more resistant in both cultivars. In addition, the effects of GA treatments on making the fruit more rigid were maintained after 35 days of postharvest storage at 0 °C. Therefore, GA treatment increased resistance without increasing tissue deformability and even reduced it, making the fruit stiffer during storage at high moisture conditions, which could render other problems, such as in-box fruit cracking, that deserve further research. Moreover, it was found that as fruit maturity advances, sensitivity to mechanical damage (induced impact and compression injury) is reduced as a result of increased fruit deformability (strain at bioyield point). Furthermore, this work provides additional information on the behaviour of rheological variables with respect to mechanical damage depending on the state of maturity of the fruit, which may lead to future research in the field.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy14112738/s1, Table S1. Effect of gibberellic acid (GA) treatments on induced mechanical damage (fruit damage index) by compression and impact tests on 'Bing' and 'Lapins' sweet cherry cultivars.

Author Contributions: Conceptualisation, J.P.Z. and A.C.-A.; methodology, A.C.-A., P.N. and J.P.Z.; software, A.C.-A.; validation, A.C.-A., J.P.Z. and P.N.; formal analysis, A.C.-A. and P.N.; investigation, J.P.Z. and A.C.-A.; resources, J.P.Z., M.S. and D.V; data curation, A.C.-A. and P.N.; writing—original draft preparation, A.C.-A.; writing—review and editing, J.P.Z., M.S., D.V. and P.N.; visualisation, A.C.-A., P.N., M.S., D.V. and J.P.Z.; supervision, J.P.Z., M.S., D.V. and P.N.; project administration, J.P.Z.; funding acquisition, J.P.Z., M.S. and D.V. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Laboratory of Physiology and Postharvest Technology of the Pontificia Universidad Católica de Chile and by the Direcció General de Ciència i Investigació of the Generalitat Valenciana, Spain, throughout the project PROMETEO/2021/089.

Data Availability Statement: The original contributions presented in the study are included in the article/Supplementary Material; further inquiries can be directed to the corresponding author.

Acknowledgments: Spanish Ministry of Science, Innovation and Universities for the scholarship of A.C.-A.; "Garcés Fruit" for allowing us to use the fields and the packaging plant for research; and the Laboratory of Physiology and Postharvest Technology of the Pontificia Universidad Católica de Chile.

Conflicts of Interest: The authors declare no conflicts of interest.

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5. Resultados y Discusión

5 RESULTADOS Y DISCUSIÓN

La cereza es un fruto muy apreciado por los consumidores tanto en los mercados nacionales como internacionales, los principales atributos de calidad en los que se fijan los compradores son el aspecto, la ausencia de defectos, el tamaño, el color, la frescura del pedicelo, la firmeza, la dulzura, la acidez y el sabor, todos estos parámetros tienen cierta variación entre los distintos cultivares (Kappel et al., 1996; Usenik et al., 2008; Díaz-Mula et al., 2009). Además, la cereza también es muy rica en compuestos bioactivos como antocianinas y fenoles, los cuales tienen propiedades antioxidantes con efectos beneficiosos para la salud (McCune et al., 2011; Gonçalves et al., 2019; Blando y Oomah, 2019). Tras la cosecha todos estos parámetros evolucionan, incluso si se mantiene la cereza en condiciones adecuadas de refrigeración, apareciendo con el paso del tiempo síntomas como pardeamiento del pedicelo, ablandamiento, deshidratación y pérdida de las características organolépticas, todos estos factores se traducen en una disminución de calidad del fruto (Serrano et al., 2009; Chockchaisawasdee et al., 2016; Zhang et al., 2021). Los elicitores o fitohormonas son una herramienta que se utiliza en agricultura y permite mejorar los parámetros de calidad de las cerezas, pero presentan resultados variables en función de una serie de factores, el factor genético suele ser importante presentando variabilidad entre las distintas variedades (Giménez et al., 2017; Saracoglu et al., 2017), incluso se ha observado variabilidad para una misma variedad entre las distintas campañas de cultivo (Valverde et al., 2015; Time et al., 2021; Ruiz-Aracil et al., 2023b).

5.1 Efecto de los elicitores en la producción y tamaño del fruto

Los tratamientos con GABA no afectaron a la producción ni al peso del fruto en ninguna de las campañas y variedades ensayadas (datos no mostrados). Sin embargo, aplicaciones precosecha de GABA en limo ‘Fino 95’ incrementaron la producción respecto al control, debido a un aumento en el número de frutos y del tamaño, sobre todo en la dosis más alta de 100 mM (Badiche et al., 2023), en limón ‘Verna’ también se observó un aumento de la producción debido al incremento en el número de frutos (Badiche-El Hilali et al., 2023). En granada tratamientos precosecha con GABA también incrementaron la producción por árbol como consecuencia del incremento en el número de frutos, en todos estos ensayos el GABA reforzó la unión entre los frutos y el árbol, reduciendo de esta manera la caída natural y por lo tanto incrementando la producción total (Lorente-Mento et al., 2023a).

Sin embargo, la melatonina fue capaz de incrementar la producción total entre un 19-38 % en la variedad 'Lapins', pero no tuvo efecto sobre la producción de 'Prime Giant' y 'Sweet Heart', aunque en estas últimas la melatonina incremento el peso del fruto un 10 % y 8 % respectivamente. Además, el porcentaje de frutos no comercializados se redujo con el tratamiento de melatonina, aumentando la proporción de cereza comercial. El incremento de producción y el aumento del peso del fruto (10-11 %) tras realizar aplicaciones foliares de melatonina en cerezo, también se ha observado incluso en condiciones estrés hídrico en los que la melatonina mejora esta situación (Hojjati et al., 2024). En otros estudios la aplicación de melatonina durante dos campañas no ha tenido influencia sobre la producción total en la variedad 'Samba' y 'Sandon Rose', incluso en 'Samba' se observó una reducción en el peso del fruto (Cortés-Montaña et al., 2024). En otras especies de frutales como el albaricoque, la aplicación de melatonina incrementó la producción por árbol como consecuencia del aumento del peso del fruto (Medina-Santamarina et al., 2021a). Todos estos efectos se atribuyen a un incremento de la tasa fotosintética y del contenido de clorofila en las hojas. El aumento del rendimiento en los cultivos bajos condiciones de estrés biótico o abiótico es mejorado por la melatonina, sin embargo en condiciones óptimas este efecto depende más de la especie, variedad, concentración aplicada y estado fenológico de la planta por lo que es necesario más investigación (Debnath et al., 2019; Colombage et al., 2023). El incremento del peso del fruto y del tamaño se ha observado también en frutos de granada tratados con melatonina (Medina-Santamarina et al., 2021b) y en uva (Meng et al., 2015), este efecto se atribuye al incremento de la fuerza sumidero de los frutos, lo que le permite almacenar más azucares alcanzando un mayor tamaño y peso en la cosecha.

El GA3 incrementó la producción total en 'Lapins' un 30 %, como consecuencia del aumento del peso del fruto que fue un 22 % superior al control, respecto a la distribución de tamaños el 97 % de los frutos tratados presentó un calibre superior a 26 mm en comparación con el 76 % de los frutos control. Se han observado incrementos similares en el peso del fruto en 'Skeena', 'Sweet Heart' y 'Staccato' (Kappel y MacDonald, 2007; Einhorn et al., 2013). Sin embargo, en la variedad 'Bing' los tratamientos con GA3 no afectaron a la producción total ni al peso del fruto, lo que podría explicarse por la alta carga obtenida en la combinación del patrón 'Gisela 12' con esta variedad, resultados similares observó Einhorn et al. (2013) en 'Sweet Heart' y 'Lapins' en años de alta carga, en los que no hubo diferencias de peso ni diámetro.

5.2 Efecto de los elicitores en los parámetros de calidad en el momento de la cosecha y durante el almacenamiento

El color, la firmeza, el contenido de sólidos solubles y la acidez de la cereza, son parámetros de calidad muy valorados por los consumidores, durante la maduración de la cereza en el árbol estos parámetros aumentan, excepto la firmeza que se reduce conforme se aproxima el momento de la recolección (Drake y Elfving, 2002; Serrano et al., 2005; Díaz-Mula et al., 2009). Por otro lado, durante el almacenamiento de los frutos aumentan las pérdidas de peso y los sólidos solubles, además disminuye la acidez y la firmeza, ocasionando que el fruto sea menos apetecible para los consumidores (Martínez-Romero et al., 2006; Díaz-Mula et al., 2012; Afonso et al., 2023). La aplicación de elicitores fue capaz de mejorar estos parámetros en el momento de la cosecha y además los mantuvo en valores superiores al control durante el periodo de conservación.

Sintetizando los datos de los distintos ensayos, a nivel general se observó que la melatonina fue capaz de incrementar los sólidos solubles entre 1 y 2 °Brix (4-14 %), la acidez total entre 0,1 y 0,2 g 100 g^{-1} (8-19 %), la firmeza entre 0,15 y 0,58 N mm^{-1} (13-60 %) y el índice de color a*/b* entre un 6 y 15 % presentando por lo tanto las cerezas tratadas una coloración más oscura. Estos parámetros fueron mejorados por los tratamientos con melatonina en el momento de la cosecha y mantenidos durante el periodo de conservación en niveles superiores al control, además durante todo el periodo de almacenamiento de 21 o 28 días en función de la variedad, se redujeron las pérdidas de peso entre un 15 % y 36 % respecto al control, que tuvo pérdidas globales del 9,4 % y 10,9 %. Aplicaciones con las mismas dosis en la variedad ‘Samba’ y ‘Sandon Rose’, no afectaron a la acidez total, sólidos solubles y firmeza, excepto la dosis 0,1 mM en la variedad ‘Sandon Rose’ (Cortés-Montaña et al., 2024). La aplicación de tres tratamientos precosecha con melatonina en la variedad de cereza ‘Hongdeng’, incrementó el contenido de sólidos solubles y disminuyó la acidez total (Xia et al., 2020), en otros estudios de cereza con árboles bien regados la aplicación precosecha de melatonina con dosis entre 100 µM y 300 µM no afectaron al contenido de sólidos solubles y acidez total (Hojjati et al., 2024), en la variedad ‘Ferrovia’ la aplicación de melatonina 0.5 mM una y dos semana antes de cosecha tampoco afectó al proceso de maduración, manteniendo valores similares de acidez total, sólidos solubles y color en el momento de la cosecha y durante 12 días de almacenamiento, en los que tampoco hubo diferencia en las pérdidas de peso (Michailidis et al., 2021). Tratamientos postcosecha mediante inmersión de cerezas en melatonina 1 mM durante 5 minutos, disminuyeron la tasa de respiración y mejoraron la calidad organoléptica, aunque estos resultados incrementaron al combinar la melatonina con quitosano (Bal, 2024), en

otros ensayos el uso de melatonina 0,1 mM durante 30 minutos también fue eficaz en retrasar la senescencia y mantener la calidad de la cereza durante el almacenamiento (Pang et al., 2023). Cerezas tratadas durante 5 minutos mediante inmersión en una solución de melatonina 100 µM, mostraron una reducción de las pérdidas de peso, que se atribuyeron a una mayor expresión de los genes relacionados con la síntesis de componentes de la cutícula y una menor expresión de los genes de las acuaporinas, lo que podría modular el transporte de agua a través de la cutícula y la membrana plasmática, reduciendo la deshidratación del fruto (Miranda et al., 2020). En otros frutos como albaricoque y granada, la melatonina incrementó los parámetros de calidad en el momento de la cosecha y los mantuvo en valores más altos que el control durante el almacenamiento (Medina-Santamarina et al., 2021a; Lorente-Mento et al., 2021).

En general la aplicación precosecha de melatonina afectó al proceso de senescencia y a los parámetros de calidad de forma variable, en función de la variedad, estado de desarrollo y dosis aplicada entre otros factores. Estos cambios se deben al efecto que tiene la melatonina en el metabolismo celular, se ha observado que es capaz de regular positivamente las vías metabólicas relacionadas con la biosíntesis de metabolitos secundarios y de los compuestos estructurales de la membrana plasmática (Hernández et al., 2023). La melatonina también incrementa el contenido de clorofila en las plantas y protege los cloroplastos del daño oxidativo, de manera que la actividad fotosintética se ve favorecida generando una mayor cantidad de fotoasimilados (Hojjati et al., 2024). Se ha demostrado que la aplicación exógena de melatonina en cereza también incrementa el contenido endógeno de esta hormona (Cortés-Montaña et al., 2024), la cual actúa como un antioxidante directo en las células, además de activar ciertas rutas metabólicas relacionadas con la postcosecha del fruto, de manera que mantiene durante más tiempo sus parámetros de calidad y es más beneficioso para el consumidor final, ya que le aporta un mayor contenido de melatonina (Grao-Cruces et al., 2023).

Teniendo en cuenta los distintos años de estudio y las tres variedades ensayadas, en general la aplicación de GABA fue capaz de incrementar los sólidos solubles entre 1 y 3,5 °Brix (5-17 %), la acidez total entre 0,1 y 0,25 g 100 g^{-1} (9-22 %) y la firmeza entre 0,12 y 0,54 N mm^{-1} (6-60 %), estos datos engloban el rango de resultados obtenidos de las distintas dosis respecto al control, tanto en el momento de la cosecha como durante 28 días de almacenamiento, en los que las pérdidas de peso se redujeron entre un 11 % y 35 % respecto al control, que tuvo pérdidas de peso globales entre el 8,9 % y 10,9 %. Por lo tanto, los tratamientos con GABA fueron capaces de incrementar la calidad en el momento de la cosecha y mantenerla en

niveles más altos que el control durante el almacenamiento. Hasta el momento no se conocen estudios con cereza en los que se han realizado aplicaciones precosecha con GABA, en otros cultivos como la granada la aplicación de este elicitor redujo las pérdidas de peso durante la conservación, aumentó la firmeza del fruto y el color de los arilos, pero no tuvo influencia sobre sólidos solubles totales y acidez total (Lorente-Mento et al., 2023b), en limón sin embargo hubo un incremento de producción pero el tratamiento no afecto a la firmeza, sólidos solubles o acidez en el momento de la cosecha (Badiche et al., 2023), en manzana la aplicación precosecha redujo la acidez total y aumentó la firmeza (Cheng et al., 2023). Tratamientos postcosecha en fresa mediante inmersión de frutos aumentaron los sólidos solubles y la acidez total durante el almacenamiento respecto a los controles (Zhang et al., 2024), en cereza cornalina la inmersión de frutos en GABA redujo el pardeamiento y mejoró el contenido de compuestos fenólicos (Aghdam et al., 2019).

Los efectos obtenidos son variables en función de la dosis, fruto empleado y forma de aplicación, pero en líneas generales este elicitor contribuye a mejorar la calidad de la cereza. A nivel celular se ha observado que el GABA actúa como una molécula energética, la cual contribuye a mejorar el estado energético en las mitocondrias (Li et al., 2021), además el GABA y la melatonina se encuentran relacionados, ya que se ha demostrado que la aplicación de melatonina incrementa el contenido de GABA, debido a que regula la actividad de las enzimas y la expresión de los genes relacionados con el metabolismo del GABA (Wu et al., 2023). El GABA también es capaz de reducir la actividad de la enzima poligalacturonasa y pectina metilesterasa, las cuales están asociadas con la degradación de la pared celular, de manera que las células con un mayor contenido de GABA podrían asociarse con tejidos más firme (Aghdam et al., 2019). También se ha observado que los frutos tratados con GABA incrementan sus niveles endógenos, por lo que estos tratamientos podrían influir en el metabolismo del GABA, además estos frutos presentarían propiedades beneficiosas para la salud de los consumidores, ya que contienen una mayor cantidad de este aminoácido natural (Cheng et al., 2023; Icer et al., 2024).

Por último, los resultados obtenidos de la aplicación de GA3 muestran que en las dos variedades estudiadas, esta fitohormona no afectó al contenido de sólidos solubles y acidez total, sin embargo en el color se observó un retraso en la maduración de 2 a 4 días en función de la dosis, retrasos similares se han obtenido en estudios previos (Choi et al., 2002b). Este retraso en la coloración del fruto se debe a un retardo en la síntesis de antocianinas, debido a la baja actividad de la enzima fenilalanina amonio liasa (PAL), la cual es inhibida por el GA3 (Ozkan et al., 2016). El retraso en la maduración y modificación de los parámetros de calidad, también se debe a la

interacción del GA3 exógeno con el ácido abscísico endógeno, lo que ocasiona la reducción de los niveles de ácido abscísico al inicio de la maduración, afectando al proceso natural de maduración de la cereza (Kondo y Danjo, 2001). Los tratamientos con GA3 incrementaron el módulo de elasticidad en el momento de la cosecha como resultado del incremento en la tensión, no se observó efecto en la deformación del tejido. Respecto a la tensión en el punto máximo los tratamientos con GA3 la incrementaron, lo que se traduce en un fruto más resistente, este efecto también se ha observado en otras variedades al realizar una única aplicación de GA3 en el endurecimiento de hueso, en color pajizo o en ambos estados fenológicos (Zoffoli et al., 2017). Tras 35 días de almacenamiento a 0°C en bolsa MAP, los frutos tratados con GA3 presentaron una mayor tensión en el punto máximo y un mayor módulo de elasticidad, pero tuvieron una menor deformación en comparación con los controles, esta situación reforzó la rigidez del tejido, el cual permanece más tensionado y podría ser más sensible a cracking, sobre todo en el ambiente saturado de humedad dentro de la bolsa MAP. El incremento de firmeza durante el almacenamiento en bolsa MAP también se ha observado en otros ensayos (Tapia García et al., 2017). Después de la salida de frío más 3 días a 15°C, el módulo de elasticidad y la tensión en el punto máximo fueron superiores en los tratamientos de GA3 que en los controles. Los tratamientos con GA3 redujeron la incidencia y severidad del bruising, Param y Zoffoli (2016) observaron que altos valores de tensión y deformación se relacionaban con frutos más resistente al daño mecánico, este ensayo muestra como el GA3 incrementa la tensión del tejido ocasionando que la cereza sea más resistente a bruising. Se observó que a medida que avanza la madurez del fruto de color 3 a color 3,5 se redujo el daño mecánico inducido, como consecuencia de un aumento de la deformación del tejido, resultados similares observó Toivonen et al. (2004) en los que conforme aumentaba la madurez la susceptibilidad a pitting se reducía.

Si se observa la firmeza en el momento de la cosecha en los frutos tratados con melatonina y GABA, se podría suponer que ambos elicitores retrasaron la maduración respecto al control, ya que se obtienen frutos más firmes, los cuales están relacionados con estados más inmaduros, en contraposición aumentaron los sólidos solubles y la acidez, parámetros directamente relacionados con un adelanto de la maduración (Serrano et al., 2005; Díaz-Mula et al., 2009). Asimismo, estos frutos mantuvieron unos parámetros de calidad organoléptica superior que los controles, haciendo que la cereza fuera más apetecible para los consumidores durante un periodo de tiempo más extenso. Un punto en común de la melatonina, el GABA y el GA3 es que consiguen incrementar la firmeza en el momento de cosecha y mantenerla en niveles superiores al control durante la conservación, este parámetro es muy valorado por los consumidores y además los niveles altos están asociados con frutos más frescos y que

presentan una mayor vida útil (Ross et al., 2009; Ricardo-Rodrigues et al., 2023; Sándor et al., 2024).

5.3 Efecto de los elicitores en los compuestos bioactivos en el momento de la cosecha y durante el almacenamiento

Los compuestos bioactivos como antocianinas y fenoles se encuentran presentes en las cerezas y son compuestos con propiedades antioxidantes que tienen beneficios para la salud, además reducen el riesgo de sufrir enfermedades cardiovasculares, neurodegenerativas y diabetes (McCune et al., 2011; Blando y Oomah, 2019; Gonçalves et al., 2024). En la cereza el color rojo intenso se debe principalmente al contenido de antocianinas, en 'Prime Giant', 'Lapins' y 'Sweet Heart' la antocianina mayoritaria es la cianidina 3-rutinósido, seguida de la pelargonidina 3-rutinósido y la cianidina 3-glucósido, las dos últimas suponen aproximadamente el 10 % de las antocianinas totales, se han obtenido resultados similares en otros trabajos (Serrano et al., 2009; Martínez-Esplá et al., 2014). Además, también se ha encontrado una alta correlación entre el índice de color a*/b* con el contenido de antocianinas, por lo que este índice es un buen indicador del contenido de antocianinas en el fruto. Dentro de los compuestos fenólicos totales, las antocianinas representan aproximadamente el 70 % (Gonçalves et al., 2021), además se ha observado una alta correlación entre las antocianinas totales y los fenoles totales en los cultivares estudiados.

La aplicación de melatonina incrementó la concentración de fenoles totales y antocianinas totales en todas las variedades estudiadas, tanto en el momento de la cosecha como durante el periodo de almacenamiento, los fenoles totales incrementaron entre 12 y 29 mg 100 g^{-1} (12-45 %) y las antocianinas totales entre 10 y 39 mg 100 g^{-1} (16-82 %). Las antocianinas individuales también incrementaron como consecuencia de los tratamientos entre un 20 % y un 40 % para cianidina 3-rutinósido y de un 10 % a 50 % para la pelargonidina 3-rutinósido en función del cultivar. De todas las variedades estudiadas 'Sweet Heart' fue la que presentó una menor concentración de compuestos bioactivos, 'Prime Giant' y 'Lapins' presentaron concentraciones similares. En otros ensayos se han observado resultados similares en los que los tratamientos precosecha con melatonina 50 µM y 100 µM, 3, 2 y 1 semana antes de la cosecha en la variedad 'Hongdeng', aplicados únicamente sobre las hojas del cerezo, tienen mayor influencia en aumentar el contenido de antocianinas totales de los frutos que tratamientos dirigidos únicamente al fruto (Xia et al., 2020). Tratamientos 100 µM, 200 µM y 300 µM aplicados cada 15 días a partir del momento en el que el árbol

estaba completamente vestido de hoja, incrementaron el contenido de antocianinas totales en arboles sin estrés hídrico y con estrés hídrico moderado (Hojjati et al., 2024). En 'Ferrovia' la aplicación de melatonina 0,5 mM, una y dos semanas antes de la cosecha no tuvo efecto sobre el contenido de antocianinas y fenoles individuales en el momento de la cosecha, pero si hubo un incremento de estos compuestos cuando la cereza se almacenó 12 días a 0°C (Michailidis et al., 2021). Por el contrario, tratamientos con melatonina en la variedad 'Prime Giant' en el estado II a una dosis de 0,1 mM no tuvieron impacto en el contenido de antocianinas, pero sin embargo a la dosis de 0,01 mM se redujo el contenido de antocianinas a la mitad, por lo que la melatonina podría tener un papel importante en el proceso de maduración del fruto (Tijero et al., 2019). Tratamientos postcosecha mediante inmersión de cerezas 'Sweet Heart' en melatonina 1 mM durante 5 minutos, mantuvieron niveles más altos de antocianinas y fenoles totales que los controles durante 28 días de almacenamiento (Bal, 2024), tratamientos similares durante 10 minutos con dosis 0,25 mM, 0,5 mM y 1 mM mantuvieron niveles superiores al control, pero los niveles más altos se encontraron en la dosis 1 mM (Bal et al., 2022). En cerezas sumergidas en melatonina 0,1 mM durante 5 minutos, se observó una regulación positiva de dos genes involucrados en los últimos pasos de la biosíntesis de antocianinas, que codifican las enzimas dihidroflavonol 4-reductasa y antocianidina 3-O-glucosiltransferasa (Miranda et al., 2020). Este aumento de los compuestos bioactivos también se ha atribuido al aumento de la actividad de la enzima PAL y a la chalcona sintasa, en las que la melatonina ejerce una regulación positiva sobre los genes que las codifican (Sharafi et al., 2021; Michailidis et al., 2021; Pang et al., 2023).

Los tratamientos con GABA incrementaron los fenoles totales y las antocianinas totales respecto al control, tanto en el momento de la cosecha como durante su almacenamiento, los fenoles totales aumentaron entre 11 y 34 mg 100 g^{-1} (16-45 %) y las antocianinas totales entre 22 y 45 mg 100 g^{-1} (36-82 %). También se analizaron las antocianinas individuales como la cianidina 3-rutinósido, la pelargonidina 3-rutinósido y la cianidina 3-glucósido que aumentaron sus niveles al tratarlas con GABA. En todas las variedades se ha observado a nivel general un incremento de las antocianinas y fenoles en los primeros 14 días de almacenamiento, descendiendo posteriormente independientemente del tratamiento, debido al proceso natural de maduración del fruto (Serrano et al., 2009; Valero et al., 2011), también se ha observado una alta correlación entre las antocianinas y los fenoles. Tratamientos precosecha con GABA 10 mM, 50 mM y 100 mM aplicados tres veces durante el desarrollo del limón, incrementaron el contenido de polifenoles el día de la cosecha y durante el almacenamiento tanto en el zumo como en el flavedo (Badiche-El Hilali et al., 2023). En granada la aplicación de cinco tratamientos de GABA 100 mM durante el desarrollo del

fruto en el árbol en dos campañas de cultivo, mantuvo las antocianinas totales y fenoles totales en niveles superiores al control en el momento de la cosecha y durante 60 días de almacenamiento frigorífico (Lorente-Mento et al., 2023b). En rosas tres aplicaciones precosecha con GABA cada 7 días con dosis de 20 mM, 40 mM y 60 mM, incrementaron el contenido total de polifenoles, carotenoides y flavonoides, aunque estos efectos fueron mayores cuando se combinó con la aplicación de cloruro de calcio (Ehsanimehr et al., 2024). Tratamientos postcosecha con GABA en tomate y carambola incrementaron los fenoles y flavonoides (Mohd Yusof et al., 2023; Li et al., 2024). El incremento de compuesto bioactivos por la aplicación de GABA se ha asociado con la activación de la vía de los fenilpropanoides, en la que se ha observado un aumento de la enzima PAL y una disminución de la enzima polifenol oxidasa (Aghdam et al., 2019; Feng et al., 2024).

5.4 Efecto de los elicitores en las enzimas antioxidantes en el momento de la cosecha y durante el almacenamiento

Las especies reactivas del oxígeno entre las que se encuentra el peróxido de hidrogeno, el radical superóxido y el radical hidróxido, se generan en la célula como consecuencia de su metabolismo, estos compuestos además se incrementan en condiciones de estrés y durante la maduración y senescencia del fruto, ocasionando peroxidación de las membranas lipídicas y daño al ADN y proteínas, todo esto acelera el proceso de senescencia del fruto (Hodges et al., 2004). Las células vegetales disponen de sistemas antioxidantes para neutralizar estas especies reactivas, por un lado tienen compuestos antioxidantes (ácido ascórbico, fenoles, carotenoides y tocoferoles) y por otro lado disponen de enzimas antioxidantes como catalasa, peroxidasa y ascorbato peroxidasa entre otras, con estos sistemas son capaces de eliminar las especies reactivas del oxígeno y reparar el daño oxidativo (Meitha et al., 2020; Sati et al., 2023).

Teniendo en cuenta los mejores resultados de los parámetros de calidad y los compuestos bioactivos, se decidió cuantificar las enzimas antioxidantes en la dosis de melatonina 0,3 mM. Se observó un incremento respecto al control de la actividad catalasa entre 15 y 40 U min^{-1} g^{-1} (15-35 %), la actividad ascorbato peroxidasa aumentó entre 8 y 20 U min^{-1} g^{-1} (7-18 %) y la actividad peroxidasa entre 3 y 9 U min^{-1} g^{-1} (5-15 %), aunque en esta última no hubo efecto en la variedad ‘Prime Giant’. En los intervalos anteriores se ha considerado tanto el día de la cosecha como el periodo de almacenamiento frigorífico. Aplicaciones con melatonina 0,1 mM y 0,2 mM en frambuesa tres días antes de la cosecha, mejoraron la actividad de las enzimas

antioxidantes y redujeron la actividad de la enzima polifenol oxidasa y el contenido de malondialdehído (Shah et al., 2024). La aplicación de melatonina 0,1 mM durante 5 minutos mediante inmersión de cerezas de la variedad 'Siah Mashhad', redujo la acumulación de peróxido de hidrogeno durante 45 días de almacenamiento y aumentó la actividad de las enzimas antioxidantes como catalasa, ascorbato peroxidasa, superóxido dismutasa y glutatión reductasa (Sharafi et al., 2021), este incremento de los sistemas antioxidantes en cereza se ha asociado a la amplia respuesta transcripcional desencadenada por la melatonina (Miranda et al., 2020). En fresa la aplicación de melatonina en postcosecha a dosis de 0,2 mM y 0,5 mM, redujo la concentración de malondialdehído y aumentó la actividad de la enzima catalasa, ascorbato peroxidasa, peroxidasa y superóxido dismutasa, alargando la vida útil de los frutos (Kahramanoğlu, 2024). Teniendo en cuenta varias especies vegetales se ha observado que la melatonina tiene propiedades antioxidantes importantes, pudiendo neutralizar las especies reactivas del oxígeno y las especies reactivas del nitrógeno, tanto de manera directa, como de manera indirecta aumentando las enzimas antioxidantes o mediante la supresión de las enzimas que promueven la oxidación, haciendo que los tejidos sean más resistentes al estrés y manteniendo los parámetros de calidad durante el almacenamiento postcosecha (Li et al., 2023; Tiwari et al., 2024).

Tras analizar los mejores resultados obtenidos del GABA con las distintas dosis para los parámetros de calidad y los compuestos bioactivos, se decidió cuantificar las enzimas antioxidantes en la dosis de GABA 50 mM. En este tratamiento se observó un incremento respecto al control de la actividad catalasa entre 25 y 98 U min^{-1} g^{-1} (19-86 %), la actividad ascorbato peroxidasa aumentó entre 13 y 40 U min^{-1} g^{-1} (12-43 %) y la actividad peroxidasa entre 3 y 16 U min^{-1} g^{-1} (4-28 %), estos datos incluyen tanto el momento de la cosecha como el periodo de almacenamiento frigorífico. En rosas tres aplicaciones precosecha con GABA cada 7 días antes de la recolección, con dosis de 20 mM, 40 mM y 60 mM, incrementaron la actividad superóxido dismutasa, catalasa y peroxidasa, además se redujo la actividad de la polifenol oxidasa, aunque estos resultados mejoraban si la aplicación se combinaba con cloruro de calcio (Ehsanimehr et al., 2024). Aplicaciones precosecha con GABA 2 mM y 4 mM en pimiento aumentaron la actividad de las enzimas antioxidantes catalasa, ascorbato peroxidasa, superóxido dismutasa y peroxidasa, tanto en plantas bien regadas como en plantas con estrés hídrico (Iqbal et al., 2023). Tratamientos postcosecha con GABA en fresa mediante inmersión de frutos en dosis de 5 mM, 10 mM y 15 mM, aumentaron la actividad catalasa y superóxido dismutasa mejorando el sistema antioxidante del fruto (Zhang et al., 2024), en papaya inmersión de frutos en 1 mM y 5 mM durante 5 minutos redujo los daños por frío, al aumentar la actividad catalasa, superóxido dismutasa, glutatión reductasa y ascorbato peroxidasa, además se redujo la

peroxidación lipídica, la fuga de electrolitos y el contenido en peróxido de hidrogeno (Khaliq et al., 2023).

Los tratamientos con GABA y melatonina han conseguido incrementar la capacidad de los tejidos para neutralizar las especies reactivas del oxígeno, debido a un incremento de la actividad de los enzimas antioxidantes y de los compuestos antioxidantes como fenoles y antocianinas, ocasionado un retraso en el proceso de senescencia y aumentando la vida útil de los frutos. Además, ambos elicitores están relacionados, se ha observado que los tratamientos con melatonina son capaces de activar la expresión de los genes de biosíntesis del GABA y suprimir los genes asociados con la degradación, generando un aumento del GABA endógeno en el fruto (Wu et al., 2023). Tanto la melatonina como el GABA son elicitores que están implicados en un gran entramado de rutas de señalización e influyen en numerosos procesos metabólicos, se ha observado que individualmente cada uno reduce los efectos negativos ocasionados por un estrés, pero sin embargo en los últimos ensayos aplicaciones conjuntas han mostrado un efecto sinérgico (Kabała et al., 2024).

5.5 Consideraciones actuales y posibles aplicaciones agronómicas

Teniendo en cuenta los resultados obtenidos, podría ser interesante realizar aplicaciones a gran escala en explotaciones comerciales, además, la melatonina, el GABA y el GA3 son compuestos naturales que se encuentran presentes en las plantas en dosis variables en función de la especie y variedad (Feng et al., 2014; Pencheva et al., 2023; Shah et al., 2023).

El GA3 aun siendo una sustancia natural se encuentra regulado por el Reglamento CE (Comunidad Europea) 1107/2009 relativo a la comercialización de productos fitosanitarios, además esta sustancia se encuentra incluida en el Anexo IV del Reglamento CE 396/2005 con carácter temporal, a la espera de la presentación del dictamen motivado por la Autoridad Europea de Seguridad Alimentaria (EFSA) (EC, 2024b). En este momento al encontrarse esta sustancia en el Anexo IV no se le exige LMR (EC, 2024c). En España actualmente hay tres productos a base de GA3 autorizados que se pueden utilizar en el cultivo del cerezo, los cuales permiten un rango de aplicación de 10 ppm hasta 40 ppm en todo el ciclo, es importante considerar el número de aplicaciones, ya que si se realizan dos, solo hay un producto que lo permite (MAPA, 2024). Teniendo en cuenta la normativa, los agricultores podrían aplicar perfectamente el tratamiento denominado como T30 y también se podría realizar una aplicación superior cercana al tratamiento T45, pero en el que se aplicarían únicamente

40 ppm como máximo en todo el ciclo de cultivo, el decidir una u otra depende del objetivo agronómico que se persiga.

En España el GABA y la melatonina son sustancias que actualmente están consideradas como complementos alimenticios, a través del reconocimiento mutuo de mercancías comercializadas legalmente en otro Estado miembro según el Reglamento (UE) 2019/515, teniendo en cuenta que para GABA la cantidad tiene que ser inferior a 500 mg y en melatonina inferior a 2 mg (AESAN, 2023). La Comisión Técnica de Nutrición, Nuevos Alimentos y Alérgenos Alimentarios evaluó las propiedades saludables del GABA en relación con la función cognitiva, pero no se demostró una relación causa efecto entre la ingesta de este compuesto y la función cognitiva, quedando sin autorización para tal efecto según lo establecido en el Reglamento (CE) 1924/2006 (EFSA Panel on Dietetic Products, Nutrition and Allergies, 2009). Esta comisión también evaluó las propiedades de la melatonina en relación con el alivio del desfase horario (jet lag) y llegó a la conclusión que la melatonina es beneficiosa para aliviar el desfase horario teniendo en cuenta una ingesta mínima de 0,5 mg por porción cuantificada (EFSA Panel on Dietetic Products, Nutrition and Allergies, 2010). Respecto a la melatonina la comisión técnica también estableció una relación causa efecto entre el consumo de melatonina y la reducción del tiempo necesario para conciliar el sueño, considerando una ingesta de 1 mg por porción cuantificada (EFSA Panel on Dietetic Products, Nutrition and Allergies, 2011), ambos efectos beneficiosos están autorizados y se pueden declarar en la etiqueta de los productos según el Reglamento (UE) 432/2012. El GABA y la melatonina son elicitores novedosos, que solo en casos puntuales se encuentran presentes en el sector agrícola, respecto al GABA comienza a aparecer en pequeña cantidad como complemento en algunos abonos y respecto a la melatonina hay productos disponibles para agricultura que provienen de extractos de plantas con un alto contenido en melatonina. Además, cuando se aplican estos elicitores en las plantas se debe de tener en cuenta que se incrementa el contenido endógeno de los frutos, tratamientos similares en dosis y número de aplicaciones con melatonina aumentaron en cereza el contenido endógeno de este compuesto de 0,002 mg kg^{-1} PF hasta 0,015 mg kg^{-1} PF en los frutos tratados (Cortés-Montaña et al., 2024), no se dispone de información sobre el incremento del contenido de GABA tras realizar tratamientos similares en cereza con este elicitor, pero en manzanas se incrementó el contenido endógeno de GABA de 30 mg kg^{-1} PF hasta 45 mg kg^{-1} PF (Cheng et al., 2023). Estos incrementos suponen una cantidad significativa, pero no llegan a aproximarse a las cantidades indicadas por porción cuantificada por la Comisión Técnica de Nutrición, Nuevos Alimentos y Alérgenos Alimentarios, para que se consideren los beneficios de salud demostrados. Aun así, las cerezas tratadas

presentan un contenido superior, aumentando la ingesta diaria de estos compuestos respecto al consumo de frutos sin tratar.

Respecto a las posibles aplicaciones agronómicas, técnicamente es posible realizar estos tratamientos a gran escala, ya que la forma de aplicación es mediante pulverización y los agricultores disponen de equipos de pulverización hidroneumáticos que utilizan para realizar aplicaciones foliares de fitosanitarios y abonos. Pero también se debe de valorar el factor económico, teniendo en cuenta el coste del producto y las aplicaciones necesarias, en la Tabla 3 se ha realizado una estimación del coste para cada uno de los elicitores, teniendo en cuenta los mismos parámetros que se han utilizado en el ensayo, es decir, manteniendo el volumen de caldo aplicado, la dosis y el mismo proveedor del producto utilizado.

Tabla 3. Características técnicas de los tratamientos y coste económico total y por aplicación para la melatonina, el ácido gamma-aminobutírico (GABA) y el ácido giberélico (GA3).

Tratamiento		Volumen	Cantidad de materia activa	Coste de materia activa	Coste por aplicación	Número de aplicaciones	Coste total
		l/ha	g/ha	€/g	€/ha	-	€/ha
Melatonina	0,1 mM	2000	46,5	85,30	3.963	3	11.888
	0,3 mM	2000	139,4	85,30	11.888	3	35.664
	0,5 mM	2000	232,3	85,30	19.813	3	59.439
GABA	10 mM	2000	2.062,4	0,385	794	3	2.382
	50 mM	2000	10.311,9	0,385	3.970	3	11.910
	100 mM	2000	20.623,8	0,385	7.940	3	23.820
GA3	15 ppm + 15 ppm	1500	22,5	0,96	22	2	43
	25 ppm + 20 ppm	1500	37,5*	0,96	36*	2	65
	30 ppm + 30 ppm	1500	45,0	0,96	43	2	86

* Se refiere a la primera aplicación de 25 ppm.

El GA3 es el producto que necesita menos cantidad de materia activa por aplicación y es el elicitor que necesita menos aplicaciones, con dos pases se consigue el efecto deseado, incluso muchas veces se realiza una única aplicación obteniendo buenos resultados, este producto se encuentra comercialmente en el mercado y es una fitohormona muy utilizada en diferentes cultivos (alcachofa, vid, peral y cítricos). Respecto al coste por unidad de materia activa el GA3 presenta un coste intermedio, pero si se tiene en cuenta la dosis empleada y el número de aplicaciones, es el elicitor

más económico para los agricultores. Respecto a la melatonina y el GABA, ambos han presentado resultados similares con ligeras diferencias en algunos parámetros, aunque respeto a compuestos bioactivos y enzimas antioxidantes, el GABA tiene valores superiores a la melatonina, por lo tanto sería la herramienta más adecuada a utilizar en campo, además es la que tiene un coste menor, pero aun así presenta unos costes inasumibles para el agricultor. Por lo tanto, en la melatonina y el GABA es necesario buscar materias primas alternativas, que aunque no tengan una pureza tan elevada como la utilizada en el ensayo, sean productos económicamente viables y efectivos, para ello se deberá evaluar su eficacia en campo para determinar si su efecto es similar a los compuestos puros.

6. Conclusiones

6 CONCLUSIONES

En esta Tesis Doctoral se ha evaluado la aplicación precosecha de elicitores novedosos en cereza, entre los que se encuentra la melatonina y el GABA, también se ha evaluado el efecto del GA3 que es un elicitor ampliamente utilizado, estas herramientas se han enfocado como estrategias precosecha para mejorar la calidad de los frutos, tanto en la cosecha como durante su almacenamiento postcosecha.

Tras evaluar los resultados obtenidos en todas la variedades y campañas, se puede concluir que tanto la melatonina como el GABA tienen efectos similares sobre la calidad de la cereza en el momento de la cosecha, ya que aumentaron los sólidos solubles totales, la acidez total, la firmeza, el contenido de fenoles totales, el contenido de antocianinas totales y la actividad de las enzimas antioxidantes (catalasa, ascorbato peroxidasa y peroxidasa), además todos estos parámetros se mantuvieron en niveles más altos en los frutos tratados que en los controles durante el almacenamiento frigorífico y se redujeron las pérdidas de peso durante este periodo. El aumento de los sistemas antioxidantes tanto enzimáticos como no enzimáticos, como consecuencia de la aplicación de melatonina y GABA permitieron que las células fueran más eficientes en la neutralización de las especies reactivas del oxígeno, retrasando el proceso senescencia y aumentando la vida útil del fruto. Además, los frutos tratados presentan un contenido más alto de compuestos bioactivos (polifenoles y antocianinas) con propiedades antioxidantes que tienen beneficios para la salud. Para ambos elicitores se llegó a la conclusión que la dosis más efectiva en el cultivo del cerezo es 50 mM para GABA y 0,3 mM para melatonina, respecto a las enzimas antioxidantes y compuestos bioactivos el GABA ha presentado resultados ligeramente superiores, por lo que sería el más interesante a la hora de utilizar en el cultivo del cerezo, además su coste es inferior al de la melatonina. Independientemente de que el coste del GABA sea inferior al de la melatonina, con los productos utilizados no es rentable la aplicación para los agricultores, por lo que se deberían de buscar otros productos que contengan estos elicitores y sean más económicos, además de verificar que se mantiene la eficacia.

El GA3 es una hormona muy utilizada en los cultivos agrícolas, en el cerezo esta herramienta es empleada por los productores para incrementar la firmeza y el tamaño del fruto, pero se dispone de poca información sobre el efecto que tiene en las propiedades reológicas del fruto. En los ensayos realizados se ha verificado que las aplicaciones de GA3 proporcionan mayor resistencia al tejido en el momento de la cosecha, debido a un incremento del módulo de elasticidad y de la tensión en el punto máximo. Además, durante el almacenamiento en bolsa MAP durante 35 días a 0°C, los tratamientos mantienen un alto módulo de elasticidad y una tensión mayor en el punto

máximo, pero sufren una reducción de la deformación del tejido ocasionando un aumento de la rigidez. También se observó que conforme avanza la madurez de los frutos, se reduce el daño mecánico inducido como consecuencia del aumento de la deformación del tejido, por lo tanto, a la hora de realizar la cosecha se debe de evaluar adecuadamente el grado de madurez de los frutos para minimizar este problema. Teniendo en cuenta los resultados obtenidos, el GA3 es una herramienta que deben seguir utilizando los productores, ya que incrementa la calidad de la cereza con un reducido coste. La dosis a emplear dependerá del objetivo perseguido, pero con dos aplicaciones de 15 ppm se han obtenido unos parámetros calidad adecuados y el retraso en la cosecha ha sido nulo en 'Lapins' y de 2 días en 'Bing', sin embargo con dosis de 45 ppm fraccionadas en dos aplicaciones, la recolección se ha retrasado 4 días en ambas variedades, que es un aspecto importante que deberán considerar los productores.

7. Futuras Líneas de Investigación

7 FUTURAS LÍNEAS DE INVESTIGACIÓN

Como futuras líneas de trabajo en base a los resultados obtenidos en esta investigación, propondría realizar los siguientes ensayos:

- Teniendo en cuenta el coste inasumible para los agricultores del GABA y la melatonina, en primer lugar se debería realizar una búsqueda de productos que contengan estos elicitores y estén disponibles a precios más bajos que los utilizados en el ensayo, en los que los elicitores presentaban una pureza igual o superior al 98 % y por lo tanto su precio es muy elevado para su empleo en agricultura. Para ello, se debería contactar con los distintos fabricantes y realizar un estudio económico del precio que pueden ofertar para su uso en agricultura, teniendo en cuenta las tres aplicaciones que hay que realizar y si el estudio de viabilidad no es favorable, no continuaría con esta línea. Junto con esta propuesta de investigación, si fueran viables económicamente estos productos, se debería realizar una nueva evaluación para corroborar los resultados obtenidos. Si el paso anterior es favorable, en último lugar se debería estudiar el comportamiento de estos elicitores con los distintos tipos de agua, fertilizantes y fitosanitarios utilizados en agricultura, para que el agricultor lo pueda mezclar en la cuba y reducir los costes de aplicación, además de simplificar el manejo agronómico.

- Monitorizar la concentración de melatonina y GABA desde fruto cuajado hasta el final de la postcosecha, para observar cómo varia la concentración de los distintos elicitores y determinar cómo incrementa su concentración tras realizar las distintas aplicaciones y se mantiene durante el tiempo. Una vez conocido el comportamiento durante este periodo, intentar optimizar las aplicaciones para observar si con un número inferior es posible hacer viable su aplicación en campo. Además, también se obtendría información importante de que cantidad de estos compuestos llegan al consumidor final en el fruto.

- Por otro lado, teniendo en cuenta las experiencias previas en otros cultivos, podría ser interesante evaluar la combinación de melatonina y GABA, para aprovechar el efecto sinérgico que se indica en algunos ensayos de la literatura (Lv et al., 2023; Rastegar et al., 2024) y verificar si este comportamiento también se da en el cultivo del cerezo.

- Respecto a la aplicación de GA3, se podría evaluar más en profundidad las posibles implicaciones de los tratamientos realizados sobre el cracking en campo, desde color pajizo hasta recolección, ya que el riesgo de lluvias siempre está presente. En postcosecha también se podría estudiar más en profundidad el cracking en bolsa MAP, ya que es una situación en la que las cerezas tratadas con GA3 presentan tejidos más rígidos y con una menor deformación que los controles, estos factores junto con la alta humedad de la bolsa MAP genera las condiciones propicias para que se desarrolle este fenómeno.

- También sería interesante evaluar el efecto de los elicitores en función de distintas cargas frutales para un mismo año, en el que el factor ambiental es igual para todos los árboles, esto se podría realizar mediante un aclareo de frutos después de cuajado dejando una carga baja, media y alta (sin aclareo). Cuando las cargas frutales son más altas el árbol está sometido a un mayor estrés, este ensayo proporcionaría información importante para ver si en situaciones de altas cargas tienen mayor potencial este tipo de elicitores.

- Teniendo en cuenta la situación de escasez hídrica que tienen gran parte de las zonas productoras del mundo y conociendo los efectos beneficiosos de estos elicitores frente al estrés abiótico, también se podría plantear un ensayo en el que la dosis de riego fuera variable para determinar si los elicitores son capaces de minimizar los efectos ocasionados por el déficit hídrico, manteniendo una producción y calidad adecuada.

Estas futuras líneas de investigación se han dado desde un punto de vista científico teniendo en cuenta la bibliografía hasta el momento, de manera que estas nuevas investigaciones aporten un mayor conocimiento sobre estos elicitores. Pero considero que también se debería tener en cuenta el coste de estas estrategias, de manera que si se obtienen resultados favorables estos se puedan aplicar al sector productivo.

8. Bibliografía

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